No. 2020-1037

UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

IMMUNEX CORP., AMGEN MANUFACTURING, LTD.,
HOFFMAN-LA ROCHE, INC.,

Plaintiffs-Appellees,

v.

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, SANDOZ GMBH,

Defendants-Appellants.

Appeal from the United States District Court for the District of New Jersey
Civil Action No. 16-1118-CCC (Cecchi, J.)

APPELLANTS' OPENING BRIEF

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CERTIFICATE OF INTEREST

Counsel for Appellants, William M. Jay, certifies the following:

1. The full name of every party or amicus represented by me is:

   Sandoz Inc.
   Sandoz International GmbH
   Sandoz GmbH

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

Sandoz Inc.:	None
Sandoz International GmbH:	None
Sandoz GmbH:	None

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

Sandoz Inc.:	Novartis AG
Sandoz International GmbH:	Novartis AG
Sandoz GmbH:	Novartis AG

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in the court (and who have not or will not enter an appearance in this case) are:

   James Richter, Merritt Westcott, Karalena Guerrieri, Noorossadat Torabi, and Thomas Melsheimer of Winston & Strawn LLP

   James Hilmert, Melinda Lackey, Michael Meneghini, and Tyler Johannes, formerly of Winston & Strawn LLP

   Eric I. Abraham of Hill Wallack LLP

   Yevgenia Kleiner and Christina Saveriano, formerly of Hill Wallack LLP

5. The title and number of any case known to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal.

Immunex Corporation et al. v. Samsung Bioepis Co., Ltd., No. 19-cv-11755 (D.N.J.)

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November 8, 2019

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STATEMENT OF RELATED CASES

No appeal in or from the same district court proceeding was previously before this or any other appellate court.

Counsel is aware of one case pending in this or any other court or agency that will be directly affected by this Court's decision in the pending appeal: *Immunex Corporation et al. v. Samsung Bioepis Co., Ltd.*, No. 19-cv-11755 (D.N.J.).

TABLE OF ABBREVIATIONS

’182 Patent	Asserted U.S. Patent No. 8,063,182 (Appx12684-12718)
’522 Patent	Asserted U.S. Patent No. 8,163,522 (Appx12719-12766)
’029 Patent	U.S. Patent No. 5,808,029 (Appx30905-30923)
’225 patent	U.S. Patent No. 7,915,225 (Appx27246-27261)
’690 Patent	U.S. Patent No. 5,605,690 (Appx27295-27321)
2004 Agreement	Accord and Satisfaction Agreement between Roche, Wyeth, Amgen, and Immunex (Appx25836-25864)
aBLA	Abbreviated biologics license application
CH2, CH3	Immunoglobulin constant region heavy chain domain 2; constant region heavy chain domain 3
Fc	Crystallizable fragment of immunoglobulin
GATT	General Agreement on Tariffs and Trade, implemented by the Uruguay Round Agreements Act
IgG	Immunoglobulin G (IgG_1: immunoglobulin G, subclass 1)
ODP	Obviousness-type double patenting
p55 receptor	TNF receptor subtype weighing approximately 55 kilodaltons
p75 receptor	TNF receptor subtype weighing approximately 75 kilodaltons
Psoriasis patents	U.S. Patent Nos. 7,915,225, 8,119,605, and 8,722,631 (Appx27246-27261; Appx27262-27277; Appx27278-27294)

Smith 1990	Smith et al., "A Receptor for Tumor Necrosis Factor Defines an Unusual Family Of Cellular and Viral Proteins," Science 248:1019-1023 (1990) (Appx26978-26982)
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TNFR:Fc	Fusion protein comprising TNFR and Fc

INTRODUCTION

At the heart of patent law is a bargain: a limited period of statutory exclusivity in exchange for disclosing innovation. 35 U.S.C. §154. Once the patent term expires, the invention "covered by the patent becomes public property." *Singer Mfg. Co. v. June Mfg. Co.*, 163 U.S. 169, 185 (1896). In the face of this rule, Plaintiff-Appellee Immunex and its corporate parent Amgen (collectively, "Immunex") are now well into their *third decade* of exclusivity for claims covering the protein etanercept, the active ingredient in Immunex's biologic product Enbrel®. On the market since 1998, Enbrel captures close to $5 billion in annual U.S. sales (Appx5791), yet it still faces no biosimilar competition. Under the decision below, that exclusivity will extend until 2029.

How did Immunex attempt such an extraordinary extension of its patent term? Another company, Plaintiff-Appellee Roche, had been working to develop proteins that would compete with Enbrel. When Roche's clinical trials for its protein failed, Immunex—anticipating the end of its lucrative exclusivity when its own etanercept patents expired—struck a deal with Roche. Their "Accord and Satisfaction" (the "2004 Agreement") gave Immunex control of the "pre-GATT" patent applications that Roche had filed in 1995. Although Immunex insisted on calling it a license, the 2004 Agreement functioned as an assignment by providing Immunex with all substantial rights to the applications—including complete control over prosecution,

the exclusive right to commercial use, the ability to sue for infringement and control infringement litigation, and the right to grant royalty-free sublicenses. Though Roche did not develop, describe, or claim etanercept, Immunex used its exclusive prosecution authority to amend the claims *and* specifications to shoehorn etanercept into the Roche applications.

The reworked applications issued to Immunex in 2011 and 2012 as U.S. Patent Nos. 8,063,182 (the "'182 patent"), and 8,163,522 (the "'522 patent"), respectively—each for a term of 17 years from issuance. These patents, through the irrevocable 2004 Agreement, give Immunex the exclusive right to commercial use of etanercept for 15 years after its original patent claiming etanercept expired and 31 years since Enbrel was first marketed—until 2029.

The FDA approved Sandoz's etanercept biosimilar in 2016, but this litigation has kept Sandoz off the market. The patents Immunex asserted here to keep Sandoz's biosimilar from the public are invalid for three reasons.

First, the patents violate the equitable prohibition on obviousness-type double patenting ("ODP"), which "forbids an individual from obtaining more than one patent on the same invention" and its obvious variants. *AbbVie Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Tr.*, 764 F.3d 1366, 1372 (Fed. Cir. 2014). Immunex cannot evade ODP by mislabeling the 2004 Agreement with Roche as a "license" rather than an assignment. The bar against ODP "prevent[s] unjustified

timewise extension of the right to exclude … *no matter how the extension is brought about*." *In re Hubbell*, 709 F.3d 1140, 1145 (Fed. Cir. 2013) (emphasis added) (quotation marks omitted).

Second, the patents-in-suit fail to satisfy the written-description requirement of 35 U.S.C. §112. Etanercept is a fusion protein combining a tumor necrosis factor receptor ("TNFR") with a molecular weight of approximately 75 kilodaltons (the "p75 receptor"), and an immunoglobulin molecule ("IgG_1"). The Roche applications were focused on a shorter "p55 receptor" invented at Roche; they were not directed toward etanercept until Immunex repurposed them. The Roche priority application does not disclose the full-length p75 receptor, much less the p75-IgG_1 etanercept protein claimed by the patents-in-suit.

Third, the asserted claims are obvious. All elements of the claimed invention were in the prior art, and Immunex concedes there was a reasonable expectation that they would work if combined. Moreover, the prior art clearly encouraged the combination, as illustrated by parallel work that created similar fusion proteins—including by Immunex itself, which actually invented etanercept.

The district court's contrary decision rested on basic legal errors. The Court should reverse the judgment below, dissolve the injunction, and allow the public to *finally* enjoy the benefits of biosimilar competition.

STATEMENT OF JURISDICTION

The district court had jurisdiction under 28 U.S.C. §§1331 and 1338, and entered final judgment for Plaintiffs on October 8, 2019. Sandoz noticed this appeal the same day. This Court has jurisdiction under 28 U.S.C. §1295(a).

STATEMENT OF ISSUES

1. Whether the patents-in-suit are invalid for ODP, where Immunex—which owns all substantial rights in those patents, including the ability to control patent prosecution—had already obtained earlier-expiring patents claiming obvious variants of the same inventions.

2. Whether the claims-in-suit are invalid for lack of written description, where the original specification did not disclose the key claimed features of etanercept and Immunex had to amend the specification to add them.

3. Whether the district court's ruling on obviousness was infected by legal error.

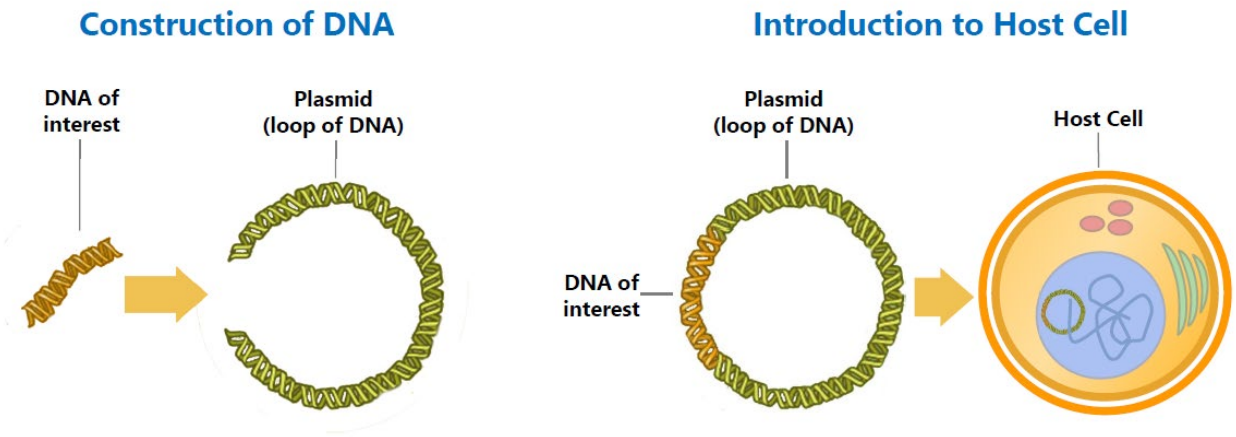
STATEMENT OF THE CASE

I. Immunex obtains patents that give it control over the etanercept franchise for more than two decades.

A. Fusion proteins.

Proteins are made up of a series of smaller molecules called amino acids. Appx4107. A protein consists of dozens to hundreds of amino acids. *Id.* Human DNA provides the code for constructing these amino acids. Appx4109-4110. As of

the priority date in 1990, scientists had learned how to use cells to make proteins from a given piece of DNA. Scientists could introduce a specific sequence into a loop of DNA, insert that DNA into a cell, and have the cell make the protein of interest (Appx4109-4110):

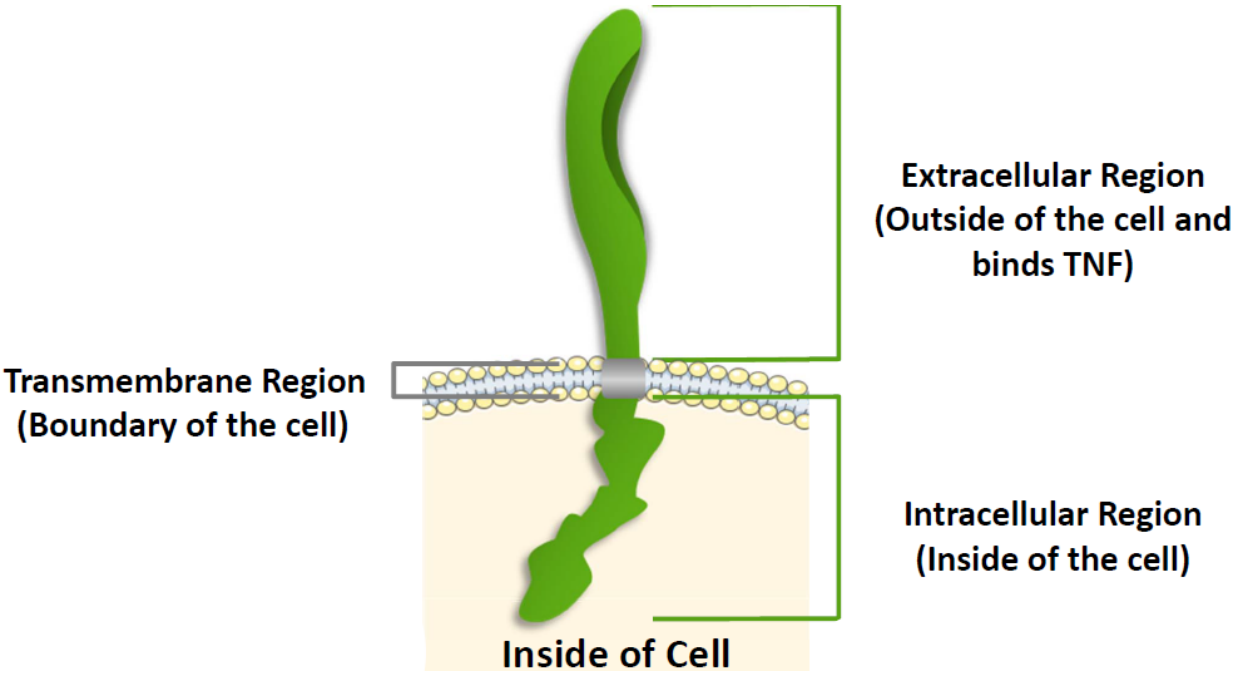


Appx7002; Appx4110-4111. This technique can also be used to combine desired parts of *different* proteins, creating new "fusion" proteins that do not occur naturally.

B. TNF and TNFRs.

Tumor necrosis factor ("TNF") is a messenger protein that helps initiate an immune response when it binds to receptors on the surface of human cells. Appx5; Appx4112-4113. While TNF is beneficial, too much TNF can trigger several known autoimmune disorders, including rheumatoid arthritis. Appx4112-4114; Appx4151-4154.

The TNFR has three parts, the most significant of which is the extracellular region, which is outside the cell and binds to TNF:



Appx7003; Appx4114-4115.

In the late 1980s to 1990, there was tremendous interest in studying whether blocking TNF from binding to its cell-surface receptors would provide a therapeutic effect. Appx4116-4117; Appx7. By the priority date, major biotech institutions were focused on using portions of the body's own TNFRs to remove TNF from the body. Appx4155-4159. At least two different TNFRs were known: a smaller p55 TNFR (weighing approximately 55 kilodaltons), and a larger p75 TNFR. Appx4115-4116.

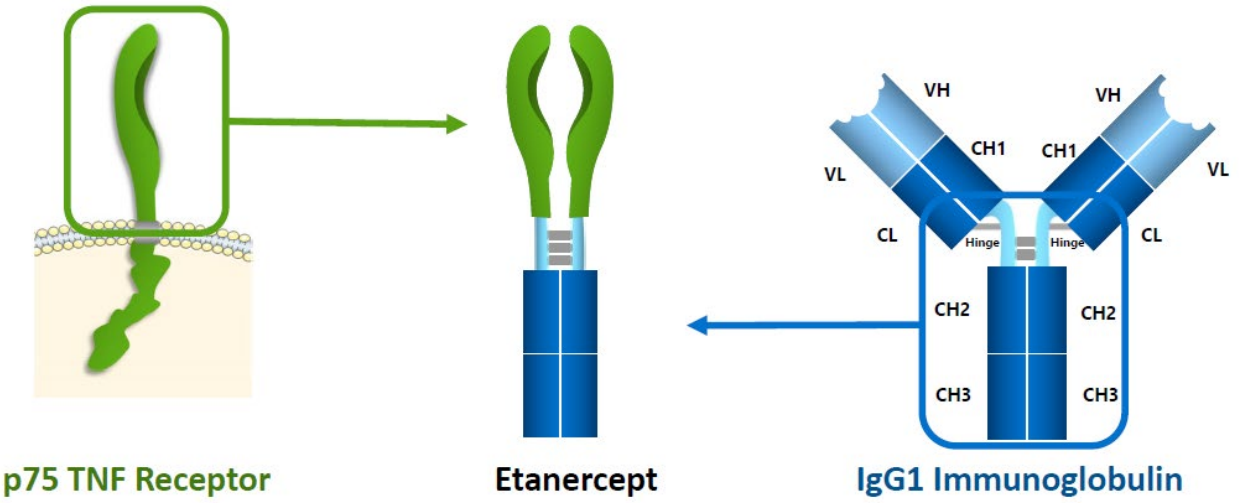
Much of the institutional research focused on cloning (*i.e.*, isolating and identifying the DNA sequence for) "soluble" forms of these TNFRs, which are portions of the extracellular region that, when cut off from the cell surface, would still bind TNF in *in vitro* assays. Appx5; Appx4116-4117. By 1990, several

researchers were also studying means to enhance the properties of soluble receptors, including TNFRs, by creating TNFR-based fusion proteins. Appx4176-4177; Appx10602. These fusion proteins were considered useful for studying TNF *in vitro*, as potential diagnostic assays, and as potential treatments for various conditions, from HIV/AIDS to rheumatoid arthritis. Appx4161-4162; Appx4209-4211; Appx4832; Appx28349; Appx28150-28151.

C. Immunex wins the race to sequence the full p75 receptor and invents etanercept.

Immunex led the work on TNFRs and TNFR fusion proteins, focusing on the p75 receptor. By October 1989, Immunex became the first to clone the full-length p75 receptor, publishing its full-length sequence in *Science* in May 1990 ("Smith 1990"). Appx26978; Appx10602; Appx28264.

Shortly thereafter, in late 1990, Immunex became the first to make the p75 TNFR-IgG_1 fusion protein now known as etanercept. Appx28266; Appx5269. Etanercept combines the extracellular portion of a p75 receptor with the hinge-CH2-CH3 portion of a human IgG_1 protein:



Appx7000; Appx4105-4106.

The Roche inventors of the patents-in-suit played *no* role in Immunex's development of etanercept. Appx28254; Appx28260; Appx5169; Appx4794.

D. Immunex obtains the reference patents.

Etanercept became the active ingredient in Immunex's product Enbrel. Appx11496. Immunex obtained a series of patents directed to etanercept and methods of using etanercept, the last expiring in 2019.

1. The '690 patent.

U.S. Patent No. 5,605,690 (the "'690 patent") was filed in 1995, issued in 1997, and expired in 2014. Appx27295. Claim 3 recites, in pertinent part, administering "a TNF-lowering amount of a chimeric antibody comprising a TNFR comprising the sequence of amino acids 3-163 of SEQ ID NO:1 fused to the constant domain of an immunoglobulin molecule." Appx27320. As described in detail below, that claim covers etanercept. *See* pp. 42-46, *infra*; Appx4145-4146.

Until its expiration, Immunex listed the '690 patent on Enbrel's label as covering Enbrel. Appx11504.

2. The psoriasis patents.

Immunex also obtained three patents on methods of treating psoriasis using etanercept. The earliest, U.S. Patent No. 7,915,225 (the "'225 patent"), issued in 2011, and all three expired on August 13, 2019. Appx27246; Appx27262; Appx27278. Claim 1 of each patent recites methods of treating psoriasis by administering a therapeutically effective dose of "TNFR:Fc," which has been defined to mean etanercept. Appx27261; Appx27277; Appx27294; *see* Appx4123-4125. These claims would have directed a skilled artisan, using routine steps, to produce etanercept. Appx4131; Appx4133-4134; *see also* Appx4110-4112.

II. Roche invents different fusion proteins.

A. The Roche inventors developed different TNFR sequences and different fusion proteins.

The Roche inventors were also interested in using TNFR fusion proteins. Appx4831-4832. But their work differed from Immunex's in two key ways.

First, the Roche inventors' efforts focused on the p55 receptor, which they cloned in October 1989. Appx26957; Appx4802; Appx3834; Appx28234. This research led to Roche's development and testing of a p55-IgG_1 fusion protein in clinical trials, starting in 1993, but those trials failed. Appx28353; Appx5752-5753.

Second, the Roche inventors tried, but failed, to clone the full-length p75 receptor. Appx4824; Appx4868-4869; Appx5070; Appx5074; Appx28415. Rather than isolating the whole p75 receptor, Roche scientists isolated only a partial p75 cDNA clone with several key mutations (the "truncated/mutated p75 receptor") Appx4824; Appx4866.

The inventors' truncated/mutated p75 receptor differed from Immunex's full-length p75 receptor. Most importantly, as compared to the sequence of 235 amino acids comprising the full p75 extracellular region, Roche's truncated/mutated p75 receptor does not include the first 48 amino acids. Appx4450; Appx4453; Appx5036; Appx4855. This accounts for a substantial portion—20 percent—of the extracellular region. Appx4453-4454; Appx5037. Roche's sequence also omits the first 22 amino acids comprising the signal sequence, which is essential to protein secretion from the cell. Appx4453-4454. In addition, that truncated/mutated p75 receptor contains four differences in amino acids. Three of those are mutations from the full-length p75 sequence that would have significantly affected the protein's properties. Appx4456-4461; Appx4855. For instance, Roche's receptor substitutes the amino acid arginine for methionine at the 196th position, which leads to a different shape and function and is associated with susceptibility to lupus. *See* Appx4458-4459. The fourth difference is that Roche's receptor has one extra amino

acid at residue 369, which leads to significant structural differences. Appx4460-4461.

Having failed to sequence the full-length p75 receptor, Roche did not seriously pursue p75 fusion proteins. Appx4794; Appx4845; Appx4848-4849. Indeed, when Roche sought to use etanercept in a study to compare its efficacy against Roche's p55-IgG_1 protein, Roche had to *borrow the protein from Immunex*. Appx10611.

B. Roche sought to patent its own TNFR, representing that it was patentably distinct from Immunex's receptor.

Roche sought and received U.S. Patent No. 5,808,029 (the "'029 patent") covering its truncated/mutated p75 receptor. Appx30923. During prosecution, Roche insisted to the PTO that its truncated/mutated p75 receptor was patentably distinct from Immunex's full-length p75 receptor. Appx31502-31503. Roche represented that Immunex's full-length p75 receptor, disclosed in Smith 1990, is "a cDNA sequence encoding a human TNF-R of about 80 kD, whereas applicants' claim a purified and isolated polynucleotide encoding an insoluble protein which has an apparent molecular weight of about 75 kilodaltons." *Id.*; *see* Appx31500. And Roche emphasized that its truncated/mutated TNFR contains the three mutations and one extra amino acid, described above. Appx31501-31502.

C. Roche's priority application for the patents-in-suit discloses fusion proteins based on *Roche's* TNFR sequences.

By April 1990, the Roche inventors had cloned the p55 receptor and the truncated/mutated p75 receptor and filed for a Swiss patent describing and claiming those proteins. Appx27350. The inventors' subsequent filings, including U.S. patent applications in the same family as the patents-in-suit, disclosed only the two TNFR sequences the Roche inventors had discovered—the p55 receptor (Figure 1) and the truncated/mutated p75 receptor (Figure 4)—and fusion proteins based on those sequences. Appx24589-24590; Appx24593-24594; Appx25139-25140; Appx25143-25144; Appx24476-24477; Appx24480-24481. Roche's priority application did not discuss the full-length p75 receptor, or a fusion protein using that receptor.

1. Roche's original specification does not disclose the full-length p75 receptor.

Given Immunex's publications, the Roche inventors could have described Immunex's full-length p75 receptor in the specification. Appx5063-5064. They did not. Instead, they chose to describe the truncated/mutated p75 receptor that they had obtained. Appx5061; Axxp5064-5065; Appx4863.

Specifically, the priority application is based on two disclosed sequences for TNFRs: Figure 1, disclosing the full p55 sequence, and Figure 4, disclosing the "[n]ucleotide sequence and deduced amino acid sequence for cDNA clones derived

from 75/65 kD TNF-BP." Appx25084. The rest of the application rests *entirely* on these sequences, and close variants thereof. There is no dispute that Figure 4 is the only p75 receptor sequence disclosed in the specification. Appx4448; Appx4961-4962. Figure 4, and smaller fragments of it, is the only p75 receptor mentioned in the Summary of the Invention. Appx25083-25084; Appx5050. The Detailed Description of the Invention likewise defines Figure 4 as *the* p75 portion of the invention, Appx25085; Appx25090; Appx4462-4463, and describes the "present invention" as TNF-binding proteins "containing the amino acid sequence depicted in Figure l or in Figure 4," Appx25085; *see also* Appx25090; Appx4464-4465. None of the examples describe a full-length p75 receptor. Example 8, for instance, describes only Roche's cloning of the truncated/mutated p75 leading up to filing the priority application. Appx25113-25114; Appx4469-4470.

2. The priority application does not disclose a p75-IgG$_1$ fusion protein that incorporates the p75 receptor.

Fusion proteins combine specific proteins (or parts thereof) at a specific place. Appx4492-4493; Appx4496-4497; Appx4515. Roche's original specification does not disclose the claimed p75-IgG$_1$ protein because it does not describe *any* of the requisite parts or how to arrange them. Appx4492-4493.

The specification describes the immunoglobulin portion of the invention as a "partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE." Appx25083-

25084; Appx4483. A skilled artisan understood that there were 11 potential immunoglobulins within these classes. Appx28617-28618; Appx27869-27870. Moreover, even after selecting a particular immunoglobulin, the specification statement encompasses a pantheon of potential hinges and a universe of variations, and does not describe the specifically-claimed exon-encoded hinge-CH2-CH3 of IgG_1. Appx4483-4484.

The specification provides only one example of DNA that could be used to make a p55-IgG_3 fusion protein. Appx4495; Appx4514; Appx4515. It was undisputed that: (1) the p55 is a very different gene product than the p75, Appx4495, and (2) IgG_3 is a different immunoglobulin from IgG_1, with a hinge four times as long and a different sequence compared to the IgG_1 hinge, *id.* Because of these differences, a skilled artisan could not have used Example 11 to make a p75-IgG_1 fusion protein. Appx4496-4497.

3. During its prosecution, Roche consistently pursued claims directed to the p55 receptor.

In May 1995, Roche filed divisional applications that led to the patents-in-suit. Appx12686; Appx12721. By filing these applications days before the Uruguay Round Agreements Act ("GATT") went into effect, Roche obtained patent terms running 17 years from issuance. Appx28330.

The divisional applications focused on the p55 receptor. Under Roche's control from May 1996 until late 2004, all claims in the '182 patent application

related only to the p55 receptor. Appx13060-13061; Appx13077-13078; Appx13098-13099; Appx13253-13255; Appx13379-13381; Appx28329-28330; Appx28331; Appx28331-28332; Appx28332-28333. Roche sought six extensions, adding over a year to the prosecution. Appx13046; Appx13058; Appx13087; Appx13108; Appx13397; Appx13416. Moreover, on three occasions between 1996 and 1998, Roche prevented the patent from issuing by ignoring communications from the PTO. Appx13056; Appx13076; Appx13096.

Similarly, the '522 patent application was filed with a preliminary amendment, in which all claims related to the p55 receptor. Appx19150; Appx19156-19158; Appx28337-28338. In August 2000, Roche amended the claims to relate to both the p55 and its truncated/mutated p75 receptor. Appx19159-19161. In response, the examiner issued a restriction, and Roche again elected the p55 receptor. Appx19528; Appx28341; Appx19575. While Roche controlled prosecution, it added almost one year to the process by requesting four extensions. Appx19580; Appx19607; Appx19761; Appx19778.

III. Immunex and Roche enter into a transaction allowing Immunex to extend its patent control over etanercept.

A. The 2004 Agreement transferred all substantial rights in the patents-in-suit to Immunex.

In 1998, after Immunex obtained FDA approval for Enbrel, Roche and Immunex entered into an agreement to cross-license their respective patents and

applications involving TNF receptors. Appx25865-25891. This agreement, which extended to the applications that became the patents-in-suit, provided Immunex with all it needed to market Enbrel. Appx5754; Appx28280; Appx25879.

Subsequently, Roche and Immunex entered into the 2004 Agreement, which turned Immunex into the *de facto* owner of the applications from which the patents-in-suit issued. Appx25836-25864. That Immunex paid Roche only $45 million—just nine days of Enbrel revenue—is consistent with the fact that Roche's applications were not directed to etanercept. Appx5790-5792.

The stated purpose of the 2004 Agreement was for Immunex "to *acquire all rights* licensed pursuant to the [1998] Roche-Immunex Agreement and to eliminate the continuing obligations to pay royalties to Roche" under the 1998 Agreement. Appx25836. The 2004 Agreement further stated that "Roche is willing to *sell such rights* in accordance with the terms of" the 2004 Agreement. *Id.* (emphasis added).

Among other things, the 2004 Agreement gave Immunex:

- The exclusive right to make, use, sell, offer for sale, and import the claimed inventions. Appx25839; Appx25839.
- The absolute right to exclude anyone, including Roche, from commercializing the claimed inventions. Appx25839.
- The complete, unfettered right to sublicense the patents. Appx25839; Appx28335; Appx5762-5763.
- The first right to sue for infringement and to then control litigation it initiated, including unilateral authority to settle and the right to collect

all damages. Appx25840; Appx5769-5770; Appx5771; Appx5775; Appx28336; Appx2833.

- The complete, unfettered right to control the prosecution of the patent applications. Appx25840; Appx28335-28336; Appx5763-5765; Appx5772.

Roche could not terminate the agreement for any reason. Appx25848.

Roche had expected to receive "an offer from [Immunex] to purchase [the] patents covering Enbrel." Appx11494; Appx28321-28322; Appx28324. But Roche "couldn't get [Immunex] to agree to have [the patents] assigned" to Immunex because Immunex "preferred a license." Appx28324-28325. Immunex had ample reason for that preference. Immunex's lead negotiator testified that he recognized that the ODP doctrine could apply to patents that became commonly owned through assignments. Appx5784. Notably, ODP law does not apply outside the United States, and the transfer of patent rights outside of North America to Wyeth in the same agreement—which are the same as the rights provided to Immunex for the U.S. patents in every material respect—was straightforwardly called an "assignment." Appx25838. Underscoring the fiction of the parties' label, Immunex could convert the "license" into a formal assignment for just $50,000. Appx25840. That clause was included at Immunex's insistence, as Roche was willing to formally assign the patent applications at no additional cost. Appx28335.

B. Immunex repurposes the patents-in-suit to focus on the p75 receptor.

In October 2004, Roche transferred its powers of attorney for the '182 and '522 patent applications to Immunex's attorneys. Appx28333-28334; Appx28341-28342; Appx13641-13648; Appx19782-19789. Immunex then repurposed the claims to cover etanercept.

In January 2005, Immunex amended the '182 application to claim either a p55 *or* a p75 TNFR:Fc fusion protein. Appx13659; Appx13665-1366. Then, in October 2005, Immunex amended the claims to remove all references to the p55 receptor, so for the first time, all claims related exclusively to the p75 TNFR:Fc fusion protein. Appx15931; Appx15933-15938; Appx5582.

In November 2006, Immunex amended the specification to include a reference to an October 2006 deposit of a plasmid related to the p75 receptor. Appx16424-Appx16425; Appx5788. This was the first time the full-length p75 receptor was incorporated into the specification. Appx24. While rewriting the '182 patent, Immunex obtained five extensions, adding 16 months to prosecution. Appx13764, Appx15932, Appx16236, Appx16720.

Immunex similarly changed the claim scope of the '522 patent. In December 2004, Immunex filed an amendment cancelling all pending claims, and filed amended claims related solely to the p75 receptor. Appx19798-19802. In August 2007, Immunex amended the specification to, for the first time, incorporate by

reference an article (Smith 1990) showing the correct amino-acid sequence for the full p75 TNFR. Appx22640; Appx5788. Additionally, Immunex amended the specification to include a reference to the plasmid deposit it made in October 2006. Appx22641. During prosecution of the '522 patent, Immunex obtained seven additional extensions, adding 18 months to prosecution. Appx19824; Appx22240; Appx22488; Appx22493; Appx23047; Appx24076; Appx24421.

The '182 patent issued in 2011 and expires on November 22, 2028. Appx12686. The '522 patent issued in 2012 and expires on April 24, 2029. Appx12721.

C. Immunex's ultimate claims focus on the etanercept compound never disclosed in the priority application.

Immunex asserts claims 11, 12, 35, and 36 of the '182 patent, all of which depend partly from claim 1, which recites a protein comprising part (a) (directed to a portion of the p75 receptor) and part (b) (directed to a portion of an IgG immunoglobulin consisting of the hinge and the CH2 and CH3 domains). Appx12717-12718. Claims 11, 35, and 36 all depend from claim 1, which limit the portion of the p75 receptor to the extracellular region and the portion of the immunoglobulin to the exon-encoded "hinge-CH2-CH3" of IgG_1, per the parties' agreed claim construction. *Id*. Claim 12 is directed to a pharmaceutical composition containing the protein of claim 11.

Immunex also asserts claims 3, 8, and 10 of the '522 patent, which are directed to a process for making the fusion protein claimed by the '182 patent. The '522 patent requires "culturing a host cell comprising a polynucleotide" that consists of only the two parts—the p75 extracellular region and the IgG_1 immunoglobulin portion—and "purifying an expression product of the polynucleotide [*i.e.*, the protein] from the cell mass or culture medium." Appx12765.

IV. The district court blesses Immunex's end-run around established patent terms.

In 2005-2006, Sandoz began to develop a biosimilar version of etanercept, now called Erelzi. Appx28383. Based on the existing patents covering etanercept, Sandoz expected that it could launch Erelzi globally in 2015. Appx4677-5678. In 2011, however, Immunex announced the issuance of the '182 patent, which would not expire until 2028. Appx12607.

In 2015, Sandoz submitted an abbreviated biologics license application ("aBLA") under 42 U.S.C. §262(k), seeking authorization to market Erelzi and designating Enbrel as the reference product. After Immunex and Sandoz completed the exchange of patent lists under §262(*l*), Immunex, Amgen, and Roche filed suit against Sandoz in the District of New Jersey, alleging that Sandoz's submission of an aBLA referencing Enbrel was an act of infringement under 35 U.S.C. §271(e)(2)(C). Sandoz argued that the asserted claims were invalid on several

grounds, including ODP, lack of written description, and obviousness. Following a bench trial, the district court concluded that the patents-in-suit are not invalid.

With respect to ODP, the court first held that the patents held by Immunex could not be used as reference patents on the theory that Roche still owned the patents-in-suit. Appx68-73. The court relied primarily on Immunex's characterization of the agreement as a license, and concluded that Roche had not transferred all substantial rights to the patents-in-suit. *Id.*

In the alternative, the district court concluded that the Immunex patents are patentably distinct from the patents-in-suit. Appx74-84. As to the psoriasis patents, the court's conclusion turned on its application of the "two-way test," which "is appropriate only in the unusual circumstance where the PTO is *solely* responsible for the delay in causing the second-filed application to issue prior to the first." *Hubbell*, 709 F.3d at 1149 (emphasis added) (quotation marks omitted). As to the '690 patent, the court rejected Sandoz's proposed construction that the fusion protein claimed by the patent is etanercept. Appx76-77.

The district court also concluded that the priority application provides adequate written-description support. Appx11-28. The court recognized that the application fails to disclose the full-length p75 sequence as part of the invention, but concluded that the disclosure of that sequence in the art, combined with oblique references to that disclosure, was enough. Appx8-19. The district court then

concluded that the application disclosed the p75-IgG_1 etanercept protein. The court reasoned that the application disclosed each part of that protein, and that a skilled artisan would have been directed by *the claims themselves* to combine those parts to create the claimed protein. Appx19-21.

Finally, the district court rejected Sandoz's argument that the asserted claims of the patents-in-suit were obvious under §103. Appx28-59.

SUMMARY OF ARGUMENT

I. The asserted claims are invalid for ODP because they are obvious over Immunex's earlier-issued patents. The district court's contrary conclusion rested on several legal errors.

A. The district court concluded that the patents-in-suit are exempt from ODP, on the theory that the 2004 Agreement did not transfer formal title to Immunex. Appx67-73. That holding was legally incorrect. The rule against ODP for commonly-owned patents applies with full force to applications acquired by assignment during prosecution. *See In re Longi*, 759 F.2d 887, 893 (Fed. Cir. 1985). And the 2004 Agreement is an assignment in all but name, because it irrevocably conveys all substantial rights in the patents-in-suit to Immunex. The meager rights retained by Roche—to practice the patents for private, research uses and to bring an infringement suit if Immunex does not sue first or grant a sublicense—are insubstantial.

The district court's alternative holding that the patents-in-suit were patentably distinct (Appx74-84) was likewise infected with legal errors. The court's conclusion as to the psoriasis patents depended *entirely* on applying the two-way test—a test that is inapplicable because both Immunex and Roche contributed to the PTO's delay in issuing the patents-in-suit through multiple extensions and major claim amendments. As to the '690 patent, the district court's conclusion that it does not claim etanercept misconstrues the claims and contradicts both the specification and prosecution history.

II. The priority application does not provide written-description support for etanercept for two reasons.

A. First, it does not describe the full-length p75 sequence, and hence does not disclose a fusion protein based on that sequence. The priority application was based entirely on the sequences identified in Figures 1 and 4 and close variants thereof—*i.e.*, the full p55 sequence and the truncated/mutated Roche p75 sequence. *Every* discussion of the p75 receptor, and *every* reference to a fusion protein based on that receptor, refers to the Roche sequence in Figure 4, *not* to the full-length p75 sequence previously discovered and used by Immunex in etanercept. The Smith reference that discloses the full-length sequence is mentioned only once in the priority application, and the parties' experts *agreed* that this passing reference

neither incorporated Smith by reference nor instructed a skilled artisan to use Smith as a substitute for Roche's Figure 4 sequence.

The district court could only find disclosure of the p75 sequence through the type of hindsight- and obviousness-based approach to written description that this Court has rejected. *See, e.g.*, *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc).

B. Second, the priority application failed to disclose the specific p75-IgG_1 etanercept protein Immunex claimed. A specification disclosing a genus of compounds only discloses a species within that genus if it provides "blaze marks" that would lead a skilled artisan to the later-claimed species. *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1326-27 (Fed. Cir. 2000). The priority application does not provide "blaze marks" to the full-length p75 sequence. Moreover, the application identifies a range of potential immunoglobulins, with an accompanying range of potential hinges. *Nothing* points a skilled artisan to the *specific* combination of features of etanercept.

The district court disputed none of this, but held that a skilled artisan would have been directed to etanercept *by the claims themselves*. That is blatant legal error. *See, e.g.*, *Novozymes A/S v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1349 (Fed. Cir. 2013).

III. The district court's obviousness analysis similarly rested on fundamental legal errors. In concluding that a skilled artisan would not have been motivated to combine the p75 receptor with an immunoglobulin to create etanercept, the court relied on the premise that the potential to stimulate inflammation would have taught away from this combination by making the fusion protein a poor candidate for treating autoimmune diseases like rheumatoid arthritis. But the asserted claims are *not* directed to a method of treatment, and it was legal error for the district court to discount evidence showing a motivation to create etanercept for *other* purposes. The district court likewise erred in assessing secondary considerations, disregarding the history of etanercept's conception and patenting *by Immunex*.

ARGUMENT

I. The patents-in-suit are invalid for ODP.

The rule against ODP enforces a fundamental bargain: a patentee receives exclusivity for an invention subject to the condition that "on the expiration of a patent the monopoly created by it ceases to exist." *Singer*, 163 U.S. at 185; *AbbVie*, 764 F.3d at 1372. Although ODP has been "described as a court created doctrine," it is "grounded in the text of the Patent Act," and in particular on §101's instruction that no one may "obtain[] more than one patent on the same invention." *AbbVie*, 764 F.3d at 1372. ODP doctrine implements this statutory policy by preventing

"separate applications or patents" from "claim[ing] inventions so alike that granting both exclusive rights would effectively extend the life of patent protection." *Hubbell*, 709 F.3d at 1145 (quotation marks omitted).

The patents-in-suit represent just such an invalid life-extension. The district court's ruling allowed Immunex to continue its patent protections after expiration of its own patents rested on several legal errors.

A. Standard of review.

ODP is a question of law, reviewed de novo. *UCB, Inc. v. Accord Healthcare, Inc.*, 890 F.3d 1313, 1324 (Fed. Cir. 2018). Underlying factual findings are reviewed for clear error. *Id.*

B. Patents owned by Immunex are proper reference patents, because Immunex is the effective owner of the patents-in-suit.

"[C]ommonly-owned applications by different inventors" are subject to the rule against ODP. *Longi*, 759 F.2d at 893. Under the 2004 Agreement, the applications that resulted in the patents-in-suit, the psoriasis patents, and the '690 patent were all "commonly-owned" by Immunex. The district court's contrary holding conflicts with precedent recognizing that an agreement that transfers all substantial rights to the patent is an assignment for purposes of federal patent policy.

1. An agreement that conveys all substantial rights to a patent is tantamount to an assignment of ownership.

Immunex has argued that its patents are not ODP references for the patents-in-suit because the 2004 Agreement supposedly conveyed only an exclusive license to the patents without formally transferring ownership. But an agreement *labeled* as a license "may be tantamount to an assignment" for purposes of federal patent law. *Aspex Eyewear, Inc. v. Miracle Optics, Inc.*, 434 F.3d 1336, 1340 (Fed. Cir. 2006). Thus, although only "[a] patentee" may sue for infringement, 35 U.S.C. §281, if an agreement "transfers 'all substantial rights' to the patent, this amounts to an assignment or a transfer of title" that provides standing for the transferee to sue in its "own name alone." *Morrow v. Microsoft Corp.*, 499 F.3d 1332, 1340 (Fed. Cir. 2007). The transferee "becomes the effective patentee," *id.* at 1340 n.6, with "effective title" to the patents-in-suit, *Keranos, LLC v. Silicon Storage Tech., Inc.*, 797 F.3d 1025, 1031 (Fed Cir. 2015). The same logic applies to the ODP context.

a. The district court questioned whether this Court's "all substantial rights" test should apply outside "the 'standing to sue' context." Appx70. Tellingly, however, the court did not suggest *any other way* to decide whether a purported licensee, whose license transaction is an assignment in every way that matters, should be treated like a patent owner for purposes of federal law.

Nor did the district court consider the textual links between the ownership inquiries in the ODP and standing contexts, which strongly support a common test.

As noted, only "[a] patentee" has statutory authorization to sue for infringement. 35 U.S.C. §281. This Court's decisions recognizing that some exclusive licensees nonetheless may sue in their own name rely on the definition of "patentee" in §100(d) to include "successors in title" to the patent. *See Karanos, LLC v. Silicon Storage Tech.*, 797 F.3d 1025, 1031 (Fed. Cir. 2015). As the Court has reasoned, when a party acquires all substantial rights to the patent, it becomes the "successor" under §100(d). *See id.* Similarly, even though only a "patentee or applicant" may file a terminal disclaimer to overcome an ODP objection, 35 U.S.C. §253(b), §100(d)'s definition of "patentee" establishes that a successor acquires authority to file a terminal disclaimer. *See In re Bowers*, 359 F.2d 886, 889 (C.C.P.A. 1966) (cited with approval in *Longi*, 759 F.2d at 894); *accord In re Borg*, 392 F.2d 642, 644 (C.C.P.A. 1968).

If anything, the argument for following the "all substantial rights" test is *stronger* in the ODP context. ODP comes from the fact that "§101 forbids an individual from obtaining more than one patent on the same invention." *AbbVie*, 764 F.3d at 1372. If Immunex would "become the effective patentee" for standing purposes, then Immunex has surely "obtained" that patent for ODP purposes.

b. Immunex argued below that courts should decide whether a party owns an application or patent for purposes of ODP by reference to *state* law. That approach is unworkable. The question of patent ownership in this context, as in the

context of statutory standing, directly implicates issues of *federal* patent policy that demand a "uniform national rule." *Rhone Poulenc Agro, S.A. v. DeKalb Genetics Corp.*, 284 F.3d 1323, 1328 (Fed. Cir. 2002).

Immunex's position also produces absurd results, because it offers an easy path for companies to circumvent ODP. On Immunex's account, a company that would face a certain ODP objection under *Longi* could avoid that objection merely by reclassifying the assignment as a license but *without changing anything of substance*. It would not matter, under this theory, whether the putative license is "tantamount to an assignment." *Alfred E. Mann Found. for Sci. Research v. Cochlear Corp.*, 604 F.3d 1354, 1358-59 (Fed. Cir. 2010). According to Immunex, if the relevant state law would accept the parties' label, then ODP cannot apply.

Immunex has no viable response to the relabel-an-assignment-as-a-license scenario because *that is this case*. As discussed, p. 17, *supra*, the executive who negotiated the 2004 Agreement for Immunex knew that commonly-owned patents are subject to ODP scrutiny under U.S. patent law. Appx5784. Whereas Roche executed a formal assignment of patent rights outside North America to Wyeth—a label that carried no invalidity risk, because ODP is not recognized outside the United States—Immunex insisted on characterizing its own substantively indistinguishable agreement as a "license," even though Roche had offered an assignment for the same price. Appx28324-28325. According to Immunex, this

strategic gambit allows it to avoid ODP while still enjoying complete control over the reference patents *and* the patents-in-suit, even during prosecution. But ODP "prevent[s] unjustified timewise extension of the right to exclude … *no matter how the extension is brought about*." *Hubbell*, 709 F.3d at 1145 (emphasis added). The Court should reject Immunex's easy-to-manipulate approach to determining ODP common ownership and instead apply the well-developed "all substantial rights" test.

2. The 2004 Agreement provided Immunex with all substantial rights to the patents-in-suit.

a. While this Court has "never established a complete list of the rights that must be examined to determine whether a patentee has transferred away sufficient rights to render another party the owner of a patent," it has described "the exclusive right to make, use, and sell" the patented invention as "*vitally important*." *Diamond Coating Techs., LLC v. Hyundai Motor Am.*, 823 F.3d 615, 619 (Fed. Cir. 2016) (quotation marks, brackets, and ellipses omitted). Likewise, the Court has identified "the nature and scope of the patentee's retained right to sue accused infringers and license the patent" as perhaps "the most important factors." *Id.* (quotation marks and brackets omitted). Those critical factors overwhelmingly support recognizing that the 2004 Agreement "transfer[s] away sufficient rights."

The 2004 Agreement granted Immunex all the classic indicia of ownership, including the two critical rights identified in *Diamond Coating*. Under Paragraph

3.1, Immunex obtained "a paid-up, irrevocable, exclusive license, with the sole right to grant sublicenses, under the [patents-in-suit] to make, have made, use, sell, offer for sale and import [the claimed inventions] for the life of such patents." Appx25839. As to litigation rights, Paragraph 3.5 provides Immunex with the first right to rectify any alleged infringement by, *e.g.*, suing the infringer or sublicensing the patents-in-suit. Appx25840-25841. Moreover, if Immunex exercises its right to sue, it has the complete rights to control the litigation—including to settle the claim on whatever terms it considers appropriate—and to pocket all proceeds from the lawsuit. *Id.*

The flip side is that Roche has been stripped of any of the traditional attributes of ownership. Here, Roche not only lost any ability to commercialize the claimed invention, but also did not "retain[] control of licensing" for the patents-in-suit. *Diamond Coating*, 823 F.3d at 620; *see* Appx25839. Likewise, Roche did not "retain[] control" of "litigation activities." *Diamond Coating*, 823 F.3d at 620. The Agreement provides Immunex with the unilateral right to initiate infringement litigation, which is then "solely within" Immunex's "control." Appx25840. Roche only has a back-up right to sue for infringement if Immunex declines to do so within 180 days of Roche's written request. Appx25841. That highly circumscribed right is "illusory" because Immunex "can render [it] nugatory by granting the alleged infringer a royalty-free sublicense." *Speedplay, Inc. v. Bebop, Inc.*, 211 F.3d 1245,

1251 (Fed. Cir. 2000). Specifically, Paragraph 3.5 authorizes Immunex "to rectify any infringement by sublicense." Appx25840. The Agreement does not place any limits on how Immunex exercises its licensing discretion, nor does it require Immunex to collect any royalties on a sublicense.

These factors alone show that the 2004 Agreement transferred all substantial rights in the patents-in-suit to Immunex. *See, e.g.*, *Diamond Coating*, 823 F.3d at 619; *Aspex*, 434 F.3d at 1342; *Alfred E. Mann*, 604 F.3d at 1360-61. But there is more. Under Paragraph 3.3, Immunex obtained the sole right to control the prosecution of the patents-in-suit—Roche did not even retain a right to review PTO submissions. Appx25840; Appx28335-28336; Appx5763-5765; Appx5768. That factor is highly significant in the ODP context, since ODP's purpose is to prevent applicants from receiving new patents that "extend the life" of their existing patents. *Hubbell*, 709 F.3d at 1145. This case shows why. Immunex used its control over prosecution to amend the applications that became the patents-in-suit in order to claim an invention—etanercept—that Roche never possessed or disclosed, but which Immunex itself had already claimed in its own patents.

b. The district court's contrary conclusion—reviewed de novo, *Lone Star Silicon Innovations LLC v. Nanya Tech Corp.*, 925 F.3d 1225, 1230 (Fed. Cir. 2019)—relied primarily on the fact that the 2004 Agreement's transfer of rights to Immunex was "expressly called a license," which the court contrasted with the

"[a]ssignment" label given to the transfer of Roche's corresponding patent rights outside of North America to Wyeth. Appx71. The court similarly looked to testimony by Immunex's lead negotiator, which reinforced that Immunex required Roche to retain formal ownership of the patents. Appx72. But the district court's reasoning runs headlong into this Court's precedent: "labels given by the parties do not control" the substantial-rights inquiry, *A123 Sys., Inc. v. Hyrdo-Quebec*, 626 F.3d 1213, 1218 (Fed. Cir. 2010), which is supposed to turn on "substance" "rather than formalities or magic words," *Lone Star*, 925 F.3d at 1229.

The need for courts to look beyond labels is especially pronounced in the ODP context, where assignees will have clear incentives to recharacterize agreements transferring patent ownership as licenses. Certainly Immunex had such an incentive here. *See* p. 17, *supra*.

Beyond its focus on the 2004 Agreement's labels, the district court identified just two rights that Roche supposedly maintained, neither of which comes close to establishing Roche's continued patent ownership.

First, the district court emphasized Roche's secondary right to sue for infringement. Appx71-72. But, as discussed, pp. 31-32, *supra*, that right was "illusory" because Immunex could undercut Roche's ability to sue by granting a royalty-free sublicense to an alleged infringer. *Speedplay*, 211 F.3d at 1251. Resisting that conclusion, the district court asserted that "Immunex could *not* end a

Roche-initiated lawsuit by granting a sublicense on its own." Appx73. But nothing in the 2004 Agreement supports the court's declaration, which conflicts with Paragraph 3.5's express grant of authority for Immunex "to rectify any … infringement" of the patents-in-suit "by sublicense." Appx25840.

Without referencing Paragraph 3.5, the district court instead relied on Paragraph 3.6, which states that Immunex "will cooperate with Roche" in a Roche-initiated suit," including by "participating as a party in the suit to the extent required by the court in order to bring suit." Appx25841. That provision, however, merely requires Immunex's participation in litigation. It does not qualify Immunex's express authority to eliminate the predicate for suit by granting a sublicense. The only other provision cited by the district court—the 2004 Agreement's mutual restrictions on further assignments without the counterparty's consent (Appx25849)—does not fill this gap. Immunex does not need *to assign* the patents-in-suit to an alleged infringer in order to vitiate Roche's ability to sue; a non-exclusive license would do. *See Carborundum Co. v. Molten Metal Equip. Innovations, Inc.*, 72 F.3d 872, 878 (Fed. Cir. 1995).[1]

[1] Roche's ability "to veto the assignment of Immunex's rights to a third party" also does not "suggest[] that the parties envisioned the agreement to be a license." Appx73. Paragraph 11.4, on which the district court relied, appears in a global section of the Agreement that also applied to Roche's agreement with Wyeth, and even Immunex recognizes that Wyeth received an assignment. Moreover, the

Even if Paragraph 3.6 could be read to implicitly restrict Immunex's otherwise unlimited right to grant sublicenses *during* a "Roche-initiated lawsuit," Appx73, Roche's secondary right to sue would remain illusory because it would still exist purely as a matter of Immunex's grace. Before Roche can file a lawsuit, Paragraph 3.5 requires Roche to make a written request to Immunex and to wait at least 180 days. Appx25841. There is no question that if Immunex grants a sublicense to the alleged infringer on day 179, Roche would lose any right to sue. Thus, just as in *Speedplay*, Immunex's sublicensing rights render Roche's secondary right to sue "illusory." 211 F.3d at 1251.

By contrast, in *Alfred E. Mann*, the patent owner's secondary right to sue was meaningful because sublicensing rights were "fettered" by the licensee's obligation to charge pass-through royalties on any sublicenses, which would flow back to the patent owner. *See* 604 F.3d at 1361-62; *see also Abbott Labs. v. Diamedix Corp.*, 47 F.3d 1128, 1132 (Fed. Cir. 1995) (similarly attributing significance to a retained right to sue, because any sublicense granted had to include royalties). No similar royalty requirement applies here.

Second, the district court pointed to Roche's reserved right "to practice the invention." Appx73. More specifically, Paragraph 3.2 allows Roche and its

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Agreement gives Immunex the same right to veto the assignment of *Roche's* rights to a third party. Appx25849.

affiliates "to practice under the [patents-in-suit] for internal, non-clinical research only." Appx25839. As this Court has explained, however, "this is not a substantial right" since it is the same right that any non-exclusive licensee might possess. *Luminara Worldwide, LLC v. Liown Elecs. Co.*, 814 F.3d 1343, 1351 (Fed. Cir. 2016). "The retained right to practice a patent is not the same as a retained right to exclude others from doing so." *Id.*

In short, Roche transferred all of its substantial rights to Immunex—which already owned multiple other etanercept patents.

C. The patents-in-suit are not patentably distinct from the '225 patent.

In conducting an ODP analysis after identifying the reference patents, the Court first "construes the claims in the earlier patent and the claims in the later patent and determines the differences." *AbbVie*, 764 F.3d at 1374 (quotation marks and brackets omitted). The Court then asks "whether those differences render the claims patentably distinct." *Id.* (quotation marks omitted). The "general rule" for deciding whether claims are patentability distinct is to apply a "one-way test," which asks whether the asserted patent claim is obvious over or anticipated by the reference-patent claim. *Hubbell*, 709 F.3d at 1149.

Under that "general rule," the obviousness of the asserted claims of the patents-in-suit over the claims of the psoriasis patents is clear beyond reasonable dispute. The patents-in-suit claim the etanercept protein (the '182 patent) and a

method of manufacturing etanercept (the '522 patent) using routine steps that were well known in the art. *See* pp. 18-20, *supra*. The psoriasis patents claim methods of using a therapeutically effective dose of etanercept to treat psoriasis. *See* p. 9, *supra*. The '225 patent, issued before both of the patents-in-suit, is unquestionably a proper ODP reference. *See Novartis Pharms. Corp. v. Breckenridge Pharm. Inc.*, 909 F.3d 1355, 1359 (Fed. Cir. 2018).[2] Thus, the issue here is whether patents claiming etanercept (the '182 patent) and a method for manufacturing etanercept (the '522 patent) were anticipated by or obvious over previously issued patent claims that presupposed etanercept's existence and described how to use etanercept to treat a specific condition. That question answers itself: because the psoriasis patent claims are effectively species of the asserted genus claims in the patents-in-suit, they are invalid for ODP. *See Perricone v. Medicis Pharm. Corp.*, 432 F.3d 1368, 1374 (Fed. Cir. 2005).

The district court did not conclude otherwise, but rather reached a different result because it employed the far more lenient (and rarely applied) "two-way test." Appx78. Under that test, "the order of issuance is, in effect, ignored," and a patentee will escape ODP if its earlier patent claims are distinct from later-issued claims. *In*

[2] The district court's extended discussion of the "[i]mpact of GATT on the Patents-in-Suit" is accordingly irrelevant to this appeal because the court acknowledged that the '225 patent could serve as a proper reference based on its date of issuance. Appx82.

re Janssen Biotech, Inc., 880 F.3d 1315, 1325 (Fed. Cir. 2018). Reversing the district court's decision to apply the two-way test—which this Court "review[s] without deference," *In re Fallaux*, 564 F.3d 1313, 1316 (Fed. Cir. 2009)—compels the conclusion that the asserted claims are invalid for ODP.

1. The district court committed legal error by applying the "two-way" test.

The two-way test is reserved for "unusual circumstances," and the standard for invoking it is strict. *Janssen*, 880 F.3d at 1325. The test is "only appropriate where (1) a second-filed application issues prior to a first-filed application, and (2) the PTO is *solely* responsible for the delay in the issuance of first-filed application." *Id.* (emphasis added) (quotation marks omitted). And "solely" really means "solely": a patentee does *not* get the benefit of the test merely by showing that "on … balance" the PTO was more responsible for the sequencing of patent issuance. *Hubbell*, 709 F.3d at 1149. If an applicant's "actions, or inactions, had a direct effect on the prosecution," then "the two-way test … *does not apply*." *In re Basell Poliolefine Italia S.P.A.*, 547 F.3d 1371, 1376 (Fed. Cir. 2008) (emphasis added); *see also Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, n.7 (Fed. Cir. 2001) (rejecting the two-way test because continuation requests filed by the applicant showed that the delay in patent issuance "was not solely caused by the PTO"); *In re Emert*, 124 F.3d 1458, 1461 (Fed. Cir. 1997) (two-way test did not apply where actions taken by the applicant, including receiving "numerous time

extensions in various filings," showed that the PTO "did not dictate the rate of prosecution").

a. The undisputed facts show that the PTO was not "solely responsible" for the delayed issuance of the patents-in-suit: Roche and Immunex unquestionably contributed to the fact that those patents issued after the '225 patent. Immunex obtained five extensions for the '182 patent and seven extensions for the '522 patent, adding at least 16 months and 18 months to the prosecutions of those patents, respectively. Appx13764; Appx15932; Appx16236; Appx16720; Appx19824; Appx22240; Appx22488; Appx22493; Appx23047; Appx24076; Appx24421. Moreover, before Immunex took control of prosecution, Roche also sought numerous extensions for both the '182 and '522 patent applications, adding another year to their prosecutions. Appx13046; Appx13058; Appx13087; Appx13108; Appx13397; Appx13416; Appx19580; Appx19607; Appx19761; Appx19778. And between 1996 and 1998, Roche repeatedly ignored communications from the PTO regarding the '182 patent, further delaying issuance. Appx13056; Appx13076; Appx13096.

Roche and Immunex also repeatedly amended the patent applications, which delayed their issuance. As discussed, Immunex made radical changes following the 2004 Agreement by shifting the focus of the claims from the p55 to the p75 receptor and amending the specifications. *See* pp. 18-19, *supra*. For example, in December

2004, Immunex filed an amendment that cancelled all pending claims of the '522 application, which Roche had prosecuted for a decade, in order to file amended claims related to the p75 receptor. Appx19798-19802. Likewise, in 2005, Immunex filed a series of amendments to the '182 patent to add claims related to the p75 receptor and then remove all references to the p55 receptor. Appx13659; Appx13665-13669; Appx15931; Appx15933-15938. Immunex also made substantial amendments to the patent specifications in 2006 and 2007 in an effort to add belated descriptions of etanercept. *See* pp. 18-19, *supra*.

The upshot of Immunex's efforts was to render much of Roche's previous decade of prosecution irrelevant, substantially delaying issuance. Immunex's contribution to the delay is underscored by the fact that Immunex did not add the asserted claims to the respective applications until *late 2010*—15 years after Roche filed the applications. Appx 18227; Appx18234; Appx18778; Appx23323-23324; Appx23362; Appx2440.

b. The district court's decision to apply the two-way test focused almost exclusively on *instances* of delay that it concluded were attributable to the PTO. Appx80. But even accepting the court's findings as to those examples, they do not support applying the two-way test. Unless the PTO was "*solely* responsible for any delays associated with [the] claims" asserted here, the one-way test applies. *Hubbell*, 709 F.3d at 1149 (emphasis added).

Although the district court purported to find "as a matter of fact" that the PTO "was solely responsible for the delay" in issuance of the patents-in-suit (Appx80), the court's analysis reveals that it applied the wrong legal standard—so the factual findings that rest on that wrong legal standard are not entitled to deference. *Pullman-Standard v. Swint*, 456 U.S. 273, 287 (1982). Specifically, the district court acknowledged that Roche and Immunex made "*several* … requests" for extensions, but it discounted those repeated extensions on the ground that they supposedly were made "in good faith." Appx80-81 (emphasis added). But an applicant's purported "good faith" is legally irrelevant. The one-way test is not a sanction; it is the *default* rule that applies outside of the "unusual circumstance" in which the PTO *alone* is responsible for the fact that an earlier-filed patent issued after the reference patent. *Janssen*, 880 F.3d at 1325.

In any event, the district court's decision to apply the two-way test could not survive even clear-error review. No possible view of the record supports a finding that Roche's and Immunex's actions and inactions during prosecution had *nothing* to do with the patents-in-suit issuing after the '225 patent.

2. The patents-in-suit are not patentably distinct from the '225 patent under the one-way test.

Applying the one-way test resolves ODP as to the '225 patent. As noted, p. 9, *supra*, claim 1 of the '225 patent recites methods of treating psoriasis by administering a therapeutically effective dose of etanercept. Those claims would

have directed a skilled artisan to produce etanercept (Appx4131), thus rendering the asserted claims of the '182 patent obvious. Moreover, to produce etanercept, a skilled artisan would have taken all of the steps described in the asserted claims of the '522 patent—*i.e.*, performing the routine steps of culturing a host cell encoding the DNA for etanercept and purifying etanercept from parts of the cell. Appx4133-4134; Appx4110-4112. Clear and convincing evidence thus establishes that the asserted claims of the patents-in-suit are obvious over claim 1 of the '225 patent when the one-way test is applied.

D. The asserted claims in the patents-in-suit are not patentably distinct from claim 3 of the '690 patent.

Claim 3 of the '690 patent covers a method of administering etanercept, and is thus not patentably distinct from the asserted claims of the patents-in-suit.

Specifically, claim 3 recites "a method for lowering the levels of active TNF-α in a mammal in need thereof which comprises administering to said mammal a TNF-lowering amount of a chimeric antibody comprising *a TNFR comprising the sequence of amino acids 3-163 of SEQ ID NO:1 fused to the constant domain of an immunoglobulin molecule*." Appx27320 (emphasis added). That claim describes etanercept: the etanercept molecule fuses the extracellular region of the TNFR ("a TNFR comprising the sequence of amino acids 3-163 of SEQ ID NO:1") to the hinge-CH2-CH3 region of IgG_1 (the constant domain of an immunoglobulin molecule"). Appx4105-4106, Appx4136-4137, Appx4145-4146.

Below, both parties agreed that claim 3 covers a protein consisting of the extracellular region of the p75 receptor fused to a portion of a human IgG_1. So the only dispute concerns the proper construction of the phrase "fused to the constant domain." Immunex argued, and the district court agreed, that that phrase describes a protein in which the TNFR is fused to "a *completely unchanged and unmodified* constant region domain for the light chain *and* for the heavy chains." Appx5272 (emphasis added). That construction is incorrect.

"Claim interpretation requires the court to ascertain the meaning of the claim to one of ordinary skill in the art at the time of invention." *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1338 (Fed. Cir. 2005). "The intrinsic evidence, *i.e.*, the patent itself, including the claims, the specification and, if in evidence, the prosecution history is the most significant source of the legally operative meaning of disputed claim language." *Id.* (quotation marks omitted). Here, evidence from the '690 patent's specification and prosecution history shows that the phrase "fused to the constant domain" in claim 3 covers the fusion of etanercept's TNFR to the hinge-CH2-CH3 region of IgG_1.

1. First, consider the '690 patent's specification. An interpretation of a patent's claims that excludes "a preferred … embodiment in the specification … is rarely, if ever, correct." *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1583 (Fed. Cir. 1996). But that is precisely what the district court has done.

Figure 1 presents a schematic representation of a "recombinant human TNFR/Fc fusion protein." Appx27297-27298; Appx27304. Both sides' experts recognized that "[t]his Figure 1 … *is* etanercept": it combines "the soluble portion, the extracellular domain of the p75 TNF receptor" with "the hinge-CH2 and CH3 domain of a human IgG." Appx4137 (emphasis added); *accord* Appx5392. A comparison of Figure 1 (Appx27297) to the schematic of etanercept in the district court's opinion (Appx6) underscores the point:

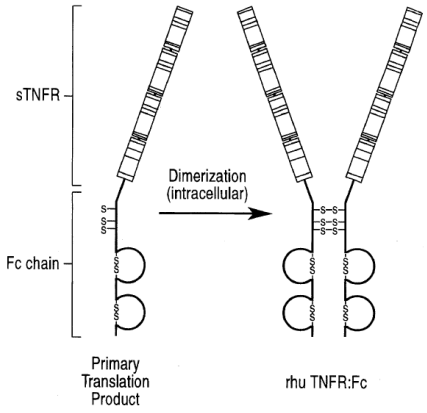

Figure 1

Etanercept

The '690 patent's examples are of similar effect. Example 2 describes the production of etanercept—referred to as a "TNFR/Fc fusion protein." Appx27310-27311 (14:55-15:60); Appx4138; Appx5392-5393. Example 4 describes the use of the p75 extracellular region and etanercept to suppress the effects of arthritic conditions, while Examples 5 and 6 describe further testing with just etanercept. Appx27312 (7:16-20:43); Appx4138-4139; Appx5393-5394.

Finally, the single paragraph in the '690 patent specification that discusses a "chimeric antibody" describes etanercept:

> A recombinant chimeric antibody molecule may also be produced having TNFR sequences substituted for the variable domains of either or both of the immunoglobulin molecule heavy and light chains and having unmodified constant region domains…. One specific example of a TNFR/Fc fusion protein is disclosed in SEQ ID NO:3 and SEQ ID NO:4.

Appx27307 (7:42–58). As both parties' experts confirmed, the TNFR/Fc fusion protein disclosed in SEQ ID NO:3 and SEQ ID NO: 4 is etanercept. Appx4139; Appx5391.

2. The prosecution history of the '690 patent points in the same direction, though Plaintiffs' expert failed even to consider it. Appx5390. The applicants amended their claims to specify that one example of a "chimeric antibody comprising a TNF receptor and the constant domain of an immunoglobulin molecule" (the phrase that now appears in claim 3) was a "soluble human TNFR is fused to the Fc region of the human immunoglobulin molecule"—*i.e.*, etanercept. Appx10016; Appx10172; Appx10219. Moreover, the applicants relied upon a declaration reporting clinical data from administering etanercept to demonstrate the utility of the claimed chimeric antibody. Appx20223; Appx10236-10237; Appx10230-10252; Appx4139-4141. At no point did the applicants disavow that the chimeric antibody includes etanercept.

3. The district court failed even to address the specification language and prosecution history discussed above. *See* Appx76-77. Instead, the court relied on a single fact: "the specification of the '690 Patent describes a chimeric antibody as a molecule 'having TNFR sequences substituted for the variable domains of either or both of the immunoglobulin heavy and light chains and having *unmodified* constant region domains.'" Appx77 (quoting Appx27307).

But etanercept *does* have unmodified constant region domains: the molecule consists of a TNFR fused to the *unmodified* hinge-CH2-CH3 region of IgG_1. *See* pp. 7-8, *supra*. According to the district court, that was not enough: *each and every constant region domain* (including CH1) must remain unmodified. Appx76-77. But that interpretation places more weight on a single passage in the specification—"having unmodified constant region domains"—than it can reasonably bear. Considering the intrinsic record as a whole, the only reasonable interpretation is that the phrase requires *some* unmodified constant region domains. Indeed, Plaintiff's own expert conceded that he did not know whether the phrase "fused to the constant domain of an immunoglobulin molecule" precludes fusing the TNFR to the hinge-CH2-CH3 portion of an immunoglobulin. Appx5400-5401.

Thus, the district court erred in holding that Claim 3 of the '690 patent does not cover etanercept. That resolves the ODP analysis, because none of Immunex's

experts disputed the obviousness of the asserted claims under this construction of claim 3.

II. The district court erred in concluding that the priority application disclosed possession of the claimed invention.

The asserted claims, added by Immunex in 2010—15 years after Roche filed the priority application—are also invalid because they lack written-description support in "the original priority application." *Novozymes*, 723 F.3d at 1344.

Roche did not have possession of etanercept. It did not invent etanercept, never even *made* etanercept, and was never even able to clone the full p75 receptor. Roche's application thus, unsurprisingly, did not describe etanercept—or any other fusion protein with the full p75 receptor.

Immunex's late-added etanercept claims survived only because the district court erroneously applied an obviousness framework to the written-description inquiry. The court looked not to the invention *actually disclosed*, but to what might have been obvious to a skilled artisan in light of those disclosures—a far lower bar than what this Court's law requires. *See Idenix Pharms. LLC v. Gilead Sci. Inc.*, 2019 WL 5583543, at *8-10 (Fed. Cir. Oct. 30, 2019) (reversing the district court's denial of JMOL on written description, where the court relied on obviousness-based arguments as a substitute for the specification's failure to describe the compound claimed).

A. Standard of review.

This Court reviews a district court's "compliance with legal standards" de novo. *Veritas Technologies LLC v. Veeam Software Corp.*, 835 F.3d 1406, 1411 (Fed. Cir. 2016). In addition, "if a district court's findings rest on an erroneous view of the law, they may be set aside on that basis." *Pullman-Standard*, 456 U.S. at 287. Absent legal error, whether a patent's specification adequately demonstrates possession of the claimed subject matter is a question of fact, reviewed for clear error. *Ariad*, 598 F.3d at 1351.

B. The district court legally erred by repeatedly looking outside the "four corners of the specification."

The written-description requirement "limits patent protection to those who actually perform the difficult work of 'invention'—that is, conceive of and complete the final invention." *Billups-Rothenberg, Inc. v. Associated Reg'l & Univ. Pathologists, Inc.*, 642 F.3d 1031, 1036 (Fed. Cir. 2011). A patent's description of the invention "must clearly allow persons of ordinary skill in the art to recognize that the inventor invented what is claimed" by demonstrating "possession of the claimed subject matter as of the filing date." *Ariad*, 598 F.3d at 1351 (quotation marks and alterations omitted). Holding the inventors to their originally-disclosed invention is particularly important for "claims added during prosecution" to ensure that they are not used to "expand the scope of [the] invention or to complete an idea." *Novozymes*, 723 F.3d at 1343-44.

Moreover, because "the hallmark of written description is disclosure," the written-description "test requires an objective inquiry into the four corners of the specification." *Ariad*, 598 F.3d at 1351. "[A] description that merely renders the invention obvious does not satisfy the [written-description] requirement." *Id.* at 1352. Similarly, a description is not adequate simply because it is later possible, "[w]orking backward from a knowledge of the claims," to put together "an amalgam of disclosures" that, "plucked selectively from the … application," can be combined together and with the prior art to create the later-claimed invention. *Novozymes*, 723 F.3d at 1349. Nor can a patentee expand an invention by initially disclosing a broad genus of compounds or characteristics, and then later claiming one species that is nowhere highlighted in the disclosure. Instead, the original disclosure must include "blaze marks" leading from the genus to the later-claimed species. *E.g.*, *Idenix*, 2019 WL 5583543, at *9.

The district court's decision flouts these basic principles. Roche's priority application never even identified each individual *piece* of the later-claimed etanercept fusion protein, let alone the "blaze marks" necessary to identify that particular protein. The district court repeatedly used hindsight to piece together what Immunex ultimately claimed—*not* what the specification describes.

C. Roche's priority application does not include written-description support for the etanercept-based claims Immunex later added.

The asserted claims all require a fusion protein with two parts: the full extracellular region of the p75 receptor and specific portions of human IgG_1, connected at a specific point. The priority application therefore must disclose a fusion protein with those specific attributes. But Roche's priority application only discloses fusion proteins that include *some* receptor portion and *some* immunoglobulin portion. Appx25091. It does *not* describe the specific combination of elements that result in etanercept.

1. The priority application described a fusion protein based on the truncated/mutated p75 DNA sequence disclosed in Figure 4, *not* the full p75 DNA sequence used in etanercept.

a. The TNFR portion of the fusion proteins disclosed in Roche's priority application was based on the DNA and amino-acid sequences disclosed in Figure 1 and Figure 4. Figure 1 discloses the p55 receptor. Appx25084. Figure 4 discloses the "[n]ucleotide sequence and deduced amino acid sequence for cDNA clones derived from 75/65 kD TNF-BP." *Id*.

All of the TNFR portions of the fusion proteins in the priority application rest on these specific DNA and amino-acid sequences, and close variations thereof. For instance, the application stated that its DNA coding for TNF-binding proteins should be "selected from the following: (a) DNA sequences as given Figure 1 or Figure 4 as well as their complementary strands, or those which include these sequences."

Appx25089. The application also identified similar DNA sequences that "hybridize" with those in Figure 1 or Figure 4, or that "code for polypeptides having exactly the same amino acid sequence." *Id*. It explained that the invention encompasses "those DNA sequences which result from deletions, substitutions and additions from one or more nucleotides of the sequences given in Figure 1 or Figure 4." Appx25090.

In discussing preferred embodiments, the specification again focused on Figures 1 and 4. It stated that "preferred first of all [are] those DNA sequences which code for such a protein having an apparent molecular weight of about 55 kD, whereby the sequence given in Figure 1 is especially preferred"; fragments of the Figure 1 sequence could also be used. *Id*. The application then disclosed that "[t]here are also preferred DNA sequences which code for a protein of about 75/65 kD, whereby those which contain the partial cDNA sequences shown in in Figure 4 are preferred." *Id.* The application never identified any DNA sequence—other than Figure 1 or 4, or close variants thereof—from which to draw the TNF-receptor portion of the fusion protein.

There is no dispute that truncated/mutated Figure 4 is *not* the full sequence for the extracellular portion of the p75 receptor that Immunex ultimately claimed. Most importantly, of the sequence of 235 amino acids that ultimately made part of the claimed fusion protein, the Figure 4 disclosure omits 20%—the first 48 amino

acids—which comprise the N-terminus of the p75 extracellular region as incorporated into etanercept. Appx4454; Appx5037. Figure 4 also omits the 22-amino-acid signal sequence, which is essential to protein secretion. Appx4453-4454. Moreover, Roche's Figure 4 sequence includes *different* amino acids than the Smith sequence in three important positions, and includes one amino acid that is not in Smith. Appx7004; Appx4456-4461. Plaintiffs' expert thus had to admit that the actual DNA or amino acid sequence ultimately used in the etanercept fusion protein Immunex later claimed "is not recited in the patent as a simple matter of fact." Appx5054-5055.

The closest the priority application comes to disclosing a fusion protein with the full p75 extracellular region is its vague reference to Smith. Specifically, after discussing how the invention encompasses "deletions, substitutions and additions" from Figure 1 or Figure 4, the application stated that "[o]ne sequence which results from such a deletion is described, for example, in Science 248, 1019-1023, (1990)," *i.e.*, Smith. Appx25090.

This vague reference to Smith as an example of a "deletion" from Figure 4 does not come close to disclosing a fusion protein that incorporates that *different* sequence for the p75 receptor, rather than the sequence repeatedly referenced in the priority application itself. Most importantly, the application does not incorporate Smith generally, or Smith's full p75 sequence specifically. It simply gives Smith as

an example of a "deletion." As Sandoz's expert explained, Smith provides such an example because Roche's Figure 4 sequence includes an alanine amino acid at residue 369 that the Smith sequence lacks. Appx4460-4461.

Plaintiffs' expert disputed this explanation, but had to acknowledge that the priority application did *not* "incorporate Smith by reference," and did *not* instruct a skilled artisan to "use Smith to complete the sequence of Figure 4." Appx5062-5063. Moreover, he agreed that if the Roche applicants had wanted to describe as their invention a fusion protein that included the p75 sequence in Smith—rather than the Figure 4 sequence that they possessed—"[n]othing stopped … the applicants from saying … our preferred sequence is Smith or we incorporate Smith by reference." Appx5064. Ultimately, Plaintiffs' expert described the priority application's reference to Smith as an example of a deletion as "making no sense": "I can't make sense of it as it says because it's on its face ridiculous." Appx5061-5062. The best he could do was to describe this reference as "refer[r]ing a person of skill in the art to Smith," and identifying it as a "landmark paper" that a skilled artisan should "go and read." Appx5063; Appx5091-5092. That is a far cry from *describing* a DNA sequence contained in Smith as part of Roche's invented fusion protein. At most, it would make such a protein-portion obvious, which is not the standard. *See Indenix*, 2019 WL 5583543, at *10.

b. Roche characterized its invention as a fusion protein that used the Figure 4 sequence, rather than the sequence disclosed in Smith, as the TNFR portion. That was no accident—Roche knew it had described a sequence that was different than Smith, and chose to include its own as the basis for its fusion protein. This is perhaps most clear from Roche's prosecution of the '029 patent, which was directed to the TNFR sequence disclosed in Figure 4. *See* p. 11, *supra*. During that prosecution, Roche distinguished its sequence from the prior-art Smith sequence precisely because its sequence was shorter and included important variations. Roche told the PTO that the Smith sequence is "a cDNA sequence encoding a human TNF-R of about 80 kD, whereas applicants claim[ed] a purified and isolated polynucleotide encoding an insoluble protein which has an apparent molecular weight of about 75 kilodaltons." Appx31502-31503; *see* Appx31500. Roche further distinguished its p75 receptor from Immunex's full-length p75 receptor because Roche's receptor contains the three amino-acid mutations and one extra amino acid described above. Appx31501-31502.

Roche's decision to focus on the distinct Figure 4 sequence was perfectly understandable. As explained, pp. 10-11, *supra*, Roche had failed to clone the full sequence disclosed in Smith, but had only identified smaller sequences, including the Figure 4 sequence and the much smaller SEQ IDs described in the priority application. *E.g.*, Appx4857. Accordingly, in describing the "final invention" that

Roche "conceive[d] of and complete[d]," *Billups-Rothenberg*, 642 F.3d at 1036, Roche naturally focused on a fusion protein with the TNFRs that Roche itself had developed, which was *not* etanercept. Indeed, when Roche needed etanercept—a fusion protein based on the full Smith sequence—for its own clinical trials, it had to *ask Immunex for it*. Appx10611.

Immunex *itself* evidently recognized that Roche had not described a fusion protein with the full p75 receptor sequence. As explained, pp. 18-19, *supra*, after Immunex took over prosecution, it amended the specifications to reference Immunex's own Smith publication—serving to highlight that Roche had *failed* to do just that in the priority application. Most blatantly, Immunex amended the specification to add the Smith sequence as Figure 5 in what ultimately became the '522 patent; to state that Smith was "incorporated by reference" into that specification; and to reference, in both patent specifications, a 2006 plasmid deposit of the full sequence never mentioned in the priority application (because it took place a decade after the priority application was filed). Appx22640-22641. These amendments to the specification do not change the written-description analysis, which focuses on "the written description of the *original priority application*." *Novozymes*, 723 F.3d at 1344 (emphasis added). But they are highly revealing as to how Immunex itself read Roche's specification: If Roche's priority application *already* described an invention that encompassed a fusion protein with the Smith

sequence, Immunex would have had no need to amend the specification to incorporate that same sequence.

c. Without even acknowledging many of Sandoz's arguments, the district court found adequate written-description support largely by concluding that a skilled artisan could have deduced the later-added claims by combining the application's disclosures with other prior-art references. That obviousness-based approach to written description was legally and factually flawed.

The district court most clearly went beyond the "four corners of the specification," *Ariad*, 598 F.3d at 1351, in concluding that a skilled artisan could have uncovered the full p75 sequence based on two small fragments of that sequence described in the priority application as SEQ ID NO: 10 and SEQ ID NO: 7. Appx14-15; Appx18-19. These two sequences disclose only 36 of the 235 amino acids that make up the full p75 sequence. Appx18. But the court nonetheless suggested that a skilled artisan could have taken these two sequences, submitted them to a third-party depository, received back the full p75 sequence, and then used the extracellular portion of *that* sequence in the fusion protein instead of the Figure 4 sequence actually described. Appx18-19. The court held that this was enough to "sufficiently describe the subject fusion protein using the known full p75 sequence." Appx19.

Even if a skilled artisan could have used these fragments to deduce the full p75 sequence in this way and then used that sequence in a fusion protein, at best that

would show that the priority application rendered the later-added claims directed to etanercept *obvious*, not that the *application itself* described a fusion protein that included the full p75 sequence. Notably, Plaintiffs' expert admitted that there is no teaching directing a skilled artisan to combine SEQ ID 10 and 7 together to get the p75 portion to use in a fusion protein. Appx5070.

The district court also relied heavily on the fact that the p75 amino acid sequence supposedly "was well known to a POSA at the time of the invention." Appx16. Again, that misses the point. The written-description problem here is not that the art was silent on p75 sequences; it is that the priority application specified *exactly* what p75 sequence Roche had in its possession, which was the sequence Roche itself had discovered and described in Figure 4. While the priority application did not need to "re-descri[be]" invoked and known prior-art concepts, *Capon v. Eshhar*, 418 F.3d 1349, 1357 (Fed. Cir. 2005), it cannot incorporate every *un*-invoked prior-art concept related to the described invention; otherwise, this Court's repeated instruction that the inquiry is limited to the "four corners of the specification" would have no meaning. *Ariad*, 598 F.3d at 1351.

Finally, the district court similarly missed the point in concluding that the specification's reference to Smith as a "deletion" would not have "deterred" a skilled artisan from looking to Smith. Appx17-18. Merely *looking* to Smith is not enough. Rather, the passage describing Smith *only* as an example of a deletion made clear

that (as Plaintiffs' expert conceded) the Roche inventors were aware of Smith and yet were *not* incorporating Smith—or the DNA sequence it disclosed—as part of Roche's invented fusion protein. Appx5063.

2. The priority application did not adequately demonstrate possession of the claimed p75-IgG$_1$ fusion protein.

Describing a broad genus of compounds is insufficient to provide written-description support for a claim directed to a specific compound. Rather, the original disclosure must provide enough direction to lead skilled artisans to "single out" the invention from the various alternatives discussed in the disclosure. *Purdue*, 230 F.3d at 1326; *see also Boston Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1367-68 (Fed Cir. 2011). As this Court explained, "one cannot disclose a forest in the original application, and then later pick a tree out of the forest and say here is my invention. In order to satisfy the written description requirement, the blaze marks directing the skilled artisan to that tree must be in the originally filed disclosure." *Purdue*, 230 F.3d at 1326. The district court's decision flouts this basic principle.

a. The priority application did not provide *any* indication that Roche had invented the *specific* p75-IgG$_1$ fusion protein that Immunex later claimed. First, it did not provide "blaze marks" suggesting that the Smith p75 sequence should be chosen for a fusion protein. To the contrary, for a skilled artisan to arrive at a fusion protein with the Smith sequence, she would have had to ignore the Figure 4 sequence repeatedly identified in the specification as "preferred"; ignore Examples 1-8 that

use Figure 4; and select the never-referenced full Smith sequence, even though many other soluble fragments of that sequence would have bound TNF.

Second, after selecting the Smith sequence, a skilled artisan would have had to select both IgG_1 and the exon-encoded version of the hinge. The specification mentioned 5 different immunoglobulin classes, associated with a wide range of potential hinges. Appx4483-4484. Indeed, Immunex argued below that "[a] POSA selecting an IgG would not have selected an exon-encoded hinge-CH2-CH3 of IgG1." Appx60334. Thus, even if the IgG_1 and the exon-encoded hinge were *described* as *possible* options within the broad genus of fusion proteins disclosed, the priority application provided no "blaze marks" that would have led a skilled artisan to their selection.

Third, to the extent the priority application provided blaze marks, they went to *different* proteins than etanercept. Example 11, the only example in the priority application directed to making a fusion protein, is directed to a p55-IgG_3 protein. As one of the Roche inventors admitted, a skilled artisan would need to alter the Example 11 method in many ways to make a p75-IgG_1 protein. Appx4844-4845; *see also* Appx4495-4497.

b. The district court's discussion of Roche's possession of Immunex's later-claimed fusion protein ignored these governing legal principles and impermissibly relied on "hindsight," "working backward from a knowledge of [the

claims] … to derive written description support from an amalgam of disclosures plucked selectively from the application." *Novozymes*, 723 F.3d at 1349.

Most blatantly, the district court relied on *the claims themselves* as evidence of the required "blaze marks." For instance, in discussing why Example 11 provided written-description support, the court stated that a skilled artisan would have modified that example to use p75 to create etanercept "based on *the claims in the Patents-in-Suit* and the specification." Appx20 (emphasis added). The court similarly found possession because "*the claim language* identifies the requisite elements of the subject invention …. and, in conjunction with the specification, provides support of possession." Appx21 (emphasis added). And the district court's ultimate finding of possession was "based on the specifications …, including the examples within the specifications, *and the claims*." Appx22 (emphasis added).

This repeated reliance on *the claims themselves* as written-description support is a flagrant use of improper hindsight. This Court held in *Ariad* that even claims in *the original application* need their own written-description support. 598 F.3d at 1349. Using claims added many years *after* the original disclosure to demonstrate possession is even more impermissible. *E.g.*, *Purdue*, 230 F.3d at 1326-27.

III. The district court's obviousness analysis was infected by legal error.

A. Standard of review.

"[W]hether a claimed invention would have been obvious is a question of law reviewed de novo." *Bayer Pharma AG v. Watson Labs, Inc.*, 874 F.3d 1316, 1321 (Fed. Cir. 2017).

B. The district court's motivation analysis disregarded the asserted claims' objective reach and instead focused on the inventors' motivation.

As of the priority date, the components of etanercept were well known and actively studied. The district court found that the prior art taught the DNA sequences of the p75 receptor and hinge-CH2-CH3 of the IgG_1, both of which are required to construct etanercept. Appx16, Appx20, Appx26. The court also found that a skilled artisan could construct etanercept and would reasonably expect etanercept to bind TNF. Appx26; Appx49. The court nevertheless held that the asserted claims were nonobvious because a skilled artisan would not have been motivated to either select the p75 receptor or combine it with an immunoglobulin based on a concern that it could stimulate inflammation and thus would be ineffective to treat inflammatory conditions like rheumatoid arthritis.[3]

[3] *See, e.g.*, Appx32-39 (addressing only whether a skilled artisan, seeking to treat rheumatoid arthritis or another pro-inflammatory disease, would be motivated to select the p75 receptor and IgGs), Appx40 ("[T]he prior art … taught that Ig fusion proteins activated effector functions leading to inflammation in the body.… Given this prior art, a POSA would have expected a fusion protein combining TNFR and IgG_1 to lead to autoimmune damage caused by effector functions."); Appx41 ("[T]he

That was legal error: the asserted claims are not directed to the treatment of any disease or condition, let alone rheumatoid arthritis or other inflammatory conditions. The sole limitation requiring any specific activity is contained in the asserted claims of the '182 patent, which only require the construct to "specifically bind TNF"—a function that, without dispute, a person of skill would have fully expected the claimed TNFR/IgG fusion protein to produce. Appx48-49. Indeed, the specification does not even mention rheumatoid arthritis, nor does it contain any data concerning the treatment of any disease.

By nonetheless centering its analysis on whether a skilled artisan would have been motivated to develop etanercept as an autoimmune-disease treatment, the district court adopted a "narrow conception of the obviousness inquiry" that precedent rejects. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 419 (2007). "[N]either the particular motivation nor the avowed purpose of the patentee controls. What matters is the objective reach of the claim." *Id.*

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prior art actually taught away from using an Ig fusion protein, such as the one proposed in Smith '760, to treat auto-immune diseases because such a construct would have likely elicited an inflammatory response in the body."); Appx44-45 (a skilled artisan would have disregard the prior-art's teaching to modify the Smith '760 protein by removing the CH1 and light chains, because the prior art would have "dissuaded a POSA from making these modifications … based on their proven increase in effector functions," which could increase inflammation).

Narrowing the motivation inquiry to exclude the full scope of the claim is reversible error here because the district court ignored the undisputed evidence that several groups working in the field at the time *were* making TNFR/IgG fusion proteins. Appx4176-4177; Appx4155-4156; Appx5170-5171; *see* Appx10602; Appx28205; Appx28199. Moreover, it was undisputed that TNFRs and TNFR/IgG fusion proteins were considered useful, not just as potential therapeutic compounds, but, importantly, as potential diagnostic and research tools. Appx4161-4162; Appx4832; Appx28349. Yet the district court did not address these potential uses, instead focusing exclusively on whether a person of skill would be motivated to make a TNFR/IgG fusion protein to treat autoimmune diseases. That is a glaring error. *See Nalpropion Pharms., Inc. v. Actavis Labs., FL, Inc.*, 934 F.3d 1344, 1354 (Fed. Cir. 2019) (rejecting patentee's motivation argument that contradicted "[t]he inescapable, real-world fact … that people of skill in the art *did combine*" two medical treatments).

C. The district court's analysis of secondary considerations was legally erroneous.

The district court also committed legal error in its decision on certain objective indicia of nonobviousness—specifically on praise, "clinical success," long-felt need, and failure of others—by incorrectly analyzing the required nexus between the claims and these asserted objective indicia. Appx52; Appx54. The court then compounded that legal error by improperly dismissing the evidence of Immunex's

simultaneous invention of etanercept (Appx57-58), resulting in a peculiar ruling that evidence of *Immunex's* success led to a finding of failure of others, when in fact Immunex—an "other"—succeeded where the inventors actually failed.

"For objective evidence to be accorded substantial weight, its proponent must establish a nexus between the evidence and the merits of the claimed invention." *In re GPAC Inc.*, 57 F.3d 1573, 1580 (Fed. Cir. 1995). Where objective evidence relates to "something other than what is both *claimed and novel* in the claim, there is no nexus to the merits of the claimed invention." *Merck & Cie v. Gnosis S.P.A.*, 808 F.3d 829, 837 (Fed. Cir. 2015).

Most particularly, the court failed to consider earlier patents claiming etanercept, including the '690 patent, and to define what was novel about the patents-in-suit compared to those earlier patents. With no evidence that objective indicia are "a result of the novel features in the … patent[s], as opposed to the other patents involved," any such indicia are "not sufficiently connected with the novel elements of the asserted claims" and thus "carry little weight." *Id.* at 838-39.

The court further legally erred in dismissing the evidence of simultaneous invention, especially by Immunex. Appx56-58. In fact, *only* Immunex combined the specific pieces set forth in the claims—the inventors never did. All the benefits etanercept allegedly offered were attributable to Immunex's work, and Immunex's patents. The court misread this Court's cases to establish a rule that one instance of

simultaneous invention is insufficient. Appx58. In the case cited, the alleged simultaneous inventors had collaborated on or seen the patented invention. *Lindemann Maschinenfabrik GmbH v. Am. Hoist & Derrick Co.,* 730 F.2d 1452, 1460 (Fed. Cir. 1984). Here, by contrast, Immunex invented etanercept independently of the inventors.

Likewise, the court legally erred in concluding that there was a "failure of others," while relying on the work of others—*i.e.*, Immunex—and ignoring the failure of the Roche inventors. Appx53-54.

CONCLUSION

This Court should reverse the district court's decision and hold that the asserted claims of the patents-in-suit are invalid.

November 8, 2019

Respectfully submitted,

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ADDENDUM TABLE OF CONTENTS

UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

IMMUNEX CORP., *et al.*, v. SANDOZ INC., *et al.*	Civil Action No.: 16-1118 (CCC) **OPINION**

CECCHI, District Judge.

This patent case was brought by Plaintiffs Immunex Corporation ("Immunex"), Amgen Manufacturing, Limited ("Amgen"), and Hoffman-La Roche, Inc. ("Roche") (collectively, "Plaintiffs") against Defendants Sandoz Inc., Sandoz International GmbH and Sandoz GmbH (collectively, "Defendants"). Specifically, this action relates to the validity of claims 11-12 and 35-36 of U.S. Patent No. 8,063,182, which covers the fusion protein etanercept, the active ingredient in Immunex's product Enbrel® (Joint Trial Exhibit ("JTX")-1[1] ("the '182 Patent")), and claims 3, 8, and 10 of U.S. Patent No. 8,163,522, which covers Enbrel®'s method of manufacture (JTX-2 ("the '522 Patent")) (collectively, the asserted claims of the "Patents-in-Suit"). *See* ECF No. 18 ¶ 9. Enbrel® is a brand name biologic drug primarily used to treat rheumatoid arthritis. Id. ¶¶ 43, 45; ECF No. 688 at 11 ¶ 38.

The Court held a two-week bench trial in this matter that began on September 11, 2018 and concluded on September 25, 2018. ECF Nos. 621-622, 627, 629-635. The parties submitted post-trial briefing and proposed findings of fact and conclusions of law through early November 2018. ECF Nos. 648 (*corrected at* 651-2 ("PFOF")), 647 (*corrected at* 649-2 *and subsequently corrected at* 650-1 ("DFOF")), 645 (*corrected at* 651-1 ("Pls. Br.")), 646 (*corrected at* 649-1 *and*

[1] JTX refers to the joint trial exhibits submitted by the parties. These exhibits have been mutually agreed to as admissible.

subsequently corrected at 650-2 ("Defs. Br.")). On November 6, 2018, the parties submitted response briefs. ECF Nos. 653 ("Pls. Reply Br."), 652 ("Defs. Reply Br."). Closing arguments were held on November 19, 2018. ECF No. 656.

Enbrel® is the first U.S. Food and Drug Administration ("FDA") approved fusion protein, approved in November 1998. PFOF ¶¶ 8, 10; DFOF ¶ 12. In August 2016, the FDA approved Defendants' biosimilar version of Enbrel®, called Erelzi™. PFOF ¶ 11; ECF No. 688 at 11 ¶¶ 41-43. Defendants do not contest infringement of the '182 Patent or the '522 Patent. ECF No. 619; PFOF ¶ 16. Therefore, the issue left for this Court to decide is whether the Patents-in-Suit are invalid based on the following legal principles: (1) lack of written description and enablement; (2) obviousness; and (3) obviousness-type double patenting.

This Opinion constitutes the Court's findings of fact and conclusions of law pursuant to Federal Rule of Civil Procedure 52(a). The findings of fact are based on the Court's observations and credibility determinations of the witnesses who testified, and a thorough review of all the evidence admitted at trial. While the Court has reviewed all of the evidence presented, given the length of the trial record, the Court includes references only to the evidence most pertinent to its analysis. For the reasons set forth below, the Court finds that the Patents-in-Suit are not invalid.

I. BACKGROUND

A. Parties

Plaintiff Roche was the first to file the patent applications that eventually issued as the Patents-in-Suit. PFOF ¶ 51. Thereafter, Plaintiffs Amgen and Immunex obtained certain rights from Roche pertaining to the Patents-in-Suit, pursuant to an agreement called the Accord and Satisfaction, which included the right to take over the prosecution of the relevant patent applications and the right to commence an infringement action. JTX-12. Plaintiff Roche is a New Jersey corporation with its principal place of business in New Jersey. ECF No. 18 ¶ 3. Plaintiff

Immunex is a Washington corporation with its principal place of business in California and is a wholly owned subsidiary of non-party Amgen Inc. Id. ¶ 1. Plaintiff Amgen is a corporation of the Territory of Bermuda with its principal place of business in Puerto Rico and is also a wholly owned subsidiary of non-party Amgen Inc. Id. ¶ 2.

Defendant Sandoz Inc. is a Colorado corporation with its principal place of business in New Jersey. Id. ¶ 4. Defendant Sandoz International GmbH is a German corporation with its principal place of business in Germany. Defendant Sandoz GmbH is an Austrian corporation with its principal place of business in Austria and is a subsidiary of Sandoz International GmbH. Id. ¶¶ 6-7. Sandoz Inc. is the United States agent for Defendants Sandoz International GmbH and Sandoz GmbH. Id. ¶ 4. All parties are in the business of developing, manufacturing, marketing, and selling biopharmaceutical products. Id.

B. Background of the Invention

The active ingredient in the biopharmaceutical drug at issue in this case is a fusion protein known as etanercept that is made by combining the extracellular region of a 75 kilodalton Human Tumor Necrosis Factor Receptor with a portion of an IgG1 immunoglobulin. This section will first provide the scientific background of the claimed invention, by explaining each component and its purpose. Next, the Court will provide the relevant research and patent history for the Patents-in-Suit.

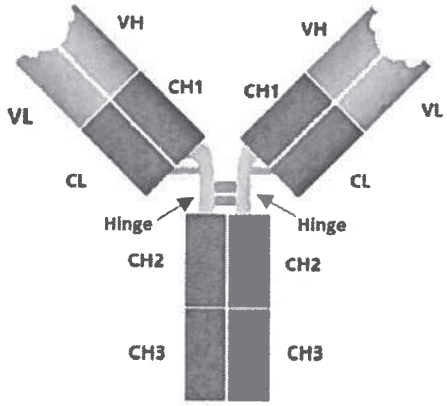
1. Scientific Background

Rheumatoid arthritis is an inflammatory auto-immune disease, i.e. a disease which occurs when "an overactive immune system attacks an individual's own body," and causes bone erosion, narrowing of joint space, and irreversible joint damage. PFOF ¶¶ 32-33. One way to treat rheumatoid arthritis is to "dampen the immune system" and to "inhibit inflammatory reactions." Id. ¶¶ 47-48. The immune system is made up of various cells and antibodies that protect the body

from foreign invaders. Id. ¶ 23. Antibodies have two primary functions: to bind foreign substances known as antigens, and to recruit other immune system components to attack antigens. Id. There are many classes and subclasses of the antibody immunoglobulin or "Ig", of which IgG is one such class. Id. ¶¶ 99, 158. There are four subclasses of human IgG: IgG1, IgG2, IgG3, and IgG4. Id.

IgG is a protein, and proteins are made up of "amino acid residues connected in a strand called a 'polypeptide,' which folds into a three-dimensional shape that imparts certain structural and functional characteristics." Id. ¶ 20. Scientists can identify protein sequences based on the order of amino acids in the protein, with the beginning portion of the sequence referred to as the "N-terminus" and the end portion referred to as the "C-terminus." Id. ¶¶ 21-22.

Structurally, an IgG protein, pictured below, consists of two heavy chains and two light chains, and each chain contains variable and constant regions. Id. ¶ 24. The constant region is the portion that interacts with other components of the immune system to elicit a response. Id. The heavy chain constant region includes the CH1, the hinge, CH2, and CH3 domains while the light chain constant region consists of the CL domain. Id. The variable region of each chain, labeled here as VH and VL, is what binds to the antigen. Id.

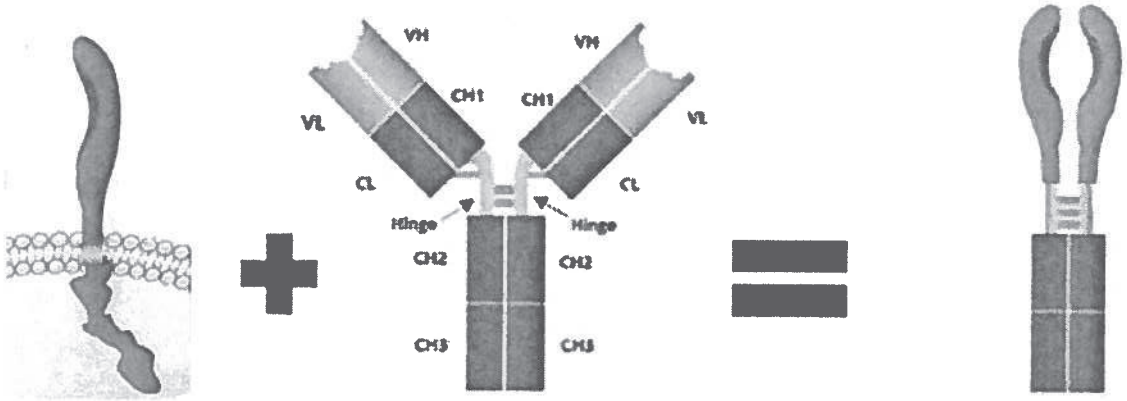


DFOF ¶ 208.

Another component of the immune system, called a cytokine, is a messenger protein that has a wide variety of functions, including to initiate an immune response. PFOF ¶ 27. The body makes dozens of distinct cytokines, one of which is the Human Tumor Necrosis Factor ("TNF"). Id. ¶¶ 27-29. TNF can be found in an insoluble (membrane-bound) or soluble (free-flowing) form. Id. ¶ 28. Originally discovered to kill tumor cells, TNF has many functions and by August 1990, scientists associated it with inflammatory diseases, such as rheumatoid arthritis. Id. ¶¶ 28-33.

TNF plays a significant role in auto-immune disorders. Id. TNF binds to certain proteins called TNF receptors ("TNFRs") that extend beyond the outer membrane of a cell. Id. ¶ 30. TNFRs have three regions: intracellular, transmembrane, and extracellular. Id. The extracellular portion of the TNFR, which is the portion that "protrudes outside the cell," can be split off to produce a "soluble" fragment of the TNFR that can bind to TNF. Id. ¶¶ 30, 76. Two types of TNFRs have been identified, one that has a molecular weight of approximately 55 kilodaltons ("p55 TNFR" or "p55") and one with a molecular weight of approximately 75 kilodaltons ("p75 TNFR" or "p75"). Id. ¶¶ 36-38.

Etanercept, the active ingredient in the biopharmaceutical drug Enbrel® at issue here, is a fusion protein that combines the extracellular region of a p75 TNFR with an IgG1. Id. ¶ 9. "A fusion protein is made by combining DNA sequences encoding parts of different proteins into one sequence, introducing that sequence into host cells, and using their natural internal machinery to produce the desired fusion protein." Id. ¶ 19. Specifically, etanercept is a "dimeric fusion protein consisting of the extracellular region of the p75 TNF receptor" which, as the parties have stipulated, is "fused to the exon-encoded 'hinge-CH2-CH3' of the constant region of a human IgG1 antibody heavy chain." Id. ¶ 9; DFOF ¶ 93; ECF No. 688 at 20 ¶ 68. Etanercept works by binding to and neutralizing excess TNF in patients with rheumatoid arthritis, thereby reducing the auto-immune inflammatory response. PFOF ¶ 244. The graphic below depicts images of a p75 TNFR and an IgG1 on the left-hand side and etanercept on the right-hand side. The Patents-in-Suit cover etanercept and the method of making etanercept. Id. ¶ 76.



p75 TNFR **IgG1 Immunoglobulin** **Etanercept**

DFOF ¶¶ 208, 214.

2. Research and Patent History

By 1990, "there was a high level of interest in studying TNF and investigating whether targeting TNF with a TNF-binding protein would provide a therapeutic benefit by inhibiting the binding of TNF to its cell-bound receptors." DFOF ¶¶ 1, 14. At that time, scientific evidence pointed to at least two TNFRs expressed by the human body: p55 and p75 TNFR. PFOF ¶¶ 37-38; DFOF ¶ 2. In April 1990, researchers at Roche (the "Roche Inventors"[2]) published the complete amino acid sequences for the p55 TNFR and the cDNAs[3] encoding it. PFOF ¶ 39; DFOF ¶¶ 15, 16; JTX-21 at 1. In May 1990, Immunex published an article containing the complete amino acid sequence for p75 and therein stated that the researchers isolated a cDNA clone of the receptor. PFOF ¶ 40; Smith, C.A., et. al., *A Receptor for Tumor Necrosis Factor Defines an Unusual Family of Cellular and Viral Proteins*, Science 248: 1019-23 (1990) (JTX-24) ("Smith 1990"); DFOF ¶ 4. Several months later in July 1990, the Roche Inventors published the complete amino acid sequence for the p75 TNFR and part of its encoding cDNA. PFOF ¶ 39; Dembic, Z. et al., *Two Human TNF Receptors Have Similar Extracellular, But Distinct Intracellular, Domain Sequences*, Cytokine 2(4): 231-37 (1990) (JTX-23) ("Dembic 1990"); DFOF ¶ 30.

Around the same time that the Roche Inventors were publishing studies on the amino acid sequences in p55 and p75 TNFR, they were also exploring the possibility of TNFR-Ig fusion proteins. PFOF ¶ 46. The Roche Inventors were ultimately successful in creating fusion proteins using both p55 and p75 TNFRs. Id. ¶ 49. The initial fusion protein used an IgG3 immunoglobulin,

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[2] The Roche Inventors were Manfred Brockhaus, Reiner Gentz, Zlatko Dembic, Werner Lesslauer, Hansruedi Lötscher, and Ernst-Jurgen Schlaeger.

[3] "cDNA" stands for complementary DNA. The Roche Inventors converted amino acid peptide sequences into DNA sequences and used those DNA sequences as probes to create primers that would allow the Roche Inventors to "fish" out cDNAs encoding TNF receptors out of a cDNA library. PFOF ¶¶ 38-39; DFOF ¶¶ 15-16.

however the Roche Inventors' "pathway of experimental work leading to a TNFR fusion protein" also contemplated fusion proteins with IgG1 and IgG2 immunoglobulins. Id. ¶¶ 50, 58-68.

On August 31, 1990, the Roche Inventors filed a patent application in Europe bearing Application No. 90116707 ("EP '707 Application") and on September 13, 1990, they filed a U.S. Patent with Application No. 07/580,013 ("'013 Application"). Id. ¶ 51. The Patents-in-Suit claim the benefit of the '013 Application and priority to the European '707 Application. Id. The Patents-in-Suit, as well as the EP '707 Application and the '013 Application, encompass a p75 TNFR-IgG1 fusion protein, but because the parties differ in their assessments of the patent specifications and validity of the claimed invention, further details on the Patents-in-Suit will be discussed below. Id. ¶¶ 50-53; DFOF ¶¶ 36-37.

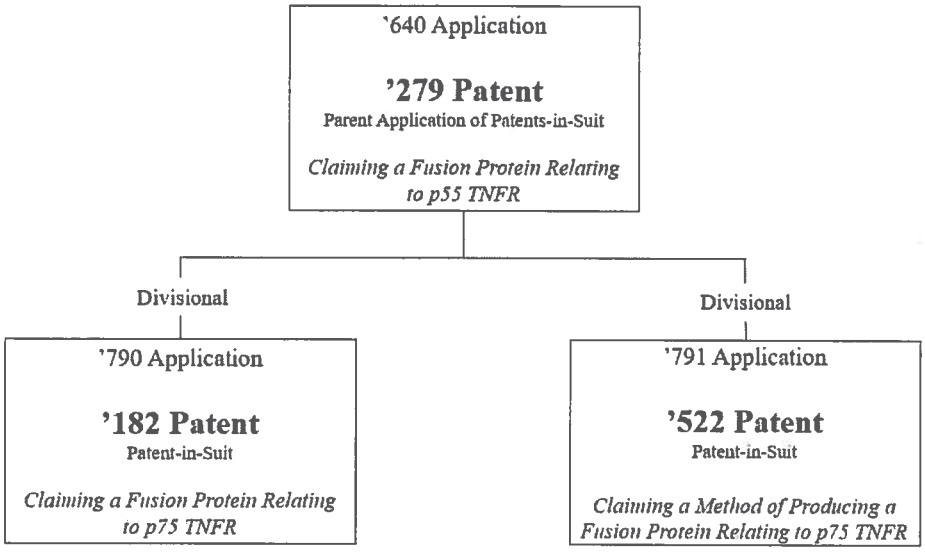
C. Patents-in-Suit and Relevant Prosecution History

1. The '182 Patent

The '182 Patent, entitled "Human TNF Receptor Fusion Protein," issued on November 22, 2011 and expires on November 22, 2028. PFOF ¶ 74; DFOF ¶ 83. The asserted claims "define a fusion protein consisting of parts of two different proteins: the extracellular region of p75 fused to all of the domains of the human IgG1 constant region other than the first domain." PFOF ¶¶ 74-76; *see also* '182 Patent (JTX-1) col. 39:60-67, 42:26-34.

The initial '013 Application was abandoned, and U.S. Application No. 08/965,640 ("'640 Application") was filed on July 21, 1993 as a continuation. PFOF ¶ 57; DFOF ¶¶ 38-39. The '640 Application was subject to a restriction requirement by the United States Patent and Trademark Office ("USPTO") and in response Roche elected to pursue claims related to the p55 fusion protein, which issued as U.S. Patent No. 5,610,279 ("'279 Patent") on March 11, 1997. PFOF ¶ 57; DFOF ¶¶ 39-40; ECF No. 688 at 6 ¶ 9. As a result of the restriction, Roche then filed two divisional applications on May 19, 1995: U.S. Application No. 08/444,790 ("'790 Application"),

which issued as the '182 Patent, and U.S. Application No. 08/444,791 (the '791 Application"), which issued as the '522 Patent. *See* PFOF ¶ 57; DFOF ¶ 41.



In 2004, prior to issuance of the '182 Patent, Amgen and Immunex acquired the exclusive right to prosecute the Patents-in-Suit, among other rights, from Roche pursuant to an Accord and Satisfaction between non-party Amgen Inc., Immunex, and Roche. JTX-12 at 4-6, Article 3, ¶¶ 3.1-3.6; *see also* PFOF ¶ 34; DFOF ¶¶ 54, 58, 62. Those rights were later consolidated in Immunex by a separate agreement. JTX-14. In 2005, Immunex amended the '790 Application in response to a USPTO office action requiring the '790 Application to come into consonance with the restriction requirement. PFOF ¶ 285; DFOF ¶ 73. The '790 Application was again amended in 2006. PFOF ¶ 144; DFOF ¶ 74. Despite the amendments, the '790 Application was rejected "for failing to comply with the written description requirement and as obvious over the applied prior art," and the rejection was appealed to the Board of Patent Appeals and Interferences ("BPAI"). Plaintiffs' Trial Exhibit ("PTX")-6.456 ("BPAI Opinion"). The BPAI reversed the examiner's rejection. PTX-6.456 at 9 (BPAI Opinion reversing rejection by examiner). The '182 Patent then issued on November 22, 2011. *See generally* '182 Patent (JTX-1).

2. The '522 Patent

The '522 Patent, entitled "Human TNF Receptor," issued on April 24, 2012 and expires on April 24, 2029. PFOF ¶ 74; DFOF ¶ 83. The asserted claims "define a method of producing [the] fusion protein" defined in the '182 Patent. '522 Patent (JTX-2) at 47-48 (claims 3, 8, 10); PFOF ¶ 75. The '522 Patent issued from the '791 Application, which was filed on May 19, 1995 as a divisional of the '640 Application, along with the '790 Application which issued as the '182 Patent. PFOF ¶ 57; DFOF ¶ 48.

Prior to the '522 Patent's issuance, Amgen and Immunex amended the '791 Application in 2004, 2007, and 2010 to include several references related to the full amino acid sequence for p75. *See, e.g.*, '522 Patent (JTX-2) col. 3:1-3, Fig. 5; DFOF ¶¶ 78-80. Like the amendments to the '182 Patent, these amendments were triggered by two USPTO actions, which rejected the '791 application for obviousness and insufficient written description. PTX-7.351. Despite the amendments, the '791 Application was still rejected, and that rejection was eventually overcome by citing the '790 Application BPAI Opinion which dealt with similar issues. PFOF ¶ 323; JTX-4 at 4952-53. The '522 Patent then issued on April 24, 2012. *See generally* '522 Patent (JTX-2).

II. ISSUES TO BE DECIDED

Prior to the commencement of trial, Defendants advised that they did not contest infringement of the Patents-in-Suit. ECF No. 619. As discussed above, the parties also stipulated that the term "all of the domains of the constant region of a human immunoglobulin IgG[1] heavy chain other than the first domain of said constant region" is construed as meaning "the exon-encoded 'hinge-CH2-CH3' region of human [IgG/IgG1]." ECF No. 688 at 20 ¶ 68. Accordingly, the question before this Court is whether the '182 and '522 Patents are invalid due to lack of written description and enablement, obviousness, and obviousness-type double patenting.

III. DISCUSSION

Issued patents are presumed valid. *See* 35 U.S.C. § 282(a). To rebut this presumption, Defendants bear the burden of proving invalidity by clear and convincing evidence. *Titan Tire Corp. v. Case New Holland, Inc.*, 566 F.3d 1372, 1376 (Fed. Cir. 2009) ("Because of this presumption, an alleged infringer who raises invalidity as an affirmative defense has the ultimate burden of persuasion to prove invalidity by clear and convincing evidence, as well as the initial burden of going forward with evidence to support its invalidity allegation.").

A. Written Description and Enablement (35 U.S.C. § 112)

A patent specification "shall contain a written description of the invention." 35 U.S.C. § 112. The specification must "reasonably convey[] to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date." *Ariad Pharm. Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010). The test for written description "requires an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art."[4] *Id.* "[W]hether a patent complies with the written description requirement will necessarily vary depending on the context. Specifically, the level of detail required . . . varies depending on the nature and scope of the claims and on the complexity and predictability of the relevant technology." *Id.* (citation omitted). When reviewing the patent according to these principles, "[w]ritten description is a question of fact, judged from the perspective of [a POSA] as of the relevant filing date." *Falko-Gunter Falkner v. Inglis*, 448 F.3d 1357, 1363 (Fed. Cir. 2006) (citing *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64 (Fed. Cir. 1991)).

[4] A person of ordinary skill in the art will hereinafter be referred to as a "POSA."

Additionally, as to enablement, a patent specification must describe "the manner and process of making and using [the invention], in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains . . . to make and use the same . . . ." 35 U.S.C. § 112. Moreover, enablement requires that the specification teach a POSA "how to make and use the full scope of the claimed invention without undue experimentation." *Martek Bioscis. Corp. v. Nutrinova, Inc.*, 579 F.3d 1363, 1378 (Fed. Cir. 2009) (citation omitted). A patentee need not "include in the specification that which is already known and available to [a POSA]" and "not every last detail is to be described, else patent specifications would turn into production specifications, which they were never intended to be." *Koito Mfg. Co. v. Turn-Key-Tech, LLC*, 381 F.3d 1142, 1156 (Fed. Cir. 2004) (citation omitted). "Enablement is a question of law involving underlying factual inquiries." *Falkner*, 448 F.3d at 1363 (citing *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997), *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988)).

Defendants argue that the Patents-in-Suit are invalid because their specifications (1) lack a sufficient written description of the invention and (2) do not enable a POSA to make or use the invention. Defs. Br. at 20-35. By contrast, Plaintiffs contend that the specifications are adequate, and that Defendants failed to prove their written description or enablement claims by clear and convincing evidence. Pls. Br. at 12-21.

In support of their arguments, the parties relied heavily on the testimony of the following four witnesses: (1) Defendants' expert Daniel Capon, Ph.D., (2) Defendants' expert Carl P. Blobel, M.D., Ph.D., (3) Plaintiffs' expert James Naismith, Ph.D, and (4) Plaintiffs' expert Hansruedi Loetscher, Ph.D.[5] For the reasons set forth below, the Court finds that Defendants

[5] Defendants' expert Daniel Capon, Ph.D. has 37 years of experience in the field of biotechnology, including at Genentech, Inc., Cell Genesys, Inc., Xenotech, Inc., and ViroLogic, Inc. Defendants'

failed to prove invalidity based on the written description and enablement requirements by clear and convincing evidence, and therefore the Patents-in-Suit are not invalid under 35 U.S.C. § 112.

1. The Specifications Meet the Written Description Requirement

Defendants argue that the specifications are deficient because they neither sufficiently describe etanercept nor convey that the Roche Inventors had possession of etanercept, and that further, the specifications in conjunction with the claims do not direct a POSA to the specific embodiment of etanercept. Defs. Br. at 20-32. Plaintiffs counter that the necessary elements of the claimed invention are adequately described throughout the specifications, were known and available prior to August of 1990, and that the specifications adequately describe the novel combination of those elements to create etanercept. Pls. Br. at 13-21. Therefore, Plaintiffs contend that the specifications demonstrate possession and the patents properly direct a POSA to etanercept. Id.

The '182 Patent claims a fusion protein consisting of the extracellular portion of the p75, as well as the exon-encoded hinge, CH2 and CH3 domains of human IgG1, while the '522 Patent claims the method of making the fusion protein. '182 Patent (JTX-1) col. 39:14- 42:34; 9/18 AM (Naismith) Tr. at 89:2-12, 91:8-14; '522 Patent (JTX-2) col. 45:44-48:4. The patent specifications of the '182 and '522 Patents identify soluble fragments of p75 TNFR as one of two TNF binding

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expert Carl P. Blobel, M.D., Ph.D. is a Professor of Medicine, Physiology, and Biophysics at the Weil Medical College of Cornell University and Virginia F. and William R. Salomon Chair in Musculoskeletal Research and Director of the Arthritis and Tissue Degeneration Program at the Hospital for Special Surgery. ECF No. 688 at 131 ¶¶ 43-44. Plaintiffs' expert James Naismith, Ph.D. is a Professor of Structural Biology at the University of Oxford in the United Kingdom who has more than 20 years of research experience on the structure and function of proteins. Id. at 126-127 ¶¶ 35-37. Dr. Naismith's post-doctoral research at the Howard Hughes Medical Institute in Dallas, Texas focused on proteins specifically involved in TNF signaling. Id. Plaintiffs' expert Hansruedi Loetscher, Ph.D., an inventor of the Patents-in-Suit, worked at F. Hoffman-La Roche AG from 1984 through 2016, where he most recently served as the Global Head of Neuroscience Discovery. Id. at 117 ¶¶ 1-3.

proteins, i.e. p55 and p75, used in TNFR-IgG fusion proteins and include both figures and examples that are referenced in the parties' arguments. There are multiple figures in the Patents-in-Suit that provide nucleotide sequences for the TNF binding protein. *See generally* '182 Patent (JTX-1); '522 Patent (JTX-2). In analyzing the specifications, it appears that Figure 1 of the specifications relates to a p55 TNFR and Figure 4 relates to a p75 TNFR.[6] Figure 4 is a "[n]ucleotide sequence . . . and deduced amino acid sequence . . . for cDNA clones derived from" a p75 TNFR, which consists of a long combination of letters representing those amino acids and related cDNA combinations. '182 Patent (JTX-1) col. 2:60-62, Fig. 4. The specifications additionally include multiple examples pertaining to a TNFR-IgG fusion protein. In the examples, both the '182 and '522 Patents notably discuss and disclose two nucleotide sequences for portions of p75—SEQ ID NO: 10 (N-terminus) and SEQ ID NO: 7 (C-terminus).

The Patents-in-Suit disclose using "especially preferred vectors" pCD4-Hγ1 (DSM 5314, deposited on Apr. 21, 1989) and pCD4-Hγ3 (DSM 5523, deposited on Sept. 14, 1989) "[f]or the expression of proteins which consist of a soluble fragment of non-soluble TNF-BP [binding protein] and an immunoglobulin fragment, i.e. all domains except the first of the constant region of the heavy chain." '182 Patent (JTX-1) col. 8:56-9:8. The specifications further state that "the present invention embraces not only allelic variants, but also those DNA sequences which result from deletions, substitutions and additions from one or more nucleotides of the sequences given in FIG. 1 or FIG. 4" and yield TNF-binding proteins. '182 Patent (JTX-1) col. 5:17-22; '522 Patent (JTX-2) col. 5:29-34. The Patents-in-Suit also reference the Smith 1990 article—the

[6] In the '522 Patent, Figure 1 is broken down into Figures 1A-1D and Figure 4 is broken down into Figures 4A-4D.

Immunex publication that includes the complete amino acid sequence for p75. '182 Patent (JTX-1) col. 5:22-24; '522 Patent (JTX-2) col. 5:34-37.

a) The Requisite Components of the Fusion Protein Were Disclosed in the Specifications and Known Prior to August 1990

The Court finds that the specifications of the Patents-in-Suit sufficiently describe the components of etanercept. A patent must include sufficient details such that a POSA could understand the subject invention and recognize that the inventor possessed it. *Ariad*, 598 F.3d at 1351. However, this requirement does not necessarily mean that the specification of the patent must include every nuanced detail.[7] Indeed, "[a] patent need not teach, and preferably omits, what is well known in the art." *Falkner*, 448 F.3d at 1365 (quoting *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1534 (Fed. Cir. 1987)); *see also Capon v. Eshhar*, 418 F.3d 1349, 1357-58 (Fed. Cir. 2005) (holding that a patent's specifications do not need to reiterate the structure, formula, or chemical name of a claimed invention to satisfy the written description requirement when that information is already known in the field). The Court will first analyze the sufficiency of the description of p75, followed by the sufficiency of the description of the IgG1 portion of the fusion protein.

[7] Defendants contend that Plaintiffs ignore the controlling precedent in *Ariad*, and improperly ask the Court to venture outside of the specifications to find the requisite written description. Defs. Reply Br. at 12-14. In other words, Defendants assume distinct requirements for an adequate written description before and after the *Ariad* decision. However, the precedent is clear that sequences disclosed in the prior art need not be repeated and the standard has not changed in that regard following *Ariad*. *See Falkner*, 448 F.3d 1357; *Capon*, 418 F.3d 1349; *see also Zoltek Corp. v. United States*, 815 F.3d 1302, 1308 (Fed. Cir. 2016) (post-*Ariad* case confirming that "written description need not include information that is already known and available to the experienced public") (internal quotation marks and citation omitted). The Court finds that the specifications meet the requirements of *Falkner* and *Capon*, which are still current and applicable law, and are not inconsistent with *Ariad*.

i. p75 Is Adequately Described

Analyzing the Patents-in-Suit, the Court finds that p75 is sufficiently described. The specifications of the Patents-in-Suit identify two TNF receptors, p55 and p75, and further note that the invention embraces allelic variants and DNA sequences resulting from deletions, substitutions, and additions of one or more nucleotides of the sequences provided in Figure 1 and/or Figure 4. '182 Patent (JTX-1) col. 4:1-5:24. Sequence identification numbers, which correspond to p75, are mentioned throughout the specification (including the examples therein) and in the claims, and Example 6 explains that the inventors isolated the p75 TNFR. Id. col. 15:31-39.

Furthermore, the prior art demonstrates that the p75 amino acid sequence was well known to a POSA at the time of the invention. The Court may look to prior art and trial testimony when determining what a POSA would have known at the time of the invention. *See, e.g.*, *Ariad*, 598 F.3d at 1351 (relying on expert testimony and examples of prior art to make written description determination); *Falkner*, 448 F.3d at 1365-66. The parties agreed that by August 1990, the p75 TNFR was well known to a POSA. PFOF ¶¶ 86-87; DFOF ¶ 2. Both the Immunex Smith article and the Roche Dembic article, which were published in May 1990 and July 1990 respectively, contain a full recitation of the p75 amino acid sequence. PFOF ¶¶ 89-91; Smith 1990 (JTX-24) at 3-4, Fig. 3B; Dembic 1990 (JTX-23) at 1-2. The Smith 1990 article, expressly referenced in the Patents-in-Suit, also notes that "[t]he entire nucleotide sequence is available upon request and has been deposited with GenBank, accession number M32315." Smith 1990 (JTX-24) at 3-4, Fig. 3B. GenBank is an amino acid repository which can match partial amino acid sequences with full corresponding sequences that have been deposited with GenBank. *See* 9/18 AM (Naismith) Tr. at 62:7-16. Sequences are provided to GenBank as "an information deposit" in which the DNA sequence letters are submitted and an "accession number" is the particular

identification number assigned to each submitted sequence. Id. at 73:17-74:1. Similarly, the Dembic 1990 article contains the entire p75 amino acid sequence.[8] *See* Dembic 1990 (JTX-23) at Fig. 1. Ultimately, neither party contests that the prior art "definitively identified two TNF receptors: the p55 and the p75" by August 1990. Defs. Br. at 21; PFOF ¶¶ 36-38. The parties further agree that Immunex scientists in May 1990 and later the Roche Inventors in July 1990 published the full-length p75 TNFR before the related European priority patent application was filed in August 1990.[9] DFOF ¶ 2; PFOF ¶¶ 39-41.

Defendants, however, argue that because the specifications refer to Smith 1990 as an example of a "deletion" when compared to Figure 4 (when it was instead the complete sequence of Figure 4), a POSA would not have considered using the Smith 1990 sequence. Defs. Br. at 25. Upon review of the disclosure, the Court does not believe a POSA would have been deterred from looking to Smith 1990 for use in the fusion protein due to the term "deletion." Just prior to that language in the specification, the invention embraces not only deletions but also all allelic variants including "substitutions and additions." '182 Patent (JTX-1) col. 5:17-24. In fact, a POSA may

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[8] The Dembic 1990 article also explains that TNFRs that have a molecular weight of either 65 kD or 75 kD are both the p75 protein because the 65 kD TNFR is simply a derivative of p75. Dembic 1990 (JTX-23) at 1. The authors of the Dembic 1990 article arrived at this conclusion because both the 65 and 75 kD TNFRs bound "the same monoclonal antibody." Id.; *see also* 9/18 AM (Naismith) Tr. at 80:9-81:5 (Dr. Naismith testifying that proteins can gain or lose weight depending on glycosylation which "is the addition of sugar molecules" and concluding that TNF receptors with molecular weights of either 65 or 75 kD are both the p75 protein used in etanercept).

[9] By April 1990, the Roche Inventors were the first to discover that there were two distinct TNFRs that specifically bound to TNF, p55 and p75. 9/17 (Loetscher) Tr. at 20:1-18, 26:8-28:8; JTX-22 at 1. In May 1990, Immunex scientists published the Smith 1990 article containing the p75's complete amino acid sequence and included a figure caption indicating that a cDNA sequence encoding the p75 had been deposited with GenBank. Smith 1990 (JTX-24) at 3, Fig. 3B; 9/17 (Loetscher) Tr. at 38:6-24; *see also* 9/13 AM (Capon) Tr. at 85:3-11. Two months later, in July 1990, the Roche Inventors published the complete amino acid sequence of p75 and a cDNA sequence encoding part of it, resulting in the Dembic 1990 article. 9/17 (Loetscher) Tr. at 33:1-33:23; Dembic 1990 (JTX-23) at 2, Fig. 1.

have been encouraged to look to an outside reference, such as the Smith 1990 article, that was expressly called out by name in the specification. 9/18 PM (Naismith) Tr. at 52:23-53:8. At trial, Plaintiffs' expert Dr. Naismith credibly testified that the Smith 1990 reference would have communicated to the ordinary artisan that "[i]f you hadn't read the paper, go and read it. They'd think it was a landmark paper."[10] Id. Thus, the Court agrees with Plaintiffs that despite the word "deletion," a POSA would have been directed to Smith 1990 and therefore the full p75 protein.

In further support of Plaintiffs' arguments, Example 7 contains the N-terminus sequence designated SEQ ID NO: 10. '182 Patent (JTX-1) col. 16:22-30. SEQ ID NO: 10 matches the first 18 amino acids at the N-terminus of the known p75 as published in Smith 1990. Id.; Smith 1990 (JTX-24) at 3, Fig. 3B. The Patents-in-Suit also include the 18 amino acid sequences close to the C-terminus of the known p75 protein designated SEQ ID NO: 7. These two disclosed nucleotide sequences for p75 would have, in addition to Figure 4 and the Smith 1990 reference, directed a POSA to the full p75 sequence at the time of the invention. *See* '182 Patent (JTX-1) col. 39:13-42:34 (claims of the '182 Patent specifically requiring the use of the protein that "comprises the amino acid sequence . . . (SEQ ID NO: 10)"), col. 4:18-20, 16:36-38 (identifying SEQ ID NO:7 as a partial amino acid sequence that makes up a preferred protein); '522 Patent (JTX-2) col. 45:44-48:4 (claims of the '522 Patent specifying the amino acid described in SEQ ID NO: 10), col. 4:31-32, 16:57-58 (listing SEQ ID NO:7 as an example of a partial amino acid

[10] Defendants misconstrue part of Dr. Naismith's testimony as indicating that he believed the Smith reference would have discouraged a POSA from using the known complete p75 TNFR sequence. Defs. Br. at 25; 9/18 PM (Naismith) Tr. at 22:19-23, 23:20-24. Plaintiffs correctly counter that because Figure 4 is a smaller sequence than the Smith 1990 sequence, a POSA would have understood the passage to suggest Smith 1990 as a source of p75 TNFR to use in the fusion protein. Pls. Br. at 21; 9/18 PM (Naismith) Tr. at 22:15-24:3, 52:19-53:8 ("I simply went and read the paper to figure out what a scientist would do . . . Smith is a complete sequence, which was known; and Figure 4 is a partial sequence of many less residues.").

sequence to be used in a preferred protein); *see also* '182 Patent (JTX-1) col. 5:17-22. With respect to the sequence identification numbers for SEQ ID NO: 10 and SEQ ID NO: 7, Plaintiffs' expert Dr. Naismith credibly testified that there was less than a one-in-a-million chance that the wrong protein would be produced by GenBank if an inquiry was made to retrieve the complete p75 sequence corresponding to one of the sequence identification numbers.[11] *See* 9/18 AM (Naismith) Tr. at 68:13-16. Moreover, Dr. Naismith testified that there was "zero chance" that any other protein would be returned by GenBank if the request included both SEQ ID NO: 10 and SEQ ID NO: 7 at that time. Id. at 68:17-25; *see also* 9/12 PM (Blobel) Tr. at 14:6-12 (Defendants' expert Dr. Blobel also testifying "if you took a sequence of this receptor, you would presumably get this receptor back. That's how it works.").[12] Accordingly, the Patents-in-Suit sufficiently describe the subject fusion protein using the known full p75 sequence.

ii. IgG1 and the Fusion Protein are Adequately Described

The disclosure of the second necessary part of etanercept was also adequate because the specification clearly refers to use of deposited vectors (including "pCD4-Hγ1") that contain DNA

[11] Defendants cite to *In re Wallach*, 378 F.3d 1330 (2004) to argue that a partial amino acid sequence is insufficient to describe the full protein when it could not be used to obtain the full protein. However, given Dr. Naismith's testimony that the partial sequences as disclosed would allow a POSA to obtain the full-length sequences from Genbank, the Court finds that the instant case is distinguishable from *Wallach.*

[12] Defendants' expert, Dr. Capon, opined that a POSA would not have been able to obtain the correct full p75 sequence from GenBank if provided with the sequence identification number or the accession number as listed in Smith 1990 because there would have been too many results. Dr. Capon, however, stated that he was not qualified to opine in that area and conceded that he had only first accessed GenBank five years after 1990. 9/13 PM (Capon) Tr. at 20:1-6, 20:18-23, 21:10-22:25 (Capon testifying that "I don't know what the requirements of accessing something from GenBank were . . . I'm not qualified to testify [about that]" and "the first time I believe I accessed GenBank was in 1995"). By contrast, Plaintiffs' expert, Dr. Naismith, limited his opinion to what a POSA would have been able to obtain "at [the] time" of the invention. *See* 9/18 AM (Naismith) Tr. at 68:21-25. The timing is significant here because the sequence match is based on the smaller number of deposits GenBank had in 1990. *See* id. at 68:2-9. Thus, the Court accords little weight to Dr. Capon's opinion on this topic.

sequences encoding the exon-defined hinge-CH2-CH3 region of a human IgG1 heavy chain as confirmed by the declaration of Defendants' expert, Jeffery Kittendorf, Ph.D., an expert in biochemistry and a Research Assistant Scientist at the University of Michigan Life Sciences Institute. ECF No. 688 at 132 ¶ 47; JTX-16 at 32-34; *see also* 9/17 (Loetscher) Tr. at 57:4-58:25.

Example 11 then provides a recipe to fuse a soluble TNF-binding fragment directly to that exon-encoded hinge-CH2-CH3 region of an IgG heavy chain, thereby providing a POSA with the full fusion protein. '182 Patent (JTX-1) col. 9:3-8; 9/17 (Loetscher) Tr. at 56:10-57:13, 58:18-59:5; 9/18 AM (Naismith) Tr. at 54:16-21, 90:10-91:7, 92:21-93:8. This example illustrates utilizing a cDNA fragment that encodes the extracellular region of a TNF-binding protein, and describes the process generally using a p55 TNFR as an illustration. 9/17 (Loetscher) Tr. at 56:5-58:24. A POSA would have followed that example and used p75 to create etanercept based on the claims in the Patents-in-Suit and the specification.[13] *See* 9/12 PM (Blobel) Tr. at 8:5-10:2, 14:6-12; 9/17 (Loetscher) Tr. at 56:5-58:24; 9/18 AM (Naismith) Tr. at 67:14-68:25, 72:15-73:1, 73:17-74:8, 94:10-14, 94:20-95:6.

Moreover, the parties agree that the IgG1 hinge-CH2-CH3 was also known in the prior art as of August 1990. DFOF ¶ 167; PFOF ¶¶ 99-100. Thus, because the p75 TNFR sequence and

[13] Defendants claim that *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341 (Fed. Cir. 2011) supports their argument that the Patents-in-Suit are invalid because they contend that the specifications do not describe the claimed fusion protein. Defs. Br. at 31; Defs. Reply Br. at 14. *Centocor* is distinguishable from the instant case for two main reasons. First, unlike in *Centocor*, the Patents-in-Suit issued from divisional applications as a result of a USPTO restriction requirement, so the specification should contain disclosures from the parent application. S*ee Pfizer, Inc. v. Teva Pharms. U.S.A., Inc.*, 518 F.3d 1353, 1359 (Fed. Cir. 2008); *Manual of Patent Examining Procedure* ("MPEP") § 201.06; *see also supra* at I.C. Second, amendments here were made as a result of that restriction requirement and in accordance with an agreement between Plaintiffs Roche, Immunex, and Amgen, and not, as in *Centocor*, in an "attempt to claim as its own the fruit of [Defendants'] innovative work." *Centocor*, 636 F.3d at 1349. Further, *Centocor* is consistent with the Court's analysis above that the written description requirement is satisfied.

the IgG1 sequence were well known and accessible to a POSA, a reproduction of the known sequences was not required to be explicitly included in the Patents-in-Suit in order to claim a novel combination of those sequences. *See Falkner*, 448 F.3d at 1368 (holding that genes and their nucleotide sequences must not be recited or incorporated by reference where "accessible literature sources . . . as of the relevant date" contain such information, because "forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification").

b) The Patents-in-Suit Demonstrate Possession

To the extent Defendants assert that Roche[14] never made the claimed p75-IgG1 fusion protein, such contention is legally insignificant. *Ariad* holds that "the written description requirement does not demand either examples or an actual reduction to practice; a constructive reduction to practice that in a definite way identifies the claimed invention can satisfy the written description requirement." 598 F.3d at 1352 (citing *Falkner*, 448 F.3d at 1366-67). Here, as discussed, the claim language identifies the requisite elements of the subject invention—the p75 fusion protein combined with the hinge-CH2-CH3 domains of IgG1—and, in conjunction with the specification, provides support of possession. Many of the examples in the Patents-in-Suit further demonstrate that the Roche Inventors had possession.[15] Accordingly, the Court is persuaded that

[14] As mentioned above (I.C.1.), Immunex acquired the rights to prosecute the Patents-in-Suit pursuant to a 2004 Accord and Satisfaction agreement between Roche and Immunex, which will be discussed in further detail below in Section III.C.2.a.

[15] Defendants assert that a POSA would not believe that the Roche Inventors had possession of a p75 fusion protein because none of the examples in the Patents-in-Suit are directed to a p75 TNFR or a p75-IgG1 fusion protein. Defs. Br. at 26-27. In opposition, Plaintiffs contend that the specifications, including the examples, disclose the known p75 protein and the p75 TNFR-IgG1 fusion protein "because [the specification in each Patent-in-Suit] identifies both parts of the claimed p75-IgG1 fusion protein . . . and describes how to combine them as the claims specify." Pls. Br. at 14-18.

the Roche Inventors had possession of the invention based on the specifications of the Patents-in-Suit, including the examples within the specifications, and the claims.

c) *Amendments to the Prosecution File History Did Not Add New Material*

The Court will now consider two amendments to the Patents-in-Suit, both of which were approved by the USPTO. First, in 2006, Amgen and Immunex, with assistance from Roche, deposited a plasmid containing a p75 cDNA with American Tissue Culture Collection ("ATCC")[16], and gave it a designation of PTA 7942. PFOF ¶¶ 93-94; DFOF ¶ 75; *see also* JTX-81 at 19-20 (Plaintiffs' witness Dr. Werner Lesslauer, one of the Roche Inventors involved in this project, testifying that Amgen deposited the p75 plasmid, Roche assisted in the deposit, and it was designated PTA 7942). That same year, Immunex amended the specification of the '790 application (which resulted in the '182 Patent) to include a reference to Immunex's PTA 7942 plasmid deposit. 9/13 AM (Capon) Tr. at 50:9-51:1; JTX-16 at 29-31. The cDNA for the PTA 7942 plasmid encodes the full-length p75 TNFR, which is identical to the sequence reported in Smith 1990. JTX-16 at 29-31. Second, in 2007, Immunex amended the specification of the '791 application (which resulted in the '522 Patent) to expressly incorporate the Smith 1990 protein by reference. Defs. Br. at 33. Immunex also inserted a new figure, Figure 5, that included the Smith 1990 sequence (in addition to the reference previously included). Id.

Defendants assert that Immunex's decision to take over the prosecution and amend the specifications of the Patents-in-Suit is a clear indication that the original specifications as filed by Roche were deficient. Id. at 32-33. In addition, Defendants assert that the USPTO did not have

[16] ATCC is a public depository where cell structures and microorganisms are deposited and made available for public access. *See* "*Who We Are*," https://lgcstandards-atcc.org/en/About/About_ATCC/Who_We_Are.aspx (last visited August 9, 2019).

complete information when it approved the amendments because the Plaintiffs informed the USPTO that the Smith 1990 protein was "99% identical" to Figure 4, when in fact Defendants contend the two proteins are meaningfully different. Id. at 33-34. Defendants argue that Plaintiffs' amendments added what amounts to "new matter" not previously included in the application, which is a ground for a patent rejection.[17] *See* 35 U.S.C. § 132 ("No amendment shall introduce new matter into the disclosure of the invention."); *see also* Defs. Reply Br. at 16 n.13.

By contrast, Plaintiffs contend that each amendment did not contain new matter and that the USPTO properly approved the valid amendments. Pls. Br. at 16 n.2, 18 n.3; PFOF ¶¶ 11-14. Plaintiffs maintain that the amendment to include the PTA 7942 plasmid, which encodes the sequence reported in Smith 1990, complies with USPTO rules because the plasmid (1) contains p75 cDNA that was identified in the original specification as variants of a "DNA sequence[] encoding the 75/65 kD," (2) was made prior to August 1990, and (3) was properly deposited with the ATCC in 2006. Pls. Br. at 16 n.2; PFOF ¶¶ 11-14.

The Court concludes that the deposited PTA 7942 plasmid was properly made part of the Patents-in-Suit and did not add new matter. The Federal Circuit has held that where information

[17] Defendants appear to have relinquished their anticipation argument, which focused on PTA 7942, because their expert on the topic, Dr. Blobel, did not provide related testimony at trial and their post-trial briefs relegate the substance of the argument to a footnote. *See* Defs. Br. at 35 n.5. Invalidity based on anticipation "requires that the same invention, including each element and limitation of the claims, was known or used by others before it was invented by the patentee." *Hoover Grp., Inc. v. Custom Metalcraft, Inc.*, 66 F.3d 299, 302 (Fed. Cir. 1995). To the extent they maintain an anticipation argument, Defendants argue that claims 35-36 of the '182 Patent, which specifically claim the 2006 PTA 7942 plasmid deposit, are invalid for anticipation because Enbrel® had been on sale and publicly available for 8 years at the time of the amendment. Defs. Br. at 35 n.5. The USPTO Board's allowance of the amendment and specific finding that it did not add new matter is "entitled to an especially weighty presumption of correctness in a subsequent validity challenge based on the alleged introduction of new matter." *See Commonwealth Sci. & Indus. Research Org. v. Buffalo Tech. (USA), Inc.*, 542 F. 3d 1363, 1380 (Fed. Cir. 2008) (quotation marks omitted); *see also* Pls. Br. at 16 n.2. Accordingly, insofar as Defendants maintain this anticipation argument, it has not been proven by clear and convincing evidence.

is properly deposited with an independent source, "[a]n accession number and deposit date add nothing to the written description of the invention" and are therefore, not considered new matter. *In re Lundak,* 773 F.2d 1216, 1223 (Fed. Cir. 1985). Further, the deposited plasmid was appropriately made part of the Patents-in-Suit as of their 1990 priority dates because as long as the plasmid was described in the application as-filed, it is not considered new and may be deposited at any time before issuance. *See In re Lundak*, 773 F.2d at 1222-23 ("Lundak's deposit with the ATCC, which was made after filing but prior to issuance of his patent, and which is referred to in his specification, meets the statutory requirements."); *see also* 37 C.F.R. § 1.804(a) (" . . . an original deposit . . . may be made . . . subject to § 1.809, during pendency of the application for patent.").[18] The Court agrees with the USPTO and finds that the properly deposited plasmid reflected one of these variants and did not add new matter. Accordingly, the Court finds that Plaintiffs' amendments adequately described the inventive concept at the time of the invention.

As to the Smith 1990 incorporation, the Court does not find that Immunex's decision to amend is proof that the original specifications were deficient. As discussed above, the Court finds that the Smith 1990 protein was sufficiently described when it was originally referred to and did not need to be amended to expressly incorporate it by reference. *See, e.g.*, *Falkner*, 448 F.3d at 1365 (finding that "the absence of incorporation by reference is not problematic."). The Court therefore finds that the amendments to the Patents-in-Suit were proper and do not alter the written description analysis.

[18] Defendants argue that the amendment occurred much sooner in time in *Lundak* than in the instant case, however, the Court has not been provided with any legal authority to suggest a time limit on specification amendments during the course of prosecution of a patent. *See* Defs. Br. at 13 n.11.

2. The Specification Enables Etanercept

Finally, Defendants argue that the claims of the Patents-in-Suit are not enabled. DFOF ¶ 180; Defs. Br. at 35. Plaintiffs assert that Defendants' enablement challenge fails because the Patents-in-Suit identify both p75 TNFR and IgG1 (which were well-known), sufficiently describe how to combine them to enable a POSA to produce etanercept, and Defendants' own experts concede that a POSA could have produced the claimed fusion protein without undue experimentation by using known methods as of August 1990. *See* Pls. Br. at 2, 21-22; *see also* 9/12 PM (Blobel) Tr. at 53:19-56:13; 9/13 PM (Capon) Tr. at 61:22-62:16.

To be enabling, "[t]he specification must 'enable one of ordinary skill in the art to practice the claimed invention without undue experimentation.'" *Transocean Offshore Deepwater Drilling, Inc. v. Maersk Contractors USA, Inc.*, 617 F.3d 1296, 1305 (Fed. Cir. 2010) (quoting *Nat'l Recovery Techs., Inc. v. Magnetic Separation Sys., Inc.*, 166 F.3d 1190, 1196 (Fed. Cir. 1999)). "Enablement is not precluded by the necessity for some experimentation such as routine screening." *In re Wands*, 858 F.2d at 736-37. However, the experimentation needed to practice the art must not be undue. *Id.* at 737. The test for undue experimentation "is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *Id.* To determine whether a disclosure would require undue experimentation, courts should consider the *Wands* factors, which include: (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *Id.*

The Court finds that Defendants have failed to show by clear and convincing evidence that the Patents-in-Suit do not meet the enablement standard. Preliminarily, both parties agree to a POSA's relative skill in the art, and each party used nearly identical definitions and qualifications for their respective hypothetical POSA. *Compare* 9/11 PM (Blobel) Tr. at 30:24-32:5 *with* 9/20 AM (Wall) Tr. at 18:6-25.[19] Specifically, the parties' experts agreed that the p75 protein and the exon-encoded hinge-CH2-CH3 portion of the IgG1 immunoglobulin sequences were known before August of 1990, which is the initial date of the applications. 9/11 PM (Blobel) Tr. at 14:19-15:5 (Dr. Blobel noting that the claims in the '182 Patent were directed at "essentially etanercept"); 9/20 AM (Wall) Tr. at 19:2-12, 92:16-93:2 (Dr. Wall explaining that the components of etanercept were known by August 1990). Both of Defendants' experts, namely Dr. Blobel and Dr. Capon, agreed that a POSA in 1990 would have been able to produce a fusion protein that is similar to etanercept. 9/12 PM (Blobel) Tr. at 55:20-56:5 (Dr. Blobel testifying that a POSA would have been able to produce a fusion protein similar to etanercept using "ordinary and routine methods utilized in the art"); 9/13 PM (Capon) Tr. at 73:5-14 (Dr. Capon testifying to the same). These experts also testified that the claim scope is both limited to and covers etanercept. 9/11 PM (Blobel) Tr. at 14:19-15:5; 9/13 PM (Capon) Tr. at 82:22-83:3. Regarding the state of the art at the time of the invention, the parties explicitly agreed that technology relating to recombinant DNA was developed by 1990 and allowed for the creation of fusion proteins like etanercept. 9/12 PM (Blobel) Tr. at 54:13-56:13 (Dr. Blobel testifying regarding the state of the art in August 1990); *see also* ECF No. 688 at 65 ¶ 247.

[19] Plaintiffs cite to expert Randolph Wall, Ph.D. as part of their enablement argument. Dr. Wall is an expert in the fields of immunology, molecular biology, and antibody engineering. (ECF No. 688 at 122 ¶ 22). Plaintiffs more heavily rely on his testimony on obviousness and therefore he is fully introduced in the obviousness section of this Opinion.

Furthermore, the Patents-in-Suit, and in particular the '522 Patent, provide a POSA with sufficient guidance on how to make etanercept. Specifically, both Patents-in-Suit explain to a POSA how to prepare a cDNA encoding the extracellular region of the known p75 protein. '182 Patent (JTX-1) col. 16:22-48, 5:22-24, 7:24-46; 9/18 AM (Naismith) Tr. at 60:13-62:6; 9/18 PM (Naismith) Tr. at 53:12-54:6; 9/20 AM (Wall) Tr. at 93:14-94:16. The specifications also provide a POSA with information regarding how to prepare a cDNA encoding all of the domains of a human IgG1 constant region, except the first, including identifying a publicly accessible exemplary vector pCD4-Hγ1. '182 Patent (JTX-1) col. 8:56-9:3; 9/20 AM (Wall) Tr. at 94:17-95:19.

Finally, Plaintiffs' witnesses Dr. Naismith and Dr. Loetscher credibly testified that the '182 Patent directs a POSA to follow the recipe set forth in Example 11 contained in the specification. 9/17 (Loetscher) Tr. at 56:5-9 (Dr. Loetscher noting that the example "describe[s] the process [of] how to make TNF receptor fusion proteins"); 9/18 AM (Naismith) Tr. at 53:22-54:2. Defendants' expert Dr. Capon even appeared to acknowledge that Example 11 in conjunction with the prior art would have enabled a POSA to construct etanercept. *See* 9/13 PM (Capon) Tr. at 72:3-73:14. Hence, as Plaintiffs submit, a POSA could have easily made the claimed fusion protein (i.e., a fusion protein that had the extracellular region of the p75 receptor with an exon-encoded hinge and the CH2-CH3 region of the IgG1 immunoglobulin) of the '182 Patent in or before August 1990 with only routine experimentation by adapting Example 11 to make the claimed fusion protein. 9/17 (Loetscher) Tr. at 58:18-59:5; 9/18 AM (Naismith) Tr. at 93:12-22 (Dr. Naismith explaining that a POSA would have been able to make Example 11 in August of 1990); 9/20 AM (Wall) Tr. at 95:17-19 (Dr. Wall testifying that a POSA would have "been able to adapt Example 11 to make the claimed fusion protein."); JTX-82 (Lesslauer Deposition) at 298:11-14, 17. The

Court finds that based on this evidence, Defendants have not met their burden of proving by clear and convincing evidence that the Patents-in-Suit fail to meet the enablement standard.

B. Obviousness (35 U.S.C. § 103)

To prove that an asserted claim of a patent is invalid as obvious under 35 U.S.C. § 103, a patent challenger bears the burden of establishing by clear and convincing evidence that the "differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a [POSA]."[20] 35 U.S.C. § 103(a); *see also Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1360-61 (Fed. Cir. 2007). Obviousness is a question of law that is predicated on several factual inquiries. *See Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17 (1966). Specifically, there are four basic factual inquiries which concern: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art;[21] (3) the differences between the claimed subject matter and the prior art; and (4) objective indicia (secondary considerations) of non-obviousness, including unexpected results, success and praise in the industry, long-felt but unsolved need, failure of others, and other indicia. *See id.*

Defendants assert that the Patents-in-Suit are invalid because they are obvious in view of prior art that would have motivated a POSA to create etanercept prior to the relevant patent

[20] The pre-America Invents Act version of 35 U.S.C. § 103 applies to the Patents-in-Suit.

[21] The parties agree as to the level of ordinary skill in the art. Defendants present that a POSA is "a scientist with an M.D. or a Ph.D. degree in biology, molecular biology, biochemistry, chemistry, or a similar field." 9/11 PM (Blobel) Tr. at 30:14-31:18. Such a person would "have one to two years of experience in the field of immunology or molecular immunology, including experience with cloning and expression of DNA, protein biochemistry on cell culture, protein purification, and immunological assays." Id. Plaintiffs offered a definition that is not materially different. *See* 9/20 AM (Wall) Tr. at 18:5-22; Pls. Br. at 24.

applications.[22] Defs. Br. at 35-43; *see also* Defs. Reply Br. at 19-22. At trial, Defendants asserted six obviousness combinations of prior art references, two of which disclose the protein sequence of, and the DNA sequence that encodes, the p75 extracellular region (Smith 1990 and Immunex's U.S. Patent No. 5,395,760 (JTX-65) (the "Smith '760 Patent")). PFOF ¶ 147. The other asserted prior art references disclose Ig fusion proteins, which combine a receptor protein with various portions of an Ig heavy chain. Id. Specifically, the first five (5) combinations are the Smith '760 in view of: (1) the Seed European Patent Application No. 0325262 ("Seed '262"); (2) Byrn, R. et al., *Biological Properties of a CD4 Immunoadhesin*, Nature 344: 667-70 (1990) ("Bryn 1990"); (3) Watson, S. et al., *A Homing Receptor-IgG Chimera as a Probe for Adhesive Ligands of Lymph Node High Endothelial Venules*, J. Cell. Bio. 110: 2221-2229 (1990) ("Watson 1990"); (4) the Karjalainen European Patent Application No. 0394827 ("Karjalainen '827"); and (5) the Capon U.S. Patent No. 5,116,964 ("Capon '964") in further view of Traunecker, A. et al., *Highly Efficient Neutralization of HIV with Recombinant CD4-immunogloblin Molecules*, Nature 339: 68-70 (1989) ("Traunecker 1989"). The sixth combination was Smith 1990 in view of Watson. Id. ¶ 147 n.3. Defendants' post-trial arguments regarding these prior art references focus on motivation. Defs. Br. at 35 ("[T]he only real dispute as to obviousness of the asserted claims concerned motivation."). The Court has examined the asserted prior art references both alone and in combination, as discussed below, to determine motivation and whether it would have been obvious to a POSA to create etanercept.

In addition, Defendants argue that certain secondary considerations prove, rather than refute, that the Patents-in-Suit are invalid for obviousness. Id. at 44-50. In support of their

[22] The Court notes that the USPTO considered these prior art references and concluded that the Patents-in-Suit were not obvious in light of these references. 9/12 AM (Blobel) Tr. at 33:25-39:4; PTX-1089 at 19; PTX-6.456 at 7-8.

obviousness arguments, Defendants primarily rely on (1) Dr. Blobel, introduced above; and (2) Arne Skerra, Ph.D, Chair of Biological Chemistry at the Technical University of Munich, Center of Life Sciences at Weihenstephan, Freising, Germany. ECF No. 688 at 131-32 ¶¶ 43, 49.[23]

Plaintiffs contend that Defendants' obviousness arguments fail because a POSA would not have been motivated to create etanercept based on the prior art and, in fact, would have actually been dissuaded by the prior art to create a TNFR-Ig fusion protein to treat inflammation. Pls. Br. at 22-23. Further, Plaintiffs counter each of Defendants' secondary consideration arguments as set forth below and contend that the secondary considerations support nonobviousness. Id. at 33-39. Plaintiffs rely on (1) Randolph Wall, Ph.D., a Distinguished Professor in the Department of Microbiology, Immunology, and Molecular Genetics at the Molecular Institute, University of California at Los Angeles (UCLA) and the David Geffen School of Medicine at UCLA, as an expert on obviousness (ECF No. 688 at 122 ¶ 22); and (2) Warner C. Greene, M.D., Ph.D., the Founder and Director of the Gladstone Institute of Virology and Immunology in San Francisco and a Distinguished Professor of Translational Medicine with over 40 years of experience in biomedical research, as an expert on etanercept's effect on the immune system (Id. at 124-25 ¶ 29).

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[23] Plaintiffs assert that the testimony of Defendants' expert Dr. Blobel should be completely disregarded because he ignored the agreed upon claim construction. Pls. Br. at 23-24; ECF No. 688 at 20 ¶ 68. While the parties agreed to construe the claim term "all of the domains of the constant region of a human immunoglobulin IgG[1] heavy chain other than the first domain of said constant region" as having a three-cysteine hinge ("the exon-encoded-hinge-CH2-CH3 region of human [IgG/IgG1]"), Dr. Blobel inconsistently testified that a two-cysteine hinge would be within the scope of the claims of the Patents-in-Suit. 9/12 AM (Blobel) Tr. at 30:19-24; ECF No. 688 at 20 ¶ 68. Although an obviousness analysis based on "an incorrect understanding of the claim construction" may be disregarded, the Court will still consider Dr. Blobel's testimony to the extent it is not inconsistent with the agreed upon claim construction, including his testimony about other fusion proteins referenced in the prior art and testimony about what would have motivated a POSA to create etanercept before August 1990. *See Cordis Corp. v. Bos. Sci. Corp.*, 658 F.3d 1347, 1357-58 (Fed. Cir. 2011).

For the reasons discussed below, the Court finds that Defendants have failed to prove by clear and convincing evidence that the Patents-in-Suit are invalid based on obviousness pursuant to 35 U.S.C. § 103.

1. Scope of the Prior Art and Differences Between the Prior Art and the Claimed Invention

The Patents-in-Suit provide for a fusion protein, etanercept ('182 Patent), consisting of the extracellular portion of a p75 TNFR combined with a three-cysteine, exon-encoded hinge-CH2-CH3 portion of an IgG1, and a method of making this fusion protein ('522 Patent). *See generally* '182 Patent (JTX-1) and '522 Patent (JTX-2). Therefore, to prove obviousness, Defendants have to show by clear and convincing evidence that the claimed invention, which consists of a precise combination of specific portions of p75 TNFR and IgG1, would have been obvious to a POSA.

Defendants point to various scientific publications and patent applications that they contend render the claimed invention obvious. Some of these prior art references relate to p75 TNFRs—Smith 1990 and Smith '760—and others disclose Ig fusion proteins without p75—Capon 1989, Traunecker 1989, Seed '262, Capon '964, Byrn 1990, and Watson 1990. DFOF ¶¶ 208-09, 217-20. Defendants contend that a POSA would have been motivated, when viewing these references alone and in combination, to select p75 and IgG1 and combine them to create etanercept. Id.; Defs. Br. at 37-41. According to Plaintiffs, these references would not have motivated a POSA to make the precise construct of etanercept because there was no clear direction in the prior art, and in fact, the prior art would have taught away from creating etanercept. Pls. Br. at 24-29. The Court will address the prior art concerning both TNFRs and Ig fusion proteins individually and then discuss the motivation to combine the two elements in the specific way necessary to create the claimed invention.

a) *The Prior Art Would Not Have Motivated a POSA to Select the Individual Components of Etanercept, and in Fact Taught Away from Using these Components*

i. *Selecting p75 TNFR*

The Patents-in-Suit identify p75 TNFR as one of the two components of etanercept, a fusion protein used to treat rheumatoid arthritis. As noted above, rheumatoid arthritis is an inflammatory autoimmune disease that arises when an overactive immune system attacks a person's own body. PFOF ¶ 32; 9/12 AM (Blobel) Tr. at 39:24-40:2. Chronic inflammation in rheumatoid arthritis patients causes bone erosion and also destroys tendons and ligaments. PFOF ¶¶ 33-34. As such, scientists studying auto-immune disorders, such as rheumatoid arthritis, in 1990 were seeking to reduce inflammation by interrupting the body's immune system. 9/20 AM (Wall) Tr. at 39:24-40:3.

According to scientists, there was a prevailing view at the time that many cytokines, including TNF, were thought to be involved in excess inflammation. PTX-34 at 6 ("It is a misconception to think that TNF[] was an obvious therapeutic target in the early 1990s since it is pro-inflammatory. . ."). As previously discussed, cytokines are messenger proteins with a wide variety of functions in the body. PFOF ¶ 27. TNF was one of dozens of cytokines known in 1990. Id. ¶ 28. Critically, the prior art demonstrates that researchers at the time were concerned that TNFRs could aggravate pro-inflammatory responses by binding TNF and then releasing it back into the body in active form, causing inflammation. 9/20 AM (Wall) Tr. at 28:24-33:15. At trial, Dr. Wall testified that this would be "a very undesirable outcome" for a POSA trying to block inflammation possibly caused by excess TNF. Id. Because the treatment of auto-immune disorders was based on trying to inhibit inflammation caused by the TNF response, a POSA would have been discouraged from using TNFR as a treatment option.

Additionally, a POSA in 1990 would have considered cytokines to be "poor therapeutic targets" and therefore TNFR would not have been an obvious choice. PFOF ¶ 149; 9/20 AM (Wall) Tr. at 20:4-10. By August of 1990, the art had identified several cytokines and discovered that these cytokines were redundant, which means that they had "overlapping functions." 9/20 AM (Wall) Tr. at 37:16-25. Because of this redundancy, a POSA would not have considered any individual cytokine to be a good therapeutic target because it was understood that if you blocked one cytokine, another cytokine would be able to fill in the missing function, thereby eliminating any beneficial effect. Id. Moreover, cytokines, including TNF, were difficult to study due to their many different roles in the body, causing their function in treating various diseases to remain unclear. Id. at 21:7-22:18. Furthermore, if a POSA targeted cytokines at all, a POSA would have looked to a different cytokine, called IL-1, to treat inflammatory diseases because IL-1 was known in August 1990 to have stronger potential as a mediator in rheumatic diseases. Id. at 23:17-24:14; PTX-10 at 8.

However, even if TNFR were chosen as the starting point, it would not have been obvious to use a p75 TNFR. The parties agree that at least two TNF receptors were known as of August 1990, namely p55 and p75. PFOF ¶¶ 36-37; DFOF ¶ 2. Much of the literature at the time showed that p55 bound TNF with five times greater strength than p75 and was superior in neutralizing TNF. PFOF ¶ 153; JTX-47 at 3; 9/18 PM (Greene) Tr. at 108:21-109:24. Equipped with this knowledge, a POSA deciding to select TNFR to treat pro-inflammatory diseases would have likely used p55. Id. Finally, even assuming a POSA decided to use p75 instead of p55, a POSA would have also had to decide between the soluble and insoluble form of p75, which could be a partial or full-length extracellular region of the p75 TNFR. PFOF ¶¶ 154, 157; 9/12 PM (Blobel) 15:21-17:6; Smith 1990 (JTX-24) at 4; Smith '760 Patent (JTX-65) col. 4:12-21, 9:17-60.

ii. Selecting IgG1

The second necessary element of etanercept is the exon-encoded, three-cysteine hinge-CH2-CH3 domain of an IgG1. At the time etanercept was being created as a possible treatment for auto-immune disorders like rheumatoid arthritis, researchers were also studying Ig fusion proteins as a viable treatment option to combat the HIV/AIDS epidemic. PFOF ¶ 171. HIV/AIDS is a disease that greatly weakens or destroys the immune system so that the immune system becomes unable to kill HIV-infected cells on its own. Id. ¶¶ 171-72. Therefore, the goal of HIV/AIDS treatment was to trigger pro-inflammatory responses in the immune system to kill the HIV-infected cells within the body. Id. ¶ 173.

By August of 1990, prior art related to HIV/AIDS research demonstrated that Ig caused increased inflammation and aggregation, the opposite objective of treatment for auto-immune conditions. According to the prior art, Ig fusion proteins were effective in eliciting pro-inflammatory responses in the body, known as effector functions. Id. ¶¶ 173-75; 9/18 PM (Greene) Tr. at 72:13-74:13, 77:17-78:2. There are two pro-inflammatory effector functions, which are separate, complex pathways by which the immune system kills other cells. PFOF ¶ 26. First, the pathway known as complement dependent cytotoxicity ("CDC") pertains to the effector functions trigged by the CH2 domain. Id. ¶ 174. Second, the pathway known as antibody dependent cellular cytotoxicity ("ADCC") refers to the effector functions triggered by the junction between the CH2 domain and the hinge. Id. The HIV/AIDS research at the time demonstrated that Ig fragments in fusion proteins successfully triggered both CDC and ADCC effector functions within the immune system. Id. ¶ 173; 9/18 PM (Greene) Tr. at 76:8-77:2.

Against this backdrop, a POSA studying auto-immune diseases would have avoided Ig because the inflammatory immune response elicited by Ig fusion proteins was extremely

undesirable. In fact, six of the asserted prior art references cited by Defendants, all of which discuss using Ig to increase inflammatory responses in the body, would have taught a POSA to look away from Ig fusion proteins as a potential treatment option for auto-immune disorders. *See* Capon 1989 (JTX-58), Traunecker 1989 (JTX-25), Seed '262 (JTX-57), Capon '964 (JTX-61), Byrn 1990 (JTX-56), and Watson 1990 (JTX-59).[24]

For example, in his 1989 article, Defendants' expert Dr. Capon reported experimental results of CD4-Ig fusion proteins that successfully triggered pro-inflammatory immune responses in HIV-infected patients by eliciting effector functions. JTX-58 at 4 (demonstrating that effector functions were "found in the constant region of the heavy chain"). The Traunecker 1989 prior art reference found a similar result with CD4-Ig fusion proteins using mouse IgG2a and mouse IgM sequences. JTX-25 at 1-2; 9/12 AM (Blobel) Tr. at 51:11-16; 9/18 PM (Greene) Tr. at 84:12-19; 9/20 AM (Wall) Tr. at 56:24-57:13. Published in July 1989, Seed '262 described CD4-Ig fusion proteins designed to treat HIV/AIDS patients and emphasized the importance of preserving

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[24] Although Watson 1990 (JTX-59) concerned studies outside of the body for which effector functions would not be relevant and therefore were not specifically discussed, similar constructs to those discussed in Watson 1990 (*e.g.*, Byrn 1990) were demonstrated through experimental evidence to have retained cell-killing effector functions. 9/20 AM (Wall) Tr. at 61:9-13, 259:12-22; JTX 59 at 3, 8.

Defendants also cite to Karjalainen '827, a European patent application published in October 1990. JTX-60 at 1; PFOF ¶ 191. The parties' experts agreed that this reference is not prior art for purposes of their analysis. 9/12 AM (Blobel) Tr. at 84:3-12; 9/20 AM (Wall) Tr. at 84:11-16. Moreover, Karjalainen '827 is exempt as prior art under § 103(c)(1) because the inventors of Karjalainen '827 and the Patents-in-Suit "were at the time the claimed invention was made . . . [both] subject to an obligation of assignment to the same person," F. Hoffmann-La Roche AG. 35 U.S.C. § 103(c)(1) ("Subject matter developed by another person . . . shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the claimed invention was made, owned by the same person or subject to an obligation of assignment to the same person."); 9/17 (Loetscher) Tr. at 21:11-13; JTX-3 at 875-79; JTX-4 at 706-10; JTX-60 at 1. In any event, Karjalainen '827 also taught use of a CD4-Ig fusion protein to elicit effector functions to treat AIDS. PFOF ¶ 192.

effector functions to properly combat HIV-infected cells. JTX-57 at 5. Similarly, Capon '964 described many different Ig fusion protein configurations that were intended to retain effector functions. JTX-61 col. 4:43-47; 9/12 AM (Blobel) Tr. at 60:24-61:13; PFOF ¶¶ 185-86. Moreover, Byrn 1990 provided experimental evidence demonstrating that a protein with only a partial Ig hinge would still successfully induce ADCC effector functions. JTX 56 at 1-2; 9/12 AM (Blobel) Tr. at 70:18-71:16; 9/18 PM (Greene) Tr. at 87:5-19. Based on these prior references, a POSA would have refrained from using Ig fusion proteins for anti-inflammatory treatments, which sought to reduce effector functions in the body.

Defendants also assert that the Patents-in-Suit are obvious in light of the combination of Watson 1990 and Smith 1990. PFOF ¶ 147 n.3. Smith 1990 disclosed the amino acid sequence of p75 TNFR but did not suggest using p75 TNFR in a fusion protein. DFOF ¶ 4. Moreover, Watson 1990 also did not contemplate a TNFR-Ig fusion protein and instead discussed a construct with a partial region of an Ig fused with a receptor known as a lymphocyte homing receptor. JTX-59 at 1-3; 9/20 AM (Wall) Tr. at 61:9-13; PFOF ¶ 194. Therefore, a POSA looking to these two prior art references either individually or in combination would not have been motivated to create etanercept.

Defendants further point to Capon '964 and additional prior art, namely Brennan 1989, to assert that researchers at the time were not concerned about the negative effects from effector functions. DFOF ¶ 226-27.[25] However, prior art published in June 1990 shows that effector functions were in fact a concern with Ig fusion proteins at the time of the invention. *See* Gerd

[25] It appears that many of the prior art references cited by the Defendants used to support the modification of Smith '760 were published prior to Smith '760. Pls. Reply Br. at 12 (noting that Traunecker 1989, Seed '262, Capon '964, and Byrn 1990 were published before Smith '760 and did not motivate the Smith '760 inventors to remove the light chain or CH1 domain).

Zettlmeissl, et al., *Expression and Characterization of Human CD4: Immunoglobulin Fusion Proteins,* DNA & Cell Biology 9: 347-53 (1990) (PTX-26 at 5-10) (discussing CD4-Ig fusion proteins created to treat HIV/AIDS and reporting that "one of the most important issues confronting" Ig fusion proteins was "the extent of autoimmune damage" caused by effector functions); *see also* 9/12 AM (Blobel) Tr. at 81:15-83:13. Additionally, a well-known immunology textbook by William E. Paul and Dr. Wall's credible testimony further demonstrate that a POSA would have expected that pro-inflammatory effector functions would have been triggered when a fusion protein, like etanercept, attached to a soluble TNF. *See* Paul, William E., *Fundamental Immunology* (2d ed., Raven Press 1989) (PTX-3); 9/20 AM (Wall) Tr. at 46:18-48:22, 49:6-53:8. Furthermore, the fact that the papers cited by Defendants did not report effector functions as problematic is reasonable in the context of HIV/AIDS research where effector functions were a desired result, rather than an obstacle. Thus, the Court finds that a POSA would have expected from the prior art that an Ig fusion protein could lead to autoimmune damage caused by effector functions. 9/20 AM (Wall) Tr. at 39:14-40:9, 59:3-18; 9/18 PM (Greene) Tr. at 90:25-91:16.

The prior art also taught that Ig fusion proteins would cause another detrimental effect, known as aggregation, in patients with inflammatory conditions. Plaintiffs' expert in immunology, Dr. Greene, opined that an Ig fusion protein would likely cause aggregation—the formation of large immune complexes in the human body—that would then lead to increased inflammation in the kidney, skin, and joints. 9/18 PM (Greene) Tr. at 98:1-16, 137:4-12. Based on the prior art, a POSA would have believed that an Ig fusion protein, like etanercept, would have likely aggregated and caused an inflammatory response, as Defendants' expert Dr. Blobel similarly opined. 9/18 PM (Greene) Tr. at 70:17-71:2; *see also* 9/12 AM (Blobel) Tr. at 53:23-54:24 (testifying that

researchers at the time were intentionally creating CD4-Ig fusion proteins to cause aggregation and attack infected cells). Therefore, a POSA would have refrained from selecting Ig for the treatment of auto-immune disorders because it was shown to increase aggregation, resulting in heightened inflammation.

Additionally, a POSA seeking to avoid using Ig at the time would have had a number of non-Ig options to achieve desirable outcomes while avoiding effector functions. PFOF ¶ 155. In fact, prior art at the time suggested joining proteins with polyethelene glycol ("PEG"), a non-Ig option that did not cause effector functions and was also associated with longer half-lives and better drug properties at that time. Id.; 9/20 AM (Wall) Tr. at 68:19-70:24; *see, e.g.,* Smith '760 Patent (JTX-65) col. 10:39-44. By August 1990, numerous PEG-modified proteins were in clinical trials and at least one PEGylated compound had been approved by the FDA. PFOF ¶ 155; 9/20 AM (Wall) Tr. at 68:22-70:24; *see* Smith '760 Patent (JTX-65) col. 10:35-53. Given that the prior art showed that Ig was increasing inflammation, PEG was a more obvious choice to use in a fusion protein than Ig.

Nevertheless, even if a POSA was undeterred by the research that predicted an inflammatory response and decided to create an Ig fusion protein, a POSA would have had numerous options when determining what type and conformation of Ig to select. While etanercept used IgG1, there were many alternative Ig constructions that a POSA could have selected, none of which was more obvious than the other. For example, a POSA would have had to choose from many known classes of immunoglobulins (Ig), such as IgG, and further choose between the subclasses of IgG, including IgG1, IgG2, IgG3, and IgG4. 9/18 AM (Naismith) Tr. at 51:11-13; *see supra* I.B.1. Moreover, a POSA would have had to consider and decide between the variety of Ig conformations in the prior art including a full hinge, an exon-encoded hinge, a two-cysteine

hinge, or no hinge. PFOF ¶ 159; 9/20 AM (Wall) Tr. at 82:24-83:8; *see also* 9/12 PM (Blobel) Tr. at 34:5-13, 39:24-40:11 (Dr. Blobel testifying that it was "not so obvious" to use a three-cysteine hinge as opposed to a two-cysteine hinge). Finally, as reflected in the prior art above, a POSA selecting Ig would have had to decide whether to use a linker, and if so, would have also had to determine which length to use. PFOF ¶ 159; 9/20 AM (Wall) Tr. at 82:1-4, 88:17-90:1. Accordingly, a POSA choosing to select Ig, despite the scientific research teaching that this was not a desirable option, would still have had many different variations and configurations of Ig to opt for when creating the fusion protein. Defendants have failed to sufficiently prove by clear and convincing evidence that it was obvious for a POSA to select IgG1, as used in etanercept, among all of these alternatives.

b) It Would Not Have Been Obvious to a POSA to Combine p75 with the Exon-Encoded Hinge-CH2-CH3 Region of IgG1

Furthermore, even assuming it was obvious to select both p75 TNFR and IgG1, a claim cannot be held obvious merely because its elements were independently known in the prior art. *KSR Int'l Co. v. Teleflex, Inv.*, 550 U.S. 398, 418-19 (2007); *Polaris Indus., Inc. v. Arctic Cat, Inc.*, 882 F.3d 1056, 1068 (Fed. Cir. 2018) (stating that the "genius of invention is often a combination of known elements which in hindsight seems preordained"). Defendants must prove by clear and convincing evidence that a POSA would have been motivated to combine the essential components from the prior art teachings to create the claimed invention, and would have had a reasonable expectation of success in doing so. *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1359-61 (Fed. Cir. 2017).

Moreover, Defendants must show by clear and convincing evidence that a POSA would have been motivated to combine the specific parts of each component that make up the claimed invention, rather than only showing it was obvious to combine p75 and IgG1. *See id.* (finding that

the required motivation is a motivation to combine prior art to achieve ***the particular claimed invention***). Merely combining p75 TNFR and IgG1 would not have resulted in etanercept because the claimed invention specifically joins the extracellular region of p75 and only a portion of IgG1, namely the exon-encoded hinge-CH2-CH3 domain. '182 Patent (JTX-1);'522 Patent (JTX-2). Therefore, Defendants must demonstrate that a POSA would have been motivated to create the precise TNRF-IgG1 construct that is etanercept.

As addressed above, the prior art cited by Defendants taught that Ig fusion proteins activated effector functions leading to inflammation in the body. *See supra* III.B.1.a.ii. Given this prior art, a POSA would have expected a fusion protein combining TNFR and IgG1 to lead to autoimmune damage caused by effector functions. 9/20 AM (Wall) Tr. at 39:14-40:9, 56:7-16; 9/18 PM (Greene) Tr. at 90:25-91:16. Therefore, for all of the reasons stated above, a POSA looking to treat an autoimmune condition, such as rheumatoid arthritis, would have been dissuaded from combining TNFR with IgG1.

Despite the prior art, Defendants assert that a POSA would have been motivated to combine p75 and IgG1 to produce etanercept because this combination was already described in the Smith '760 Patent. Defs. Br. at 37. However, this argument fails because, as discussed below, (1) Smith '760 was an unconstructed, untested chimeric antibody that would not have been an obvious starting point; (2) the Smith '760 construct was distinct from etanercept; and (3) a POSA would not have been motivated to modify Smith '760 in the precise ways necessary to create etanercept.

i. A POSA Would Not Have Ignored the Prior Art Concerning Effector Functions in Ig Fusion Proteins Because of the Smith '760's Hypothetical Antibody

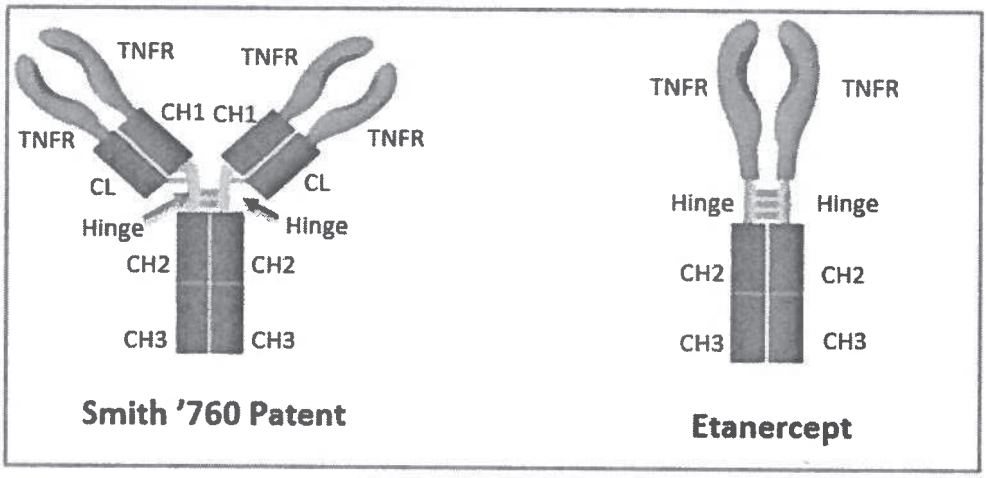
First, the Smith '760 Patent, filed in May 1990, described a hypothetical construction of a TNFR-IgG1 chimeric antibody that was never made. PFOF ¶ 164; DFOF ¶ 210; 9/12 PM (Blobel) Tr. at 84:5-7. There is no prior art that suggests exactly how the Smith '760's construct may have

been used, much less that it was known to have desirable therapeutic properties. Defendants argue that a POSA would have obviously looked to the Smith '760 fusion protein because this protein was expected to have advantageous properties, including an "extended *in vivo* half-life, ease of purification, and enhanced TNF binding." Defs. Br. at 38. However, as outlined above, the prior art actually taught away from using an Ig fusion protein, such as the one proposed in Smith '760, to treat auto-immune diseases because such a construct would have likely elicited an inflammatory response in the body. *See supra* III.B.1.a.ii. The speculative expectations of Smith '760's unconstructed chimeric antibody would not have been enough to compel a POSA to ignore the numerous experimental studies that revealed that Ig proteins elicited an inflammatory response and use the Smith '760's fusion protein as a starting point to create an anti-inflammatory drug. It is not obvious that a POSA would have selected this idea as a possible solution for patients with pro-inflammatory conditions when the therapeutic effects of this chimeric antibody were uncertain, at best.

ii. Etanercept Is Distinct from Smith '760 Such That it Cannot Render the Patents-in-Suit Obvious

Second, etanercept is not an obvious variant of the Smith '760 Patent because the Patents-in-Suit claim a distinct fusion protein. Smith '760 teaches fusing a portion of TNFR to a human IgG1 containing both the CH1 and the light chain (*see generally* Smith '760 Patent (JTX-65)), whereas the Patents-in-Suit require the removal of the CH1 and the light chain from the constant region domain of IgG1 (*see* '182 Patent (JTX-1) col. 39:12-42:34; '522 Patent (JTX-2) col. 45:44-48:4). The Smith '760 Patent also discussed a number of ways to construct the fusion site of the TNFR, none of which suggested directly fusing the TNFR to the hinge. *See* Smith '760 Patent (JTX-65) col. 10:33-56; PFOF ¶ 166. The Patents-in-Suit directly fused the extracellular region

of p75 to the exon-encoded hinge-CH2-CH3 region of IgG1. '182 Patent (JTX-1) col. 39:12-42:34; '522 Patent (JTX-2) col. 45:44-48:4.



In contrast to Smith '760, etanercept specifically uses only a portion of IgG1, namely the partial exon-encoded hinge-CH2-CH3. Defendants have not pointed to any prior art that recommends using the exon-encoded hinge-CH2-CH3 of IgG1 for such a fusion protein, or any reference that advises fusing this portion to the extracellular region of p75. This concept was not taught in the prior art, rendering etanercept a distinct, nonobvious construction from Smith '760. *Compare* '182 Patent (JTX-1) and '522 Patent (JTX-2) *with* Smith '760 Patent (JTX-65) at 10:53-68. Defendants have failed to show why a POSA would have been motivated to combine the specific parts of IgG1 and p75 that make up the claimed invention.

Moreover, the specific construct within Smith '760 that Defendants compare to the distinct construct of etanercept, as pictured above, was only one of many contemplated in Smith '760. In hindsight, Defendants assert that this one construct contemplated in Smith '760 would have obviously motivated a POSA to create etanercept. This assertion ignores the fact that had a POSA looked to Smith '760 in its entirety, the POSA would have had to consider and select among a broad array of options as the patent suggested a variety of different constructs to pursue, none of

which were ever actually constructed or determined to be preferred. The Smith '760 Patent embraces many variations, including both monovalent and polyvalent forms of TNFR, and further reports a wide variety of choices for the polyvalent forms. *See* Smith '760 Patent (JTX-65) at 13; PFOF ¶¶ 165-67. Among these possibilities was combining p75 with PEG, which as mentioned above was a widely used and FDA approved non-Ig construct. It appears Defendants focused on a single construct "out of the sea" of alternatives based on hindsight reasoning notwithstanding other potential constructs contemplated in Smith '760 that refuted their assertions. *See WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1337 (Fed. Cir. 2016). Therefore, Defendants have failed to show by clear and convincing evidence that a POSA looking to Smith '760 for motivation would have decided on the specific construct of p75 and IgG1.

iii. A POSA Would Not Have Been Motivated to Modify Smith '760 by Removing the Light Chain, Removing the CH1 Domain, and Directly Fusing the p75 Protein to the Exon-Encoded Hinge-CH2-CH3 Region

Third, a POSA would not have been motivated to alter the Smith '760 fusion protein in the specific ways necessary to create etanercept. To modify Smith '760 and construct etanercept, a POSA would need to have been motivated to remove the CH1 domain, eliminate the light chain, and directly fuse the extracellular region of p75 to the exon-encoded hinge-CH2-CH3.

As to the removal of the CH1 domain and the light chain, a POSA would not have been motivated to make these modifications to Smith '760 based on the patent itself. First, Smith '760 specifically states that its construct must have "unmodified constant region domains[,]" signifying to a POSA that the light chain and CH1 should not be modified if the POSA wished to maintain all of the alleged advantageous properties of Smith '760. *See* Smith '760 Patent (JTX-65) col. 10:53-57; Defs. Br. at 38. Accordingly, a POSA looking to Smith '760 for motivation would have been discouraged from altering the constant region by removing the light chain and CH1.

However, even if a POSA were to ignore this statement, there was no clear evolution in the prior art that would have taught a POSA to eliminate the light chain or the CHI domain. If anything, the prior art would have dissuaded a POSA from making these modifications to Smith '760 based on their proven increase in effector functions.

Furthermore, in analyzing the cited prior art beyond Smith '760, it would not have been obvious to a POSA to remove the CH1 and light chain because there was no clear direction in the prior art. When the prior art provides no reason to select, among several unpredictable alternatives, the exact route that would guide and/or motivate a POSA to the patented invention, then it is not obvious. *Ortho-McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364 (Fed. Cir. 2008). Researchers at the time, many of whom were seeking treatment for HIV/AIDS patients, were modifying fusion proteins in a number of different ways and no one way was known to definitively work better than the other. For example, with respect to removal of the light chain, Defendants point to Dr. Capon's 1989 article, mentioned above, that disclosed several CD4-Ig fusion proteins, including proteins that retained the light chains and those that lacked the light chain. DFOF ¶ 216; JTX-58 at 1-2. Additionally, prior art references that contemplated removing the CH1 domain, such as the Seed '262 and Capon '964 publications, disclosed a variety of constructs, including proteins with the CH1 domain and those that deleted it. JTX-57 at 10; JTX-61 at 28; 9/20 AM (Wall) Tr. at 79:12-21. No one arrangement could have been considered to be predictable in its effect as even Dr. Capon found the results of his own constructs to be "surprising." JTX-58 at 4-5; 9/12 AM (Blobel) Tr. at 46:8-11. Therefore, the prior art was in a state of uncertainty and had many variables, such that creating etanercept using only the CH2-CH3 domain of the IgG1 immunoglobulin would not have been obvious.

In fact, a POSA would have been disincentivized to remove the CH1 chain because the prior art established that Ig fusion proteins without the CH1 domain created additional effector functions, thereby intensifying the inflammatory response. 9/20 AM (Wall) Tr. at 78:20-79:6. According to the prior art, HIV/AIDS researchers were removing CH1 to successfully *increase* the effector functions—an undesired response for an anti-inflammatory drug. 9/18 PM (Greene) Tr. at 84:12-19 (Dr. Greene explaining that removal of the CH1 domain was shown to cause an inflammatory response). For example, Byrn 1990 provided experimental evidence that CD4-Ig fusion proteins lacking CH1 would trigger the ADCC effector function as desired for HIV/AIDS treatments. JTX-56 at 1-2; 9/12 AM (Blobel) 70:18-71:15. Such a result would have been contrary to a goal of reducing inflammation, and therefore a POSA would have been dissuaded to alter the Smith '760 protein in this way. Moreover, Defendants point to Traunecker 1989, which found that the pro-inflammatory response of the Ig fusion protein remained strong despite eliminating the CH1 domain in mouse fusion proteins. JTX-25; 9/20 AM (Wall) Tr. at 78:18-79:1. Based on this finding, a POSA seeking new therapies for auto-immune disorders would not have been motivated to remove the CH1 domain because Traunecker 1989 showed that removing CH1 retained the inflammatory effects. *See* 9/20 AM (Wall) Tr. at 78:20-79:1.

Defendants specifically aver that a POSA would have been motivated to eliminate the CH1 domain and the light chain of the Smith '760 protein, as was done in etanercept, because these deletions were known to improve the secretion of fusion proteins, a desirable feature because it allowed the fusion protein to leave the cell. *See* Defs. Br. at 39; DFOF ¶ 325. However, Dr. Capon's 1989 paper that Defendants use to support this argument reported that secretion problems actually arose when the light chain was removed from CD4 fusion proteins. JTX-58 at 2. Furthermore, even if the deletion of the CH1 domain did increase secretion, a POSA would have

likely avoided eliminating the CH1 domain because removal was known to elicit effector functions and increase inflammation, as discussed above.

Lastly, a POSA would not have been motivated to remove the linker and directly fuse the p75 extracellular region to the full exon-encoded hinge. Again, Defendants cite to a number of references teaching multiple variations of what fragments a POSA could use, including many references that recommend using a partial hinge and/or a linker. *See, e.g.*, JTX-57 at 10:57-11:2 (Seed '262 describing the use of a five amino acid linker); JTX-56 at 1-2 (Byrn 1990 using a partial two-cysteine hinge); JTX-25 at 1 (Traunecker 1989 contemplating the removal of the entire hinge). Based on the uncertainty in the art, it would not have been obvious to a POSA to remove the linker or to use the full exon-encoded hinge. *See Ortho-McNeil Pharm.*, 520 F.3d at 1364 (holding that the patented invention was not obvious where a POSA had no reason to select the exact route among several unpredictable alternatives).

Defendants have failed to show by clear and convincing evidence that it would have been obvious to a POSA to create etanercept by precisely combining specific portions of TNFR and IgG1 prior to August 1990.

2. Objective Indicia of Nonobviousness

As part of its obviousness analysis, the Court must also consider evidence regarding objective considerations of nonobviousness when present. *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1075-77 (Fed. Cir. 2012). Secondary considerations such as unexpected results, success, long felt but unsolved needs, and the failure of others may be relevant indicia of nonobviousness. *See Graham*, 383 U.S. at 17-18; *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc.*, 471 F.3d 1369, 1380 (Fed. Cir. 2006). Moreover, evidence of copying, simultaneous invention, and licensing may also be considered. *See Diamond Rubber Co. v. Consol. Rubber Tire Co.*, 220 U.S. 428, 441 (1911); *Geo. M. Martin Co. v. Alliance Mach. Sys.*

Int'l LLC, 618 F.3d 1294, 1304 (Fed. Cir. 2010); *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1539 (Fed. Cir. 1983).

The parties have both presented evidence of certain objective indicia that they argue support their obviousness arguments, all of which are discussed below. A number of witnesses opined on these objective indicia including, for the Plaintiffs, (1) Dr. Naismith, as mentioned above; (2) Dr. Greene, as mentioned above; and (3) Dr. Fleischmann, an expert in the field of rheumatic diseases and disorders, who is the Founder and Co-Medical Director of the Metroplex Clinical Research Center in Dallas, Texas, and a Clinical Professor in the Department of Internal Medicine at the University of Texas, Southwestern Medical Center at Dallas (ECF No. 688 at 121 ¶¶ 19-21); and for the Defendants, (1) Dr. Blobel, as mentioned above; and (2) Dr. Skerra, as mentioned above. While both parties offered evidence of objective indicia to support their positions, the burden always remains on Defendants to prove by clear and convincing evidence that the claimed invention is obvious. *In re Cyclobenzaprine Hydrochloride,* 676 F.3d at 1075-79 (concluding that, when considering secondary considerations of nonobviousness, the burden never shifts to the patentee to prove nonobviousness and instead always remains on the party challenging the patent to prove by clear and convincing evidence that the patent at issue is obvious).

As to the objective indicia, Defendants challenge whether there is a sufficient nexus between the merits of the claimed invention and the objective evidence. Plaintiffs contend that the appropriate nexus is present and such evidence is commensurate in scope with the claims. *See Tokai Corp. v. Easton Enters., Inc.*, 632 F.3d 1358, 1369-70 (Fed. Cir. 2011) (concluding that, to establish a nexus to the merits of a claimed invention, the offered secondary consideration must actually result from what is both claimed and novel in the patent); *see also Dome Patent L.P. v. Rea*, 59 F. Supp. 3d 52, 86 (Fed. Cir. 2014) (holding that objective evidence of secondary

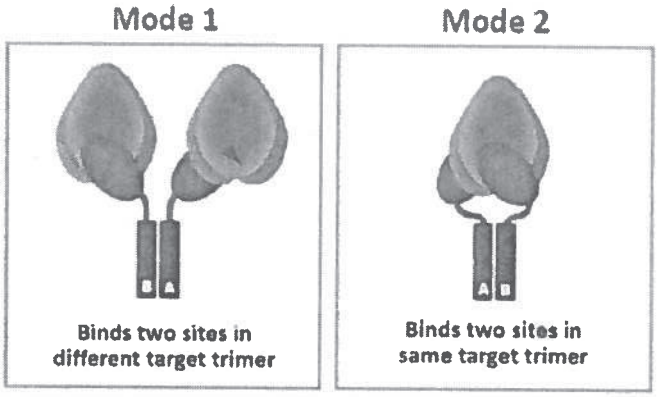
considerations must be proportional to the scope of the claims to be probative of nonobviousness). Here, the Court finds that the secondary considerations discussed below have a sufficient nexus to, and are commensurate in scope with, the claimed invention because the proffered evidence is linked to etanercept, which the Court has found was adequately described in the Patents-in-Suit. To the extent more specific arguments concerning nexus and scope were made by the parties, such assertions are addressed in the relevant sections below.

a) Unexpected Results

Unexpected or surprising results can support nonobviousness. To demonstrate unexpected results, a party must "show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995). "The principle applies most often to the less predictable fields, such as chemistry, where minor changes in a product or process may yield substantially different results." *Id.* Plaintiffs assert that etanercept exhibits three unexpected properties: (1) a lack of aggregation with TNF due to Mode 2 binding; (2) a superior binding affinity to, and inhibition of, TNF; and (3) little to no effector functions. Pls. Br. at 33. Defendants disagree that these properties were unexpected. Defs. Br. at 45-48.

First, the Court finds that etanercept's ability to bind in Mode 2 with little to no aggregation was an unexpected result. For background, in order for etanercept to be effective, the TNFR in etanercept has to bind to TNF. 9/20 PM (Fleischmann) Tr. at 149:11-17. Etanercept is a bivalent fusion protein, which means that it has two binding sites. DFOF ¶ 238. Dr. Naismith explained that fusion proteins like etanercept can potentially bind to TNF in either one of two ways: (1) Mode 1 binding, which occurs when a bivalent fusion protein binds two TNF cytokines at each of its two separate binding sites, (9/18 AM (Naismith) Tr. at 110:13-21 (explaining that etanercept has two "hand[s]," and that in "Mode 1" binding each hand would attach to a different TNF

molecule)); or (2) Mode 2 binding, which occurs when a bivalent fusion protein binds one TNF with both binding sites.[26] DFOF ¶ 238; 9/24 AM (Skerra) Tr. at 39:25-41:24; 9/18 AM (Naismith) Tr. at 110:10-111:13; Defendants' Trial Exhibit-84 at 5. While Mode 1 binding is very common in protein constructs similar to etanercept, (*see* 9/18 AM (Naismith) Tr. at 112:1-5), Mode 2 binding, which occurs in etanercept, is much rarer because the receptors have to be precisely arranged for Mode 2 binding to work. *See* id. at 110:22-111:8.



DFOF ¶ 238.

Despite the fact that Mode 2 binding was uncommon in proteins similar to etanercept, etanercept surprisingly engages in Mode 2 binding, which is one of the reasons why it effectively treats rheumatoid arthritis.[27] 9/18 AM (Naismith) Tr. at 114:19-115:23. Plaintiffs' expert Dr. Naismith credibly explained that a POSA in 1990 would have expected etanercept to bind in Mode 1 because Mode 1 had fewer limitations and, as a result, was much more likely in antibodies similar

[26] The Court notes that the parties' experts also discussed Mode 3 binding, which is an intermediate or transient step that could lead to either Mode 1 or Mode 2 binding. *See* 9/24 AM (Skerra) Tr. at 39:25-41:24; 9/18 AM (Naismith) Tr. at 111:9-13; DFOF ¶ 238.

[27] Defendants' expert, Dr. Skerra, who testified that a POSA would not have expected aggregation, was later impeached on this point because he based his opinions on a molecule that was different from etanercept and ultimately agreed that a POSA would have expected a molecule with etanercept's exact construction to have caused aggregation. 9/24 AM (Skerra) Tr. at 81:6-84:1.

to etanercept. Id. at 111:14-114:25. Etanercept's unexpected ability to bind in Mode 2 has important consequences. If etanercept had engaged in Mode 1 binding, aggregation would have resulted in the body—an undesired result for rheumatoid arthritis treatment as it leads to further inflammation. Id. at 112:13-19, 114:21-25. Mode 2 binding, however, results in little to no aggregation. 9/18 AM (Naismith) Tr. at 110:22-111:8. Hence, based on the state of the art in 1990, the Court finds that there is sufficient evidence to support the fact that etanercept's lack of aggregation due to Mode 2 binding was an unexpected and crucial result.

Second, a POSA would not have expected etanercept to bind fifty times stronger to TNF or to exhibit superior TNF-neutralizing properties. PFOF ¶ 254; PTX-73 at 4; 9/18 AM (Naismith) Tr. at 116:7-118:3. According to Defendants' expert, Dr. Capon, a POSA at the time would have thought that the binding power of an Ig fusion protein, such as etanercept, would have been weak.[28] JTX-58 at 2 (stating that Ig fusion proteins exhibit binding that is "indistinguishable" from binding as exhibited by soluble receptors, which were known to have weak binding strength at the time). Therefore, the fact that etanercept has strong binding capabilities would have been surprising to a POSA at the time. Moreover, etanercept's Mode 2 binding led to increased neutralization of TNF because etanercept bound to TNF more efficiently, reducing the amount of TNF left in the cells and thereby decreasing TNF's inflammatory effect. 9/18 AM (Naismith) Tr. at 117:17-118:3. A POSA would have also been surprised by etanercept's ability to powerfully neutralize TNF given that etanercept's ability to bind without aggregation was unexpected. Id. at 117:9-19 (Dr. Naismith concluding that prior to August 1990, a POSA would not have expected

[28] Defendants, relying on *Galderma Labs., L.P. v. Tolmar, Inc.*, 737 F.3d 731, 739 (Fed. Cir. 2013), argue that etanercept's ability to strongly bind to, and effectively neutralize, TNF was not unexpected because TNF was already known to bind and neutralize soluble TNFRs. Defs. Br. at 46. However, this argument fails because the ability of TNF to effectively bind to *etanercept*—a TNFR-Ig fusion protein—rather than to a soluble TNFR, was unknown and unexpected. *See* JTX-58 at 2; 9/18 AM (Naismith) Tr. at 116:10-16, 117:7-118:3.

etanercept to produce the 1000-fold efficacy in TNF neutralization that etanercept is now known to produce).

Third, prior to August 1990, a POSA would not have expected etanercept to produce little to no undesired effector functions. 9/18 PM (Greene) Tr. at 91:17-93:12, 100:10-101:10 (Dr. Greene testifying that it was a "surprise" and "unexpected result" that etanercept produced little or no CDC or ADCC effector functions. As discussed at length above, this result would have been unknown to a POSA prior to August 1990 and supports the assertion that etanercept produced unexpected results. Id. at 70:17-71:2 (Dr. Greene comparing etanercept to the prior art and testifying that, given the results of the testing with the CD4 fusion proteins, a POSA would have expected etanercept to exhibit effector functions); *see also supra* III.B.1.a. Accordingly, the Court concludes that this evidence of unexpected results weighs in favor of finding that the claims of the Patents-in-Suit are nonobvious.

b) Praise and Clinical Success

"Evidence that the industry praised a claimed invention or a product which embodies the patent claims weighs against an assertion that the same claim would have been obvious. Industry participants, especially competitors, are not likely to praise an obvious advance over the known art." *WBIP, LLC*, 829 F.3d at 1334. The Court may also look to evidence of Enbrel®'s clinical success. *See KSR*, 550 U.S. at 415 (concluding that with respect to the question of obviousness, courts should take "an expansive and flexible approach[,]" and noting that *Graham* "set forth a broad inquiry and invited courts, where appropriate, to look at any secondary considerations that would prove instructive"). Here, Plaintiffs offered ample evidence of praise and clinical success. In fact, Defendants' counsel conceded this at the beginning of trial. 9/11 AM (Opening) Tr. at 49:20-25 (Defendants' counsel stating that they were "not going to dispute that Enbrel[®], the product, the etanercept product . . . has not been . . . commercially successful[,] . . . clinically

successful[,] … [and] praised."). In any event, the evidence at trial confirmed that etanercept has been highly praised as a drug that has "changed the practice of medicine." 9/14 (McCamish) Tr. at 41:13-17. Enbrel® has been widely prescribed since its approval in 1998 and the number of prescriptions rose rapidly through 2008, despite shortages in supply and the entry of two major competitors into the market. 9/21 (Vellturo) Tr. at 14:22-15:19.

Nevertheless, Defendants assert that Enbrel®'s success and praise is unpersuasive because Enbrel®'s achievements are not sufficiently connected to the asserted claims of the Patents-in-Suit. *See* DFOF ¶ 335; 9/11 AM (Opening) Tr. at 49:25-50:10. The testimony at trial, however, established that Enbrel®'s success was largely rooted in the unexpected ability of etanercept, the claimed invention, to bind and neutralize TNF and its stability in the human body. 9/20 PM (Fleischmann) Tr. at 148:16-149:20 (Dr. Fleischmann testifying that the success of etanercept was due to its molecular properties and efficacy). Therefore, the Court concludes that there is a sufficient nexus between the claimed invention, etanercept, and Enbrel® because the drug's successes result from the effectiveness and novelty of etanercept, Enbrel®'s active ingredient. *WBIP, LLC*, 829 F.3d at 1331 (holding that a nexus can be presumed when the asserted objective indicia is tied to a specific product and the product is the invention claimed in the patent).

Moreover, as to whether this secondary consideration is reasonably commensurate in scope with the claims, Defendants contend that the evidence of Enbrel®'s success and praise ignores etanercept's failures in treating other conditions, such as Crohn's disease. Defs. Br. at 49. The Court has considered this argument and nonetheless concludes that Enbrel®'s success in treating rheumatoid arthritis—the focus of the litigation and the only use for which etanercept was FDA-approved in 1998—is probative of nonobviousness as etanercept was highly praised and extremely

successful in helping vast numbers of rheumatoid arthritis patients.[29] 9/20 PM (Fleischmann) Tr. at 148:16-150:20. Thus, praise and clinical success also weighs in favor of nonobviousness.

c) Long-Felt Need and Failure of Others

"Evidence is particularly probative of obviousness when it demonstrates both that a demand existed for the patented invention, and that others tried but failed to satisfy that demand." *In re Cyclobenzaprine Hydrochloride*, 676 F.3d at 1082-83. In order to show satisfaction of long-felt need, one must establish that (1) a POSA recognized a problem that existed for a long period of time without a solution, (2) the long felt need had not been satisfied by another before the claimed invention, and (3) the invention in fact satisfied the long-felt need. *See Newell Cos., Inc. v. Kenney Mfg. Co.*, 864 F.2d 757, 768 (Fed. Cir. 1988); *In re Cavanagh*, 436 F.2d 491, 495-96 (C.C.P.A. 1971); *In re Gershon*, 372 F.2d 535, 538-39 (C.C.P.A. 1967).

The trial testimony showed that there was a long-felt need for a better treatment for rheumatoid arthritis and that Enbrel® was the first drug to successfully satisfy this need. Prior to Immunex's sale of Enbrel®, a drug known as methotrexate "was a drug of choice" to treat rheumatoid arthritis. 9/20 PM (Fleischmann) Tr. at 131:22-24, 135:21-136:3 (Dr. Fleischmann testifying that methotrexate was the best drug available to treat rheumatoid arthritis in the mid-1990s, and that it was "the gold standard"). However, methotrexate could help only a small minority of patients. Pls. Br. at 35; 9/20 PM (Fleischmann) Tr. at 146:20-147:9. Although other research groups tried for decades to inhibit inflammation in the body, they failed to develop an effective solution before the claimed invention. 9/11 PM (Blobel) Tr. at 66:13-67:6. After

[29] The Court has similarly considered that Plaintiffs presented data focusing on Enbrel®'s success during its first ten years on the market. The Court has weighed this evidence accordingly and finds that the evidence of Enbrel®'s success over this ten-year span is persuasive of nonobviousness. *See* Defs. Br. at 49-50.

Enbrel® was introduced into the market, approximately 70% of patients with rheumatoid arthritis found relief from this treatment. 9/20 PM (Fleischmann) Tr. at 139:2-17.

In analyzing Plaintiffs' evidence as to this factor, the Court finds that a nexus is established because the community's long-felt need for an effective, wide-reaching rheumatoid arthritis drug was satisfied by the claimed invention itself. *See* id. at 139:8-24, 149:2-9, 146:20-147:9, 151:3-17. Enbrel® was able to satisfy this need because of etanercept's ability to effectively neutralize and bind TNF while suppressing pro-inflammatory effector functions. 9/20 AM (Wall) Tr. at 87:11-24. Accordingly, the Court finds that Plaintiffs have presented sufficient evidence to show that etanercept met a long-felt need that many others failed to successfully address prior to etanercept.

d) Copying

There is no dispute that Defendants' biosimilar has the same amino acid sequences and structure as Enbrel®. *See* DFOF ¶¶ 258-59. Plaintiffs ask the Court to find Defendants' copying as probative of nonobviousness. Pls. Br. at 12. Defendants draw a comparison to Hatch-Waxman Act Abbreviated New Drug Application ("ANDA") cases with generic drugs, and counter that copying a biologic drug should not be evidence of nonobviousness for creation of a biosimilar because "copying by Sandoz reflects its efforts to meet the FDA standards for approval of biosimilar products." Defs. Br. at 50, DFOF ¶¶ 258-59, 337.

It is well settled that the copying of an invention can be indicative of nonobviousness. *Diamond Rubber*, 220 U.S. at 440-41 (finding "imitation" of a certain tire as a "concession to its advance beyond the prior art and of its novelty and utility"). In the pharmaceutical realm, however, copying is generally not considered evidence of nonobviousness for matters in the ANDA context. *See, e.g.*, *Bayer Healthcare Pharms., Inc. v. Watson Pharms., Inc.*, 713 F.3d 1369, 1377 (Fed. Cir.

2013) ("evidence of copying in the ANDA context is not probative of nonobviousness because a showing of bioequivalence is required for FDA approval") (citation omitted).[30]

In order to obtain FDA approval for a biosimilar under the Biologics Price Competition and Innovation Act ("BPCIA"), "the applicant may piggyback on the showing made by the [original] manufacturer of a previously licensed biologic (reference product)" if the applicant can "show that its product is 'highly similar' to the reference product and that there are no 'clinically meaningful differences' between the two in terms of 'safety, purity, and potency.'" *Sandoz Inc. v. Amgen Inc.*, 137 S. Ct. 1664, 1670 (2017) (quoting 42 U.S.C. § 262(i)(2)(A), (B) and citing § 262(k)(2)(A)(i)(l)). Specifically, as compared to the original biologic, the biosimilar is permitted to have "minor differences in clinically inactive components," but must be "interchangeable with the reference product." 42 U.S.C. § 262(i)(2)(A), (k)(4). This is similar to the ANDA process for FDA approval of generic drugs, which requires "a generic drug company [to] submit information to show, inter alia, that its generic drug and the relevant listed drug share the same active ingredients and are bioequivalent." *Caraco Pharm. Labs., Ltd. v. Forest Labs., Inc.*, 527 F.3d 1278, 1282 (Fed. Cir. 2008) (citing 21 U.S.C. § 355(j)(2)(A)(ii), (iv)). At trial, Plaintiffs presented testimony by deposition from Graham B. Jones, Ph.D., their expert on the FDA's practices and policies regarding demonstrating biosimilarity, which was not inconsistent with the Court's

[30] Plaintiffs cite to *Merck Sharp & Dohme Corp. v. Hospira, Inc.*, an ANDA case in which the Federal Circuit found copying evidence of nonobviousness where the alleged infringer copied the "process of ***manufacturing*** the drug" in the patent. 874 F.3d 724, 726, 731 (Fed. Cir. 2017) (emphasis in original). The Court finds the facts of this case distinguishable from *Merck*. Here, Defendants presented credible testimony that they began developing their biosimilar in 2006, prior to the issuance of the Patents-in-Suit and prior to the BPCIA, and that they developed the biosimilar by utilizing etanercept's amino acid sequence directly from the commercial product Enbrel® due to an understanding that the amino acid sequence would need to be identical to etanercept for approval as a biosimilar. *See* 9/14 (McCamish) Tr. at 17:17-18:15, 84:15-85:6; JTX-83 (Alliger Deposition) at 9:85-10:90; DFOF ¶¶ 258-59; Defs. Br. at 50.

analysis in this Opinion. *See generally* JTX-87 (Jones Deposition); DFOF at xv.[31] Given the BPCIA abbreviated pathway for FDA approval and the testimony on the active ingredient at issue here, the Court finds that the same logic for not considering copying in ANDA cases would apply in this circumstance.[32] Thus, this factor cannot be used herein as evidence of nonobviousness.

e) Simultaneous Invention

Evidence of an independently made, simultaneous invention may be used in "some rare instances" to provide objective indicia of obviousness by showing that persons of ordinary skill in the art identified the same particular solution to a known problem. *Geo. M. Martin*, 618 F.3d at 1304 (citations and internal quotations omitted); *see Lindemann Maschinenfabrik GmbH v. Am. Hoist & Derrick Co.*, 730 F.2d 1452, 1460 (Fed. Cir. 1984). "Unlike the ultimate determination of obviousness, which requires courts to answer the hypothetical question of whether an invention 'would have been obvious,' 35 U.S.C. § 103, simultaneous invention demonstrates what others in the field ***actually accomplished***." *Trustees of Columbia Univ. v. Illumina, Inc.*, 620 F. App'x 916, 930 (Fed. Cir. 2015) (emphasis in original). Defendants assert four instances of alleged simultaneous invention of etanercept by: (1) Dr. Beutler at the University of Texas; (2) Dr.

[31] Jones testified that theoretically a proposed biosimilar could "encode a different primary amino acid sequence than the reference product," however the FDA guidance calls for evaluation "on a case-by-case basis." JTX-87 (Jones Deposition) at 5:33-34, 7:49-50, 8:54. Jones confirmed that he had not reviewed either of the Patents-in-Suit nor was he offering an opinion on whether Sandoz specifically "was required to use the same primary amino acid sequence as Enbrel® to obtain licensure of its etanercept product under the abbreviated pathway." Id. at 4:28. Furthermore, Jones could not "provide any examples of a biosimilar drug that's been approved by the FDA with an expression construct that encodes a different primary amino acid sequence as its reference product." Id. at 8:56.

[32] The Court notes that even if this factor could be used as evidence of nonobviousness in favor of Plaintiffs, such finding would not have any material impact on the outcome of the Court's obviousness analysis.

Ashkenazi at Genentech; (3) Dr. Lauffer of Behringwerke, who was working in collaboration with Immunex; and (4) Dr. Goodwin of Immunex. *See* DFOF ¶¶ 10, 223, 228, 233.

Dr. Beutler, Dr. Ashkenazi, and Dr. Lauffer did not make etanercept, but rather different fusion proteins, and therefore their constructs cannot be used as evidence of simultaneous invention. *See Endo Pharms. Inc. v. Actavis Pharms., LLC*, 922 F.3d 1365, 1378 n.14 (Fed. Cir. 2019), *aff'g Endo Pharms. Inc. v. Amneal Pharms., LLC*, 224 F. Supp. 3d 368, 381 (D. Del. 2016) (finding that alleged evidence of simultaneous invention can be disregarded for obviousness if it is not the same compound as the claimed invention); *see also Shire Orphan Therapies LLC v. Fresenius Kabi USA, LLC*, No. 15-1102, 2018 WL 2684097, at *20 (D. Del. June 5, 2018). Dr. Beutler of the University of Texas was working on a fusion protein that consisted of the extracellular region of p55 fused to a mouse IgG1 with a two-cysteine hinge. *See* JTX-67 col. 7:5-8; DFOF ¶ 235; 9/18 PM (Greene) Tr. at 103:12-21; *see also* 9/20 AM (Wall) Tr. at 89:19-90:1. Dr. Ashkenazi at Genentech similarly constructed a fusion protein with p55 and a partial two-cysteine hinge. JTX-69 at 1; PFOF ¶ 267; DFOF ¶ 233. Behringwerke's Dr. Lauffer made a fusion protein with p75 and a three-cysteine hinge but deleted the last five amino acids of the C-terminus of the TNFR and also added a linker. PFOF ¶¶ 269-71. The record does not demonstrate that Dr. Lauffer or anyone at Behringwerke contemplated using the full extracellular region of p75 or removing the linker, as was done in etanercept. Based on the evidence presented, the Court finds that the constructs of Dr. Beutler, Dr. Ashkenazi, and Dr. Lauffer do not support a finding of obviousness because these inventions did not contemplate etanercept.

Roche's patent applications were already pending when Immunex created etanercept in November or December 1990. PFOF ¶¶ 51, 263. Immunex's subsequent decision to license the Patents-in-Suit from Roche demonstrates etanercept's inventive nature and undermines an

obviousness finding. *See* id. ¶¶ 69-70; DFOF ¶ 228. Moreover, a single instance of simultaneous invention cannot alone support a finding of obviousness for the following reasons. First, if one instance of simultaneous invention were sufficient to show obviousness, any claims involved in an interference would be unpatentable for obviousness, making interference proceedings futile. *Lindemann Maschinenfabrik GmbH*, 730 F.2d at 1460 (Fed. Cir. 1984) (concluding that because the statute governing interference "recognizes the possibility of near simultaneous invention by two or more equally talented inventors working independently, that occurrence may or may not be an indication of obviousness when considered in light of all the circumstances"). Second, even when evidence of simultaneous invention exists, the unexpected success of the claimed invention can preclude a finding of obviousness because surprising results demonstrate the true novelty of the invention, even if multiple inventors happened to discover it within a similar time period. *See Regents of Univ. of Cal. v. Broad Inst., Inc.*, 903 F.3d 1286, 1291, 1295-96 (Fed. Cir. 2018) (declining to find obviousness despite strong evidence of six different simultaneous inventions because the results of the claimed invention were unpredictable and unexpected, thereby outweighing any potential probativeness of the simultaneous inventions). Accordingly, the Court finds that the Defendants' argument concerning the factor of simultaneous invention fails to support obviousness.

f) Licensing

The licensing of a patent is also objective indicia that a patent is not obvious. *See Stratoflex*, 713 F.2d at 1539 ("Recognition and acceptance of the patent by competitors who take licenses under it to avail themselves of the merits of the invention is evidence of nonobviousness."). Here, Defendants concede that Immunex obtained a license for the Patents-in-Suit from Roche in 1998. DFOF ¶ 52; JTX-13. As such, the Court finds that the licensing factor also weighs in favor of nonobviousness.

Accordingly, for all of the above reasons, the Court finds that Defendants have failed to prove by clear and convincing evidence that the Patents-in-Suit are obvious.

C. Obviousness-Type Double Patenting

The judicially-created doctrine of obviousness-type double patenting prevents a party from extending their right to exclude by obtaining a later patent with claims that are not patentably distinct from claims in a commonly-owned previous patent. *In re Longi*, 759 F.2d 887, 892 (Fed. Cir. 1985). "The purpose of the rule against double patenting is to prevent an inventor from effectively extending the term of exclusivity by the subsequent patenting of variations that are not patentably distinct from the first-patented invention." *Applied Materials, Inc. v. Advanced Semiconductor Materials Am., Inc.*, 98 F.3d 1563, 1568 (Fed. Cir. 1996); *see also Procter & Gamble Co. v. Teva Pharms. USA, Inc.*, 566 F.3d 989, 999 (Fed. Cir. 2009). Thus, a preliminary step to find that the rule against obviousness-type double patenting was violated is to assess whether the patents or patent applications have a common inventor or common ownership. *See Applied Materials, Inc.*, 98 F.3d at 1568; *In re Longi*, 759 F.2d at 895.

Double patenting entails a two-pronged analysis. "First, as a matter of law, a court construes the claim in the earlier patent and the claim in the later patent and determines the differences." *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 968 (Fed. Cir. 2001) (citing *Ga.-Pac. Corp. v. U.S. Gypsum Co.*, 195 F.3d 1322, 1326 (Fed. Cir. 1999)). "Second, the court determines whether the differences in subject matter between the two claims render the claims patentably distinct." *Id.* (citing *Ga.-Pac. Corp.*, 195 F.3d at 1327). If the later claim is an "obvious variant" or obvious modification of the earlier claim, then the later claim is invalid for double patenting. *In re Basell Poliolefine Italia S.P.A.*, 547 F.3d 1371, 1378-79 (Fed. Cir. 2008).

An analysis of step two requires a determination of whether or not the claims are "patentably distinct," by "ask[ing] whether the identified difference renders the claims of the . . .

[two] patents non-obvious to a person of ordinary skill in the art in light of the prior art." *Amgen Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1361 (Fed. Cir. 2009); *see also Pfizer, Inc. v. Teva Pharms. USA, Inc.*, 518 F.3d 1353, 1363 (Fed. Cir. 2008); *In re Kaplan*, 789 F.2d 1574, 1580 (Fed. Cir. 1986). "This part of the obviousness-type double patenting analysis is analogous to an obviousness analysis under 35 U.S.C. § 103, except that" the alleged invalidating reference patent itself "is not considered prior art" for purposes of the analysis. *Amgen*, 580 F.3d at 1361. Specifically, an obviousness-type double patenting analysis requires an inquiry into the scope and content of the prior art, the level of skill in the art, and what would have been obvious to a POSA. *See Studiengesellschaft Kohle mbH v. N. Petrochemical Co.*, 784 F.2d 351, 355 (Fed. Cir. 1986).

Defendants argue that the Patents-in-Suit (*i.e.*, the '182 and '522 Patents) should be invalidated because Immunex has used the Patents-in-Suit to "obtain[] an unjustified timewise extension of its etanercept monopoly" in violation of 35 U.S.C. § 121. Defs. Br. at 6-15. Specifically, Defendants contend that the Patents-in-Suit are invalid for obviousness-type double patenting over (1) Roche's '279 Patent; (2) Immunex's U.S. Patent No. 5,605,690 ("the '690 Patent"); and (3) three Immunex patents aimed at psoriasis and psoriatic arthritis, U.S. Patent Nos. 7,915,225 ("'225 Patent"), 8,119,605 ("'605 Patent"), and 8,722,631 ("'631 Patent") (collectively, "the Finck Patents"). Id. Plaintiffs counter that Defendants' challenges fail because (1) a safe harbor provision applies to the Roche '279 Patent, preventing an obviousness-type double patenting violation; (2) Defendants employ an incorrect doctrine to find common ownership over Immunex's '690 Patent and Finck Patents, which is required before even conducting the traditional two-step analysis; and (3) the Patents-in-Suit are patentably distinct from the Roche '279 Patent, Immunex's '690 Patent, and Immunex's Finck Patents. Pls. Br. at 39-50.

In support of their arguments on obviousness-type double patenting, Defendants relied, to a large extent, on the following two of their witnesses: (1) Dr. Blobel, previously introduced in sections III.A and III.B, who is an expert in biophysics, particularly focusing on arthritis and tissue degeneration; and (2) John Parise, who testified via deposition and was Roche's former Senior Counsel and Managing Attorney involved in drafting and negotiating the 2004 Accord and Satisfaction on behalf of Roche. ECF No. 688 at 131 ¶ 43, 137 at ¶ 71; DFOF ¶¶ xvi, 66. Plaintiffs relied heavily on (1) expert Stephen G. Kunin, J.D., an attorney who is the former Deputy Commissioner for Patent Examination Policy in the Office of the Commissioner for Patents in the USPTO and an expert in USPTO policies, practices, and procedures; and (2) Stuart Watt, former Vice President of Law and Intellectual Property Officer at Amgen, who was involved in the prosecution of the Patents-in-Suit and the negotiation and drafting of licensing agreements for the company. ECF No. 688 at 128 ¶ 39, 137 ¶ 73.

The Court will first address Defendants' arguments with respect to Roche's '279 Patent, followed by Immunex's '690 Patent, and finally Immunex's Finck Patents. For the reasons set forth, the Court agrees with Plaintiffs that the Patents-in-Suit are not invalid for obviousness-type double patenting.

1. The '182 Patent Is Not Invalid in View of Roche's '279 Patent

Defendants argue that the '182 Patent should be invalidated based on Roche's '279 Patent.[33] Defs. Br. at 6-15. There is no dispute that Roche is the owner of both the '279 Patent and the '182 Patent and therefore common ownership exists. However, Plaintiffs contend that any

[33] Defendants stipulated at trial that the Safe Harbor provision of 35 U.S.C. § 121 protects the '522 Patent against a challenge based on Roche's '279 Patent. Pls. Br. at 39; 9/21 Tr. at 9:7-16 (defense counsel acknowledging, at trial, that the Safe Harbor provision protects the '522 Patent from any challenge based on the '279 Patent). The analysis herein will therefore solely focus on the validity of the '182 Patent as it relates to Roche's '279 Patent.

challenge based on the '279 Patent must fail because of the safe harbor provision in 35 U.S.C. § 121 ("Safe Harbor"). The Safe Harbor provision protects applicants from obviousness-type double patenting invalidity when they are forced to pursue inventions in separate patent applications as a result of a "restriction requirement" set by the USPTO, here in the filing of related divisional applications. *See* 35 U.S.C. § 121; Pls. Br. at 39-41. The Court will first examine Plaintiffs' argument that the Safe Harbor provision protects the '182 Patent from being invalidated by Roche's '279 Patent for obviousness-type double patenting and then go through the traditional obviousness-type double patenting analysis comparing the asserted claims of the '182 Patent to Roche's '279 Patent claims.

a) Roche's '279 Patent

i. Background on the '279 Patent

Roche's first patent application covering the claimed invention, the '013 Application, was filed in September 1990. PFOF ¶ 51; DFOF ¶ 38. That application was abandoned and the '640 Application, which also covered the claimed invention, was filed in July 1993. PFOF ¶ 57; DFOF ¶¶ 38-39. During patent prosecution, the USPTO placed a restriction requirement on the '640 Application, requiring Roche to "elect one of three distinct inventions" and choose "between the p55 and p75 protein." DFOF ¶ 40. Roche elected to pursue claims related to the p55 fusion protein, which resulted in the '279 Patent being issued in March 1997. PFOF ¶ 57; DFOF ¶¶ 39-40. In order to pursue the non-elected claims, i.e. those related to the p75 fusion protein, Roche was required to file separate divisional applications. In May 1995, Roche filed the '790 Application, which eventually issued as the '182 Patent. PFOF ¶ 57.

ii. The Safe Harbor Provision Protects the Claims of the '182 Patent in View of the '279 Patent

The Court finds that the Safe Harbor provision protects the claims of the '182 Patent from Defendants' invalidity argument based on the '279 Patent. Under the Safe Harbor provision, a patent cannot be invalidated for obviousness-type double patenting if the subject patent was issued from a divisional application that was filed as a result of a requirement for restriction. 35 U.S.C. § 121; *see Symbol Techs., Inc. v. Opticon, Inc.*, 935 F.2d 1569, 1579 (Fed. Cir. 1991). There are three requirements for invoking the protection of the Safe Harbor provision: (1) a restriction requirement, (2) a divisional application filed as a result of the restriction requirement, and (3) consonance with the restriction requirement. 35 U.S.C. § 121.

At trial, Plaintiffs' expert Steven G. Kunin explained the USPTO's policy, practice, and procedure related to the Safe Harbor protection afforded to "applicants who are forced to file multiple patent applications." 9/21 (Kunin) Tr. at 69:18-20. Based on his experience with the USPTO for more than thirty-four years, ten of which were spent as the Deputy Commissioner, Mr. Kunin described the procedure for restriction requirements and divisional applications. Id. at 66:5-68:9, 69:21-70:24. He explained that if an "applicant claimed more than one independent and distinct invention" in a parent application, the applicant would be forced to file a divisional patent application to ensure "administrative efficiency and effectiveness." Id. at 69:23-70:7. If the applicant still wished to obtain a patent for the other inventions initially included in the parent application, the applicant would need to file a "divisional application." Id. at 70:8-24. This divisional application would be prohibited from rejection on obviousness-type double patenting grounds over the claims of the parent application based on the Safe Harbor provision. Id. The Safe Harbor provision was created for the specific purpose of preventing "unfairness by penalizing the applicant who would do . . . what the examiner had requested by electing an invention, filing

a divisional and seeking the examination of the withdrawn claims in the parent in the divisional." Id. at 70:25-71:7.

As to the instant case, Mr. Kunin testified that the USPTO placed a restriction requirement on the '640 Application (which became the '279 Patent) during its prosecution. PFOF ¶¶ 278-82; DFOF ¶¶ 40-41; *see also* 9/21 (Kunin) Tr. at 69:23-70:2 (Mr. Kunin testifying generally that when "the examiner required the applicant to elect only one of those inventions for search and examination" it is "known as a restriction requirement"). According to the restriction requirement, Roche was obligated to choose between prosecuting claims of either p55 or p75 TNFR. PFOF ¶ 280; DFOF ¶ 50. Roche elected claims relating to p55 TNFR, which resulted in the '279 Patent. PFOF ¶ 285; DFOF ¶ 50. Thereafter, the p75 TNFR claims were pursued in a divisional application that led to the '182 Patent. PFOF ¶ 285. Plaintiffs therefore meet the first two requirements because there was both a restriction on the application underlying the '279 Patent and a divisional application filed as a result of that restriction.

Defendants do not challenge the fact that there was a restriction on the application for the '279 Patent and that the Patents-in-Suit were the result of divisional applications filed based on that restriction. DFOF ¶¶ 40-41, 45 ("[d]uring the prosecution of the '279 patent, the examiner issued a restriction requirement" and "[f]ollowing the restriction requirement, Roche filed divisional applications from the '279 patent application," one of "which led to the '182 patent"). The focus of Defendants' Safe Harbor challenge for the '182 Patent therefore appears to be based on the third requirement of consonance. Id. ¶¶ 298-301. Consonance is a judge-made principle that states that the divisional application cannot reclaim the invention, which was elected and examined in the parent. *See Symbol Techs.*, 935 F.2d at 1579 ("Consonance requires that the line of demarcation between the 'independent and distinct inventions' that prompted the restriction

requirement be maintained.") (quoting *Gerber Garment Tech., Inc. v. Lectra Sys., Inc.*, 916 F.2d 683, 688 (Fed. Cir. 1990)); *see also* 9/21 (Kunin) Tr. at 76:5-8. In other words, just as the parent patent application must elect a distinct invention as a result of the restriction requirement, so too must the subsequent divisional application refrain from claiming the elected invention from the parent application. Where the principle of consonance is violated, the Safe Harbor provision "will not apply to remove the parent [patent] as a reference" in an obviousness-type double patenting analysis. *See Symbol Techs.*, 935 F.2d at 1579.

Here, Roche's '279 Patent elected claims relate to p55 TNFR from the original patent application as a result of the restriction requirement. Immunex and Amgen then amended the subsequent '790 Application (a divisional of the '279 Patent application) which became the '182 Patent, to include claims for p75. *See* PTX-6.280. That amendment was made in response to a rejection by the USPTO, approximately ten years after the application for the '182 Patent was originally filed[34] and brought the claims into consonance with the restriction requirement. PTX-6.332; 9/21 (Kunin) Tr. at 87:19-90:9 (Mr. Kunin explaining the patent prosecution history and when the patent applications were brought into consonance). Defendants take the position that the amount of time it took for Roche to amend the claims of the '182 Patent to bring them into consonance with the restriction requirement should result in invalidity of the patent, "because the applicants failed to maintain consonance throughout the prosecution of the '182 patent application." (DFOF ¶¶ 298-99).

[34] As discussed further below, the Court notes that Plaintiffs' expert Mr. Kunin testified that he reviewed the prosecution history and prior to the amendment, there "was something like three years, in which the applicant submitted like six status requests because the Office hadn't been working on them" and also "the '182 patent . . . was lost for a couple of years" by the USPTO. 9/21 (Kunin) Tr. at 104:15-105:18.

The USPTO allows application amendments at any time and does not provide temporal limits for the Safe Harbor provision to apply. *See* 35 U.S.C.§ 121 (including no time limits as to when Safe Harbor applies, so long as "divisional application is filed before the issuance of the patent on the other application"); *see also* 9/21 Tr. (Kunin) at 90:22-91:5 (Mr. Kunin testifying that "[t]here's nothing in [the relevant section that] talks about time limits. So long as the applicant is still permitted to amend claims, then if the claims during that period prior to issuance are amended to bring them back into consonance, then the safe harbor will apply."). Moreover, an inquiry into whether the Safe Harbor rule applies requires analysis of the issued claims. *Boehringer Ingelheim Int'l GmbH v. Barr Labs, Inc.*, 592 F.3d 1340, 1354 (Fed. Cir. 2010) (explaining that, when doing a Safe Harbor analysis, the proper inquiry is on the issued claims). Defendants have not presented case law or trial testimony to indicate by clear and convincing evidence that the timing of the amendment or the content of pre-amendment application claims bear any legal significance. Based on its analysis of the issued claims, the Court concludes that the Safe Harbor provision protects the '182 Patent such that it cannot be invalidated for obviousness-type double patenting because the '182 Patent was (1) the result of a divisional application, (2) based on a restriction requirement issued by the USPTO, and (3) in consonance with that restriction requirement.

b) The Claims of the '279 Patent Are Patentably Distinct from the '182 Patent

Even assuming the Safe Harbor provision did not protect the '182 Patent from invalidity based on obviousness-type double patenting, the Court nonetheless finds that the '182 Patent is patentably distinct from the '279 Patent and therefore not invalid for obviousness-type double patenting. To determine whether the claims are patentably distinct, the Court must compare the two patents at issue and decide whether the '182 Patent is an obvious modification of the earlier-

issued '279 Patent. If the later claim is an "obvious variant" or obvious modification of the earlier claim, according to a POSA, then the later claim is invalid for non-statutory double patenting. *In re Basell Poliolefine Italia S.P.A.*, 547 F.3d at 1378-79. The Court concludes that the claims of the '182 Patent are patentably distinct from the '279 Patent for the reasons stated herein.

The '279 Patent relates to an "invention [that] is concerned with non-soluble proteins and soluble or insoluble fragments thereof, which bind TNF, in homogenous form." '279 Patent (JTX-5) at "Abstract". All claims of the '279 Patent relate to a p55 TNFR. Id. at col. 24:11-21. Claim 1 is for a p55 TNFR and all of the remaining claims in the '279 Patent depend on Claim 1. Hence, the '279 Patent involves a p55 TNFR that is fused to an immunoglobulin. Id.

In contrast, the '182 Patent claims, in part, an insoluble human TNFR that "has an apparent molecular weight of about 75 kilodaltons," which specifically binds human TNF. '182 Patent (JTX-1) col. 39:18-19. Throughout Defendants' contentions regarding the patent specification, Defendants acknowledge that p55 TNFR is distinct from p75 TNFR. DFOF ¶ 125. During prosecution of the '279 Patent application, the USPTO required Roche to elect either the p55 or the p75, acknowledging that p55 and p75 were patentably distinct. JTX-9 at 118 ("The proteins are unobvious in view of each other . . . ."); *see also* 9/21 (Kunin) Tr. at 83:5-84:19. Accordingly, the Court finds that there are significant distinctions between the '279 and '182 Patents such that the patents would not have been modifications obvious to a POSA in 1990. Therefore, the Court concludes that the '182 Patent is not invalid for obviousness-type double patenting based on the '279 Patent.

2. The Patents-in-Suit Are Not Invalid over the '690 Patent and the Finck Patents

Defendants argue that the Patents-in-Suit are obvious over Immunex's '690 Patent and Immunex's Finck Patents (consisting of the '225, '605, and '631 Patents) (collectively, the

"Immunex Patents"). Defs. Br. at 6-20. However, common ownership is required for obviousness-type double patenting. *In re Longi*, 759 F.2d at 893-95. While Roche is the recorded owner of the Patents-in-Suit, Defendants contend that the 2004 Accord and Satisfaction was tantamount to an assignment to Immunex, making Immunex a common owner of the Patents-in-Suit and the Immunex Patents. Defs. Br. at 6-15. Specifically, Defendants claim that the Accord and Satisfaction transferred "all substantial rights" from Roche to Immunex, resulting in Immunex's ownership of the Patents-in-Suit and an impermissible extension of Plaintiffs' monopoly over etanercept. Id. at 7-14. Plaintiffs argue that the Accord and Satisfaction did not transfer ownership from Roche to Immunex, and instead granted a license. Pls. Br. at 41-47. Plaintiffs further aver that even if the Patents-in-Suit were commonly owned, Defendants have not met their burden to show that the Patents-in-Suit are patentably indistinct from the Immunex Patents. Id. at 47-50.

The Court will first address the common ownership issue and then discuss the '690 Patent and the Finck Patents.

a) The Accord and Satisfaction Does Not Create Common Ownership

In 1999, Immunex licensed Roche's pending patent applications, which became the Patents-in-Suit, effective back to the FDA approval date of Enbrel® in 1998. PFOF ¶ 70. Under the license, Immunex was required to pay Roche "tens of millions of dollars." Id. Non-party Amgen Inc. acquired Immunex in 2002. Id. ¶ 71. Later, Roche entered into the Accord and Satisfaction with Amgen Inc. and its affiliates, including Immunex, which was executed on June 7, 2004. JTX-12; PFOF ¶ 71. Thereunder, Amgen Inc. and Immunex fully paid their outstanding royalty obligations to Roche and received an exclusive license to the Patents-in-Suit.[35] PFOF ¶

[35] At that time, the applications for the Patents-in-Suit were still pending and had not yet been issued.

71. Immunex and Amgen Inc. received the following rights as they pertained to the eventual Patents-in-Suit and their then-pending applications: (1) an "irrevocable, exclusive license, with the sole right to grant sublicenses" of the Patents-in-Suit; (2) the exclusive right to practice under the Patents-in-Suit in North America; (3) the exclusive right to prosecute the Patents-in-Suit; (4) the right to select outside counsel for the prosecution of the Patents-in-Suit; (5) the first right to bring an infringement action in connection with the Patents-in-Suit; and (6) the right to retain all profits that result from any infringement litigation brought by Amgen Inc. or Immunex. JTX-12 at 4-7 (§§ 3.1-3.6). Roche retained the rights to (1) sue for infringement if Amgen Inc. does not, (2) choose its partners under the license agreement, and (3) use the inventions for non-clinical research. PFOF ¶¶ 304-06; DFOF ¶¶ 53, 62. The rights conferred by Roche through the Accord and Satisfaction were later consolidated in Immunex by a separate agreement, and Immunex "sublicensed exclusive rights related to Enbrel®'s commercialization to Amgen."[36] JTX-14; 9/24 PM (Watt) Tr. at 28:20-29:8; JTX-15 at 3; PFOF ¶¶ 4-5.

To use the Immunex Patents to invalidate the Patents-in-Suit, Immunex must first be a common owner to both sets of patents, in accordance with the obviousness-type double patenting doctrine. *In re Longi*, 759 F.2d at 892. Defendants' argument of common ownership is that the 2004 Accord and Satisfaction transferred "all substantial rights" from Roche to Amgen and Immunex and any rights that Roche did retain were illusory. Defs. Br. at 7-20; *see also Speedplay, Inc. v. BeBop, Inc.*, 211 F.3d 1245, 1249-50 (Fed. Cir. 2000). Defendants therefore ask the Court to find first that the transfer of all substantial rights is the legal equivalent of common ownership,

[36] While the Accord and Satisfaction was negotiated with non-party Amgen Inc., the rights were later consolidated in Immunex. JTX-14. For ease of reference the Court will refer to Immunex, which is a party to this action and currently retains the rights discussed in the Accord and Satisfaction.

which is necessary for obviousness-type double patenting invalidation, and second that the Accord and Satisfaction transferred all substantial rights.

Defendants' cases in support of their common ownership argument all analyze indicia of common ownership for the purpose of determining whether a party had what is referred to as "prudential standing" to sue, and not ownership for the purpose of obviousness-type double patenting. *See Diamond Coating Techs., LLC v. Hyundai Motor Am.*, 823 F.3d 615, 618-19 (Fed. Cir. 2016); *Luminara Worldwide, LLC v. Liown Elecs. Co.*, 814 F.3d 1343, 1349-50 (Fed. Cir. 2016); *Speedplay*, 211 F.3d at 1249-50; *Vaupel Textlimaschinen KG v. Meccanica Euro Italia SPA*, 944 F.2d 870, 875 (Fed. Cir. 1991); *EMC Corp. v. Pure Storage, Inc.*, 165 F. Supp. 3d 170, 178 (D. Del. 2016). For example, although the Federal Circuit in *Diamond Coating* made observations about what constitutes ownership, Defendants correctly concede that the observations were made in the context of deciding whether the plaintiff had standing or the right to sue under the subject patent, which is not the question currently before this Court.[37] *See Diamond Coating*, 823 F.3d at 617-19; s*ee also Speedplay*, 211 F.3d at 1250.

Here, the matter is not within the "standing to sue" context, and thus the ownership caselaw presented by Defendants is not directly applicable. However, even assuming those cases apply, the Court finds that Roche remained the owner of the Patents-in-Suit because the Accord and Satisfaction did not confer all substantial rights on Immunex. First, the Court finds that the parties specifically intended for the Accord and Satisfaction to be a license such that Roche would remain

[37] Defendants have not cited to, nor has this Court found, any caselaw that has extended or applied the "all substantial rights" test to render a patent *invalid* pursuant to the obviousness-type double patenting doctrine. The purpose of the doctrine of obviousness-type double patenting is to prevent the same inventor and/or owner of an invention from extending their patent terms over the same invention or an obvious variant thereof. *Gilead Scis., Inc. v. Natco Pharma Ltd.*, 753 F.3d 1208, 1212 (Fed. Cir. 2014) ("[T]he doctrine of double patenting was primarily designed to prevent . . . harm [to the public] by limiting a patentee to one patent term per invention or improvement.").

the owner of the Patents-in-Suit. "To determine whether an exclusive license is tantamount to an assignment, [the Court] 'must ascertain the intention of the parties [to the license agreement] and examine the substance of what was granted.'" *Alfred E. Mann Found. For Sci. Research v. Cochlear Corp.*, 604 F.3d 1354, 1359 (Fed. Cir. 2010) (quoting *Mentor H/S, Inc. v. Med. Device All., Inc.*, 240 F.3d 1016, 1017 (Fed. Cir. 2001)); *see also AsymmetRx, Inc. v. Biocare Med., LLC*, 582 F.3d 1314, 1319 (Fed. Cir. 2009) ("To determine whether an assignment of patent rights was made, we must examine whether the agreement transferred all substantial rights to the patents and whether the surrounding circumstances indicated an intent to do so.") (internal citations omitted)). A district court's interpretation of a contract presents a question of law. *Alfred E. Mann*, 604 F.3d at 1359. To the extent that determining the intention of the parties to the license agreement requires evaluation of evidence outside of the contract, the district court's evaluation presents a question of fact. *Id.*

The evidence during trial demonstrated that the parties agreed to draft the Accord and Satisfaction as a license, and not an assignment of all rights. On the face of the Accord and Satisfaction itself, the transfer of rights in North America to Amgen is expressly called a "[l]icense," in contrast to the transfer of rights outside of North America to non-party Wyeth BV, which is expressly called an "[a]ssignment." *Compare* JTX-12 at 4 ("Article 3 License to Amgen" granting "to Amgen and its Affiliates a paid-up, irrevocable, exclusive license, with the sole right to grant sublicenses") *with* id. at 3 ("Article 2 Assignment to Wyeth BV" stating Roche "hereby agrees to assign, and will cause its Affiliates to assign"). Under the Accord and Satisfaction with respect to the Patents-in-Suit in North America, Roche maintained a second right to sue for infringement, including a right to determine whether an assignment or sublicence would be granted to cure the infringement, and retained the right to practice the invention. PFOF ¶¶ 304-05; DFOF

¶¶ 53, 62. In contrast, the Accord and Satisfaction expressly assigned to non-party Wyeth BV "all right, title and interest in and to" the Patents-in-Suit outside of North America and acknowledges that "Wyeth BV has succeeded to all of Roche's and its Affiliates' right, title, interest, benefit, and standing to receive all rights and benefits" pertaining to the Patents-in-Suit outside of North America. JTX-12 at 3-4.

Moreover, the Court heard the testimony of Stuart Watt, Amgen's Vice President of Law and Intellectual Property Officer, who engaged in negotiations with Roche on behalf of Immunex and Amgen. *See* 9/24 PM (Watt) Tr. at 20:21-23, 25:15-18. Watt credibly testified that it was more valuable to Immunex for Roche to remain as the owner of the Patents-in-Suit. *See* id. at 29:11-22. Watt stated that, based on his past litigation experience, it was important for Roche to have an obligation to participate in litigation as a party, rather than have the mere contractual duty, which could easily be breached. Id. at 29:15-31:14. The fact that the parties thoughtfully negotiated and ultimately agreed to draft the portion of the Accord and Satisfaction pertaining to North America and Amgen as a license presents strong evidence that the parties intended for the Accord and Satisfaction to be treated as a license, rather than an assignment.

While Defendants believe the rights retained by Roche for the Patents-in-Suit in North America are "illusory" or insignificant, the Court disagrees. As explained at trial, Roche still possessed the power to bring a patent infringement action if the Immunex Plaintiffs failed to do so. *See* 9/24 PM (Watt) Tr. at 39:2-25. The Federal Circuit has found that a second right to sue is in fact a substantial right retained. *Alfred E. Mann*, 604 F.3d at 1361-62. According to the language of the Accord and Satisfaction, if Roche initiates a suit for infringement, the suit is solely within the control of Roche but Immunex has a duty to cooperate during the suit. *See* JTX-12 at 6 (§ 3.6). Importantly, while Immunex had the right to sublicense, Immunex could *not* end a

Roche-initiated lawsuit by granting a sublicense on its own. *See* id. Moreover, Roche could veto the assignment of Immunex's rights to a third party, which suggests that the parties envisioned the agreement to be a license. *See* id. at 14 (§ 11.4). This scenario is distinguishable from a situation where the licensee can grant a license to end a licensor-initiated lawsuit. *See, e.g.*, *Speedplay*, 211 F.3d at 1251. Ultimately, Roche's own enforcement capabilities, in the event Immunex chooses not to sue, are not nullified by Immunex's separate right to sublicense.

Furthermore, Roche maintained the right to practice the invention. JTX-12 at 4 (§ 3.2) (Roche "reserves for itself and its Affiliates the right to practice" the invention in North America "for internal, non-clinical research"). In *AsymmetRx, Inc.*, the licensor also retained the right to practice the patents "for academic research" and the court noted that as one factor in finding that the licensor did not transfer all substantial rights. 582 F.3d at 1320 (considering the retained "right to make and use the [patented compound] for its own academic research purposes," in ultimate conclusion that rights conveyed were a license). Because Roche retained not only a right to sue for infringement, but a right to veto assignments or sublicenses, and the right to practice the patent, the Court finds that Roche did not convey all substantial rights.

In sum, should the "all substantial rights" test have a place in this case, Roche has nonetheless retained certain substantial rights and accordingly, ownership of the Patents-in-Suit did not transfer to Immunex. As stated above, common ownership or having at least one common inventor is a required element for the Patents-in-Suit to be invalid under the obviousness-type double patenting doctrine. *In re Longi*, 759 F.2d at 893-95. Hence, Defendants cannot establish common ownership and/or inventorship to support invalidity of the Patents-in-Suit pursuant to the doctrine of double patenting over the '690 and the Finck Patents based on Defendants' all substantial rights argument.

b) *The Claims of the Immunex Patents Are Patentably Distinct from the Patents-in-Suit*

Due to the Court's finding of no common ownership, the remaining portions of this Opinion are not necessary to the Court's ultimate conclusion on obviousness-type double patenting. Nevertheless, even assuming the Court had found common ownership, the Court finds that the Immunex Patents (the '690 and Finck Patents) are patentably distinct from the Patents-in-Suit and therefore the Patents-in-Suit are not invalid. According to the law of double patenting, the Court must first ask: "[i]s the same invention being claimed twice?" *Gen. Foods Corp. v. Studiengesellschaft Kohle mbH*, 972 F.2d 1272, 1278 (Fed. Cir. 1992) (citing *In re Vogel*, 422 F.2d 438, 442 (C.C.P.A. 1970)). If the answer to the first question is no, then the Court must ask: "[d]oes any claim in the application define merely an obvious variation of an invention claimed in the patent asserted as supporting double patenting?" *Id.* If the answer to that question is no, there is no double patenting. *Id.* That is, if the claim at issue "defines *more* than an obvious variation, it is *patentably distinct*" and any double patenting argument would fail. *Id.* When conducting this analysis, the claims must be read as a whole. *Id.*

When construing a claim in an earlier patent against a claim in a later patent, the Court needs to determine whether the differences in subject matter between the two claims render the claims patentably distinct. *Eli Lilly & Co.*, 251 F.3d at 968 (citing *Ga.-Pac. Corp.*, 195 F.3d at 1326). If, according to a POSA, the later claim is an obvious modification of the earlier claim, then the later claim is invalid for non-statutory double patenting. *In re Basell Poliolefine Italia S.P.A.*, 547 F.3d at 1378-79.

The Court will first address Defendants' claims as to the '690 Patent, and then will examine the claims regarding the Finck Patents.

i. The '690 Patent Is Patentably Distinct from the Patents-in-Suit

The '690 Patent, entitled "Methods of Lowering Active TNF-α Levels in Mammals Using Tumor Necrosis Factor Receptor," issued on February 25, 1997 and expired on February 25, 2014. '690 Patent (JTX-42); DFOF ¶ 97. There is no dispute that Immunex is the proper owner of the '690 Patent. ECF No. 688 at 36 ¶¶ 147-48. The parties dispute whether the asserted claims from the Patents-in-Suit are invalid in view of Claim 3 of the '690 Patent. Primarily, the parties dispute the meaning of the term "fused to the constant domain of an immunoglobulin" contained in the '690 Patent. Defendants argue that the '690 Patent's claim scope includes etanercept because the claimed chimeric antibody could have been fused to an immunoglobulin in the same way described in the Patents-in-Suit. Defs. Br. at 17. Plaintiffs disagree and state that the Patents-in-Suit do not cover the fusion of a TNFR to "the constant domain of an immunoglobulin" because etanercept's construction requires the removal of a portion of the constant domain, namely CH1 and the light chain of the IgG1 immunoglobulin. Pls. Br. at 48. In the alternative, Defendants assert that even if the claim is not construed to cover etanercept exactly, the prior art would have led a POSA to modify the claimed protein to create etanercept. Defs. Br. at 15. By contrast, Plaintiffs contend that Claim 3 of the '690 Patent does not include etanercept and therefore the inventions claimed in the Patents-in-Suit are patentably distinct. Pls. Br. at 48. The Court finds that Defendants have not demonstrated by clear and convincing evidence that the claims in the '690 Patent are patentably indistinct from the claims in the Patents-in-Suit—the '182 and '522 Patents.

Claim 3 of the '690 Patent is directed to "a method for lowering the levels of active TNF-α" by using a chimeric antibody[38] consisting of "a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1 fused to the constant domain of an immunoglobulin molecule." '690 Patent (JTX-42) col. 33:66-34:54. In other words, Claim 3 "requires that the p75 TNF receptor has to be fused to the constant domain of an immunoglobulin molecule" which "would include CH1, the hinge, CH2, CH3 and the constant region on the variable region." 9/12 PM (Blobel) Tr. at 69:15-18, 70:2-7. In fact, the specification of the '690 Patent describes a chimeric antibody as a molecule "having TNFR sequences substituted for the variable domains of either or both of the immunoglobulin heavy and light chains and having *unmodified* constant region domains." '690 Patent (JTX-42) col. 7:42-46 (emphasis added).

In comparison, the Patents-in-Suit claim a fusion protein with "all of the domains of the constant region of a human immunoglobulin IgG heavy chain *other than the first domain of said constant region*" ('182 Patent (JTX-1) col. 39:13-25) (emphasis added) and methods of making it ('522 Patent (JTX-2)). Critically, both the '182 and the '522 Patents exclude the CH1 and the light chain of the IgG1 immunoglobulin. *See* '182 Patent (JTX-1) col. 39:13-25; '522 Patent (JTX-2) col. 46:59-47:3. The Patents-in-Suit cover the fusion of p75 to the hinge-CH2-CH3 of the constant domain of IgG1. Id.

Therefore, the Court finds that the chimeric antibody of the '690 Patent could not have been etanercept because the constant region domains include CH1. In other words, the '690 Patent requires the use of the CH1 domain and light chain of the IgG1, while the Patents-in-Suit specifically require the removal of both of these items. *Compare* '690 Patent (JTX-42) col. 33:66-

[38] As defined in the '690 Patent, a chimeric antibody is a "molecule having TNFR sequences substituted for the variable domains of either or both of the immunoglobulin heavy and light chains and having unmodified constant region domains." 9/12 (Blobel) AM Tr. at 24:9-18.

34:54 *with* '182 Patent (JTX-1) col. 39:46-49 and '522 Patent (JTX-2) col. 45:57-60. Thus, the Patents-in-Suit are patentably distinct from the '690 Patent.

Lastly, Defendants argue that even if Claim 3 of the '690 Patent was strictly construed to include the complete constant domain for the light chain and the heavy chains, etanercept only differs in the removal of the light chain and the CH1 domain from the IgG1, which would have been obvious to a POSA. Defs. Br. at 15. However, as the Court previously stated above, it would not have been obvious to a POSA to modify the constant region domain in this way and combine it with a p75 TNFR. *See supra* III.B.

For these reasons, the Court finds that Defendants have failed to prove by clear and convincing evidence that the Patents-in-Suit are invalid in light of the '690 Patent based on obviousness type double patenting.

ii. The Patents-in-Suit Are Not Invalid In View of the Finck Patents

The patents referred to collectively as the Finck Patents are comprised of the following three patents: (1) the '225 Patent entitled "Soluble Tumor Necrosis Factor Receptor Treatment of Medical Disorders," issued on March 29, 2011; (2) the '605 Patent entitled "Soluble Tumor Necrosis Factor Receptor Treatment of Medical Disorders," issued on February 21, 2012; and (3) the '631 Patent entitled "Soluble Tumor Necrosis Factor Receptor Treatment of Medical Disorders," issued on May 13, 2014. JTX-39 ("'225 Patent"); JTX-40 ("'605 Patent"); JTX-41 ("'631 Patent"). The Finck Patents will expire on August 13, 2019 and there is no dispute that Immunex is the proper owner.[39] Defendants claim that the Patents-in-Suit are invalid for obviousness-type double patenting in view of the Finck patents. Defs. Br. at 14-15. The parties

[39] The Finck Patents expire on the same day because each is subject to a terminal disclaimer pursuant to 37 CFR § 1.321.

disagree as to whether the one-way test or two-way test shall be used to compare the Finck Patents and the Patents-in-Suit.[40] Part of that issue is a question of how and to what extent the amendments to the General Agreement on Tariffs and Trade ("GATT") impact an obviousness-type double patenting analysis.[41] Next, the parties disagree as to whether the Patents-in-Suit are patentably distinct from the Finck Patents. These arguments will be addressed in turn.

a. The Two-Way Test Shall Apply to Analysis of the Finck Patents

Invalidity for obviousness-type double patenting is a question of law based on underlying factual inquiries. *See Eli Lilly & Co. v. Teva Parenteral Meds., Inc.*, 689 F.3d 1368, 1376 (Fed. Cir. 2012). Under the "one-way" test, the court determines whether the asserted patent claim is patentably distinct from—i.e., obvious over or anticipated by—the reference patent claim. *See In re Berg*, 140 F.3d 1428, 1432 (Fed. Cir. 1998). For purposes of the two-way analysis, "the order of issuance is, in effect ignored, and the relevant determination becomes whether the improvement is patentably distinct from the generic invention." *In re Braat*, 937 F.2d 589, 593-94 (Fed. Cir. 1991); *see also In re Hubbell*, 709 F.3d 1140, 1149 (Fed. Cir. 2013). The two-way test is a "narrow exception to the general rule of the one-way test" and is only applied when "(1) a second-filed application issues prior to a first-filed application, and (2) 'the [US]PTO is solely responsible for

[40] Plaintiffs alternatively argue that even under the one-way test, Defendants have failed to prove by clear and convincing evidence that any claims in the Patents-in-Suit are invalid for obviousness-type double patenting over the Finck Patents. PFOF ¶ 325. Plaintiffs contend that even if the Finck Patents were proper obviousness-type double patenting references, their claims could not have been rendered invalid given that the Finck Patents' claims are directed to a method of treatment with etanercept whereas the claims of the Patents-in-Suit are directed to a compound and the method of composition. Pls. Br. at 49. The facts on patentable distinctness, discussed infra, may be considered in accordance with either test.

[41] The Uruguay Round Agreements Act was enacted on December 8, 1994. *See* Pub. L. No. 103-465, 108 Stat. 4809. This Act implemented various agreements during the Uruguay Round of General Agreement on Tariffs and Trade. *Id.* The Act is commonly referred to as "GATT."

the delay' in the issuance of the first-filed application." *In re Janssen Biotech, Inc.*, 880 F.3d 1315, 1325 (Fed. Cir. 2018); *see also Smith & Nephew, Inc. v. Arthrex, Inc.*, 355 F. App'x 384, 388 n.4 (Fed. Cir. 2009).

The two-way test arose to "prevent rejections for obviousness-type double patenting when the applicants filed first for a basic invention and later for an improvement, but, through no fault of the applicants, the [US]PTO decided the applications in the reverse order of filing." *In re Hubbell*, 709 F.3d at 1149 (quoting *In re Berg*, 140 F.3d at 1432). "The two-way exception can only apply when the applicant could not avoid separate filings, and even then, only if the [US]PTO controlled the rates of prosecution to cause the later filed species claims to issue before the claims for a genus in an earlier application." *In re Berg*, 140 F.3d at 1435. Whether the one-way or two-way test applies is a question of law, but the determination can be based on underlying factual findings.[42] *See In re Emert*, 124 F.3d 1458, 1460 (Fed. Cir. 1997).

The applications for the Patents-in-Suit were both filed in May 1995, however the '182 Patent issued in November 2011, and the '522 Patent issued in April 2012. The Finck Patent applications, which describe a method of treating psoriasis and psoriatic conditions, were filed four years after the applications for the Patents-in-Suit, in August 1999. However, the '225 Finck Patent issued in March 2011 prior to the issuance of the Patents-in-Suit, the '605 Finck Patent issued in February 2012, after the '182 Patent but prior to the '522 Patent, and the '631 Finck Patent issued in May 2014, after the Patents-in-Suit. As the Court has already determined that the Patents-in-Suit and Finck Patents lack the requisite common ownership for an obviousness-type

[42] The Court notes that the Finck Patents are the only patents as to which a two-way test argument has been made. The other patents analyzed for obviousness-type double patenting were all earlier-filed and earlier-issued compared to the Patents-in-Suit and therefore were evaluated under a one-way test.

double patenting analysis, the Court need not look any further to address the issue of patentable distinctness. Nevertheless, the Court has reviewed the evidence presented at trial and the prosecution file history and determines that if common ownership existed, the two-way test should apply.

At trial, Plaintiffs' expert Mr. Kunin reviewed the prosecution history for the Patents-in-Suit and testified that there was a period of "something like three years" where Roche submitted "six status requests because the Office hadn't been working" on the applications for the Patents-in-Suit. 9/21 (Kunin) Tr. at 104:21-105:1; *see also* JTX-4 at 354-55. He later testified that the application for the "'182 Patent . . . was lost for a couple of years" by the USPTO. 9/21 (Kunin) Tr. at 105:2-18. Then, in August 2010, a Director at the relevant USPTO Technology Center sent a letter to Plaintiffs' legal representative acknowledging that a petition decision mailed in August 2007 "relied upon an image file wrapper which mistakenly contained papers from an unrelated application." JTX-4 at 4239. The letter additionally acknowledged that "only one substantive office action has been set forth in the last five years" and therefore "the Examiner has been advised to treat this application as special and expedite its prosecution to conclusion." Id. at 4240. Further, the applications for the Patents-in-Suit faced several rejections from the patent examiners, which ultimately were found to be unjustified and reversed by the BPAI on appeal. *See* BPAI Opinion, PTX-6.456 (reversing all of the Examiner's rejections, and finding Plaintiffs' "evidence . . . convincing to rebut the Examiner's . . . rejection" as well as stating the BPAI was "persuaded by Appellants' argument"); PFOF ¶¶ 321-22. While Plaintiffs did make several proper requests for extensions, the Court finds that, to the extent the earlier-filed Patents-in-Suit were issued after the later-filed Finck Patents, as a matter of fact the USPTO was solely responsible for the delay that resulted. *See* PFOF ¶¶ 321-25. The Court additionally finds that, based on the record presented,

Plaintiffs acted in good faith to diligently prosecute the Patents-in-Suit. Therefore, the Court will apply the two-way test.

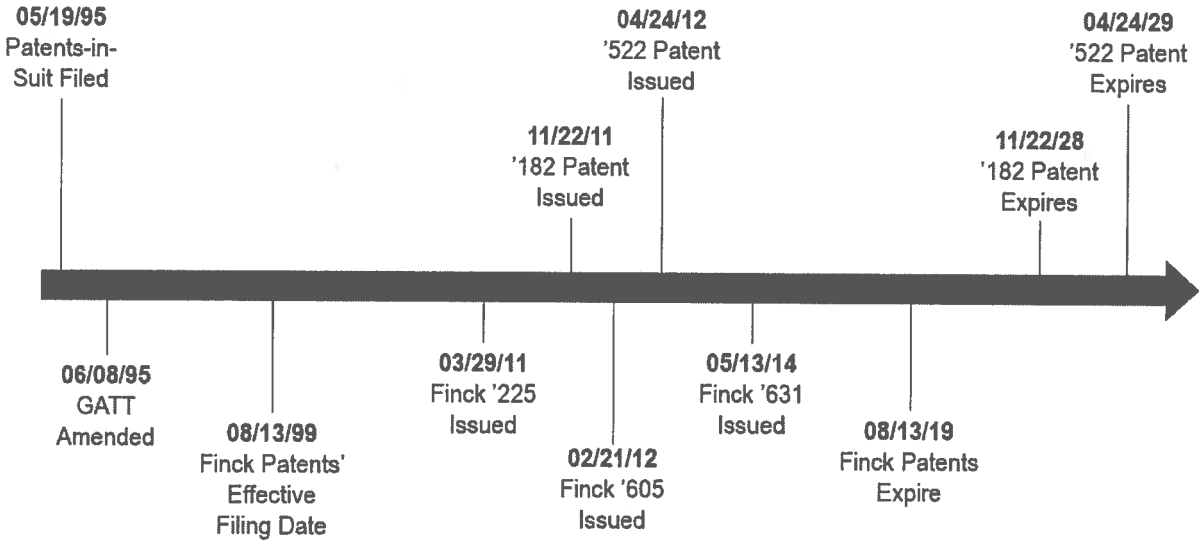
b. Impact of GATT on the Patents-in-Suit

The parties also disagree about the impact of GATT on the obviousness-type double patenting analysis. In particular, the parties argue as to whether an earlier-expiring post-GATT patent can cut short the statutory term of a pre-GATT later-expiring patent. Pls. Br. at 47-48; Defs. Reply Br. at 9-10. Among other things, GATT changed the term of a patent and how to calculate its expiration. *See Avanir Pharms., Inc. v. Actavis S. Atl. LLC*, 987 F. Supp. 2d 504, 516 n.20 (D. Del. 2013). Prior to GATT, "[p]atents claiming priority to applications filed ***before*** June 8, 1995, . . . have a patent term which is the greater of 20 years from the date of the filing of the application or 17 years from the date of the grant of the patent, subject to any terminal disclaimers." *Id.* (citing 35 U.S.C. § 154(a)(2)) (emphasis in original). However, due to the GATT amendment, "[p]atents that issued from applications filed ***after*** June 8, 1995 receive a 20-year term" from the effective filing date. *Id.* (citing 35 U.S.C. § 154(a)(2)) (emphasis in original).

Here, the applications for the Patents-in-Suit were filed pre-GATT in May 1995, and therefore were granted a patent term of seventeen years from the date of issuance (from November 2011 until November 2028 for the '182 Patent and from April 2012 to April 2029 for the '522 Patent). The Finck Patents, however, were filed post-GATT, and therefore will expire in August 2019, twenty years from the earliest effective filing date of August 1999 for the applications. Defendants' arguments focus on Claim 1 and the term TNFR:Fc in the Finck Patents, which is identical in each Finck Patent. Based on the times of filing, issuance, and expiration, at least one Finck Patent would properly serve as a reference patent for the Patents-in-Suit for an obviousness-

type double patenting analysis, which is all that is needed because the claim terms at issue in the Finck Patents are identical in each one.[43]

Next, because obviousness-type double patenting is "intended to address *unjustifiable* extensions of patent terms," a post-GATT later-granted and earlier-expiring patent cannot cut short the term of a pre-GATT "valid, earlier-granted patent with a longer term." *Abbott Labs. v. Lupin Ltd.*, No. 09-152, 2011 WL 1897322, at *9-10 (D. Del. May 19, 2011) (citing *Brigham & Women's Hosp. Inc. v. Teva Pharms. USA Inc.*, 2011 WL 63895 (D. Del. Jan. 7, 2011). Here as in *Abbott*, an act of Congress, rather than "improper gamesmanship by the patentee" or "strategic abuse of



[43] An obviousness-type double patenting analysis requires a comparison between the earlier patent, referred to as the reference patent, and the later patent. *See Eli Lilly & Co.*, 251 F.3d at 968. For patent applications filed pre-GATT, the ***issuing date*** is used to ascertain which patent was earlier and which was later. *Gilead,* 753 F.3d at 1214-15 (Fed. Cir. 2014). For applications filed post-GATT, however, the patent ***expiration date*** determines the earlier and later patents. *Id.* at 1216. In the instant matter, because the validity challenge is to the Patents-in-Suit, which are not subject to GATT, the issuance date should determine the reference patent. *See id.* at 1214-15 (finding that issuing date is used in an obviousness-type double patenting analysis for patents to which GATT does not apply). Looking to the issuance dates, the '225 Finck Patent is the only one which issued prior to both of the Patents-in-Suit and therefore is the only Finck Patent which could be properly considered an "earlier patent" for an obviousness-type double patenting analysis. The Court notes that alternatively looking to expiration date, all of the Finck Patents could serve as reference patents because they expire prior to the Patents-in-Suit, and therefore under either analysis at least one Finck Patent properly serves as the reference patent.

the patent system[,]" led to the Patents-in-Suit having a longer patent term and the expiration date for the Patents-in-Suit is "the same as it would have been had the [Finck Patents] never issued." *Id.* The Court therefore finds that the statutory term for the Patents-in-Suit may not be cut short to mirror the statutory term for the Finck Patents.

c. The Finck Patents Are Patentably Distinct from the Patents-in-Suit

At issue here is the patentable distinctness of Claims 11 and 35 of the '182 Patent and Claims 3 and 8 of the '522 Patent in comparison to Claim 1 of the Finck Patents in light of the definition of etanercept in the Finck specification. DFOF ¶¶ 92-95. The Patents-in-Suit claim etanercept itself and the method of making it. *See generally* '182 Patent (JTX-1); '522 Patent (JTX-2). In contrast, the Finck Patents cover a method of treating psoriasis and psoriatic conditions with etanercept. *See, e.g.,* '225 Patent (JTX-39) col. 21:33-36. For example, Claim 1 of the '225 Finck Patent claims "a method for treating a patient having psoriasis comprising administering to the patient a therapeutically effective dose of TNFR:Fc [i.e. etanercept], wherein the patient attains at least fifty percent improvement in PASI score." *See, e.g.,* id.

The Court first notes that a biologic manufacturer "may hold multiple patents covering the biologic, its therapeutic uses, and the processes used to manufacture it." *Sandoz*, 137 S. Ct. at 1670. Here, the '182 Patent claims the compound etanercept, the '522 Patent claims a process used to manufacture etanercept, and the Finck Patents claim a therapeutic use of treating psoriasis and psoriatic variants using etanercept. In support of their argument that the Finck Patents and the Patents-in-Suit are not patentably distinct, Defendants cite to *Geneva Pharm., Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373 (Fed. Cir. 2003), wherein the court found that a claimed compound for which a POSA "would recognize a single use" was not distinct from a patent that "simply claims that use as a method." *See id.* at 1385-86*; see also Astellas Pharma, Inc. v.*

Ranbaxy Inc., No. 05-2563, 2007 WL 576341, at *6 (D.N.J. Feb. 21, 2007). In that case, however, the court determined that the claimed use of the compound was not only an inherent property of the compound but its sole use. *Geneva Pharm.*, 349 F.3d at 1385. *Geneva* is distinguishable from the instant case because, while the Finck Patents use etanercept for the treatment of psoriasis and related conditions, psoriasis treatment is neither an inherent property nor the sole use of etanercept.[44] *See* PFOF ¶ 316; *cf. Geneva Pharm.*, 349 F.3d at 1385.

Plaintiffs contend that practicing the claimed invention of the Patents-in-Suit to make etanercept would not result in the practice of the Finck Patents, because merely making etanercept would not result in treating psoriasis. *See* PFOF ¶ 316. Reviewing the Finck Patents and the Patents-in-Suit, the treatment methods for psoriasis and psoriatic conditions contained in the Finck Patents are not found in the Patents-in-Suit. Therefore, based on the Court's analysis, the Finck Patents' claim to a psoriasis treatment method using etanercept cannot be used to invalidate the Patents-in-Suit. *See In re Braat*, 937 F.2d at 593-94. Furthermore, the Finck Patents and the Patents-in-Suit could not have been combined into a single application because they do not share common owners. *See supra* III.C.2.a. Accordingly, the Court finds that Defendants have not demonstrated by clear and convincing evidence that the Patents-in-Suit are invalid for obviousness type double patenting.

[44] Furthermore, Plaintiffs argue that etanercept can be made using methods other than the one detailed in the '522 Patent, namely "by using a host cell" other than the type specified in the patent and therefore the Finck Patents' treatment method could be accomplished without infringing on the '522 Patent. *See* PFOF ¶ 316.

IV. CONCLUSION

For the foregoing reasons, the Court finds that Defendants have failed to show by clear and convincing evidence that the Patents-in-Suit are invalid. An appropriate Order accompanies this Opinion.

Dated: August 9, 2019

HON. CLAIRE C. CECCHI
United States District Judge

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

IMMUNEX CORPORATION; AMGEN MANUFACTURING, LIMITED; and HOFFMANN-LA ROCHE INC.;	)	Civil Action No.: 2:16-cv-01118-CCC-MF
Plaintiffs,	)	
v.	)	
SANDOZ INC.; SANDOZ INTERNATIONAL GMBH; and SANDOZ GMBH;	)	
Defendants.	)	

FINAL JUDGMENT AND ORDER OF PERMANENT INJUNCTION

THIS MATTER was brought by Plaintiffs, Immunex Corporation, Amgen Manufacturing, Limited (collectively, "Immunex"), and Hoffmann-La Roche Inc. ("Roche") (collectively, "Plaintiffs"), against Defendants, Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH (collectively, "Defendants"). After a bench trial, the Court issued its Opinion and Order in the above-captioned case on August 9, 2019 (ECF No. 689, 690).

It is ORDERED, ADJUDGED, and DECREED as follows:

1. The Court has jurisdiction over the subject matter of the above-captioned case pursuant to 28 U.S.C. §§ 1331 and 1338(a).

2. The Court has personal jurisdiction over the parties, and venue is proper as to all parties pursuant to 28 U.S.C. §§ 1391(b), (c), and 1400(b).

3. The Court retains jurisdiction to enforce or supervise performance under this Final Judgment and Order of Permanent Injunction.

I. The Patents-In-Suit

4. Sandoz Inc.'s submission of abbreviated Biologics License Application ("aBLA") No. 761042 infringed claims 11-12 and 35-36 of U.S. Patent No. 8,063,182 (the "'182 Patent").

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5. Defendants' making, using, offering to sell, or selling of any product containing the fusion protein known as etanercept and described in the Court's opinion in this case at ECF No. 689 at 6 ("etanercept") within the United States, or Defendants' importation of any product containing etanercept into the United States, will infringe claims 11-12 and 35-36 of the '182 Patent.

6. For the reasons stated in the Court's August 9, 2019 Opinion (ECF No. 689), the Court finds that Defendants failed to prove that claims 11-12 and 35-36 of the '182 Patent are invalid or unenforceable.

7. Judgment is hereby entered against Defendants regarding infringement of the '182 Patent.

8. Any claim of infringement of any claims of the '182 Patent other than claims 11-12 and 35-36 is hereby dismissed with prejudice.

9. Sandoz Inc.'s submission of aBLA No. 761042 infringed claims 3, 8, and 10 of U.S. Patent No. 8,163,522 (the "'522 Patent").

10. Defendants' making, using, offering to sell, or selling of any product containing etanercept within the United States, or Defendants' importation of any product containing etanercept into the United States, will infringe claims 3, 8, and 10 of the '522 Patent.

11. For the reasons stated in the Court's August 9, 2019 Opinion (ECF No. 689), the Court finds that Defendants failed to prove that claims 3, 8, and 10 of the '522 Patent are invalid or unenforceable.

12. Judgment is hereby entered against Defendants regarding infringement of the '522 Patent.

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13. Any claim of infringement of any claims of the '522 Patent other than claims 3, 8, and 10 is hereby dismissed with prejudice.

14. Based on the stipulation dated October 7, 2019, Defendants, and each of them, and each of their affiliates, subsidiaries, successors, and partners, and all of their officers, agents, servants, employees, and attorneys, and all persons and entities acting on behalf or at the direction of, or in active concert or participation or privity with any of them, are hereby enjoined from making, using, offering to sell, or selling within the United States, or importing into the United States any product containing etanercept. This paragraph does not restrict Defendants' activities that fall within the scope of 35 U.S.C. § 271(e)(1). This permanent injunction shall terminate no later than the later of the expiration of any infringed and valid claim of the '182 Patent on November 22, 2028 or any infringed and valid claim of the '522 Patent on April 24, 2029.

II. Immunex Patents

15. Pursuant to the stipulation filed June 7, 2018 (ECF No. 510), any claim of infringement under 35 U.S.C. § 271(b) or 35 U.S.C. § 271(e)(2)(C) of any claim in U.S. Patent No. 7,915,225, U.S. Patent No. 8,119,605, or U.S. Patent No. 8,722,631 is hereby dismissed with prejudice. (ECF No. 1 ¶¶ 114-167.)

III. Prior Preliminary Injunctions

16. Prior stipulated preliminary injunctions ECF Nos. 95, 96, and 509 are hereby terminated. Paragraph 5(b) of Confidential ECF No. 510 remains in effect until the issuance of a mandate from the Federal Circuit. Paragraph 8 of Confidential ECF No. 510 remains in effect until the conclusion of (or expiration of time to seek) review by the United States Supreme Court of the Federal Circuit's decision and shall terminate if any one of claims 11-12 and 35-36 of the '182 Patent or claims 3, 8, and 10 of the '522 Patent is not rendered invalid following the

conclusion of (or expiration of time to seek) such review. The remainder of Confidential ECF No. 510 is hereby terminated.

17. Pursuant to Fed. R. Civ. P. 58(a), this is the **FINAL JUDGMENT** of the Court.

SO ORDERED:

Dated: October 8, 2019

Hon. Claire C. Cecchi, U.S.D.J.

Case: 20-1037 Document: 48 Page: 170 Filed: 11/08/2019

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America, and if the invention is a process, of the right to exclude others from using, offering for sale or selling throughout the United States of America, or importing into the United States of America, products made by that process, for the term set forth in 35 U.S.C. 154(a)(2) or (c)(1), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b). See the Maintenance Fee Notice on the inside of the cover.

David J. Kappos

Director of the United States Patent and Trademark Office

JOINT EXHIBIT

JTX-1

MAINTENANCE FEE NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the United States Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

PATENT TERM NOTICE

If the application for this patent was filed on or after June 8, 1995, the term of this patent begins on the date on which this patent issues and ends twenty years from the filing date of the application or, if the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121, or 365(c), twenty years from the filing date of the earliest such application ("the twenty-year term"), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b), and any extension as provided by 35 U.S.C. 154(b) or 156 or any disclaimer under 35 U.S.C. 253.

If this application was filed prior to June 8, 1995, the term of this patent begins on the date on which this patent issues and ends on the later of seventeen years from the date of the grant of this patent or the twenty-year term set forth above for patents resulting from applications filed on or after June 8, 1995, subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b) and any extension as provided by 35 U.S.C. 156 or any disclaimer under 35 U.S.C. 253.

(12) **United States Patent**
Brockhaus et al.

(10) **Patent No.: US 8,063,182 B1**
(45) **Date of Patent: Nov. 22, 2011**

(54) **HUMAN TNF RECEPTOR FUSION PROTEIN**

(75) Inventors: **Manfred Brockhaus**, Bettingen (CH); **Reiner Gentz**, Rheinfelden (DE); **Dembic Zlatko**, Basel (CH); **Werner Lesslauer**, Basel (CH); **Hansruedi Lotscher**, Mohlin (CH); **Ernst-Jurgen Schlaeger**, Efringen-Kirchen (DE)

(73) Assignee: **Hoffman-LaRoche Inc.**, Nutley, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/444,790**

(22) Filed: **May 19, 1995**

Related U.S. Application Data

(60) Division of application No. 08/095,640, filed on Jul. 21, 1993, now Pat. No. 5,610,279, which is a continuation of application No. 07/580,013, filed on Sep. 10, 1990, now abandoned.

(30) **Foreign Application Priority Data**

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(52) **U.S. Cl.** **530/350**; 514/12.2; 530/387.3; 536/23.5; 930/144

(58) **Field of Classification Search** 530/324, 530/350; 424/192.1; 536/23.5
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,593,002	A		6/1986	Dulbecco
4,675,285	A		6/1987	Clark et al.
4,729,326	A		3/1988	Richter
4,769,326	A		9/1988	Rutler
4,770,995	A		9/1988	Rubin et al.
4,816,567	A		3/1989	Cabilly et al.
4,894,439	A		1/1990	Dorin et al.
4,912,044	A		3/1990	Jacob et al.
4,935,233	A		6/1990	Bell et al.
4,948,875	A		8/1990	Tanaka et al.
4,963,354	A		10/1990	Shepard et al.
4,965,271	A		10/1990	Mandell et al.
5,055,447	A		10/1991	Palladino et al.
5,073,627	A		12/1991	Curtis et al.
5,075,222	A		12/1991	Hannum et al.
5,098,702	A		3/1992	Zimmerman et al.
5,098,833	A		3/1992	Lasky et al.
5,116,964	A	*	5/1992	Capon et al. 536/23.5
5,118,500	A		6/1992	Hanel et al.
5,136,021	A		8/1992	Dembinski et al.
5,155,027	A		10/1992	Sledziewski et al.
5,211,945	A		5/1993	Wallach et al.
5,223,395	A		6/1993	Gero
5,225,538	A		7/1993	Capon et al.
5,258,498	A		11/1993	Huston et al.
5,264,416	A		11/1993	Park et al.
5,270,038	A		12/1993	Klimpel et al.
5,336,603	A		8/1994	Capon et al.
5,344,915	A	*	9/1994	LeMaire et al. 530/350
5,350,683	A		9/1994	Sims et al.
5,359,032	A		10/1994	Dayer et al.
5,359,037	A		10/1994	Wallach et al. 530/388.22
5,395,760	A	*	3/1995	Smith et al. 435/365
5,428,130	A		6/1995	Capon et al.
5,447,851	A		9/1995	Beutler et al.
5,455,165	A		10/1995	Capon et al.
5,478,925	A		12/1995	Wallach et al.
5,512,544	A		4/1996	Wallach et al.
5,514,582	A		5/1996	Capon et al.
5,599,905	A		2/1997	Mosley et al.
5,605,690	A		2/1997	Jacobs et al.
5,610,279	A		3/1997	Brockhaus et al.
5,633,145	A		5/1997	Feldmann et al.

(Continued)

FOREIGN PATENT DOCUMENTS

AU 58976 1/1991

(Continued)

OTHER PUBLICATIONS

Miles JS, et al. Principles of DNA cloning. British Medical Journal. 1989 299:1019-1022.*
Olsson, I, et al., Isolation and charaterization of a tumor necrosis factor binding protein from urine. Eur J Haematol. 1989;42:270-275.*
Dembic et al, 1990. Cytokine. 2(4): 231-7.*
Chan et al. 2000, Science, 288: 2351-2354.*
Smith et al, 1989. Journal of Biological Chemistry. 14646-15652.*
Heller et al., JBC 265(10): 5708-5717 (1990).

(Continued)

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(57) **ABSTRACT**

The present invention is concerned with non-soluble proteins and soluble or insoluble fragments thereof, which bind TNF, in homogeneous form, as well as their physiologically compatible salts, especially those proteins having a molecular weight of about 55 or 75 kD (non-reducing SDS-PAGE conditions), a process for the isolation of such proteins, antibodies against such proteins, DNA sequences which code for non-soluble proteins and soluble or non-soluble fragments thereof, which bind TNF, as well as those which code for proteins comprising partly of a soluble fragment, which binds TNF, and partly of all domains except the first of the constant region of the heavy chain of human immunoglobulins and the recombinant proteins coded thereby as well as a process for their manufacture using transformed pro- and eukaryotic host cells.

36 Claims, 6 Drawing Sheets

U.S. PATENT DOCUMENTS

5,639,597	A		6/1997	Lauffer et al.
5,695,953	A		12/1997	Wallach et al.
5,705,364	A		1/1998	Etcheverry et al.
5,712,155	A		1/1998	Smith et al. 735/320.1
5,721,121	A		2/1998	Etcheverry et al.
5,808,029	A		9/1998	Brockhaus et al.
5,811,261	A	*	9/1998	Wallach et al. 435/69.1
5,863,786	A		1/1999	Feldmann et al.
5,447,851	A		7/1999	Beutler et al.
5,945,397	A		8/1999	Smith et al.
5,981,701	A	*	11/1999	Wallach et al. 530/350
RE36,755	E		6/2000	Smith et al.
6,143,866	A		11/2000	Brewer et al.
6,165,476	A		12/2000	Strom et al.
6,201,105	B1		3/2001	Smith et al.
6,221,675	B1		4/2001	Hauptmann et al.
6,271,346	B1		8/2001	Hauptmann et al.
6,294,352	B1		9/2001	Hauptmann et al.
6,417,158	B1		7/2002	Hauptmann et al.
6,541,610	B1		4/2003	Smith
6,541,620	B1		4/2003	Brewer et al.
6,572,852	B2		6/2003	Smith et al.
6,858,409	B1		2/2005	Thompson et al.
7,253,264	B1		8/2007	Lauffer et al.
2003/0064480	A1		4/2003	Lauffler et al.

FOREIGN PATENT DOCUMENTS

EP	120694		10/1984
EP	227110		7/1987
EP	230574		8/1987
EP	269455		6/1988
EP	315 062		10/1988
EP	308 378		3/1989
EP	0 314 317		5/1989
EP	325 224		7/1989
EP	325262		7/1989
EP	0334165		9/1989
EP	393 438		4/1990
EP	0 394 827		10/1990
EP	398 327		11/1990
EP	412 486		2/1991
EP	414178		2/1991
EP	418 014		3/1991
EP	417563		3/1991
EP	422 339		4/1991
EP	433 900		6/1991
EP	460846		12/1991
EP	464 533		1/1992
EP	471701		2/1992
EP	526452		2/1993
EP	526905		2/1993
EP	0 567 566	B1	11/1993
EP	568925		11/1993
EP	606869		7/1994
GB	2218101		11/1989
GB	2 246 569		2/1992
JP	61-293924		12/1986
JP	61-293924	A2	12/1986
JP	02-154695		6/1990
WO	WO 89/02922	*	4/1989
WO	WO 89 09622		10/1989
WO	9013575		11/1990
WO	WO 91/02078		2/1991
WO	91/03553		3/1991
WO	WO 91/17184		11/1991
WO	WO 91/08298		12/1991
WO	WO 92/08495		5/1992
WO	WO 92/13095		8/1992
WO	WO 93/07863		4/1993
WO	WO 93/19777		10/1993
WO	WO 94/06476		3/1994

OTHER PUBLICATIONS

Abstract by Heller et al., Napa Valley Conference (1989).
Olsson et al., Eur. J. Haematol., 42: 270-275 (1989).
Nophar et al., EMBO J., 9:3269-3278 (1990).
Evans, T.J. et al., J. Exp. Med. 180, pp. 2173-2179 (1994).
Traunecker, A. et al., Nature, 339, pp. 68-70 (1989).
Dembic, Z., et al., Cytokine, 2, pp. 231-237 (1990).
Byrn, et al., Nature, 344, pp. 667-670 (1990).
Peppel, K., et al., J. Exp. Med., 174, pp. 1483-1489 (1991).
Zettlmeissl, G., et al., DNA & Cell Biology, 9, pp. 347-353 (1990).
Loetscher et al, *J. of Bio. Chem.*, 266(27), pp. 18324-18329, (1991).
Nature Biotechnology, 15 (1997) p. 13.
Fisher, et al., N. Engl. J. of Med. 334, pp. 1697-1702 (1996).
Hsu, et al., J. Biol. Chem. 268, pp. 16430-16436 (1993).
Mohler et al., Soluble Tumor NecrosisFfactor (TNF) Receptors are Effective Therapeutic Agents in Lethal Endotoxemia and Function Simultaneously as Both TNF Carriers and TNF Antagonists. J. Immunol., 151:1548-1561 (1993).
Sell, Immunology, Immunopathology and Immunity, 4th Edition, Elsevier Science Publishing Co., New York, 1987, at pp. 85-91.
Bringman, Monoclonal antibodies to human tumor necrosis factors alpha and beta: application for affinity purification, immunoassays, and as structural probes. Hybridoma, 6(5):489-507 (1987).
Paul et al., Fundamental Immunology, 2nd Edition, Paul, ed., Raven Press, New York, 1989, at pp. 679-701 and pp. 735-764.
Smith et al., A Receptor for Tumor Necrosis Factor Defines an Unusual Family of Cellular and Viral Proteins, *Science*, 248:1019-1023, (1990).
Wingfield et al., Tumour Necrosis Factor is a Compact Trimer, FEBS Lett. 211: 179-84 (1987).
Smith and Baglioni, The ActiveFform of Tumor Necrosis Factor is a Trimer J. Biological Chemistry, 262:6951-4 (1987).
Larsson and Mosbach, Affintiy Precipitation of Enzymes FEBS Lett. 98(2):333-338 (1979).
Irwin and Tipton, Chapter 22, "Affinity Precipitation Methods" in Methods in Mol. Bio., 59:217 (1996).
Kohno et al., Presentation 1495, poster 271 presented at American College of Rheumatology Annual Meeting, Nov. 13-17, 2005, San Diego, CA.
Klein, Immunology, 1st Edition, Klein ed., Blackwell Scientific Publications, Cambridge, MA 1990 at pp. 446-447.
Byrn et al., Biological properties of a CD4 Immunoadhesin, Nature, 344:667-670 (Apr. 1990).
Traunecker et al., Highly Efficient Neutralization of HIV with Recombinant CD-4-Immunoglobulin Molecules, Nature 339:68-70, 1989.
Capon, Designing CD4 Immunoadhesions for AIDS Therapy, Nature, 337:525-531 (1989).
Barone et al., Comparative Analysis of the Ability of Entanercept and Inflixamab to Lyse TNF-Expressing Cells in a Complement-Dependent fashion, Arthritis Rheum., v42(9) supplement, Sep. 1999.
Khare et al, Poster 715 presented at the Annual Meeting of the Society for Investigative Dermatology (SID), May 3-5, 2006, Philadelphia, PA.
Nesbitt, et al., "Mechanism of Action of Certolizumab Pegol (Cdp870): In Vitro Comparison With Other Anti-Tumor Necrosis Factor α Agents", *Inflamm Bowel Dis*, 13: 1323-1332 (Nov. 2007).
Aruffo et al., "CD44 Is the Principal Cell Surface Receptor for Hyaluronate," Cell 61:1303-1313 (1990).
Brennan et al., "Inhibitory Effects of TNF α Antibodies on Synovial Cell Interleukin-1 Production in Rheumatoid Arthritis," The Lancet 2(8657):244-247 (1989).
Monnat, "Molecular Analysis of Spontaneous Hypoxanthine Phosphoribosyltransferase Mutations in Thioguanine-resistant HL-60 Human Leukemia Cells," Cancer Res. 49:81-87 (1989).
Shalaby et al., "The Involvement of Human Tumor Necrosis Factors-α and -βin the Mixed Lymphocyte Reaction," J. Immunol. 141:449-503 (1988).
Watson et al., "A Homing Receptor—IgG Chimera as a Probe for Adhesive Ligands of Lymph Node High Endothelial Venules," J. Cell Biol. 110:2221-2229 (1990).
Gen Seq DBase Printout.
Stauber et al., "Human tumor necrosis factor-α receptor—purification by immunoaffinity chromatography and initial characterization", J. Bio. Chem. 263:19098-19104 (1988).
Seckinger et al., "Purification and biologic characterization of a sp cific tum r necrosis fact r α inhibitor", J. Bio. Chem. 264:11966-11973 (1989).

Engelmann et al., "A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity", J. Bio. Chem. 264:11974-11980 (1989).
Hohmann et al., "Two different cell types have different major r cept rs f r human tumor necr sis factor (TNFα)", J. Bio. Chem. 264:14927-14934 (1989).
Smith t al., "A receptor for tum r necrosis factor defines an unusual family of cellular and viral proteins", Scienc 248:1019-1023 (1990).
Heller et al., "Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor", Proc. Natl. Acad. Sci. U.S.A. 87:6151-6155 (1990).
Novick et al., "Soluble cytokine receptors are present in normal human urine", J. Exp. Med. 170:1409-1414 (1989).
Engelmann et al., "Two tum r necr sis factor-binding proteins purified from human urine", J. Bio. Chem. 265:1531-1536 (1990).
Schall et al., "Molecular cloning and expression of a receptor for human tumor necrosis factor", Cell 61:361-370 (1990).
Seckinger et al., "Tumor necrosis factor inhibitor: purification, NH_3-terminal amino acid sequence and evidence for anti-inflammatory and immunomodulatory activities", Eur. J. Immunol. 20:1167-1174 (1990).
Hohmann et al., "Expression of the types A and B tumor necrosis factor (TNT) recept rs is independently r gulated, and both receptors mediate activation of the transcription factor NF-kB", J. Bi . Chem. 265:22409-22417 (1990).
Espevik et al., "Characterization of binding and biological effects of monoclonal antibodies against a human tumor necrosis factor receptor", J. Exp. Med. 171:415-426 (1990).
Porteu and Nathan, "Shedding of tumor necrosis factor receptors by activated human neutrophils", J. Exp. Med. 172:599-607 (1990).
Engelmann et al., "Antibodies to a s luble f rm f a tumor necrosis factor (TNF) receptor have TNF-like activity", J. Bio. Chem. 265:14497-14504 (1990).
Seckinger et al., "Characterization of a tumor necrosis factor α (TNF-α) inhibitor: evidence of immunological cross-activity with the TNF receptor", Proc. Natl. Sci. USA 87:5188-5192 (1990).
Gray et al., "Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein", Proc. Natl. Sci. USA 87:7380-7384 (1990).
Loetscher et al., "Molecular cloning and expression of the human 55 kd tum r necrosis fact r rec ptor", Cell 61:351-359 (1990).
Peppel et al., "Chimaeric TNF-receptor—IgG molecule acts as soluble inhibitor of TNF mediated cytotoxicity", Journal of Cellular Biochem., Abstract, 20th Annual Meetings, Keystone Symposia on Molecular and Cellular Biology, p. 118, Supplement 15F (1991).
Olsson et al., "Isolation and characterization of a tumor necrosis factor binding pr tein fr m urin ", Eur. J. Ha matol. 42:270-275 (1989).
Capon et al., "Desiging CD4 immunoadhesins for AIDS therapy", Nature 337:525-530 (Feb. 9, 1989).
Abstract 92-009794/02 (1992) for EP 464 533.
U.S. Appl. No. 08/444,791—Office Action mailed Jun. 8, 2010.
U.S. Appl. No. 08/444,791—Office Action mailed Oct. 15, 2010.
Declaration of Taruna Arora, PH.D. Under 37 C.F.R. 1.132 with Exhibits A-D, Dec. 16, 2010.
Heller et al., "Amplified Expression of Tumor Necrosis Factor Receptor in Cells Transfected with Epstein-Barr Virus Shuttle Vector cDNA Libraries," J. Biol. Chem. 265(10): 5708-5717 (1990).
Arora et al., "Differences in Binding and Effector Functions Between Classes of TNF Antagonists," Cytokine 45: 124-131 (2009).
Furst et al., "Tumor Necrosis Factor Antagonists: Different Kinetics and/or Mechanisms of Action May Explain Differences in the Risk for Developing Granulomatous Infection," Semin. Arthritis Rheum. 36: 159-167 (2006).
Mitoma et al., "Mechanisms for Cytotoxic Effects of Anti-Tumor Necrosis Factor Agents on Transmembrane Tumor Necrosis Factor α-Expressing Cells," Arthr. & Rheum. 58(5): 1248-1257 (2008).
Strangfeld et al., "Risk of Herpes Zoster in Patients with Rheumatoid Arthritis Treated with Anti-TNF-α Agents," JAMA 301(7): 737-744 (2009).
Wallis et al., "Granulomatous Infectious Diseases Associated with Tumor Necrosis Factor Antagonists," Clin. Inf. Dis. 38: 1261-1265 (2004).
Wallis et al., "Reactivation of Latent Granulomatous Infections by Infliximab," Clin. Inf. Dis. 41(Suppl 2): S1-S5 (2005).
Winzor et al., "Evaluation of Equilibrium Constants from Precipitin Curves: Interaction of α -Crystallin with an Elicited Monoclonal Antibody," Arch. Biochem. Biophys. 268(1): 221-226 (1989).
Avis, Kenneth, Parental Preparations, Remington Pharmaceutical Sciences, Chapter 85, pp. 1518-1541 (1985).
Center for Drug Evaluation and Research, Guidelines on Sterile Drug Products Produced by Aseptic Processing, Published by the FDA Jun. 1987.
Feldmann et al., "Cytokine production in the rheumatoid joint: implications for treatment," Ann. Rheum. Dis. 49: 480-486 (1990).
Hoogenboom et al., "Construction and Expression of Antibody-Tumor Necrosis Factor Fusion Proteins," Molecular Immunol. 28(9): 1027-1037 (1991).
Smitii, "cDNA Expression: Cloning of the Receptor for Human Tumor Necrosis Factor," Presentation Programme, 29th Midwinter Conference of Immunologists (Jan. 27-30, 1990).
U.S. Appl. No. 08/484,783, filed Jun. 7, 1995, Kohno et al.
U.S. Appl. No. 07/555,274, filed Jul. 19, 1990, Kohno et al.
Final Office action dated Jun. 24, 2011, in U.S. Appl. No. 08/444,791 (Brockhaus, et al.).
U.S. Appl. No. 08/478,995, Lauffler, Leander et al.
Abraham et al., p55 Tumor Necrosis Factor Receptor Fusion Protein in the Treatment of Patients With Severe Sepsis and Septic Shock: JAMA, 19:1531-1538 (1997).
Abraham et al., Lenercept (p55 Tumor Necrosis Factor Receptor Fusion Protein) In Severe Sepsis and Early Septic Shock: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase III Trial With 1,342 Patients, Crit Care Med, 29:503-510 (2001).
Aggarwal et al., Characterization of Receptors for Human Tumour Necrosis Factor and Their Regulation by γ-Interferon, Nature, 318:665-667 (1985).
Aggarwal et al., Induction of Receptors for Tumor Necrosis Factor-α by Interferons Is Not a Major Mechanism for Their Synergistic Cytotoxic Response, J. Biol. Chem., 262:10000-10007 (1987).
Aggarwal et al., Human tumour necrosis factors: structure and receptor interactions, in Tumor necrosis factor and related cytotoxins, pp. 39-51, (Ciba Foundation symposium 131), Wiley, Chichester (1987).
Arenzana-Seisdedos et al., Immunoregulatory Mediators in the Pathogenesis of Rheumotoid Arthritis, Scand. J. Rheumatol., Supplement 66:13-17 (1987).
Aruffo et al., Molecular Cloning of a CD28 cDNA by a High-Efficiency COS Cell Expression System, Proc. Natl. Acad. Sci. USA, 84:8573-8577 (1987).
Ashkenazi et al., Protection Against Endotoxic Shock by a Tumor Necrosis Factor Receptor Immunoadhesin, Proc. Natl. Acad. Sci., U.S.A. 88:10535-10539 (1991).
Ayala, Modem Genetics, Benjamin/Cummings, Publ. Co., Menlo Park CA, p. 45, (1980).
Baglioni et al., Binding of Human Tumor Necrosis Factor to High Affinity Receptors on HeLa and Lymphoblastoid Cells Sensitive to Growth Inhibition, J. Biol. Chem., 260:13395-13397 (1985).
Benjamini et al., Antibody Structure, in Immunology: A Short Course, 3rd ed., Wiley-Liss New York, 61-69 (1996).
Branellec et al., TNF: Antitumoral Agent at the Border Lines of Immunity and Inflammation, Path. Biol., 39:230-239 (1991).
Brockhaus et al., Identification of Two Types of Tumor Necrosis Factor Receptors on Human Cell Lines by Monoclonal Antibodies, Proc. Natl. Acad. Sci. USA, 87:3127-3131 (1990).
Carter et al., Purification, Cloning, Expression and Biological Characterization of an Interleukin-1 Receptor Antagonist Protein, Nature, 344:633-638 (1990).
Carpenter et al., Epidermal Growth Factor, J. Biol. Chem., 265:7709-7712 (1990).
Carpenter, Receptors for Epidermal Growth Factor and Other Polypeptide Mitogens, Ann. Rev, Biochem., 56:881-914 (1987).
Casadei et al., Expression and Secretion of Aequorin as a Chimeric Antibody by Means of a Mammalian Expression Vector, Proc. Natl. Acad. Sci., U.S.A. 87:2047-2051 (1990).
Coffman et al., The Role of Helper T Cell Products in Mouse B Cell Differentiation and Isotype Regulation, Immunol. Rev., 102:5-28 (1988).

Creasey et al., A High Molecular Weight Component of the Human Tumor Necrosis Factor Receptor is Associated With Cytotoxicity, Proc. Natl. Acad. Sci. USA, 84:3293-3297 (1987).
Dayer, Chronic Inflammatory Joint Diseases: Natural Inhibitors of Interleukin 1 and Tumor Necrosis Factor α, J. Rheumatol, 18 (Suppl. 27): 71-75 (1991).
Dower et al., Human Cytokine Receptors, J. Clin. Immunol., 10:289-299 (1990).
Eisenberg et al., Primary Structure and Functional Expression From Complementary DNA of a Human Interleukin-1 Receptor Antagonist, Nature, 343:341-346 (1990).
Ellison et al., The Nucleotide Sequence of a Human Immunoglobulin Cγ1 Gene, Nucleic Acids Res. 10(13): 4071-79 (1982).
Esmon, The Roles of Protein C and Thrombomodulin in the Regulation of Blood Coagulation, J. Biol. Chem., 264:4743-4746 (1989).
European Search Report for EP 97 12 0664, dated Mar. 9, 1998.
Fell et al., Genetic Construction and Characterization of a Fusion Protein Consisting of a Chimeric F(ab') With Specificity for Carcinomas and Human IL-2, J. Immunol., 146:2446-2452 (1991).
Fernandez-Botran et al., A Soluble, High-Affinity, Interleukin-4-Binding Protein is Present in the Biological Fluids of Mice, Proc. Natl. Acad. Sci., 87:4202-4206 (1990).
Fernandez-Botran, Soluble Cytokine Receptors: Their Role in Immunoregulation, The FASEB Journal, 5:2567-2574 (1991).
Ferrante et al., Inhibition of Tumour Necrosis Factor Alpha (TNF-α)-Induced Neutrophil Respiratory Burst by a TNF Inhibitor, Immunology, 72:440-442 (1991).
Fisher et al., Cloning and Expression of Human Tissue Factor cDNA, Thrombosis Research, 48:89-99 (1987).
Foley et al., An Inhibitor of the Toxicity of Tumour Nectrosis Factor in the Serum of Patients With Sarcoidosis, Tuberculosis and Crohn's Disease, Clin. Exp. Immunol, 80:395-399 (1990).
Fomsgaard et al., Preliminary Study on Treatment of Septic Shock Patients With Antilipopolysaccharide IgG from Blood Donors, Scand. J. Infect. Dis., 21:697-708 (1989).
Garcia et al., High Sensitivity of Transgenic Mice Expressing Soluble TNFR1 Fusion Protein to Mycobacterial Infections: Synergistic Action of TNF and IFN-γ in the Differentiation of Protective Granulomas, Eur. J. Immunol., 27:3182-3190 (1997).
Gascoigne et al., Secretion of a Chimeric T-Cell Receptor-Immunoglobulin Protein, Proc. Natl. Acad. Sci USA, 84:2936-2940 (1987).
Gehr et al., Both Tumor Necrosis Factor Receptor Types Mediate Proliferative Signals in Human Mononuclear Cell Activation, J. Immunol., 149:911-917 (1992).
Gillies et al., Targeting Human Cytotoxic T Lymphocytes to Kill Heterologous Epidermal Growth Factor Receptor-Bearing Tumor Cells, J. Immunol., 144:1067-1071 (1991).
Goodman, Identification of Antigenic Determinants, in Basic & Clinical Immunol., 24-25 (1982).
Goodman, Immunogenicity & Antigenic Specificity, in Basic & Clinical Immunol., 101-108 (1991).
Goodwin et al., Molecular cloning and Expression of the Type 1 and Type 2 Murine Receptors for Tumor Necrosis Factor, Molecular and Cellular Biology, 11:3020-3026 (1991).
Gray et al, Cloning and Expression of cDNA for Human Lymphotoxin, a Lymphokine With Tumour Necrosis Activity, Nature, 312:721-724 (1984).
Grundmann et al., Characterization of cDNA Coding for Human Factor XIIIa, Proc. Natl. Acad. Sci. USA, 83:8024-8028 (1986).
Haak-Frendscho et al., Inhibition of TNF by a TNF Receptor Immunoadhesin, J. Immunol., 152:1347-1353 (1994).
Hannum et al., Interleukin-1 Receptor Antagonist Activity of a Human Interleukin-1 Inhibitor, Nature, 343:336-340 (1990).
Heflin et al., Prevention by Granulocyte Depletion of Increased Vascular Permeability of Sheep Lung Following Endotoxemia, J. Clin. Invest., 68:1253-1260 (1981).
Himmler et al., Molecular Cloning and Expression of Human and Rat Tumor Necrosis Factor Receptor Chain (p60) and Its Soluble Derivative, Tumor Necrosis Factor-Binding Protein, DNA and Cell Biology, 9:705-715 (1990).
Hobart, The Immune System: A Course on the Molecular and Cellular Basis of immunity, Blackwell Scientific Pubs, p. 7 (1975).
Holtmann et al., Down Regulation of the Receptors for Tumor Necrosis Factor by Intereukin 1 and 4β-Phorbol-12-Myristate-13-Acetate, J. Immunol., 139:1161-1167 (1987).
Idzerda et al., Human Interleukin 4 Receptor Confers Biological Responsiveness and Defines a Novel Receptor Superfamily, J. Exp. Med., 171:861-873 (1990).
Imamura et al., Expression of Tumor Necrosis Factor Receptors on Human Monocytes and Internalization of Receptor Bound Ligand, J. Immunol., 139:2989-2992 (1987).
Ishikura et al., Differential Biologic Effects Resulting From Bimodal Binding of Recombinant Human Tumor Necrosis Factor to Myeloid Leukemia Cells, Blood, 73:419-424 (1989).
Israel et al., Binding of Human TNF-α to High-Affinity Cell Surface Receptors: Effect of IFN, Immunology Letters, 12:217-224 (1986).
Jacobs et al., Pharmacokinetic Parameters and Biodistribution of Soluble Cytokine Receptors, International Review of Expermental Pathology, 34B:123-135 (1993).
Jones et al, Structure of Tumour Necrosis Factor, Nature, 338:225-228 (1989).
Kaczmarski et al., The Cytokine Receptor Superfamily, Blood Reviews, 5:193-203 (1991).
Kaushansky, Structure-Function Relationships of the Hematopoietic Growth Factors, Proteins: Structure, Function & Genetics, 12:1-9 (1992).
Keegan et al., The Interleukin-4 Receptor. Signal Transduction by a Hematopoietin Receptor, Journal of Leukocyte Biology, 55:272-279 (1994).
Keegan et al., Interleukin 4 Receptor: Signaling Mechanisms, Immunology Today, 15:423-432 (1994).
Kleinau et al., Importance of CD23 for Collagen-Induced Arthritis: Delayed Onset and Reduced Severity in CD23-Deficient Mice, J. Immunol., 162:4266-4270 (1999).
Klinkert et al., TNF-α Receptor Fusion Protein Prevents Experimental Auto-Immune Encephalomyelitis and Demyelination in Lewis Rats: an Overview, The Journal of Neuroimmunology, 72:163-168 (1997).
Kohno et al., A Second Tumor Necrosis Factor Receptor Gene Product Can Shed a Naturally Occurring Tumor Necrosis Factor Inhibitor, Proc. Natl. Acad. Sci. USA, 87:8331-8335 (1990).
Kruse et al., Conversion of Human Interleukin-4 Into a High Affinity Antagonist by a Single Amino Acid Replacement, The EMBO Journal, 11:3237-3244 (1992).
Kull et al., Cellular Receptor for ^{125}I-Labeled Tumor Necrosis Factor: Specific Binding, Affinity Labeling, and Relationship to Sensitivity, Proc. Natl. Acad. Sci. USA, 82:5756-5760 (1985).
Landolfi, A Chimeric IL-2/Ig Molecule Possesses the Functional Activity of Both Proteins, J. Immunol., 146:915-919 (1991).
Langner et al., Structural and Functional Analysis of a TNF Receptor-Immunoglobulin Fusion Protein, New Advances on Cytokines, 349-354 (1992).
Leberthon et al., Enhanced Tumor Uptake of Macromolecules Induced by a Novel Vasoactive Interleukin 2 Immunoconjugate, Cancer Research, 51:2694-2698 (1991).
Lesslauer et al., Recombinant Soluble Tumor Necrosis Factor Receptor Proteins Protect Mice From Lipopolysaccharide-Induced Lethality, Eur. J. Immunol., 21:2883-2886 (1991).
Liabakk et al., A Rapid and Sensitive Immunoassay for Tumor Necrosis Factor Using Magnetic Monodisperse Polymer Particles, Journal of Immunological Methods, 134:253-259 (1990).
Loetscher et al., Efficacy of a Chimeric TNFR-IgG Fusion Protein to Inhibit TNF Activity in Animal Models of Septic Shock, Endotoxin Research Series, 2:455-462•1993).
Loetscher et al., Two distinct human TNF receptors: purification, molecular cloning and expression, in Tumor Necrosis Factor: Structure-Function Relationship and Clinical Application, (3rd International Conference, 1992.
Maliszewski et al., Cytokine Receptors and B Cell Functions: Recombinant Soluble Receptors Specifically Inhibit IL-1 and IL-4 Induced Cell Activities In Vitro, J. Immunol., 144:3028-3033 (1990).
Mohler et al., Soluble Tumor Necrosis Factor (TNF) Receptors Are Effective Therapeutic Agents in Lethal Endotoxemia and Function Simultaneously as Both TNF Carriers and TNF Antagonists, J. Immunol., 151:1548-1561 (1993).

Mori et al., Attenuation of Collagen-Induced Arthritis in 55-kDa TNF Receptor Type 1 (TNFR1)-IgG1-Treated and TNFR1-Deficient Mice, J. Immunol., 157:3178-3182 (1996).
Morrissey et al., Molecular Cloning of the cDNA for Tissue Factor, the Cellular Receptor for the Initiation of the Coagulation Protease Cascade, Cell: 50:129-135 (1987).
Morrison, In Vitro Antibodies: Strategies for Production and Application, Annu. Rev. Immunol., 10:239-265 (1992).
Mosley et al., The Murine Interleukin-4 Receptor: Molecular Cloning and Characterization of Secreted and Membrane Bound Forms, Cell: 59:335-348 (1989).
Novotny et al., A Soluble, Single-Chain T-Cell Receptor Fragment Endowed With Antigen-Combining Properties, Proc. Natl. Acad. Sci. USA, 88:8646-8650 (1991).
Okayama et al., High-Efficiency Cloning of Full-Length cDNA, Molecular and Cellular Biology, 2:161-170 (1982).
Okayama et al., A cDNA Cloning Vector That Permits Expression of cDNA Inserts in Mammalian Cells, Molecular and Cellular Biology, 3:280-289 (1983).
Old, Tumor Necrosis Factor, 2nd Intl Conference on Tumor Necrosis Factor & Related Cytokines, Napa, CA, 1-30 (1989).
Paborsky et al., Purification of Recombinant Human Tissue Factor, Biochemistry, 28:8072-8077 (1989).
Parrillo, Pathogenetic Mechanisms of Septic Shock, New Eng. J. Med., 328:1471-1477 (1993).
Peetre et al., A Tumor Necrosis Factor Binding Protein is Present in Human Biological Fluids, Eur. J. Haematol. 41:414-419 (1988).
Pennica et al., Human Tumour Necrosis Factor: Precursor Structure, Expression and Homology to Lymphotoxin, Nature, 312:724-729 (1984).
Piguet et al., Evolution of Collagen Arthritis in Mice is Arrested by Treatment With Anti-Tumor Necrosis (TNF) Antibody or a Recombinant Soluble TNF Receptor, Immunology, 77 (4):510-514 (1992).
Redfield'et al., Secondary Structure and Topology of Human Interleukin 4 in Solution, Biochemistry, 30:11029-11035 (1991).
Rubin, Binding Receptor Characters Zako and Expression, and Intracellular Events. 2nd Intl Conference on Tumor Necrosis Factor & Related Cytokines, Napa, CA, 94-96 (1989).
Ruddle et al., An Antibody to Lymphotoxin and Tumor Necrosis Factor Prevents Transfer of Experimental Allergic Encephalomyelitis, J. Exp. Med., 172:1193-1200 (1990).
Rutka et al., The Effects of Human Recombinant Tumor Necrosis Factor on Glioma-Derived Cell Lines: Cellular Proliferation, Cytotoxicity, Morphological and Radioreceptor Studies, Int. J. Cancer., 41:573-582 (1988).
Saxne et al., Detection of Tumor Necrosis Factor α But Not Tumor Necrosis Factor β in Rheumatoid Arthritis Synovial Fluid and Serum, Arthritis & Rheumatism, 31:1041-1045 (1988).
Scallon et al., Functional Comparisons of Different Tumour Necrosis Factor Receptor/IgG Fusion Proteins, Cytokine, 7:759-770 (1995).
Scarpati et al., Human Tissue Factor: cDNA Sequence and Chromosome Localization of the Gene, Biochemistry, 26:5234-5238 (1987).
Schleiffenbaum et al., The Tumor Necrosis Factor Receptor and Human Neutrophil Function, J. Clin. Invest., 86:184-195 (1990).
Schnee et al., Construction and Expression of a Recombinant Antibody-Targeted Plasminogen Activator, Proc. Natl. Acad. Sci. USA, 84:6904-6908 (1987).
Seckinger et al., A Human Inhibitor of Tumor Necrosis Factor α, J. Exp. Med. 167:1511-1516 (1988).
Shalaby et al., Receptor Binding and Activation of Polmorphonuclear Neutrophils by Tumor Necrosis Factor-Alpha, Journal of Leukocyte Biology, 41:196-204 (1987).
Shalaby et al., Binding and Regulation of Cellular Function by Monoclonal antibodies Against Human Tumor Necrosis Factor Receptors, J. Exp. Med. 172: 1517-1520 (1990).
Sheehan et al., Generation and Characterization of Hamster Monoclonal Antibodies That Neutralize Murine Tumor Necrosis Factors, Journal of Immunology, 142:3884-3893 (1989).
Shin et al., Expression and Characterization of an Antibody Binding Specificity Joined to Insulin-Like Growth Factor 1: Potential Applications for Cellular Targeting, Proc. Natl. Acad. Sci., 87:5322-5326 (1990).
Sims et al., cDNA Expression Cloning of the IL-1 Receptor, a Member of the Immunoglobulin Superfamily, Science, 241:585-589 (1988).
Sims et al., Cloning the Interleukin 1 Receptor From Human T Cells, Proc. Natl. Acad. Sci., 86:8946-8950 (1989).
Smith et al., The Active Form of Tumor Necrosis Factor is a Trimer, J. Biol. Chem., 262:6951-6954 (1987).
Smith et al., Blocking of HIV-1 Infectivity by a Soluble, Secreted Form of the CD4 Antigen, Science, 238:1704-1707 (1987).
Smith et al., Multimeric Structure of the Tumor Necrosis Factor Receptor of HeLa Cells, J. Biol. Chem., 264:14646-14652 (1989).
Spicer et al., Isolation of cDNA Clones Coding for Human Tissue Factor: Primary Structure of the Protein and cDNA, Proc. Natl. Acad. Sci., 84:5148-5152 (1987).
Staines et al., Collagen Arthritis—What Can It Teach Us?, British Journal of Rheumatology, 33:798-807 (1994).
Strader et al., Structural Basis of β-Adrenergic Receptor Function, The FASEB Journal, 3:1825-1832 (1989).
Suggs et al., Use of Synthetic Oligonucleotides as Hybridization Probes: Isolation of Cloned cDNA Sequences for Human β_2-Microglobulin, Proc. Natl. Acad. Sci. U.S.A., 78:6613-6617 (1981).
Tauber et al., Toxicity in Neuronal Cells Caused by Cererospinal Fluid fFom Pneumococcal and Gram-Negative Meningitis, The Journal of Infectious Diseases, 166:1045-1050 (1992).
Thoma et al., Identification of a 60-kD Tumor Necosis Factor (TNF) Receptor as the Major Signal Transducing Component in TNF Responses, J. Exp. Med. 172: 1019-23 (1990).
Tsujimoto et al., Characterization and Affinity Crosslinking of Receptors for Tumor Necrosis Factor on Human Cells, Archives of Biochemistry and Biophysics, 249:563-568 (1986).
Tsujimoto et al, Interferon-γ Enhances Expression of Cellular Receptors for Tumor Necrosis Factor, J. Immunol., 136:2441-2444 (1986).
Tsujimoto et al, Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells, Proc. Natl. Acad. Sci. 82: 7626-30 (1985).
Ulich et al., Intratracheal Administration of Endotoxin and Cytokines, Clinical Immunology & Immunopathology., 72:137-140 (1994).
Unglaub et al., Downregulation of Tumor Necrosis Factor (TNF) Sensitivity Via Modulation of TNF Binding Capacity by Protein Kinase C Activators, J. Exp. Med. 166:1788-1797 (1987).
Van Der Poll et al., Pretreatment with a 55-kDa Tumor Necrosis Factor Receptor-Immunoglobulin Fusion Protein Attenuates Activation of Coagulation, but not of Fibrinolysis, during Lethal Bacteremia in Baboons, The Journal of Infectious Diseases., 176:296-299 (1997).
Van Zee et al., Protection Against Lethal *Escherichia coli* Bacteremia in Baboons (*Papio anubis*) by Pretreatment With a 55-kDa TNF Receptor (CD120a)-Ig Fusion Protein, Ro 45-2081, J. Immunol., 156:2221-2230 (1996).
Wallach et al., Soluble and Cell Surface Receptors for Tumor Necrosis Factor, Progress, Inflammation Research & Therapy, 51-57 (1991).
Wallach et al., Cell surface and soluble TNF receptors, in Tumor Necrosis Factor: Structure-Function Relationship and Clinical Application, (3rd International Conference on Tumor Necrosis Factor and Related Cytokines, Makuhari, Chiba, Nov. 21-25, 1990), Osawa and Bonavida, eds., Basel, Karger, pp. 47-57 (1992).
Wilks, The CD4 Receptor: Post Binding Events, Conformational Change and the Second Site, Molec. Aspects Med., 12:255-265 (1991).
Yamasaki et al., Cloning and Expression of the Human Interleukin-6 (BSF-2/IFNβ 2) Receptor, Science, 241:825-282 (1988).
Yonehara et al., A Cell-Killing Monoclonal Antibody (Anti-Fas) to a Cell Surface Antigen Co-Downregulated With the Receptor of Tumor Necrosis Factor, J. Exp. Med., 169:1747-1765 (1989).
Yoshie et al., Binding and Crosslinking of ^{125}I-Labeled Recombinant Human Tumor Necrosis Factor to Cell Surface Receptors, J. Biochem., 100: 531-541(1986).
Official Communication relating to an Opposition in EP Application No. 99 100 703.0, Jan. 31, 2006.
H. Engelmann et al., Jour. of Biological Chemistry, vol. 264, Nr. 20, p. 11974-11980, (Jul. 1989).

P.W. Gray et al, Proc. of the Nat'l Acad. of Sci., vol. 87, p. 7380-7884, (Oct. 1990).
H. Engelmann et al., Jour. of Biological Chemistry, vol. 264, p. 531-1536, (Jan. 1990).
H. Loetscher et al,. Cell, vol. 61, p. 351-359 (Apr. 1990).
T.J. Schall et al., Cell, vol. 61, p. 361-370 (Apr. 1990).
R. A. Heller et al., Proc. of the Nat'l Acad. of Sci., vol. 87, pp. 6151-6155, (Aug. 1990).
C. A. Smith et al, Science, vol. 248, pp. 1019-1023, (May 1990).
P. Seckinger et al, Eur. J. Immunology, vol. 20, pp. 1167-1174 (1990).
D. Novick et al, J. Exp. Med., vol. 170, pp. 1409-1414, (1988).
P. Seckinger et al., Jour. of Biological Chem., vol. 264, pp. 22966-11973 (Jul. 1989).
Folks et al., Tumor necrosis factor α induces expression of human immunodeficiency virus in a chronically infected T-cell clone, PNAS, 86:2365-8 (1989).
Lee, Generation of cDNA probes directed by amino acid sequence: cloning of urate oxidase, Science, 239:1288-1291 (1988).
Moreland et al., Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein, New England Journal of Medicine, 337:141-147 (1997).
Sandborn et al., Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial, Gastroenterol., 121:1088-94 (2001).
Wozney, Using purified protein to clone its genes, Methods in Enzymology, 182:738-751 (1990).
Inflammation & Infection: Rheumatoid Arthritis, Drug & Market Development, 67-70 (2003).
Letter to the EPO with new Auxiliary request 3 in appeal proc. against EP 0 471 701, Dec. 31, 2001.
Notification of the Examiner during the prosecution of EP 99100703. 0, Oct. 24, 2000.
Zavoico, Drug & Market Development, 10(7):235-243 (1999).
Heller et al., Abstract at the 2nd International Conference on Tumor Necrosis Factor and Related Cytokines, Napa Valley, California, Jan. 15-20, 1989.
Loetscher et al., Two Distinct Human TNF Receptors: Purification, Molecular Cloning and Expression, 3rd International Conference on Tumor Necrosis Factor and Related Cytokines, Makuhari, Chiba, Japan, Nov. 21-25, 1990.
Inflammation & Infection: Rheumatoid Arthritis, Drug & Market Development, 67-70 (Mar. 2003).
Barone et al., Arthritis Rheum., 42(9)Suppl, Sep. 1999, Abstract 116.
IPS Notice of Opposition to EP 0 939 121 and Statement in Support of the Opposition against EP 0939121, Dec. 30, 2003.
Patentee's Response to Notice of Opposition Request for Oral Hearing, dated Aug. 3, 2004 (with translation).
Answer of the Opponent on the Admissibility of the Oppositions, dated Nov. 3, 2004.
Response to Patentee's Written Submission, dated Jul. 22, 2005.
Opponent's Observations Under R 71a EPC, dated Sep. 22, 2006.
AHP Comments on Oral Hearing and Preliminary Opinion, dated Sep. 22, 2006.
AHP Response to Opponent's Observations, dated Oct. 6, 2006.
US 6,224,867, 05/2001, Smith et al. (withdrawn)

* cited by examiner

Figure 1

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-185  GAATTCGGGGGGGTTCAAGATCACTGGGACCAGGCCGTGATCTCTATGCCCGAGTCTCAA
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 -65  CCAGCACTGCCGCTGCCACACTGCCCTGAGCCCAAATGGGGGAGTGAGAGGCCATAGCTG
          -28.
 -30        MetGlyLeuSerThrValProAspLeuLeuLeuProLeuValLeuLeuGluLeu
  -5  TCTGGCATGGGCCTCTCCACCGTGCCTGACCTGCTGCTGCCGCTGGTGCTCCTGGAGCTG
                                          +1
 -10  LeuValGlyIleTyrProSerGlyValIleGlyLeuValProHisLeuGlyAspArgGlu
  55  TTGGTGGGAATATACCCCTCAGGGGTTATTGGACTGGTCCCTCACCTAGGGGACAGGGAG
                                                  ***
  10  LysArgAspSerValCysProGlnGlyLysTyrIleHisProGlnAsnAsnSerIleCys
 115  AAGAGAGATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAAATAATTCGATTTGC

  30  CysThrLysCysHisLysGlyThrTyrLeuTyrAsnAspCysProGlyProGlyGlnAsp
 175  TGTACCAAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGAT

  50  ThrAspCysArgGluCysGluSerGlySerPheThrAlaSerGluAsnHisLeuArgHis
 235  ACGGACTGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACAC

  70  CysLeuSerCysSerLysCysArgLysGluMetGlyGlnValGluIleSerSerCysThr
 295  TGCCTCAGCTGCTCCAAATGCCGAAAGGAAATGGGTCAGGTGGAGATCTCTTCTTGCACA

  90  ValAspArgAspThrValCysGlyCysArgLysAsnGlnTyrArgHisTyrTrpSerGlu
 355  GTGGACCGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAA
                    ***                 ***
 110  AsnLeuPheGlnCysPheAsnCysSerLeuCysLeuAsnGlyThrValHisLeuSerCys
 415  AACCTTTTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGC

 130  GlnGluLysGlnAsnThrValCysThrCysHisAlaGlyPhePheLeuArgGluAsnGlu
 475  CAGGAGAAACAGAACACCGTGTGCACCTGCCATGCAGGTTTCTTTCTAAGAGAAAACGAG

 150  CysValSerCysSerAsnCysLysLysSerLeuGluCysThrLysLeuCysLeuProGln
 535  TGTGTCTCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAG

 170  IleGluAsnValLysGlyThrGluAspSerGlyThrThrValLeuLeuProLeuValIle
 595  ATTGAGAATGTTAAGGGCACTGAGGACTCAGGCACCACAGTGCTGTTGCCCCTGGTCATT

 190  PhePheGlyLeuCysLeuLeuSerLeuLeuPheIleGlyLeuMetTyrArgTyrGlnArg
 655  TTCTTTGGTCTTTGCCTTTTATCCCTCCTCTTCATTGGTTTAATGTATCGCTACCAACGG

 210  TrpLysSerLysLeuTyrSerIleValCysGlyLysSerThrProGluLysGluGlyGlu
 715  TGGAAGTCCAAGCTCTACTCCATTGTTTGTGGGAAATCGACACCTGAAAAAGAGGGGGAG
                                          ***
 230  LeuGluGlyThrThrThrLysProLeuAlaProAsnProSerPheSerProThrProGly
 775  CTTGAAGGAACTACTACTAAGCCCCTGGCCCCAAACCCAAGCTTCAGTCCCACTCCAGGC

 250  PheThrProThrLeuGlyPheSerProValProSerSerThrPheThrSerSerSerThr
 835  TTCACCCCCACCCTGGGCTTCAGTCCCGTGCCCAGTTCCACCTTCACCTCCAGCTCCACC

 270  TyrThrProGlyAspCysProAsnPheAlaAlaProArgArgGluValAlaProProTyr
 895  TATACCCCCGGTGACTGTCCCAACTTTGCGGCTCCCCGCAGAGAGGTGGCACCACCCTAT

 290  GlnGlyAlaAspProIleLeuAlaThrAlaLeuAlaSerAspProIleProAsnProLeu
 955  CAGGGGGCTGACCCCATCCTTGCGACAGCCCTCGCCTCCGACCCCATCCCCAACCCCCTT
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Figure 1 (cont.

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 330  LeuTyrAlaValValGluAsnValProProLeuArgTrpLysGluPheValArgArgLeu
1075  CTGTACGCCGTGGTGGAGAACGTGCCCCCGTTGCGCTGGAAGGAATTCGTGCGGCGCCTA

 350  GlyLeuSerAspHisGluIleAspArgLeuGluLeuGlnAsnGlyArgCysLeuArgGlu
1135  GGGCTGAGCGACCACGAGATCGATCGGCTGGAGCTGCAGAACGGGCGCTGCCTGCGCGAG

 370  AlaGlnTyrSerMetLeuAlaThrTrpArgArgArgThrProArgArgGluAlaThrLeu
1195  GCGCAATACAGCATGCTGGCGACCTGGAGGCGGCGCACGCCGCGGCGCGAGGCCACGCTG

 390  GluLeuLeuGlyArgValLeuArgAspMetAspLeuLeuGlyCysLeuGluAspIleGlu
1255  GAGCTGCTGGGACGCGTGCTCCGCGACATGGACCTGCTGGGCTGCCTGGAGGACATCGAG

 410  GluAlaLeuCysGlyProAlaAlaLeuProProAlaProSerLeuLeuArg
1315  GAGGCGCTTTGCGGCCCCGCCGCCCTCCCGCCCGCGCCCAGTCTTCTCAGATGAGGCTGC
1375  GCCCCTGCGGGCAGCTCTAAGGACCGTCCTGCGAGATCGCCTTCCAACCCCACTTTTTTC
1435  TGGAAAGGAGGGGTCCTGCAGGGGCAAGCAGGAGCTAGCAGCCGCCTACTTGGTGCTAAC
1495  CCCTCGATGTACATAGCTTTTCTCAGCTGCCTGCGCGCCGCCGACAGTCAGCGCTGTGCG
1555  CGCGGAGAGAGGTGCGCCGTGGGCTCAAGAGCCTGAGTGGGTGGTTTGCGAGGATGAGGG
1615  ACGCTATGCCTCATGCCCGTTTTGGGTGTCCTCACCAGCAAGGCTGCTCGGGGGCCCCTG
1675  GTTCGTCCCTGAGCCTTTTTCACAGTGCATAAGCAGTTTTTTTTGTTTTTGTTTTGTTTT
1735  GTTTTGTTTTTAAATCAATCATGTTACACTAATAGAAACTTGGCACTCCTGTGCCCTCTG
1795  CCTGGACAAGCACATAGCAAGCTGAACTGTCCTAAGGCAGGGGCGAGCACGGAACAATGG
1855  GGCCTTCAGCTGGAGCTGTGGACTTTTGTACATACACTAAAATTCTGAAGTTAAAAAAAA
1915  AACCCGAATTC
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Figure 2A

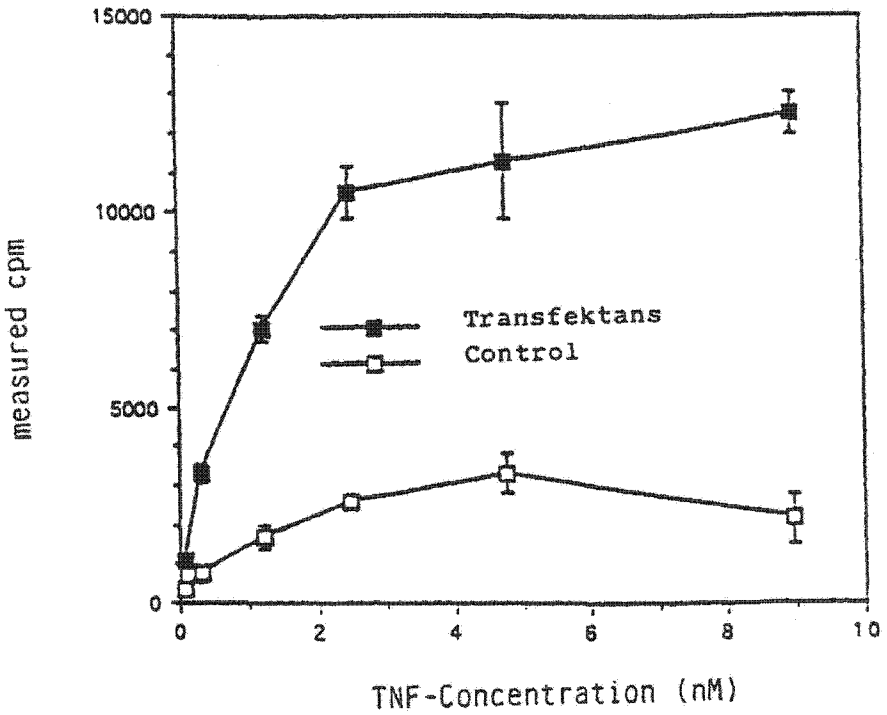

Figure 2B

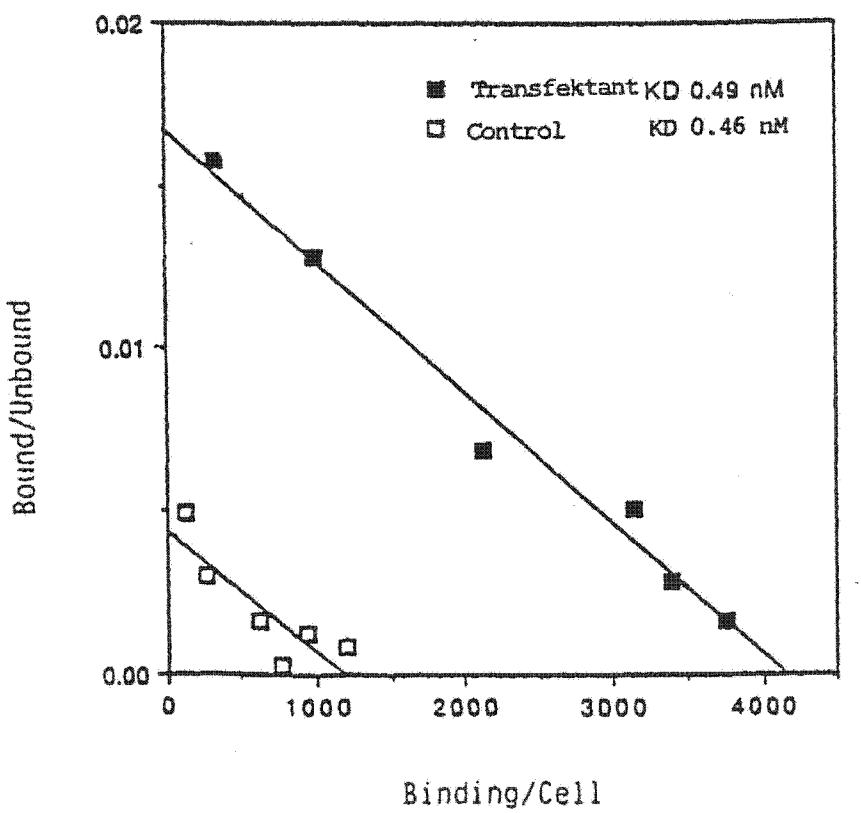

Figure 3

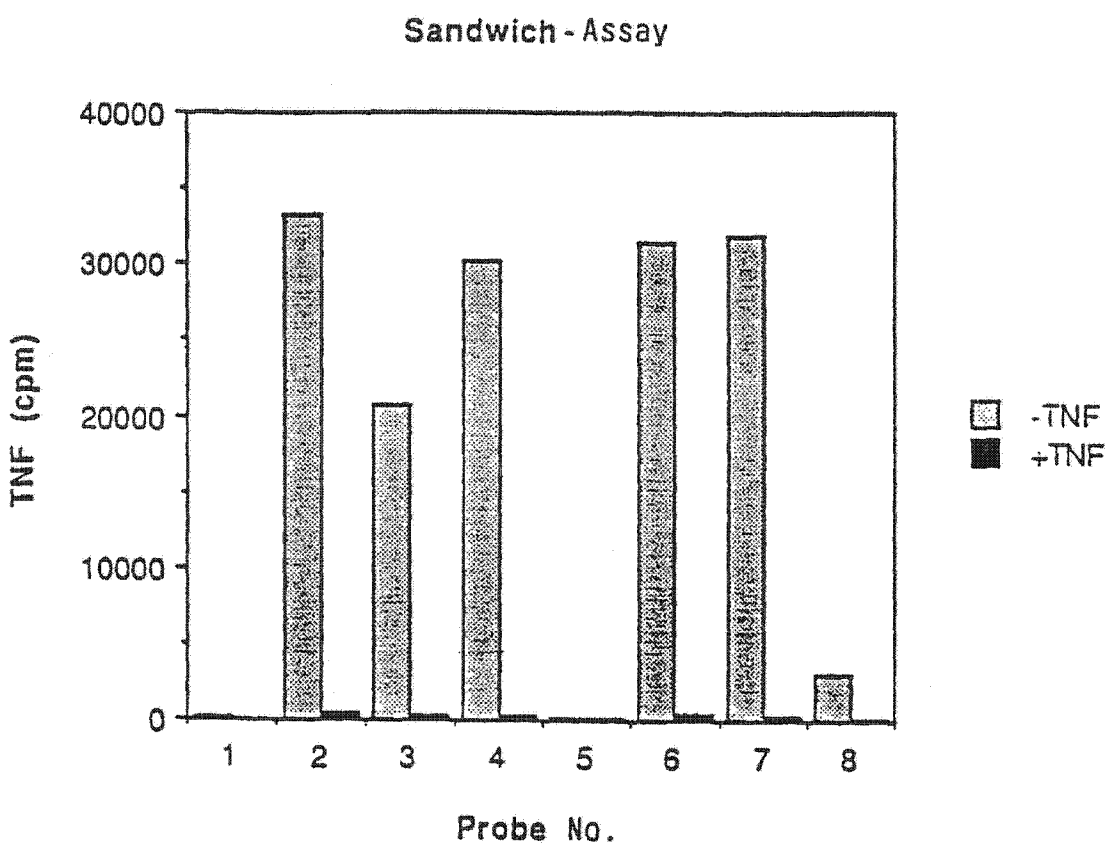
Sandwich - Assay
40000
30000
20000
10000
0
TNF (cpm)
1
2
3
4
5
6
7
8
Probe No.
-TNF
+TNF

Figure 4

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  1  SerAspSerValCysAspSerCysGluAspSerThrTyrThrGlnLeuTrpAsnTrpVal
  1  TCGGACTCCGTGTGTGACTCCTGTGAGGACAGCACATACACCCAGCTCTGGAACTGGGTT

 21  ProGluCysLeuSerCysGlySerArgCysSerSerAspGlnValGluThrGlnAlaCys
 61  CCCGAGTGCTTGAGCTGTGGCTCCCGCTGTAGCTCTGACCAGGTGGAAACTCAAGCCTGC

 41  ThrArgGluGlnAsnArgIleCysThrCysArgProGlyTrpTyrCysAlaLeuSerLys
121  ACTCGGGAACAGAACCGCATCTGCACCTGCAGGCCCGGCTGGTACTGCGCGCTGAGCAAG

 61  GlnGluGlyCysArgLeuCysAlaProLeuProLysCysArgProGlyPheGlyValAla
181  CAGGAGGGGTGCCGGCTGTGCGCGCCGCTGCCGAAGTGCCGCCCGGGCTTCGGCGTGGCC

 81  ArgProGlyThrGluThrSerAspValValCysLysProCysAlaProGlyThrPheSer
241  AGACCAGGAACTGAAACATCAGACGTGGTGTGCAAGCCCTGTGCCCCGGGGACGTTCTCC

101  AsnThrThrSerSerThrAspIleCysArgProHisGlnIleCysAsnValValAlaIle
301  AACACGACTTCATCCACGGATATTTGCAGGCCCCACCAGATCTGTAACGTGGTGGCCATC

121  ProGlyAsnAlaSerArgAspAlaValCysThrSerThrSerProThrArgSerMetAla
361  CCTGGGAATGCAAGCAGGGATGCAGTCTGCACGTCCACGTCCCCCACCCGGAGTATGGCC

141  ProGlyAlaValHisLeuProGlnProValSerThrArgSerGlnHisThrGlnProSer
421  CCAGGGGCAGTACACTTACCCCAGCCAGTGTCCACACGATCCCAACACACGCAGCCAAGT

161  ProGluProSerThrAlaProSerThrSerPheLeuLeuProMetGlyProSerProPro
481  CCAGAACCCAGCACTGCTCCAAGCACCTCCTTCCTGCTCCCAATGGGCCCCAGCCCCCCA

181  AlaGluGlySerThrGlyAspPheAlaLeuProValGlyLeuIleValGlyValThrAla
541  GCTGAAGGGAGCACTGGCGACTTCGCTCTTCCAGTTGGACTGATTGTGGGTGTGACAGCC

201  LeuGlyLeuLeuIleIleGlyValValAsnCysValIleMetThrGlnValLysLysLys
601  TTGGGTCTACTAATAATAGGAGTGGTGAACTGTGTCATCATGACCCAGGTGAAAAAGAAG

221  ProLeuCysLeuGlnArgGluAlaLysValProHisLeuProAlaAspLysAlaArgGly
661  CCCTTGTGCCTGCAGAGAGAAGCCAAGGTGCCTCACTTGCCTGCCGATAAGGCCCGGGGT

241  ThrGlnGlyProGluGlnGlnHisLeuLeuIleThrAlaProSerSerSerSerSerSer
721  ACACAGGGCCCCGAGCAGCAGCACCTGCTGATCACAGCGCCGAGCTCCAGCAGCAGCTCC

261  LeuGluSerSerAlaSerAlaLeuAspArgArgAlaProThrArgAsnGlnProGlnAla
781  CTGGAGAGCTCGGCCAGTGCGTTGGACAGAAGGGCGCCCACTCGGAACCAGCCACAGGCA
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Figure 4 (cont.)

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 281  ProGlyValGluAlaSerGlyAlaGlyGluAlaArgAlaSerThrGlySerSerAlaAsp
 841  CCAGGCGTGGAGGCCAGTGGGGCCGGGGAGGCCCGGGCCAGCACCGGGAGCTCAGCAGAT

 301  SerSerProGlyGlyHisGlyThrGlnValAsnValThrCysIleValAsnValCysSer
 901  TCTTCCCCTGGTGGCCATGGGACCCAGGTCAATGTCACCTGCATCGTGAACGTCTGTAGC

 321  SerSerAspHisSerSerGlnCysSerSerGlnAlaSerSerThrMetGlyAspThrAsp
 961  AGCTCTGACCACAGCTCACAGTGCTCCTCCCAAGCCAGCTCCACAATGGGAGACACAGAT

 341  SerSerProSerGluSerProLysAspGluGlnValProPheSerLysGluGluCysAla
1021  TCCAGCCCCTCGGAGTCCCCGAAGGACGAGCAGGTCCCCTTCTCCAAGGAGGAATGTGCC

 361  PheArgSerGlnLeuGluThrProGluThrLeuLeuGlySerThrGluGluLysProLeu
1081  TTTCGGTCACAGCTGGAGACGCCAGAGACCCTGCTGGGGAGCACCGAAGAGAAGCCCCTG

 381  ProLeuGlyValProAspAlaGlyMetLysProSer
1141  CCCCTTGGAGTGCCTGATGCTGGGATGAAGCCCAGTTAACCAGGCCGGTGTGGGCTGTGT
1201  CGTAGCCAAGGTGGCTGAGCCCTGGCAGGATGACCCTGCGAAGGGGCCCTGGTCCTTCCA
1261  GGCCCCCACCACTAGGACTCTGAGGCTCTTTCTGGGCCAAGTTCCTCTAGTGCCCTCCAC
1321  AGCCGCAGCCTCCCTCTGACCTGCAGGCCAAGAGCAGAGGCAGCGAGTTGTGGAAAGCCT
1381  CTGCTGCCATGGCGTGTCCCTCTCGGAAGGCTGGCTGGGCATGGACGTTCGGGGCATGCT
1441  GGGGCAAGTCCCTGAGTCTCTGTGACCTGCCCCGCCCAGCTGCACCTGCCAGCCTGGCTT
1501  CTGGAGCCCTTGGGTTTTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTCTCCCCCTGGGC
1561  TCTGCCCAGCTCTGGCTTCCAGAAAACCCCAGCATCCTTTTCTGCAGAGGGGCTTTCTGG
1621  AGAGGAGGGATGCTGCCTGAGTCACCCATGAAGACAGGACAGTGCTTCAGCCTGAGGCTG
1681  AGACTGCGGGATGGTCCTGGGGCTCTGTGCAGGGAGGAGGTGGCAGCCCTGTAGGGAACG
1741  GGGTCCTTCAAGTTAGCTCAGGAGGCTTGGAAAGCATCACCTCAGGCCAGGTGCAGTGGC
1801  TCACGCCTATGATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATCACCTGAGGTTAGGA
1861  GTTCGAGACCAGCCTGGCCAACATGGTAAAACCCCATCTCTACTAAAAATACAGAAATTA
1921  GCCGGGCGTGGTGGCGGGCACCTATAGTCCCAGCTACTCAGAAGCCTGAGGCTGGGAAAT
1981  CGTTTGAACCCGGGAAGCGGAGGTTGCAGGGAGCCGAGATCACGCCACTGCACTCCAGCC
2041  TGGGCGACAGAGCGAGAGTCTGTCTCAAAAGAAAAAAAAAAAGCACCGCCTCCAAATGCT
2101  AACTTGTCCTTTTGTACCATGGTGTGAAAGTCAGATGCCCAGAGGGCCCAGGCAGGCCAC
2161  CATATTCAGTGCTGTGGCCTGGGCAAGATAACGCACTTCTAACTAGAAATCTGCCAATTT
2221  TTTAAAAAAGTAAGTACCACTCAGGCCAACAAGCCAACGACAAAGCCAAACTCTGCCAGC
2281  CACATCCAACCCCCCACCTGCCATTTGCACCCTCCGCCTTCACTCCGGTGTGCCTGCAG
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HUMAN TNF RECEPTOR FUSION PROTEIN

This is a division of application Ser. No. 08/095,640, filed Jul. 21, 1993; now U.S. Pat. No. 5,610,279, which is a continuation application of Ser. No. 07/580,013, filed Sep. 10, 1990, now abandoned. This application claims priority under 35 U.S.C. §119 to application Serial Numbers 3319/89, 746/90 and 1347/90, filed on Sep. 12, 1989, Mar. 8, 1990 and Apr. 20, 1990, respectively, all in Switzerland. This application also claims priority under 35 U.S.C. §119 to European Patent Application Number 90116707.2—(now Patent Number EP 0417563), filed Aug. 31, 1990.

BACKGROUND OF THE INVENTION

Tumor necrosis factor α (TNFα, also cachectin), discovered as a result of its hemorragic-necrotizing activity on certain tumors, and lymphotoxin (TNFβ) are two closely related peptide factors [3] from the class of lymphokines/cytokines which are both referred to herein-after as TNF [see references 2 and 3]. TNF possesses a broad cellular spectrum of activity. For example, TNF has inhibitory or cytotoxic activity on a series of tumor cell lines [2,3], stimulates the proliferation of fibroblasts and the phagocytic/cytotoxic activity of myeloic cells [4, 5, 6], induces adhesion molecules in endothelial cells or exerts an inhibitory activity on the endothelium [7, 8, 9, 10], inhibits the synthesis of specific enzymes in adipocytes [11] and induces the expression of histo-compatibility antigens [12]. Many of these TNF activities are produced via induction of other factors or by synergistic effects with other factors such as interferons or interleukins [13-16].

TNF is involved in pathological conditions such as shock states in meningococcal sepsis [17], the development of autoimmune glomerulonephritis in mice [18] and cerebral malaria in mice [19] and human beings [41]. The toxic effects of endotoxin appear to be mediated by TNF [20]. Furthermore, TNF can trigger interleukin-1 fever [39]. On the basis of its pleiotropic functional properties, TNF in interaction with other cytokines is involved in additional pathological conditions as a mediator of immune response, inflammation, and other processes.

These biological effects are mediated by TNF via specific receptors. According to present knowledge not only TNFα, but also TNFβ bind to the same receptors [21]. Different cell types differ in their number of TNF receptors [22, 23, 24]. Generally known TNF-binding proteins (TNF-BP) have been detected by covalent bonding to radioactively labelled TNF [24-29], and the following apparent molecular weights of the TNF/TNF-BP complexes obtained have been determined to be: 95/100 kD and 75 kD [24], 95 kD and 75 kD [25], 138 kD, 90 kD, 75 kD and 54 kD [26], 100±5 kD [27], 97 kD and 70 kD [28] and 145 kD [29]. One such TNF/TNF-BP complex was isolated by anti-TNF-antibody immune affinity chromatography and preparative SDS-polyacrylamide gel electrophoreses (SDS-PAGE) [27]. The reductive cleavage of this complex and subsequent SDS-PAGE analysis gave several bands which were not tested for TNF-binding activity. Since the specific conditions which must be used for the cleavage of the complex lead to inactivation of the binding protein [31], the latter has also not been possible. The separation of soluble TNF-BP from human serum or urine by ion exchange chromatography and gel filtration (molecular weight in the region of 50 kD) was described by Olsson et al. [30].

Brockhaus et al. [32] obtained an enriched TNF-BP preparation from membrane extracts of HL_{60} cells by TNFα-ligand affinity chromatography and HPLC which, in turn, was used as an antigen preparation for the production of monoclonal

antibodies against TNF-BP. Using such an immobilized antibody (immune affinity chromatography) Loetscher and Brockhaus obtained an enriched preparation of TNF-BP [31] from an extract of human placenta using TNFα-ligand affinity chromatography and HPLC, which gave a strong broad band at 35 kD, a weak band at about 40 kD and a very weak band in the region between 55 kD and 60 kD on SDS-PAGE analysis. Moreover, the gel showed a protein background smear in the region of 33 kD to 40 kD. The significance of these protein bands was, however, not clear due to the heterogenicity of the starting material which was used (placenta tissue; combined material from several placentas). In the state of the art TNF-BP have already been characterized by a N-terminal partial sequence [European Patent Application, Publication No. 308 378], whereby this sequence differs from the N-terminal partial sequence according to formula (IA) in accordance with the invention. Moreover, the TNF-binding proteins described in the state of the art are soluble, i.e. non-membrane bound, TNF-BP and not membrane-bound, i.e. insoluble, TNF-BP isolated from urine.

SUMMARY OF THE INVENTION

This invention comprises insoluble, homogenous proteins or soluble or insoluble fragments thereof, capable of binding tumor necrosis factor-(TNF).

This invention also comprises TNF-binding proteins containing amino acid sequences of FIG. **1** or FIG. **4**, proteins containing fragments of these sequences, and proteins analagous to the sequences of FIG. **1** or FIG. **4** or to fragments thereof.

This invention % further comprises DNA sequences encoding the proteins: described above, proteins encoded by these sequences, and antibodies to any of these proteins.

This invention comprises. DNA sequences which combine two partial DNA sequences, one sequence encoding soluble fragments of TNF binding proteins and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE, and the recombinant proteins encoded by these sequences.

This invention additionally comprises vectors containing the above DNA sequences, and host systems transfected with such vectors.

This invention finally comprises a process for the isolation of an insoluble homogenous protein capable of binding TNF.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. Nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) for cDNA clone derived from 55 kD TNF-BP. The 19 amino acid transmembrane region is underlined. Hypothetical glycosylation sites are identified by asterisks.

FIG. **2**. Binding analysis of COS cells transfected with plasmid pN123. Panel 2A—binding of transfected cells to ^{125}I-TNFα. Panel 2B—Scatchard plot of binding data.

FIG. **3**. Sandwich assays of cells transfected with plasmid pK19. Culture supernatants of cells transfected 30 with pK19 were incubated with anti-55 kD TNF-BP antibody followed by ^{125}I-TNFα. Columns 1, 5, and 8 are controls. Columns 2, 3, 4, 5, and 6 are five parallel transfections.

FIG. **4**. Nucleotide sequence (SEQ ID NO: 3) and deduced amino acid sequence (SEQ ID NO: 4) for cDNA clones derived from 75/6510 TNF-BP.

DETAILED DESCRIPTION OF THE INVENTION

The TNF-binding proteins of the present invention are homogenous, insoluble proteins and soluble or insoluble

fragments of such proteins which are capable of binding TNF. These proteins have the ability to bind TNF as measured by standard assays.

The TNF-binding proteins of the present invention include homogenous proteins containing the amino acid sequence depicted in FIG. 1 (SEQ ID NO: 2) or in FIG. 4 (SEQ ID NO: 4), proteins containing fragments of either sequence, and analogues of any such proteins for example proteins containing amino acid sequences analogous to the amino acid sequences of FIG. 1 (SEQ ID NO: 2) or FIG. 4 (SEQ ID NO: 4) or to fragments thereof. An analogue is a protein in which one or more amino acids of the sequences depicted in FIG. 1 (SEQ ID NO: 2) or in FIG. 4 (SEQ ID NO: 4) have had their side-groups chemically modified in a known manner, or those in which one or more amino acids have been replaced or deleted, without thereby eliminating TNF-binding ability. Such analogues may be produced by known methods of peptide chemistry, or by known methods of recombinant DNA technology, such as planned mutagenesis.

The TNF binding activity of the proteins of the present invention may be determined using the assay described in Example 1.

TNF-binding proteins of this invention are obtained as follows:

TNF binding proteins may be isolated from tissues and purified to homogeneity, or isolated from cells which contain membrane-bound TNF binding protein, and purified to homogeneity. One possible method for growing cells and isolating cell extract is described in Example 2, however, other cells types and other growth and isolation methods are well known in the art. Purification of TNF-binding proteins from cell extracts may be performed using the methods described in Examples 4, 5, and 6 in combination with the assay described in Example 1. TNF-binding proteins isolated and purified by these methods were sequenced by well-known methods, as described in Example 7. From these amino acid sequences, DNA probes were produced and used to obtain mRNA encoding TNF binding proteins from which cDNA was made, all by known methods described in Examples 8 and 11. Other well-known methods for producing cDNA are known in the art and may effectively be used. In general, any TNF-binding protein can be isolated from any cell or tissue expressing such proteins using a cDNA probe such as the probe described above, isolating mRNA and transcribing the mRNA into cDNA. Thereafter, the protein can be produced by inserting the cDNA into an expression vector as described in Example 9, such as a virus, plasmid, cosmid, or other vector, inserting the expression vector into a cell, such as the COS cell-described in Example 9 or the insect cell described in Example 10, proliferating the resulting cells, and isolating the expressed TNF-binding protein from the medium or from cell extract as described above. Alternatively, TNF-binding proteins may be chemically synthesized using the sequence described above and an amino acid synthesizer, or manual synthesis using chemical conditions well known to form peptide bonds between selected amino acids. Analogues and fragments of TNF-binding proteins may be produced by the above methods. In the case of analogues, the proteins may be chemically modified, or modified by genetic engineering as described above. These fragments and analogues may then be tested for TNF-binding activity using methods such as the assay of Example 1.

Finally, monoclonal antibodies directed against TNF-binding proteins, such as the antibodies described in Example 3, may be produced by known techniques, and used to isolate TNF-binding proteins.

In more detail, the proteins of the present invention are non-soluble proteins, i.e. for example membrane proteins or so-called receptors, and soluble or non-soluble, fragments thereof, which bind TNF (TNF-BP), in homogeneous form, as well as their physiologically compatible salts. Preferred proteins are those which according to SDS-PAGE under non-reducing conditions are characterized by apparent molecular weights of about 55 kD, 51 kD, 38 kD, 36 kD and 34 kD or 75 kD and 65 kD, especially those with about 55 kD and 75 kD. Furthermore, there are preferred those proteins which are characterized by containing at least one of the following amino acid partial sequences:

(IA) Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5)

(IB) Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6)

(IIA) Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7)

(IIB) Val-Phe-Cys-Thr (SEQ ID NO: 8)

(IIC) Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9)

(IID) Leu-pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys (SEQ ID NO: 10)

(IIE) Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

(IIF) Leu-Cys-Ala-Pro (SEQ ID NO: 12)

(IIG) Val-Pro-His-Leu-Pro-Ala-Asp (SEQ ID NO: 13)

(IIH) Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14)

in which X stands for an amino acid residue which could not be unequivocally determined.

A process for the isolation of the TNF-BP in accordance with the invention is also an object of the present invention. This process comprises carrying out essentially the following purification steps in sequence: production of a cell or tissue extract, immune affinity chromatography and/or single or multiple ligand affinity chromatography, high resolution liquid chromatography (HPLC) and preparative SDS-polyacrylamide gel electro phoresis (SDS-PAGE). The combination of the individual purification steps, which are known from the state of the art, is essential to the success of the process in accordance with the invention, whereby individual steps have been modified and improved having regard to the problem to be solved. Thus, for example, the original combined immune affinity chromatography/TNFα-ligand affinity chromatography step originally used for the enrichment of TNF-BP from human placenta [31] has been altered by using a BSA-Sepharose 4B pre-column. For the application of the cell or membrane extract, this pre-column was connected in series with the immune affinity column followed by the ligand affinity column. After the application of the extract the two aforementioned columns were coupled, each eluted and the TNF-BP-active fractions were purified again via a ligand affinity column. The use of a detergent-containing solvent mixture for the performance of the reversed-phase HPLC step is essential to the invention.

Further, an industrial process for the production of high cell densities of mammalian cells from which TNF-BP can be isolated is also an object of the present invention. Such a process comprises using a medium, which has been developed for the specific growth requirements of the cell line used, in combination with a perfusion apparatus as described e.g. in detail in Example 2. By means of such a process there can be produced, for example, in the case of HL-60 cells up to more than 20-fold higher cell densities than usual.

In addition thereto, the present invention is also concerned with DNA sequences coding for proteins and soluble or non-soluble fragments thereof, which bind TNF. Thereunder there are to be understood; for example, DNA sequences coding for non-soluble proteins or soluble as well as non-soluble fragments thereof, which bind TNF, such DNA sequences being selected from the following:

(a) DNA sequences as given FIG. **1** or FIG. **4** as well as their complementary strands, or those which include these sequences;

(b) DNA sequences which hybridize with sequences defined under (a) or fragments thereof;

(c) DNA sequences which, because of the degeneracy of the genetic code, do not hybridize with sequences as defined under (a) and (b), but which code for polypeptides, having exactly the same amino acid sequence.

That is to say, the present invention embraces not only allelic variants, but also those DNA sequences which result from deletions, substitutions and additions from one or more nucleotides of the sequences given in FIG. **1** or FIG. **4**, whereby in the case of the proteins coded thereby there come into consideration, just as before, TNF-BP. One sequence which results from such a deletion is described, for example, in Science 248, 1019-1023, (1990).

There are preferred first of all those DNA sequences which code for such a protein having an apparent molecular weight of about 55 kD, whereby the sequence given in FIG. **1** is especially preferred, and sequences which code for non-soluble as well as soluble fragments of such proteins. A DNA sequence which codes, for example, for such a non-soluble protein fragment extends from nucleotide −185 to 1122 of the sequence given in FIG. **1**. DNA sequences which code for soluble protein fragments are, for example, those which extend from nucleotide −185 to 633 or from nucleotide −14 to 633 of the sequence given in FIG. **1**. There are also preferred DNA sequences which code for a protein of about 75/65 kD, whereby those which contain the partial cDNA sequences shown in FIG. **4** are preferred. Especially preferred DNA sequences in this case are the sequences of the open reading frame of nucleotide 2 to 1,177. The peptides IIA, IIC, IIE, IIF, IIG and IIH are coded by the partial cDNA sequence in FIG. **4**, whereby the insignificant deviations in the experimentally determined amino acid sequences are based on the cDNA-derived sequence with highest probability from the limited resolution of the gas phase sequencing. DNA sequences which code for insoluble (deposited on Oct. 17, 2006 with the American Type Culture Collection under Accession No. PTA 7942) as well as soluble fractions of TNF-binding proteins having an apparent molecular weight of 65 kD/75 kD are also preferred. DNA sequences for such soluble fragments can be determined on the basis of the amino acid sequences derived from the nucleic acid sequences coding for such non-soluble TNF-BP.

The invention is also concerned with DNA sequences which comprise a combination of two partial DNA sequences, with one of the partial sequences coding for those soluble fragments of non-soluble proteins which bind TNF (see above) and the other partial sequence coding for all domains other than the first domain of the constant region of the heavy chain of human immunoglobulins such as IgG, IgA, IgM or IgE, in particular IgG_1 or IgG_3 subtypes.

The present invention is also concerned with the recombinant proteins coded by any of DNA sequences described above. Of course, there are thereby also included such proteins in whose amino acid sequences amino acids have been exchanged, for example by planned mutagenesis, so that the activity of the TNF-BP or fragments thereof, namely the binding of TNF or the interaction with other membrane components participating in the signal transfer, have been altered or maintained in a desirable manner. Amino acid exchanges in proteins and peptides which do not generally alter the activity of such molecules are known in the state of the art and are described, for example, by H. Neurath and R. L. Hill in "The'Proteins" (Academic Press, New York, 1979, see especially FIG. **6**, page 14). The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse. The present invention is also concerned with vectors which contain any of the DNA sequences described above in accordance with the invention and which are suitable for the transformation of suitable pro- and eukaryotic host systems, whereby there are preferred those vectors whose use leads to the expression of the proteins which are coded by any of the DNA sequences described above in accordance with the invention. Finally, the present invention is also concerned with pro- and eukaryotic host systems transformed with such vectors, as well as a process for the production of recombinant compounds in accordance with the invention by cultivating such host systems and subsequently isolating these compounds from the host systems themselves or their culture supernatants.

An object of the present invention are also pharmaceutical preparations which contain at least one of these TNF-BPs or fragments thereof, if desired in combination with other pharmaceutically active substances and/or non-toxic, inert, therapeutically compatible carrier materials.

Finally, the present invention is concerned with the use of such a TNF-BP on the one hand for the production of pharmaceutical preparations and on the other hand for the treatment of illnesses, preferably those in which TNF is involved in their course.

Starting materials for the TNF-BP in accordance with the invention are quite generally cells which contain such TNF-BP [in membrane-bound form] and which are generally accessible without restrictions to a person skilled in the art, such as, for example, HL60 [ATCC No. CCL 240], U 937 [ATCC No. CRL 1593], SW 480 [ATCC No. CCL 228] and HEp2 cells [ATCC No. CCL 23]. These cells can be cultivated according to known methods of the state of the art [40] or, in order to produce high cell densities, according to the procedure already described generally and described in detail in. Example 2 for HL60 cells. TNF-BP can then be extracted from the cells, which are centrifuged-off from the medium and washed, according to known methods of the state of the art using suitable detergents, for example Triton X-114, 1-0-n-octyl-β-D-glucopyranoside (octylglucoside) or 3-[(3-cholylamido-propyl)-dimethylammonio]-1-propane sulphonate (CHAPS), especially using Triton X-100. For the detection of such TNF-BP there can be Used the usually used detection methods for TNF-BP, for example a polyethylene glycol-1-induced precipitation of the ^{125}I-TNF/TNF-BP complex [27], especially filter-binding tests with radioactively labelled TNF according to Example 1. In order to produce the TNF-BP in accordance with the invention, the general methods of the state of the art used for the purification of proteins, especially of membrane proteins, such as, for example, ion exchange chromatography, gel filtration, affinity chromatography, HPLC and SDS-PAGE can be used. Especially preferred methods for the production of TNF-BP in accordance with the invention are affinity chromatography, especially with TNF-a as the ligand bound to the solid phase, and immune-affinity chromatography, HPLC and SDS-PAGE. The elution of TNF-BP bands which are separated

using SDS-PAGE can be effected according to known methods of protein chemistry, for example using electroelution according to Hunkapiller et al. [34], whereby according to present knowledge the electro-dialysis times given there generally have to be doubled. Thereafter, traces of SDS which still remain can then be removed in accordance with Bosserhoff et al. [50].

The thus-purified TNF-BP can be characterized by methods of peptide chemistry which are known in the state of the art, such as, for example, N-terminal amino acid sequencing of enzymatic well as chemical peptide cleavage. Fragments obtained by enzymatic or chemical cleavage can be separated according to usual methods such as, for example, HPLC and can themselves be subjected to further N-terminal sequencing. Such fragments which themselves bind TNF can be identified using the afore-mentioned detection methods for TNF-BP and are likewise objects of the present invention.

Starting from the thus-obtained amino acid sequence information or the DNA and amino acid sequences given in FIG. 1 as well as in FIG. 4 there can be produced, taking into consideration the degeneracy of the genetic code, according to methods known in the state of the art suitable oligonucleotides [51]. By means of these, again according to known methods of molecular biology [42,43], cDNA or genomic DNA banks can be searched for clones which contain nucleic acid sequences coding for TNF-BP. More-over, using the polymerase chain reaction (PCR) [49] cDNA fragments can be cloned by completely degenerating the amino acid sequence of two spaced apart relatively short segments while taking into consideration the genetic code and introducing into their complementarity suitable oligo-nucleotides as a "primer", whereby the fragment lying between these two sequences can be amplified and identified. The determination of the nucleotide sequence of a such a fragment permits an independent determination of the amino acid sequence of the protein fragment for which it codes. The cDNA fragments obtainable by PCR can also, as already described for the oligonucleotides themselves, be used according to known methods to search for clones containing nucleic acid sequences coding for TNF-BP from cDNA or genomic DNA banks. Such nucleic acid sequences can then be sequenced according to known methods [42]. On the basis of the thus-determined sequences and of the already known sequences for certain receptors, those partial sequences which code for soluble TNF-BP fragments can be determined and cut out from the complete sequence using known methods [42].

The complete sequence or such partial sequences can then be integrated using known methods into vectors described in the state of the art for their multiplication and expression in prokaryotes [42]. Suitable prokaryotic host organisms are, for example, gram-negative and gram-positive bacteria such as, for example, *E. coli* strains such as *E. coli* HB101 [ATCC No. 33 694] or *E. coli* W3110 [ATCC No. 27 325] or *B. subtilis* strains.

Furthermore, nucleic acid sequences in accordance with the invention which code for TNF-BP as well as for TNF-BP fragments can be integrated using known methods into suitable vectors for reproduction and expression in eukaryotic host cells, such as, for example, yeast, insect cells and mammalian cells. Expression of such sequences is preferably effected in mammalian and insect cells.

A typical expression vector for mammalian cells contains an efficient promoter element in order to produce a good transcription rate, the DNA sequence to be expressed and signals for an efficient termination and polyadenylation of the transcript. Additional elements which can be used are "enhancers" which lead to again intensified transcription and sequences which e.g. can bring about a longer half life of the mRNA. For the expression of nucleic acid sequences in which the endogenous sequence fragment coding for a signal peptide is missing, there can be used vectors which contain such suitable sequences which code for signal peptides of other known proteins. See, for example, the vector pLJ268 described by Cullen, B. R. in Cell 46, 973-982 (1986) as well as Sharma, S. et al. in "Current Communications in Molecular Biology", edt. by Gething, M. J., Cold Spring Harbor Lab. (1985), pages 73-78.

Most of these vectors which are used for a transient expression of a particular DNA sequence in mammalian cells contain the replication source of the SV40 virus. In cells which express the T-antigen of the virus (e.g. COS cells), these vectors are reproduced abundantly. A transient expression is, however, not limited to COS cells. In principle any transfectable mammalian cell line can be used for this purpose. Signals which can bring about a strong transcription are e.g. the early and late promoters of SV40, the promoter and enhancer of the "major immediate-early" gene of HCMV (human cytomegalovirus), the LTR's ("long terminal repeats") of retroviruses such as, for example, RSV, HIV and MMTV. There can, however, also be used signals of cellular genes such as e.g. the promoters of the actin and collagenase genes.

Alternatively, however, stable cell lines which have the specific DNA sequence integrated into the genome (chromosome) can also be obtained. For this, the DNA sequence is cotransfected together with a selectable marker, e.g. neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthin guanine phosphoribosyl transferase (hgpt). The DNA sequence stably incorporated in the chromosome can also be reproduced abundantly. A suitable selection marker for this is, for example, dihydrofolate reductase (dhfr). Mammalian cells (e.g. CHO cells), which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transinfection has been effected. In this manner cell lines which contain more than a thousand copies of the desired DNA sequence can be obtained.

Mammalian cells which can be used for the expression are e.g. cells of the human cell lines Hela [ATCC CCL2] and 293 [ATCC CRL 1573] as well as 3T3 [ATCC CCL 163] and L cells, e.g. [ATCC CCL 149], (CHO) cells [ATCC CCL 61], BHK [ATCC CCL 10] cells as well as the CV 1 [ATCC CCL 70] and the COS cell lines [ATCC CRL 1650, CRL 1651].

Suitable expression vectors include, for example, vectors such as pBC12MI [ATCC 67 109], pSV2dhfr [ATCC 37 146], pSVL [Pharmacia, Uppsala, Sweden], pRSVcat [ATCC 37 152] and pMSG [Pharmacia, Uppsala, Sweden]. The vectors "pK19" and "pN123" used in Example 9 are especially preferred vectors. These can be isolated according to known methods from *E. coli* strains HB101(pK19) and HB101 (pN123) transformed with them [42]. These *E. coli* strains have been deposited on the 26 Jan. 1990 at the Deutschen Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) in Braunschweig, FRG, under DSM 5761 for HB101 (pK19) and DMS 5764 for HB101(pN123). For the expression of proteins which consist of a soluble fragment of non-soluble TNF-BP and an immunoglobulin fragment, i.e. all domains except the first of the constant region of the heavy chain, there are especially suitable pSV2-derived vectors as described, for example, by German, C. in "DNA Cloning" [Vol. II., edt. by Glover, D. M., IRL Press, Oxford, 1985]. The vectors pCD4-Hp (DSM 5315, deposited on 21 Apr. 1989), pDC4-Hγ1 (DSM 5314, deposited on 21 Apr. 1989) and pCD4-Hγ3 (DSM 5523, deposited on 14 Sep. 1989) which have been deposited at the Deutschen Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) in Braunsch-

weig, FRG, and which are described in detail in European Patent Application No. 90107393.2 are especially preferred vectors. This European Patent Specification and the equivalent Applications referred to in Example 11 also contain data with respect to the further use of these vectors for the expression of chimeric proteins (see also Example 11) and for the construction of vectors for the expression of such chimeric proteins with other immunoglobulin fragments.

The manner in which these cells are transfected depends on the chosen expression system and vector system. An overview of these methods is to be found e.g. in Pollard et al., "DNA Transformation of Mammalian Cells" in "Methods in Molecular Biology" [Nucleic Acids Vol. 2, 1984, Walker, J. M., ed, Humana, Clifton, N.J.]. Further methods are to be found in Chen and Okayama ["High-Efficiency Transformation of Mammalian Cells by Plasmid DNA", Molecular and Cell Biology 7, 2745-2752, 1987] and in Feigner [Feigner et al., "Lipofectin: A highly efficient, lipid-mediated. DNA-transfection procedure", Proc. Nat. Acad. Sci. USA 84, 7413-7417, 1987].

The baculovirus expression system, which has already been used successfully for the expression of a series of proteins (for an overview see Luckow and Summers, Bio/Technology 6, 47-55, 1988), can be used for the expression in insect cells. Recombinant proteins can be produced in authentic form or as fusion proteins. The thus-produced proteins can also be modified such as, for example, glycosylated (Smith et al., Proc. Nat. Acad. Sci. USA 82, 8404-8408, 1987). For the production of a recombinant baculovirus which expresses the desired protein there is used a so-called "transfer vector". Under this there is to be understood a plasmid which contains the heterologous DNA sequence under the control of a strong promoter, e.g. that of the polyhedron gene, whereby this is surrounded on both sides by viral sequences. The vectors "pN113", "pN119" and "pN124" used in Example 10 are especially preferred vectors. These can be isolated according to known methods from *E. coli* strains HB101(pN113), HB101(pN119) and HB101(pN124) transformed with them. These *E. coli* strains have been deposited on the 26 Jan. 1990 at the Deutschen Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) in Braunschweig, FRG, under DSM 5762 for HB101(pN113), DSM 5763 for HB101 (pN119) and DSM 5765 for HB101(pN124). The transfer vector is then transfected into the insect cells together with DNA of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. An overview of the baculovirus expression system and the methods used therein is to be found in Luckow and Summers [52].

Expressed TNF-BP as well as its non-soluble or soluble fractions can then be purified from the cell mass or the culture supernatants according to methods of protein chemistry which are known in the state of the art, such as, for example, the procedure already described on pages 5-6.

The TNF-BP obtained in accordance with the invention can also be used as antigens to produce polyclonal and monoclonal antibodies according to known-techniques [44, 45] or according to the procedure described in Example 3. Such antibodies, especially monoclonal antibodies against the 75 kD TNF-BP species, are also an object of the present invention. Those antibodies which are directed against the 75 kD TNF-BP can be used for the isolation of TNF-BP by modifications of the purification procedure described in detail in Examples 4-6 which are familiar to a person skilled in the art.

On the basis of the high binding affinity of TNF-BP in accordance with the invention for TNF (K_d value in the order of 10^{-9}-10^{-10} M), these or fragments thereof can be used as diagnostics for the detection of TNF in serum or other body fluids according to methods known in the state of the art, for example in solid phase binding tests or in combination with anti-TNF-BP antibodies in so-called "sandwich" tests.

Moreover, TNF-BP in accordance with the invention can be used on the one hand for the purification of TNF and on the other hand for the detection of TNF agonists and TNF antagonists according to procedures which are known in the state of the art.

The TNF-BP in accordance with the invention as well as their physiologically compatible salts, which can be manufactured according to methods which are known in the state of the art, can also be used for the production of pharmaceutical preparations, primarily those for the treatment of illnesses in which TNF is involved in their course. For this purpose, one or more of the said compounds, where desired or required in combination with other pharmaceutically active substances, can be processed in a known manner with the usually used solid or liquid carrier. materials. The dosage of such preparations can be effected having regard to the usual criteria in analogy to already used preparations of similar activity and structure.

Since the invention has been described hereinbefore in general terms, the following Examples are intended to illustrate details of the invention, but they are not intended to limit its scope in any manner.

Example 1

Detection of TNF-Binding Proteins

The TNF-BP were detected in a filter test with human radioiodinated ^{125}I-TNF. TNF (46, 47) was radioactively labelled with Na^{125}I (IMS40, Amersham, Amersham, England) and iodo gene (#28600, Pierce Eurochemie, Oud-Beijerland, Netherlands) according to Fraker and Speck [48]. For the detection of the TNF-BP, isolated membranes of the cells or their solubilized, enriched and purified fractions were applied to moist nitrocellulose filter (0.45µ, BioRad, Richmond, Calif., USA). The filters were then blocked in buffer solution with 1% skimmed milk powder and subsequently incubated with $5 \cdot 10^5$ cpm/ml of ^{125}I-TNFα ($0.3\text{-}1.0 \cdot 10^8$ cpm/µg) in two batches with and without the addition of 5 µg/ml of non-labelled TNFα, washed and dried in the air. The bound radio-activity was detected semiquantitatively by autoradiography or counted in a gamma-counter. The specific ^{125}I-TNF-α binding was determined after correction for unspecific binding in the presence of unlabelled TNF-α in excess. The specific TNF-binding in the filter test was measured at various TNF concentrations and analyzed according to Scatchard, whereby a K_d value of $\cdot 10^{-9}$-10^{-10} M was determined.

Example 2

Cell Extracts of HL-60 Cells

HL60 cells [ATCC No. CCL 240] were cultivated on an experimental laboratory scale in a RPMI 1640 medium [GIBCO catalogue No. 074-01800], which contained 2 g/l $NaHCO_3$ and 5% foetal calf serum, in a 5% CO_2 atmosphere, and subsequently centrifuged.

The following procedure was used to produce high cell densities on an industrial scale. The cultivation was carried out in a 75 l Airlift fermenter (Fa. Chemap, Switzerland) with a working volume of 58 l. For this there was used the cassette membrane system "PROSTAK" (Millipore, Switzerland)

with a membrane surface of 0.32 m^2(1 cassette) integrated into the external circulation circuit. The culture medium (see Table 1) was pumped around with a Watson-Marlow pump, Type 603U, with 5 l/min. After a steam sterilization of the installation, whereby the "PROSTAK" system was sterilized separately in autoclaves, the fermentation was started with growing HL-60 cells from a 20 l Airlift fermenter (Chemap). The cell cultivation in the inoculation fermenter was effected in a conventional batch process in the medium according to Table 1 and an initial cell titre of 2×10^5 cells/ml. After 4 days the HL60 batch was transferred with a titre of 4.9×10^6 cells/ml into the 75 l fermenter. The pH value was held at 7.1 and the pO_2 value was held at 25% saturation, whereby the oxygen introduction was effected through a microporous frit. After initial batch fermentation, on the 2nd day the perfusion at a cell titre of 4×10^6 cells/ml was started with 30 l of medium exchange per day. On the filtrate side of the medium the conditioned medium was removed and replaced by the addition of fresh medium. The added medium was fortified as follows: Primatone from 0.25% to 0.35%, glutamine from 5 mM to 6 mM and glucose from 4 g/l to 6 g/l. The perfusion rate was then increased on the 3rd and 4th day to 72 l of medium/day and on the 5th day to 100 l of medium/day. The fermentation had finished after 120 hours of continuous cultivation. Exponential cell growth up to 40×10^6 cells/ml took place under the given fermentation conditions. The duplication time of the cell population was 20-22 hours to 10×10^6 cells/ml and then increased to 30-36 hours with increasing cell density. The proportion of living cells lay at 90-95% during the entire fermentation period. The HL-60 batch was then cooled down in the fermenter to about 12° C. and the cells were harvested by centrifugation (Beckman centrifuge [Model J-6B, Rotor JS], 3000 rpm, 10 min., 4° C.).

TABLE 1

HL-60 medium	
Components	Concentrations mg/l
$CaCl_2$ (anhydrous)	112.644
$Ca(NO_3)_2 \cdot 4H_2O$	20
$CuSO_4 \cdot 5H_2O$	$0.498 \cdot 10^{-3}$
$Fe(NO_3)_3 \cdot 9H_2O$	0.02
$FeSO_4 \cdot 7H_2O$	0.1668
KCl	336.72
KNO_3	0.0309
$MgCl_2$ (anhydrous)	11.444
$MgSO_4$ (anhydrous)	68.37
NaCl	5801.8
Na_2HPO_4 (anhydrous)	188.408
$NaH_2PO_4 \cdot H_2O$	75
$Na_2SeO_3 \cdot 5H_2O$	$9.6 \cdot 10^{-3}$
$ZnSO_4 \cdot 7H_2O$	0.1726
D-Glucose	4000
Glutathion (red.)	0.2
Hepes buffer	2383.2
Hypoxanthin	0.954
Linoleic acid	0.0168
Lipoic acid	0.042
Phenol Red	10.24
Putrescine 2HCl	0.0322
Na pyruvate	88
Thymidine	0.146
Biotin	0.04666
D-Ca pantothenate	2.546
Choline chloride	5.792
Folic acid	2.86
i-Inositol	11.32
Niacinamide	2.6
Nicotinamide	0.0074
para-Aminobenzoic acid	0.2

TABLE 1-continued

HL-60 medium	
Components	Concentrations mg/l
Pyridoxal HCl	2.4124
Pyridoxin HCl	0.2
Riboflavin	0.2876
Thiamin HCl	2.668
Vitamin B_{12}	0.2782
L-Alanine	11.78
L-Aspartic acid	10
L-Asparagine H_2O	14.362
L-Arginine	40
L-Arginine HCl	92.6
L-Aspartate	33.32
L-Cystine 2HCl	62.04
L-Cysteine HCl • H_2O	7.024
L-Glutamic acid	36.94
L-Glutamine	730
L-Glycine	21.5
L-Histidine	3
L-Histidine HCl • H_2O	27.392
L-Hydroxypyroline	4
L-Isoleucine	73.788
L-Leucine	75.62
L-Lysine HCl	102.9
L-Methionine	21.896
L-Phenylalanine	43.592
L-Proline	26.9
L-Serine	31.3
L-Threonine	53
L-Tryptophan	11.008
L-Tyrosine • 2Na	69.76
L-Valine	62.74
Penicillin/streptomycin	100 U/ml
Insulin (human)	5 µg/ml
Tranferrin (human)	15 µg/ml
Bovine serum albumin	67 µg/ml
Primatone RL (Sheffield Products, Norwich NY, USA)	0.25%
Pluronic F68 (Serva, Heidelberg, FRG)	0.01%
Foetal calf serum	0.3-3%

The centrifugate was washed with isotonic phosphate buffer (PBS; 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 8.0 g/l NaCl, 2.16 g/l $Na_2HPO_4.7H_2O$), which had been treated with 5% dimethylformamide, 10 mM benzamidine, 100 U/ml aprotinin, 10 µM leupeptin, 1 µM pepstatin, 1 mM o-phenanthroline, 5 mM iodoacetamide, 1 mM phenyl-methylsulphonyl fluoride (referred to hereinafter as PBS-M). The washed cells were extracted at a density of $2.5\cdot10^8$ cells/ml in PBS-M with Triton X-100 (final concentration 1.0%). The cell extract was clarified by centrifugation (15,000×g, 1 hour; 100,000×g, 1 hour).

Example 3

Production of Monoclonal (TNF-BP) Antibodies

A centrifugation supernatant from the cultivation of HL60 cells on an experimental laboratory scale, obtained according to Example 2, was diluted with PBS in the ratio 1:10. The diluted supernatant was applied at 4° C. (flow rate: 0.2 ml/min.) to a column which contained 2 ml of Affigel 10 (Bio Rad Catalogue No. 153-6099) to which had been coupled 20 mg of recombinant human TNF-α [Pennica, D. et al. (1984) Nature 312, 724; Shirai, T. et al. (1985) Nature 313, 803; Wang, A. M. et al. (1985) Science 228, 149] according to the recommendations of the manufacturer. The column was washed at 4° C. and a throughflow rate of 1 ml/min firstly with 20 ml of PBS which contained 0.1% Triton X 114 and there-

after with 20 ml of PBS. Thus-1-enriched TNF-BP was eluted at 22° C. and a flow rate of 2 ml/min with 4 ml of 100 mM glycine, pH 2.8, 0.1% decyl-maltoside. The eluate was concentrated to 10 µl in a Centricon 30 unit [Amicon].

10 µl of this eluate were mixed with 20 µl of complete Freund's adjuvant to give an emulsion. 10 µl of the emulsion were injected according to the procedure described by Holmdahl, R. et al. [(1985), J. Immunol. Methods 32, 379] on each of days 0, 7 and 12 into a hind paw of a narcotized Balb/c mouse.

The immunized mice were sacrificed on day 14, the popliteal lymph nodes were removed, minced and suspended by repeated pipetting in Iscove's medium (IMEM, GIBCO Catalogue No. 074-2200) which contained 2 g/l $NaHCO_3$. According to a modified procedure of De St. Groth and Scheidegger [J. Immunol. Methods (1980), 35, 1] 5×10^7 cells of the lymph nodes were fused with 5×10^7PAI mouse myeloma cells (J. W. Stocker et al., Research Disclosure, 217, May 1982, 155-157) which were in logarithmic growth. The cells were mixed, collected by centrifugation and resuspended in 2 ml of 50% (v/v) polyethylene glycol in IMEM at room temperature by slight shaking and diluted by the slow addition of 10 ml of IMEM during careful shaking for 10 minutes. The cells were collected by centrifugation and resuspended in 200 ml of complete medium [IMEM+20% foetal calf serum, glutamine (2.0 mM), 2-mercaptoethanol (100 µl), 100 µM hypoxanthine, 0.4 µM aminopterine and 16 µM thymidine (HAT)]. The suspension was distributed on 10 tissue 10 culture dishes each containing 96 wells and incubated at 37° C. for 11 days without changing the medium in an atmosphere of 5% CO_2 and a relative humidity of 98%.

The antibodies are distinguished by their inhibitory action on the binding of TNF to HL60 cells or by their binding to antigens in the filter test according to Example 1. The following procedure was used to detect the biological activity of anti(TNF-BP) antibodies: 5×10^6 HL60 or U937 cells were incubated in complete RPMI 1640 medium together with affinity-purified monoclonal anti-(TNF-BP) antibodies or control antibodies (i.e. those which are not directed against TNF-BP) in a concentration range of 1 ng/ml to 10 µg/ml. After incubation at 37° C. for one hour the cells were collected by centrifugition and washed with 4.5 ml of PBS at 0° C. They were resuspended in 1 ml of complete RPMI 1640 medium (Example 2) which additionally contained 0.1% sodium azide and ^{125}I-TNFα (10^6 cpm/ml) with or without the addition of unlabelled TNFα (see above). The specific radioactivity of the ^{125}I-TNFα amounted to 700 Ci/mmol. The cells were incubated at 4° C. for 2 hours, collected and washed 4 times at 0° C. with 4.5 ml of PBS which contained 1% BSA and 0.001% Triton X 100 (Fluka). The radioactivity bound to the cells was measured in a γ-scintillation counter. The cell-bound radioactivity of cells which had not been treated with anti-(TNF-BP) antibodies was determined in a comparative experiment (approximately 10 000 cpm/5×10^6 cells).

Example 4

Affinity Chromatography

For the further purification, a monoclonal anti-(55 kD TNF-BP) antibody (2.8 mg/ml gel), obtained according to Example 3, TNFα (3.9 mg/ml gel) and bovine serum albumin (BSA, 8.5 mg/ml gel) were each covalently coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the directions of the manufacturer. The cell extract obtained according to Example 2 was passed through the thus-prepared columns which were connected in series in the following sequence: BSA-Sepharose pre-column, immune affinity column (anti-(55 kD-TNF-BP) antibody), TNFα-ligand affinity column. After completion of the application the two last-mentioned columns were separated and washed individually with in each case 100 ml of the following buffer solutions: (1) PBS, 1,0% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin; (2) PBS, 0.1% Triton X-100, 0.5M NaCl, 10 mM ATP, 10 mM benzamidine, 100 U/ml aprotinin; and (3) PBS, 0.1% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin. Not only the immune affinity column, but also the TNFα-ligand affinity column were then each eluted with 100 mM glycine pH 2.5, 100 mM NaCl, 0.2% decylmaltoside, 10 mM benzamidine, 100 U/ml aprotinin. The fractions of each column which were active in the filter test according to Example 1 were thereafter combined and neutralized with 1M Tris pH 8.0.

The thus-combined TNF-BP active fractions of the immune affinity chromatography on the one hand and of the TNFα-ligand affinity chromatography on the other hand were, for further purification, again applied to in each case one small TNFa-ligand affinity column. Thereafter, these two columns were washed with in each case 40 ml of (1) PBS, 1.0% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin, (2) PBS, 0.1% Triton X-100, 0.5M NaCl, 10 mM ATP, 10 mM benzamidine, 100 U/ml aprotinin, (3) PBS, 0.1% Triton X-100, (4) 50 mM Tris pH 7.5, 150 mM NaCl, 1.0% NP-40, 1.0% desoxycholate, 0.1% SDS, (5) PBS, 0.2% decyl-maltoside. Subsequently, the columns were eluted with 100 mM glycine pH 2.5, 100 mM NaCl, 0.2% decylmaltoside. Fractions of 0.5 ml from each column were collected and the fractions from each column which were active according to the filter test (Example 1) were combined and concentrated in a Centricon unit (Amicon, molecular weight exclusion 10,000).

Example 5

Separation by Means of HPLC

The active fractions obtained according to Example 4 were each applied according to their different source (immune or ligand affinity chromatography) to C1/C8 reversed phase HPLC columns (ProRPC, Pharmacia, 5×20 mm) which had been equilibrated with 0.1% trifluoroacetic acid, 0.1% octylglucoside. The columns were then eluted with a linear acetonitrile gradient (0-80%) in the same buffer at a flow of 0.5 ml/min. Fractions of 1.0 ml were collected from each column and the active fractions from each column were combined (detection according to Example 1).

Example 6

Separation by Means of SDS-PAGE

The fractions which were obtained according to Example 5 and which were active according to the filter test (Example 1) were further separated by SDS-PAGE according to [34]. For this purpose, the samples were heated to 95° C. for 3 minutes in SDS sample buffer and subsequently separated electrophoretically on a 12% acrylamide separation gel with a 5% collection gel. The following standard proteins were used as a reference for the determination of the apparent molecular weights on the SDS-PAGE gel: phosphorylase B (97.4 kD), BSA (66.2 kD), ovalbumin (42.7 kD), carboanhydrase (31.0 kD), soya trypsin inhibitor (21.5 kD) and lysozyme (14.4 kD).

Under the mentioned conditions there were obtained for samples which has been obtained according to Example 4 by TNF-α-ligand affinity chromatography of immune affinity chromatography eluates and which had been further separated by HPLC according to Example 5 two bands of 55 kD and 51 kD as well as three weaker bands of 38 kD, 36 kD and 34 kD. These bands were transferred electro-phoretically during 1 hour at 100 V in 25 mM Tris, 192 mM glycine, 20% methanol on to a PVDF membrane (Immobilon, Millipore, Bedford, Mass. USA) in a Mini Trans Blot System (BioRad, Richmond, Calif., USA). Thereafter, the PVDF membrane was either protein-stained with 0.15% Serva-Blue (Serva, Heidelberg, FRG) in methanol/water/glacial acetic acid (50/40/10 parts by volume) or blocked with skimmed milk powder and subsequently incubated with ^{125}I-TNFα according to the filter test conditions described in Example 1 in order to detect bands having TNF-BP activity. This showed that all bands produced in the protein staining bonded TNFα specifically. In the Western blot according to Towbin et al. [38] all of these bands also bonded the monoclonal anti-55 kD-TNF-BP antibody produced according to Example 3. In this case, a procedure according to that described in Example 1 with Na^{125}I radioactively-labelled, affinity-purified (mouse immuno-globulin-Sepharose-4B affinity column) rabbit-anti-mouse-immunoglobulin antibody was used for the autoradiographic detection of this antibody.

Samples which had been obtained according to Example 4 by two-fold TNF-α-ligand affinity chromatography of the throughput of the immune affinity chromatography and which had been further separated by HPLC according to Example 5 showed under the above-specified SDS-PAGE and blot transfer conditions two additional bands of 75 kD and 65 kD, both of which bonded TNF specifically in the filter test (Example 1). In the Western blot according to Towbin et al. (see above) the proteins of these two bands did not react with the anti-(55 kD TNF-BP) antibody produced according to Example 3. They reacted, however, with a monoclonal antibody which had been produced starting from the 75 kD band (anti-75 kD TNF-BP antibody) according to Example 3.

Example 7

Amino Acid Sequence Analysis

For the amino acid sequence analysis, the fractions which had been obtained according to Example 5 and which were active according to the filter test (Example 1) were separated using the SDS-PAGE conditions described in Example 6, but now reducing (SDS sample buffer with 125 mM dithiothreitol). The same bands as in Example 6 were found, but because of the reducing conditions of the SDS-PAGE in comparison to Example 6 all showed an about 1-2 kD higher molecular weight. These bands were then transferred according to Example 6 on to PVDF membranes and stained with 0.15% Serva-Blue in methanol/water/glacial acetic acid (50/400/10 parts by volume) for 1 minute, decolorized with methanol/water/glacial acetic acid (45/48/7 parts by volume), rinsed with water, dried in air and thereafter cut out. The conditions given by Hunkapiller [34] were adhered to in all steps in order to avoid N-terminal blocking. Initially, the purified TNF-BP were used unaltered for the amino acid sequencing. In order to obtain additional sequence information, the TNF-BP after reduction and S-carboxymethylation [Jones, B. N. (1986) in "Methods of Protein Micro-characterisation", J. E. Shively, ed., Humana Press, Clifton N.J., 124-125] were cleaved with cyanogen bromide (Tarr, G. E. in "Methods of Protein Micro-characterisation", 165-166, loc. cit.), trypsin and/or protease K and the peptides were separated by HPLC according to known methods of protein chemistry. Thus-prepared samples were then sequenced in an automatic gas phase microsequencing apparatus (Applied Biosystems Model 470A, ABI, Foster City, Calif., USA) with an on-line automatic HPLC PTH amino acid analyzer (Applied Biosystems Model 120, ABI see above) connected to the outlet, whereby the following amino acid sequences were determined:

1. For the 55 kD band (according to non-reducing SDS-PAGE):

   Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5),

   and

   Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6) in which X stands for an amino acid residue which could not be determined,

2. for the 51 kD and 38 kD bands (according to non-reducing SDS-PAGE):

   Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu (SEQ ID NO: 15)

3. for the 6510 band (according to non-reducing SDS-PAGE)

   In the N-terminal sequencing of the 6510 band two parallel sequences were determined up to the 15th residue without interruption. Since one of the two sequences corresponded to a partial sequence of ubiquitin [36,37], the following sequence was derived for the 65 kD band:

   Leu-Pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys. (SEQ ID NO: 10)

   in which X stands for an amino acid residue which could not be determined.

Additional peptide sequences for 75(65)kDa-TNF-BP were determined:

Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

and

Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7) and Val-Phe-Cys-Thr (SEQ ID NO: 8)

and

Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9) and Leu-Cys-Ala-Pro (SEQ ID NO: 12)

and

Val-Pro-His-Leu-Pro-Ala-Asp (SEQ ID NO: 13)

and

Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14), in which X stands for an amino acid residue which could not be determined.

Example 8

Determination of Base Sequences of Complementary DNA (cDNA)

Starting from the amino acid sequence according to formula IA there were synthesized having regard to the genetic code for the amino acid residues 2-7 and 17-23 corresponding completely degenerated oligonucleotides in suitable complementarity ("sense" and "antisense" oligonucleotides). Total cellular RNA was isolated from HL60 cells [42,43] and the first cDNA strand was synthesized by oligo-dT priming or by priming with the "antisense" oligonucleotide using a cDNA synthesis kit (RPN 1256, Amersham, Amersham; England) according to the instructions of the manufacturer. This cDNA strand and the two synthesized degenerate "sense" and "antisense" oligonucleotides were used in a polymerase chain reaction (PCR, Perkin Elmer Cetus, Norwalk, Conn., USA

according to the instructions of the manufacturer) to synthesize as a cDNA fragment the base sequence coding for the amino acid residues 8-16 (formula IA). The base sequence of this cDNA fragment accorded to: 5'-AGGGAGAA-GAGAGATAGTGTGTGTCCC-3' (SEQ ID NO: 16). This cDNA fragment was used as a probe in order to identify according to a known procedure a cDNA clone coding for the 55 kD TNF-BP in a Xgt11-cDNA gene bank from human placenta (42, 43). This clone was then cut according to usual methods from the X-vector and cloned in the plasmids pUC18 (Pharmacia, Uppsala, Sweden) and pUC19 (Pharmacia, Uppsala, Sweden) and in the M13 mp 18/M13 mp 19 bacteriophage (Pharmacia, Uppsala, Sweden) (42, 43). The nucleotide sequence of this cDNA clone was determined using a Sequenase kit (U.S. Biochemical, Cleveland, Ohio, USA) according to the details of the manufacturer. The nucleotide sequence and the amino acid sequence derived therefrom for the 55 kD TNF-BP and its signal peptide (amino acid "−28" to amino acid "0") is given in FIG. **1** using the abbreviations for bases such as amino acids usual in the state of the art. From sequence comparisons with other already known receptor protein sequences there can be determined a N-terminal domain containing approximately 180 amino acids and a C-terminal domain containing 220 amino acids which are separated from one another by a transmembrane region of 19 amino acids (underlined in FIG. **1**) which is typical according to the sequence comparisons. Hypothetical glycosylation sites are characterized in FIG. **1** by asterisks above the corresponding amino acid.

Essentially analogous techniques were used to identify 75/65 kD TNF-BP-coding partial cDNA sequences, whereby however, in this case genomic human DNA and completely degenerated 14-mer and 15-mer "sense" and "antisense" oligonucleotides derived from peptide IIA were used in order to produce a primary 26 by cDNA probe in a polymerase chain reaction. This cDNA probe was then used in a HL-60 cDNA library to identify cDNA clones of different lengths. This cDNA library was produced using isolated HL60 RNA and a cDNA cloning kit (Amersham) according to the details of the manufacturer. The sequence of such a cDNA clone is given in FIG. **4**, whereby repeated sequencing lead to the following correction. A threonine coded by "ACC" not "TCC", has to be at position 3 instead of the serine.

Example 9

Expression in COS 1 Cells

Vectors starting from the plasmid "pN11" were constructed for the expression in COS cells. The plasmid "pN11" contains the efficient promoter and enhancer of the "major immediate-early" gene of human cytomegalovirus ("HCMV"; Boshart et al., Cell 41, 521-530, 1985). After the promoter there is situated a short DNA sequence which contains several restriction cleavage sites, which are present only once in the plasmid ("polylinker"), inter alia the cleavage sites for HindIII, BalI, BamHI and PvuII (see sequence).

PvuII
5'-AAGCTTGGCCAGGATCCAGCTGACT-
GACTGATCGCGAGATC-3' (SEQ ID NO: 17)
3'-TTCGAACCGGTCCTAGGTCGACTGACT-
GACTAGCGCTCTAG-5' (SEQ ID NO: 18)

After these cleavage sites there are situated three translation stop codons in all three reading frames. After the polylinker sequence there is situated the 2nd intron and the polyadenylation signal of the preproinsulin gene of the rat (Lomedico et al., Cell 18, 545-558, 1979). The plasmid also contains the replication origin of the SV40 virus and a fragment from pBR322 which confers *E. coli*-bacteria ampicillin resistance and permits the replication of the plasmid in *E. coli*.

For the construction of the expression vector "pN123", this plasmid "pN11" was cleaved the restriction endo-nuclease PvuII and subsequently treated with alkaline phosphatase. The dephosphorylated vector was thereafter isolated from an agarose gel (V1). The 5'-projecting nucleotides of the EcoRI-cleaved 1.3 kb fragment of the 55 kD TNF-BP-cDNA (see Example 8) were filled in using Klenow enzyme. Subsequently, this fragment was isolated from an agarose gel (F1). Thereafter, V1 and F1 were joined together using T4-ligase. *E. coli* HB101 cells were then transformed with this ligation batch according to known methods [42]. By means of restriction analyses and DNA sequencing according to known methods [42] there were identified transformants which had been transformed with a plasmid and which contained the 1.3 kb EcoRI fragment of the 55 kD TNF-BP-cDNA in the correct orientation for expression via the HCMV-promoter. This vector received the designation "pN123".

The following procedure was used for the construction of the vector "pK19". A DNA fragment which contained only the cDNA coding for the extracellular part of the 55 kD TNF-BP (amino acids −28 to 182 according to FIG. **1**) was obtained by PCR technology (Saiki et al., Science 230, 1350-1354, 1985, see also Example 8). The following oligonucleotides were used in order to amplify the cDNA from "pN123" coding for the extracellular part of the 55 kD TNF-BP:

BAMHI 5'-CACAGGGATCCATAGCTGTCTG-
GCATGGGCCTCTCCAC-3' (SEQ ID NO: 19)
ASP718
3'-CGTGACTCCTGAGTCCGTGGTGTAT-
TATCTCTAGACCA TGGCCC-5' (SEQ ID NO: 20)

By means of these oligonucleotides there were also introduced two stop codons of the translation after amino acid 182. The thus-amplified DNA fragment was cleaved with BamHI and Asp718, the thereby resulting projecting ends were filled in using Klenow enzyme and this fragment was subsequently isolated from an agarose gel (F2). F2 was then ligated with V1 and the entire batch was used for the transformation of *E. coli* HB101, as already described. Transformants which had been transformed with a plasmid containing the DNA fragment in the correct orientation for the expression via the HCMV-promoter were identified by DNA sequencing (see above). The plasmid isolated therefrom received the designation "pK19".

Transfection of the COS cells with the plasmids "pN123" or "pK19" was carried out according to the lipofection method published by Feigner et al. (Proc. Natl. Acad. Sci. USA 84, 7413-7417, 1987). 72 hours after the transfection had been effected the cells transfected with "pN123" were analyzed for binding with ^{125}I-TNFα according to known methods. The results of the Scatchard analysis [Scatchard, G., Ann. N.Y. Acad. Sci. 51, 660, 1949] of the thus-obtained binding data (FIG. **2**A) is given in FIG. **2**B. The culture supernatants of the cells transfected with "pK19" were investigated in a "sandwich" test. For this purpose, PVC microtitre plates (Dynatech, Arlington, Va., USA) were sensitized with 100 µl/well of a rabbit-anti-mouse immunoglobulin (10 µg/ml PBS). Subsequently, the plates were washed and incubated (3 hours, 20° C.) with an anti-55 kD TNF-BP antibody which had been detected by its antigen binding and isolated according to Example 3, but which did not inhibit the TNF-binding to cells. The plates were then again washed and incubated overnight at 4° C. with 100 µl/well of the culture supernatant (diluted 1:4 with buffer A containing 1% skimmed milk powder: 50 mM Tris/HCl pH 7.4, 140 mM

NaCl, 5 mM EDTA, 0.02% Na azide). The plates were emptied and incubated at 4° C. for 2 hours with buffer A containing ^{125}I-TNFα (10^6 cpm/ml, 100 μl/well) with or without the addition of 2 μg/ml of unlabelled TNF. Thereafter, the plates were washed 4 times with PBS, the individual wells were cut out and measured in a λ-counter. The results of 5 parallel transfections (columns # 2, 3, 4, 6 and 7), of two control transfections
with the pN11 vector (columns # 1, 5) and of a control with HL60 cell lysate (column # 8) are given in FIG. 3.

Example 10

Expression in Insect Cells

The plasmid "pVL941" (Luckow and Summers, 1989, "High Level Expression of Nonfused Foreign Genes with *Autographa california* Nuclear Polyhedrosis virus Expression Vectors", Virology 170, 31-39) was used for the expression in a baculovirus expression system and was modified as follows. The single EcoRI restriction cleavage site in "pVL941" was removed by cleaving the plasmid with EcoRI and the projecting 5'-end was filled in with Klenow enzyme. The plasmid pVL941/E obtained therefrom was digested with BamHI and Asp718 and the vector trunk was subsequently isolated from an agarose gel. This fragment was ligated with a synthetic oligonucleotide of the following sequence:

BamHI EcoRI Asp718
5'-GATCCAGAATTCATAATAG-3' (SEQ ID NO: 21)
3'-GTCTTAAGTATTATCCATG-5' (SEQ ID NO: 22)

E. coli HB101 was transformed with the ligation batch and transformants containing a plasmid in which the oligonucleotide had been incorporated correctly were identified by restriction analysis and DNA sequencing according to known methods (see above); this plasmid was named "pNR704". For the construction of the transfer vector "pN113", this plasmid "pNR704" was cleaved with EcoRI, treated with alkaline phosphatase and the thus-1-produced vector trunk (V2) was subsequently isolated from an agarose gel. The 1.3 kb fragment of the 55 kD TNF-BP-cDNA cleaved with EcoRI as above was ligated with fragment V2. Transformants obtained with this ligation batch, which contained a plasmid containing the cDNA insert in the correct orientation for the expression via the polyhedron promoter, were identified (see above). The vector isolated therefrom received the designation "pN113".

The following procedure was used for the construction of the transfer vector "pN119". The 1.3 kb EcoRI/EcoRI fragment of the 55 kD TNF-BP cDNA in the "pUC19" plasmid (see Example 8) was digested with BanI and ligated with the following synthetic oligonucleotide:

BanI Asp718
5'-GCACCACATAATAGAGATCTGGTACCGGGAA-3' (SEQ ID NO: 23)
3'-GTGTATTATCTCTAGACCATGGCCC-5' (SEQ ID NO: 24)

Two stop codons of the translation after amino acid 182 and a cleavage site for the restriction endo-nuclease Asp718 are incorporated with the above adaptor. After carrying out ligation the batch was digested with EcoRI and Asp718 and the partial 55 kD TNF-BP fragment (F3) was isolated. Furthermore, the plasmid "pNR704", likewise cleaved with Asp718 and EcoRI, was ligated with F3 and the ligation batch was transformed into *E. coli HB*101. The identification of the transformants which contained a plasmid in which the partial 55 kD TNF-BP cDNA had been correctly integrated for the expression was effected as already described. The plasmid isolated from these transformants received the name "pN119".

The following procedure was used for the construction of the transfer vector "pN124". The cDNA fragment coding for the extracellular part of the 55 kD TNF-BP, described in Example 9, was amplified with the specified oligo-nucleotides with the aid of PCR technology as described in Example 9. This fragment was cleaved with BamHI and Asp718 and isolated from an agarose gel (F4). The plasmid "pNR704" was also cleaved with BamHI and Asp718 and the vector trunk (V4) was isolated (see above). The fragments V4 and F4 were ligated, *E. coli* HB101 was transformed therewith and the recombinant transfer vector "pN124" was identified and isolated as described.

The following procedure was used for the transfection 10 of the insect cells. 3 μg of the transfer vector "pN113" were transfected with 1 μg of DNA of the *Autographa californica* nuclear polyhedrosisvirus (AcMNPV) (EP 127839) in Sf9 cells (ATCC CRL 1711). Polyhedron-negative viruses were identified and purified from "plaques" [52]. Sf9 cells were again infected with these recombinant viruses as described in [52]. After 3 days in the culture the infected cells were investigated for TNF-binding using ^{125}I-TNFα. For this purpose, the transfected cells were washed from the cell culture dish with a Pasteur pipette and resuspended at a cell density of 5×10^6 cells/ml of culture medium [52] which contained 10 ng/ml of ^{125}I-TNF-α, not only in the presence of, but also in the absence of 5 μg/ml of non-labelled TNF-α and incubated on ice for 2 hours. Thereafter, the cells were washed with pure culture medium and the cell-bound radio-activity was counted in a γ-counter (see Table 2).

TABLE 2

Cells	Cell-bound radioactivity per 10^6 cells
Non-infected cells (control)	60 cpm
Infected cells	1600 ± 330 cpm [1)]

[1)] Average and standard deviation from 4 experiments

Example 11

Analogously to the procedure described in Example 9, the cDNA fragment coding for the extracellular region of the 55 kDa TNF-BP was amplified in a polymerase chain reaction, but now using the following oligonucleotides as the primer:
Oligonucleotide 1:
Sst I 5'-TAC GAG CTC GGC CAT AGC TGT CTG GCA TG-3' (SEQ ID NO: 25)
Oligonucleotide 2:
Sst I 5'-ATA GAG CTC TGT GGT GCC TGA GTC CTC AG-3' (SEQ ID NO: 26)

This cDNA fragment was ligated in the pCD4-Hγ3 vector [DSM 5523; European Patent Application No. 90107393.2; Japanese Patent Application No. 108967/90; U.S. Pat. No. 51,077,390] from which the CD4-cDNA had been removed via the SstI restriction cleavage sites. SstI cleavage sites are situated in vector pCD4-Hγ3 not only in front of, but also behind the CD4-partial sequence fragment. The construction was transfixed in J558 myeloma cells (ATCC No. TIB6) by means of protoplast fusion according to Oi et al. (Procd. Natl. Acad. Sci. USA 80, 825-829, 1983). Transfectants were selected by adding 5 μg/ml of mycophenolic acid and 250 g/ml of xanthin (Traunecker et al., Eur. J. Immunol. 16, 851-

854 [1986]) in basic medium (Dulbecco's modified Eagle's Medium, 10% foetal calf serum, 5×10^{-5}M 2-mercaptoethanol). The expression product secreted by the transfixed cells could be purified using usual methods of protein chemistry, e.g. TNF-BP-antibody affinity chromatography. Unless not already specifically indicated, standard procedures as described e.g. by Freshney, R. I. in "Culture of Animal Cells", Alan R. Liss, Inc., New York (1983) were used for the cultivation of the cell lines employed, for the cloning, for the selection or for the expansion of the cloned cells.

REFERENCES

1. G. E. Nedwin, S. L. Naylor, A. Y. Sakaguchi, D. Smith, Jarrett-Nedwin, D. Pennica, D. V. Goeddel and P. W. Gray: Nucl. Acids Res. 12, 6361, 1985
2. B. Beutler and A. Cerami: New England J. Med. 316, 379, 1987
3. L. J. Old: Science 230, 630, 1985
4. G. Trinchieri, M. Kobayashi, M. Rosen, R. Loudon, M. Murphy and B. Perussia: J. exp. Med. 164, 1206, 1986
5. J. Vilcek, V. J. Palombella, D. Henriksen-de Stefano, C. Swenson, R. Feinman, M. Hirai and M. Tsujimoto: J. exp. Med. 163, 632, 1986
6. B. J. Sugarman, B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino and H. M. Shepard: Science 230, 943, 1985
7. J. R. Gamble, J. M. Harlan, S. J. Klebanoff and M. A. Vadas: Proc. Natl. Acad. Sci. USA 82, 8667, 1985
8. N. Sato, T. Goto, K. Haranaka, N. Satomi, H. Nariuchi, Y. Mano and Y. Sawasaki: J. Natl. Cancer Inst. 76, 1113, 1986
9. A. H. Stolpen, E. C. Guinan, W. Fiers and J. S. Pober: Am. J. Pathol. 123, 16, 1986
10. J. S. Pober, L. A. Lapierre, A. H. Stolpen, T. A. Brock, T. A. Springer, W. Fiers, M. P. Bevilacqua, D. L. Mendrick and M. A Gimbrone: J. Immunol. 138, 3319, 1987
11. M. Kawakami, P. Pekala, M. Lane and A. Cerami: Proc. Natl. Acad. Sci. USA 22, 912, 1982
12. T. Collins, L. A. Lapierre, W. Fiers, J. L. Strominger and J. S Pober: Proc. Natl. Acad. Sci. USA 83, 446, 1986
13. G. H. W. Wong and D. V. Goeddel: Nature 323, 819, 1986
14. J. W. Lowenthal, D. W. Ballard, E. B•hnlein and W. C. Greene: Proc. Natl. Acad. Sci. USA ja, 2331, 1989
15. M. J. Lenardo, C. M. Fan, T. Maniatis and D. Baltimore: Cell 51, 287, 1989
16. A. E. Goldfeld and T. Maniatis: Proc. Natl. Acad. Sci. USA 86, 1490, 1989
17. A. Waage, A. Halsteuren and T. Espevik: Lancet, Febr. 14, 1987, 355,
18. C. O. Jacob and H. O. McDevitt: Nature 331, 356, 1988
19. G. E. Grau, L. F. Fajardo, P. Piguet, B. Allet, P. Lambert and P. Vassalli: Science 237, 1210, 1987
20. B. Beutler, I. W. Milsark and A. C. Cerami: Science 229, 869, 1985
21. B. B. Aggarwal, T. E. Eessalu and P. E. Hass: Nature 318, 665, 1985
22. M. Tsujimoto, Y. K. Yip and J. Vilcek: Proc. Natl. Acad. Sci. USA 82, 7626, 1985
23. C. Baglioni, S. McCandless, J. Tavernier and W. Fiers: J. Biol. Chem. 260, 13395, 1985
24. P. Hohmann, R. Remy, M. Brockhaus and A. P. G. M. van Loon: J. Biol. Chem., im Druck
25. F. C. Kull, S. Jacobs and P. Cuatrecasas: Proc. Natl. Acad. Sci. USA 82, 5756, 1985
26. A. A. Creasy, R. Yamamoto and Ch. R. Vitt: Proc. Natl. Acad. Sci. USA 84, 3293, 1987
27. G. B. Stauber, R. A. Aiyer and B. B. Aggarwal: J. Biol. Chem. 263, 19098, 1988
28. K. Hirano, K. Yamamoto, Y. Kobayashi and T. Osawa: J. Biochem. 105, 120, 1989
29. Y. Niitsu, N. Watanabe, H. Sone, H. Neda, N. Yamauchi, M. Maeda and I. Urushizaki: J. Biol. Resp. Modifiers 7, 276, 1988
30. I. Olsson, A. Grubb, U. Gullberg, M. Lantz, E. Nilsson, C. Peetre and H. Thysell: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. January 1989
31. H. R. Loetscher and M. Brockhaus: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. Januar 1989
32. M. Brockhaus, H. Loetscher, H.-P. Hohmann and W. Hunziker: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. January 1989
33. C. R. Cantor and P. R. Schimmel, in Biophysical Chemistry, W.H. Freeman, ed., San Francisco, 1980, p. 850
34. M. W. Hunkapiller, E. Lujan, F. Ostrander, L. E. Hood: Methods Enzymol. 91, 227, 1983
35. U. K. Lammli: Nature 227, 680, 1970
36. T. St. John, W. M. Gallatin, M. Siegelman. H. T. Smith, V. A. Fried and I. L. Weissman: Science 231, 845, 1986
37. M. Siegelman, M. W. Bond, W. M. Gallatin, T. St. John, H. T. Smith, V. A. Fried and I. L. Weissman: Science 231, 823, 1986
38. H. Towbin, T. Staehelin and J. Gordon: Proc. Natl. Acad. Sci. USA 76, 4350, 1979
39. Dinarello, Ch. A., in Lymphokines, Vol. 14, E. Pick, ed., p. 1, Academic Press, London, 1987
40. D. J. Merchant, R. H. Kahn and W. H. Murphy: Handbook of Cell and Organ Culture, Burgess Publ. Co., Minneapolis, 1969
41. G. E. Grau, T. E. Taylor, M. E. Molyneux, J. J. Wirima, P. Vassalli, M. Hommel and P. Lambert: New Engl. J. Med. 320, 1586, 1989
42. J. Sambrook, E. F. Fritsch and T. Maniatis: Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989
43. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman and K. Struhl: Current Protocols in Molecular Biology 1987-1988, S. Wiley and Sons, New York, 1987
44. E. Harlow and D. Lane: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Publications, New York, 1988
45. S. Fazekas de St. Groth and D. Scheidegger: J. Immunol. Methods 35, 1, 1980
46. D. Pennica and D. V. Goeddel, in Lymphokines, Vol. 13, D. R. Webb and D. V. Goeddel, eds. p. 163, Academic Press, London, 1987
47. J. Tavernier, L. Franzen, A. Marmenout, J. van der Heyden, R. Muller, M. Ruysschaert, A. van Vliet, R. Banden and W. Fiers, in Lymphokines, Vol. 13, D. R. Webb and D. V. Goeddel, eds., p. 181, Academic Press, London
48. P. J. Fraker and J. C. Speck: Biochem. Biophys. Res. Commun. 80, 849, 1987
49. D. H. Erlich, D. H. Gelfand, R. K. Saiki: Nature 331, 61, 1988
50. Bosserhoff, J. Wallach and R. W. Frank: J. Chromatogr. 473, 71, 1989
51. R. Lathe: J. Mol. Biol. 183, 1, 1985
52. Luckow and Summers, "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures", Texas Agricultural Experimental Station, Texas A & M University, Bulletin No. 1555, 2nd edition, 1988

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<213> ORGANISM: Homo sapiens

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agaccaggaa ctgaaacatc agacgtggtg tgcaagccct gtgccccggg gacgttctcc    300

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cccttgtgcc tgcagagaga agccaaggtg cctcacttgc ctgccgataa ggcccggggt    720

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Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
20 25 30

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
35 40 45

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
50 55 60

Arg Leu Cys Ala Pro Leu Pro Lys Cys Arg Pro Gly Phe Gly Val Ala
65 70 75 80

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
85 90 95

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
100 105 110

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
115 120 125

Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
130 135 140

His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Ser
145 150 155 160

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
165 170 175

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Phe Ala Leu Pro Val
180 185 190

Gly Leu Ile Val Gly Val Thr Ala Leu Gly Leu Leu Ile Ile Gly Val
195 200 205

Val Asn Cys Val Ile Met Thr Gln Val Lys Lys Lys Pro Leu Cys Leu
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Gln Arg Glu Ala Lys Val Pro His Leu Pro Ala Asp Lys Ala Arg Gly
225 230 235 240

Thr Gln Gly Pro Glu Gln Gln His Leu Leu Ile Thr Ala Pro Ser Ser
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Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser Ala Leu Asp Arg Arg Ala
            260                 265                 270

Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala
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Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser Ala Asp Ser Ser Pro Gly
    290                 295                 300

Gly His Gly Thr Gln Val Asn Val Thr Cys Ile Val Asn Val Cys Ser
305                 310                 315                 320

Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln Ala Ser Ser Thr Met
                325                 330                 335

Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro Lys Asp Glu Gln Val
            340                 345                 350

Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser Gln Leu Glu Thr Pro
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Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro Leu Pro Leu Gly Val
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Pro Asp Ala Gly Met Lys Pro Ser
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<210> SEQ ID NO 5
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<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa = unknown amino acid

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1               5                   10                  15

Gln Gly Lys Tyr Ile His Pro Gln Xaa Asn Ser Ile
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<210> SEQ ID NO 6
<211> LENGTH: 15
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 6

Ser Thr Pro Glu Lys Glu Gly Glu Leu Glu Gly Thr Thr Thr Lys
1               5                   10                  15


<210> SEQ ID NO 7
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 7

Ser Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys
1               5                   10                  15

Pro Leu


<210> SEQ ID NO 8
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<213> ORGANISM: Artificial sequence
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Val Phe Cys Thr
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<213> ORGANISM: Artificial sequence
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Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala Gly Glu Ala
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<223> OTHER INFORMATION: Xaa = unknown amino acid

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Leu Pro Ala Gln Val Ala Phe Xaa Pro Tyr Ala Pro Glu Pro Gly Ser
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Thr Cys

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<212> TYPE: PRT
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<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = unknown amino acid

<400> SEQUENCE: 11

Ile Xaa Pro Gly Phe Gly Val Ala Tyr Pro Ala Leu Glu
1 5 10

<210> SEQ ID NO 12
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 12

Leu Cys Ala Pro
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<210> SEQ ID NO 13
<211> LENGTH: 7
<212> TYPE: PRT
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 13

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Val Pro His Leu Pro Ala Asp
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<210> SEQ ID NO 14
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<220> FEATURE:
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<222> LOCATION: (9)..(10)
<223> OTHER INFORMATION: Xaa = unknown amino acid
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<400> SEQUENCE: 14

Gly Ser Gln Gly Pro Glu Gln Gln Xaa Xaa Leu Ile Xaa Ala Pro
1 5 10 15

<210> SEQ ID NO 15
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<212> TYPE: PRT
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<220> FEATURE:
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<400> SEQUENCE: 15

Leu Val Pro His Leu Gly Asp Arg Glu
1 5

<210> SEQ ID NO 16
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<400> SEQUENCE: 16

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<210> SEQ ID NO 17
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<212> TYPE: DNA
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<210> SEQ ID NO 18
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<223> OTHER INFORMATION: Antisense primer

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<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic primer

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<400> SEQUENCE: 21

gatccagaat tcataatag 19

<210> SEQ ID NO 22
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 22

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<210> SEQ ID NO 23
<211> LENGTH: 31
<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 23

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<210> SEQ ID NO 24
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 24

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<210> SEQ ID NO 25
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 25

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<210> SEQ ID NO 26

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<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 26

atagagctct gtggtgcctg agtcctcag 29

The invention claimed is:

1. A protein comprising

(a) a human tumor necrosis factor (TNF)-binding soluble fragment of an insoluble human TNF receptor, wherein the insoluble human TNF receptor (i) specifically binds human TNF, (ii) has an apparent molecular weight of about 75 kilodaltons on a non-reducing SDS-polyacrylamide gel, and (iii) comprises the amino acid sequence LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10); and

(b) all of the domains of the constant region of a human immunoglobulin IgG heavy chain other than the first domain of said constant region;

wherein said protein specifically binds human TNF.

2. The protein of claim 1, wherein the soluble fragment comprises the peptides LCAP (SEQ ID NO: 12) and VFCT (SEQ ID NO: 8).

3. The protein of claim 2, wherein the soluble fragment further comprises the peptide LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10).

4. The protein of claim 1, wherein said human immunoglobulin IgG heavy chain is IgG_1.

5. The protein of claim 4, wherein said domains of the constant region of the human immunoglobulin heavy chain consist essentially of the immunoglobulin amino acid sequence encoded by pCD4-Hγ1 vector (deposited at Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5314).

6. A pharmaceutical composition comprising the protein of claim 4 and a pharmaceutically acceptable carrier material.

7. The protein of claim 1, wherein the protein is purified.

8. The protein of claim 1, wherein the protein is produced by CHO cells.

9. The protein of claim 1, wherein the protein consists of (a) the soluble fragment of the receptor and (b) all of the domains of the constant region of the human immunoglobulin IgG heavy chain other than the first domain of the constant region.

10. The protein of claim 1, wherein said domains of the constant region of the human immunoglobulin heavy chain consist essentially of the immunoglobulin amino acid sequence encoded by pCD4-Hγ1 vector (deposited at Deutschen Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5314) or by pCD4-Hγ3 vector (deposited at Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5523).

11. The protein of claim 1, wherein the protein consists essentially of the extracellular region of the insoluble human TNF receptor and all the domains of the constant region of a human IgG_1 immunoglobulin heavy chain other than the first domain of the constant region.

12. A pharmaceutical composition comprising the protein of claim 11 and a pharmaceutically acceptable carrier material.

13. A protein comprising

(a) a human tumor necrosis factor (TNF)-binding soluble fragment of an insoluble human TNF receptor, wherein the insoluble human TNF receptor (i) specifically binds human TNF, (ii) has an apparent molecular weight of about 75 kilodaltons on a non-reducing SDS-polyacrylamide gel, and (iii) comprises the amino acid sequences LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10), LCAP (SEQ ID NO: 12), VFCT (SEQ ID NO: 8), NQPQAPGVEASGAGEA (SEQ ID NO: 9) and VPHLPAD (SEQ ID NO: 13),

wherein the soluble fragment comprises the peptides LCAP (SEQ ID NO: 12) and VFCT (SEQ ID NO: 8); and

(b) all of the domains of the constant region of a human IgG_1 heavy chain other than the first domain of the constant region;

wherein said protein specifically binds human TNF.

14. The protein of claim 13, wherein the protein is purified.

15. The protein of claim 13, wherein the protein is produced by CHO cells.

16. The protein of claim 13, wherein the protein consists of (a) the soluble fragment of the receptor and (b) all of the domains of the constant region of the human IgG_1 heavy chain other than the first domain of the constant region.

17. The protein of claim 13, wherein the soluble fragment further comprises the peptide LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10).

18. A protein encoded by a polynucleotide which comprises two nucleic acid subsequences,

(a) one of said subsequences encoding a human TNF-binding soluble fragment of an insoluble human TNF receptor protein having an apparent molecular weight of about 75 kilodaltons on a non-reducing SDS-polyacrylamide gel, said soluble fragment comprising the amino acid sequence LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10), and

(b) the other of said subsequences encoding all of the domains of the constant region of the heavy chain of a human IgG immunoglobulin other than the first domain of said constant region,

wherein said protein specifically binds human TNF.

19. The protein of claim 18, wherein the soluble fragment comprises the peptides LCAP (SEQ ID NO: 12) and VFCT (SEQ ID NO: 8).

20. The protein of any one of claim 18 or 19, wherein said human immunoglobulin heavy chain is IgG_1.

21. The protein of claim 20, wherein said domains of the constant region of the human immunoglobulin heavy chain consist essentially of the immunoglobulin amino acid sequence encoded by the DNA insert of pCD4-Hγ1 vector (deposited at Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5314).

22. The protein of claim 18, wherein the protein is purified.

23. The protein of claim 18, wherein the protein is produced by CHO cells.

24. The protein of claim 18, wherein the protein consists of (a) the soluble fragment of the receptor and (b) all of the domains of the constant region of the human IgG_1 heavy chain other than the first domain of the constant region.

25. The protein of claim 18, wherein said domains of the constant region of the human immunoglobulin heavy chain consist essentially of the immunoglobulin amino acid sequence encoded by pCD4-Hγ1 vector (deposited at Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5314) or by pCD4-Hγ3 vector (deposited at Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG under No. DSM 5523).

26. A protein consisting of

(a) a human tumor necrosis factor (TNF)-binding soluble fragment of an insoluble human TNF receptor, wherein the insoluble human TNF receptor (i) specifically binds human TNF, and (ii) has an apparent molecular weight of about 75 kilodaltons on a non-reducing SDS-polyacrylamide gel and (iii) comprises the amino acid sequence LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10),

wherein the soluble fragment comprises the peptides LCAP (SEQ ID NO:12) and VFCT (SEQ ID NO:8), and

(b) all of the domains of the constant region of a human IgG_1 heavy chain other than the first domain of the constant region,

wherein the protein specifically binds human TNF, and

wherein the protein is produced by CHO cells.

27. The protein of claim 26, wherein the soluble fragment comprises the peptide LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10).

28. The protein of claim 26, wherein the protein is purified.

29. A pharmaceutical composition comprising the protein of any of claim 1, 18, 26, or 27 and a pharmaceutically acceptable carrier material.

30. A protein comprising

(a) human tumor necrosis factor (TNF) binding soluble fragment of the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC on Oct. 17, 2006 under accession number PTA 7942,

(b) all of the domains of the constant region of a human immunoglobulin IgG heavy chain other than the first domain of said constant region;

wherein said protein specifically binds human TNF.

31. The protein of claim 30, consisting of the soluble fragment and all the domains of the constant region of the human immunoglobulin IgG heavy chain other than the first domain of said constant region.

32. The protein of claim 30, wherein the protein is expressed by a mammalian host cell.

33. The protein of claim 32, wherein the mammalian host cell is a CHO cell.

34. The protein of claim 32, consisting of the soluble fragment and all the domains of the constant region of the human immunoglobulin IgG heavy chain other than the first domain of said constant region.

35. The protein of claim 30, wherein the protein consists essentially of the extracellular region of the human tumor necrosis factor (TNF) receptor amino acid sequence encoded by the cDNA insert, and all the domains of the constant region of a human IgG_1 immunoglobulin heavy chain other than the first domain of the constant region.

36. A pharmaceutical composition comprising the protein of claim 35 and a pharmaceutically acceptable carrier material.

* * * * *

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America, and if the invention is a process, of the right to exclude others from using, offering for sale or selling throughout the United States of America, or importing into the United States of America, products made by that process, for the term set forth in 35 U.S.C. 154(a)(2) or (c)(1), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b). See the Maintenance Fee Notice on the inside of the cover.

David J. Kappos

Director of the United States Patent and Trademark Office

JOINT EXHIBIT

JTX-2

MAINTENANCE FEE NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the United States Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

PATENT TERM NOTICE

If the application for this patent was filed on or after June 8, 1995, the term of this patent begins on the date on which this patent issues and ends twenty years from the filing date of the application or, if the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121, or 365(c), twenty years from the filing date of the earliest such application ("the twenty-year term"), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b), and any extension as provided by 35 U.S.C. 154(b) or 156 or any disclaimer under 35 U.S.C. 253.

If this application was filed prior to June 8, 1995, the term of this patent begins on the date on which this patent issues and ends on the later of seventeen years from the date of the grant of this patent or the twenty-year term set forth above for patents resulting from applications filed on or after June 8, 1995, subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b) and any extension as provided by 35 U.S.C. 156 or any disclaimer under 35 U.S.C. 253.

US008163522B1

(12) **United States Patent**
Brockhaus et al.

(10) **Patent No.: US 8,163,522 B1**
(45) **Date of Patent: Apr. 24, 2012**

(54) **HUMAN TNF RECEPTOR**

(75) Inventors: **Manfred Brockhaus**, Bettingen (CH); **Reiner Gentz**, Rheinfelden (DE); **Dembic Zlatko**, Basel (CH); **Werner Lesslauer**, Basel (CH); **Hansruedi Lotscher**, Mohlin (CH); **Ernst-Jurgen Schlaeger**, Efringen-Kirchen (DE)

(73) Assignee: **Hoffman-LaRoche Inc.**, Nutley, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/444,791**

(22) Filed: **May 19, 1995**

Related U.S. Application Data

(60) Division of application No. 08/095,640, filed on Jul. 21, 1993, now Pat. No. 5,610,279, which is a continuation of application No. 07/580,013, filed on Sep. 10, 1990, now abandoned.

(30) **Foreign Application Priority Data**

Sep. 12, 1989	(CH)	3319/89
Mar. 8, 1990	(CH)	746/90
Apr. 20, 1990	(CH)	1347/90
Aug. 31, 1990	(EP)	90116707

(51) **Int. Cl.**
C12N 15/12 (2006.01)
C12N 15/63 (2006.01)
C12N 5/10 (2006.01)
C12P 21/00 (2006.01)

(52) **U.S. Cl.** **435/69.9**; 536/23.4; 435/320.1; 435/325; 435/358

(58) **Field of Classification Search** 530/350, 530/385, 388.22, 387.1, 866, 867, 391.1; 636/23.53, 23.1; 435/69.7, 70.21, 320.1; 536/23.4

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,593,002	A		6/1986	Dulbecco
4,675,285	A		6/1987	Clark et al.
4,729,326	A		3/1988	Richter
4,769,326	A		9/1988	Rutler
4,770,995	A		9/1988	Rubin et al.
4,816,567	A		3/1989	Cabilly et al.
4,894,439	A		1/1990	Dorin et al.
4,912,044	A		3/1990	Jacob et al.
4,935,233	A		6/1990	Bell et al.
4,948,875	A		8/1990	Tanaka et al.
4,963,354	A		10/1990	Shepard et al.
4,965,271	A		10/1990	Mandell et al.
5,055,447	A		10/1991	Palladino et al.
5,073,627	A		12/1991	Curtis et al.
5,075,222	A		12/1991	Hannum et al.
5,098,702	A		3/1992	Zimmerman et al.
5,098,833	A		3/1992	Lasky et al.
5,116,964	A		5/1992	Capon et al.
5,118,500	A		6/1992	Hanel et al.
5,136,021	A		8/1992	Dembinski et al.
5,155,027	A		10/1992	Sledziewski et al.
5,211,945	A		5/1993	Wallach et al.
5,223,395	A		6/1993	Gero
5,225,538	A		7/1993	Capon et al.
5,258,498	A		11/1993	Huston et al.
5,264,416	A		11/1993	Park et al.
5,270,038	A		12/1993	Klimpel et al.
5,336,603	A		8/1994	Capon et al.
5,344,915	A	*	9/1994	LeMaire et al. 530/350
5,350,683	A		9/1994	Sims et al.
5,359,032	A		10/1994	Dayer et al.
5,395,760	A	*	3/1995	Smith et al. 435/240.1
5,428,130	A	*	6/1995	Capon et al. 530/350
5,447,851	A		9/1995	Beutler et al.
5,455,165	A		10/1995	Capon et al.
5,478,925	A		12/1995	Wallach et al.
5,512,544	A		4/1996	Wallach et al.
5,514,582	A		5/1996	Capon et al.
5,599,905	A		2/1997	Mosley et al.
5,605,690	A		2/1997	Jacobs et al.
5,610,279	A		3/1997	Brockhaus et al.
5,633,145	A		5/1997	Feldmann et al.
5,639,597	A		6/1997	Lauffer et al.

(Continued)

FOREIGN PATENT DOCUMENTS

AU	58976	1/1991

(Continued)

OTHER PUBLICATIONS

Lee et al., Science, vol. 239: 1284-1291, Generation of cDNA Probes Directed by Amino Acid Sequence: Cloning of Urate Oxidase, Mar. 11, 1988.*

Wozney, Methods in Enzymology, vol. 182:738-751, "Using Purified Protein to Clone Its Gene", 1990.*

Monnat, Jr "Molecular Analysis of Spontaneous Hypoxanthine Phosphoribosyltransferase Mutations in Thioguanine-resistant HL-60 Human Leukemia Cells" Cancer Research, 49:81-87, 1989.*

Peppel, K., et al., J. Exp. Med., 174, pp. 1483-1489 (1991).

Zettlmeissl, G., et al., DNA & Cell Biology, 9, pp. 347-353 (1990).

Loetscher et al, *J. of Bio. Chem.*, 266(27), pp. 18324-18329 (1991).

Nature Biotechnology, 15 (1997) p. 13.

Stauber et al., "Human tumor necrosis factor-α receptor-purification by immunoaffinity chromatography and initial characterization", J. Bio. Chem. 263:19098-19104 (1988).

(Continued)

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(57) **ABSTRACT**

The present invention is concerned with non-soluble proteins and soluble or insoluble fragments thereof, which bind TNF, in homogeneous form, as well as their physiologically compatible salts, especially those proteins having a molecular weight of about 55 or 75 kD (non-reducing SDS-PAGE conditions), a process for the isolation of such proteins, antibodies against such proteins, DNA sequences which code for non-soluble proteins and soluble or non-soluble fragments thereof, which bind TNF, as well as those which code for proteins comprising partly of a soluble fragment, which binds TNF, and partly of all domains except the first of the constant region of the heavy chain of human immunoglobulins and the recombinant proteins coded thereby as well as a process for their manufacture using transformed pro- and eukaryotic host cells.

10 Claims, 16 Drawing Sheets

U.S. PATENT DOCUMENTS

5,695,953	A	12/1997	Wallach et al.
5,705,364	A	1/1998	Etcheverry et al.
5,712,155	A	1/1998	Smith et al.
5,721,121	A	2/1998	Etcheverry et al.
5,808,029	A	9/1998	Brockhaus et al.
5,811,261	A	9/1998	Wallach et al.
5,863,786	A	1/1999	Feldmann et al.
5,945,397	A	8/1999	Smith et al.
5,981,701	A	11/1999	Wallach et al.
RE36,755	E	6/2000	Smith et al.
6,143,866	A	11/2000	Brewer et al.
6,165,476	A	12/2000	Strom et al.
6,201,105	B1	3/2001	Smith et al.
6,221,675	B1	4/2001	Hauptmann et al.
6,271,346	B1	8/2001	Hauptmann et al.
6,294,352	B1	9/2001	Hauptmann et al.
6,541,610	B1	4/2003	Smith
6,541,620	B1	4/2003	Brewer et al.
6,572,852	B2	6/2003	Smith et al.
6,858,409	B1	2/2005	Thompson et al.
7,253,264	B1	8/2007	Lauffer et al.
2003/0064480	A1	4/2003	Lauffler et al.

FOREIGN PATENT DOCUMENTS

EP	120694		10/1984
EP	227110		7/1987
EP	230574		8/1987
EP	269455		6/1988
EP	315 062		10/1988
EP	308 378		3/1989
EP	314317		5/1989
EP	325 224		7/1989
EP	325262		7/1989
EP	0334165		9/1989
EP	393 438		4/1990
EP	0 394 827	A1	10/1990
EP	398 327		11/1990
EP	412 486		2/1991
EP	414178		2/1991
EP	418 014		3/1991
EP	422 339		4/1991
EP	433 900		6/1991
EP	460846		12/1991
EP	526452		2/1993
EP	526905		2/1993
EP	0 567 566	B1	11/1993
EP	568925		11/1993
EP	606869		7/1994
GB	2218101	A	10/1989
GB	2218101		11/1989
GB	2 246 569		2/1992
JP	61-293924		12/1986
JP	02-154695		6/1990
WO	89/02922	*	4/1989
WO	WO 89 09622		10/1989
WO	WO 91/02078		2/1991
WO	91/03553		3/1991
WO	WO 91/17184		11/1991
WO	WO 91/08298		12/1991
WO	WO 92/08495		5/1992
WO	WO 92/13095		8/1992
WO	WO 93/07863		4/1993
WO	WO 93/19777		10/1993
WO	WO 94/06476		3/1994

OTHER PUBLICATIONS

S ckinger et al., "Purification and bi logic charact rization of a specific tumor necrosis factor α inhibitor", J. Bio. Chem. 264:11966-11973 (1989).

Engelmann et al., "A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity", J. Bio. Chem. 264:11974-11980 (1989).

Hohmann et al., "Two different cell types have different major rec ptors for human tumor necrosis factor (TNFα)", J. Bio. Ch m. 264: 14927-14934 (1989).

Smith et al., "A rec ptor for tumor necrosis factor defines an unusual family of cellular and viral prot ins", Science 248:1019-1023 (1990).

Heller et al., "Complementary DNA cloning of a receptor for tumor necrosis factor and demonstraction of a shed form of the receptor", Proc. Natl. Acad. Sci. U.S.A. 87:6151-6155 (1990).

Novick et al., "Soluble cytokine receptors are present in normal human urine", J. Exp. Med. 170:1409-1414 (1989).

Engelmann et al., "Two tumor necrosis factor-binding proteins purified from human urine", J. Bio. Chem. 265:1531-1536 (1990).

Schall et al., "Molecular cloning and expression of a receptor for human tumor necrosis factor", Cell 61:361-370 (1990).

Seckinger et al., "Tumor necrosis factor inhibitor: purification, NH_2-terminal amino acid sequence and evidence for anti-inflammatory and immunomodulatory activities", Eur. J. Immunol. 20:1167-1174 (1990).

Hohmann et al., "Expression of the types A and B tumor necrosis factor (TNT) receptors is independently regulated, and both receptors mediate activiation of the transcription factor NF-kB", Bio. Ch m. 265:22409-22417 (1990).

Espevik et al., "Characterization of binding and biological effects of monoclonal antibodies against a human tumor necrosis factor receptor", J. Exp. Med. 171:415-426 (1990).

Porteu and Nathan, "Shedding of tumor necrosis factor receptors by activated human neutrophils", J. Exp. Med. 172:599-607 (1990).

Eng lmann et al., "Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity", J. Bio. Chem. 265:14497-14504 (1990).

Seckinger et al., "Characterization of a tumor necrosis factor α (TNF-α) inhibitor: evidence of immunological cross-activity with the TNF receptor", Proc. Natl. Sci. USA 87:5188-5192 (1990).

Gray et al., "Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein", Proc. Natl. Sci. USA 87:76380-7384 (1990).

Loetscher et al., "Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor", Cell 61:351-359 (1990).

Peppel et al., "Chimaeric TNF-receptor-IgC molecule acts as soluble inhibitor of TNF mediated cytotoxicity", Journal of Cellular Biochem., Abstract, 20th Annual Meetings, Keystone Symposia on Molecular and Cellular Biology, p. 118, Suppl m nt 15F (1991).

Olsson et al., "Isolation and characterization of a tumor necrosis factor binding protein from urine", Eur. J. Ha matol. 42:270-275 (1989).

Capon et al., "Designing CD4 immunoadhesions for AIDS therapy", Nature 337:525-530 (Feb. 9, 1989).

Abstract 92-009794/02 (1992) for EP 464 533.

Official Communication relating to an Opposition in EP Application No. 99 100 703.0.

U.S. Appl. No. 08/478,995, Lauffler, Leander et al.

Abraham et al., p55 Tumor Necrosis Factor Receptor Fusion Protein in the Treatment of Patients With Severe Sepsis and Septic Shock: ΔΔΔΔΔ JAMA, 19:1531-1538 (1997).

Abraham et al., Lenercept (p55 TuMor Necrosis Factor Receptor Fusion Protein) in Severe Sepsis and Early Septic Shock: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase III Trial With 1,342 Patients, Crit Care Med,. 29:503-510 (2001).

Aggarwal et al., Characterization of Receptors for Human Tumour Necrosis Factor and Their Regulation by γ-Interferon, Nature, 318:665-667 (1985).

Aggarwal et al., Induction of Receptors for Tuthor Necrosis Factor-α by Interferons Is Not a Major Mechanism for Their Synergistic Cytotoxic Response, J. Biol. Chem., 262:10000-10007 (1987).

Aggarwal et al., Human tumour necrosis factors: structure and receptor interactions, in Tumor necrosis factor and related cytotoxins, pp. 39-51, (Ciba Foundation symposium 131), Wiley, Chichester (1987).

Arenzana-Seisdedos et al., Immunoregulatory Mediators in the Pathogenesis of Rheumotoid Arthritis, Scand. J. Rheumatol., Supplement 66:13-17 (1987).

Aruffo et al., Molecular Cloning of a CD28 cDNA by a High-Efficiency COS Cell Expression. System, Proc. Natl. Acad. Sci. USA, 84:8573-8577 (1987).

Ashkenazi et al., Protection Against Endotoxic Shock by a Tumor Necrosis Factor Receptor Immunoadhesin, Proc. Natl. Acad. Sci., U.S.A. 88:10535-10539 (1991).

Ayala, Modem Genetics, Benjamin/Cummings, Publ. Co., Menlo Park CA, p. 45, (1980).

Baglioni et al., Binding of Human Tumor Necrosis Factor to High Affinity Receptors on HeLa and Lymphoblastoid Cells Sensitive to Growth Inhibition, J. Biol. Chem., 260:13395-13397 (1985).
Benjamini et al., Antibody Structure, in Immunology: A Short Course, 3rd ed., Wiley-Liss New York, 61-69 (1996).
Branellec et al., TNF: Antitumoral Agent at the Border Lines of Immunity and Inflammation, Path. Biol., 39:230-239 (1991).
Brockhaus et al., Identification of Two Types of Tumor Necrosis Factor Receptors on Human Cell Lines by Monoclonal Antibodies, Proc. Natl. Acad. Sci. USA, 87:3127-3131 (1990).
Carter et al., Purification, Cloning, Expression and Biological Characterization of an Interleukin-1 Receptor Antagonist Protein, Nature, 344:633-638 (1990).
Carpenter et al., Epidermal Growth Factor, J. Biol. Chem., 265:7709-7712 (1990).
Carpenter, Receptors for Epidermal Growth Factor and Other Polypeptide Mitogens, Ann. Rev. Biochem., 56:881-914 (1987).
Casadei et al., Expression and Secretion of Aequorin as a Chimeric Antibody by Means of a Mammalian Expression Vector, Proc. Natl. Acad. Sci., U.S.A. 87:2047-2051 (1990).
Coffman et al., The Role of Helper T Cell Products in Mouse B Cell Differentiation and Isotype Regulation, Immunol. Rev., 102:5-28 (1988).
Creasey et al., A High Molecular Weight Component of the Human Tumor Necrosis Factor Receptor is Associated With Cytotoxicity, Proc. Natl. Acad. Sci. USA, 84:3293-3297 (1987).
Dayer, Chronic Inflammatory Joint Diseases: Natural Inhibitors of Interleukin 1 and Tumor Necrosis Factor α, J. Rheumatol, 18 (Suppl. 27): 71-75 (1991).
Dower et al., Human Cytokine Receptors, J. Clin. Immunol., 10:289-299 (1990).
Eisenberg et al., Primary Structure and Functional Expression From Complementary DNA of a Human Interleukin-1 Receptor Antagonist, Nature, 343:341-346 (1990).
Ellison et al., The Nucleotide Sequence of a Human Immunoglobulin Cγ1 Gene, Nucleic Acids Res. 10(13): 4071-79 (1982).
Esmon, The Roles of Protein C and Thrombomodulin in the Regulation of Blood Coagulation, J. Biol. Chem., 264:4743-4746 (1989).
European Search Report for EP 97 12 0664, dated Mar. 9, 1998.
Fell et al., Genetic Construction and Characterization of a Fusion Protein Consisting of a Chimeric F(ab') With Specificity for Carcinomas and Human IL-2, J. Immunol., 146:2446-2452 (1991).
Fernandez-Botran et al., A Soluble, High-Affinity, Interleukin-4-Binding Protein is Present in the Biological Fluids of Mice, Proc. Natl. Acad. Sci., 87:4202-4206 (1990).
Fernandez-Botran, Soluble Cytokine Receptors: Their Role in Immunoregulation, The FASEB Journal, 5:2567-2574 (1991).
Ferrante et al., Inhibition of Tumour Necrosis Factor Alpha (TNF-α)-Induced Neutrophil Respiratory Burst by a TNF Inhibitor, Immunology, 72:440-442 (1991).
Fisher et al., Cloning and Expression of Human Tissue Factor cDNA, Thrombosis Research, 48:89-99 (1987).
Fisher et al., Treatment of Septic Shock with the Tumor Necrosis Factor Receptor: Fc Fusion Protein, New Eng. J. Med., 334:1697-1702 (1996).
Foley et al., An Inhibitor of the Toxicity of Tumour Nectrosis Factor in the Serum of Patients with Sarcoidosis, Tuberculosis and Crohn's Disease, Clin. Exp. Immunol, 80:395-399 (1990).
Fomsgaard et al., Preliminary Study on Treatment of Septic Shock Patients With Antilipopolysaccharide IgG from Blood Donors, Scand. J. Infect. Dis., 21:697-708 (1989).
Garcia et al., High Sensitivity of Transgenic Mice Expressing Soluble TNFR1 Fusion Protein to Mycobacterial Infections: Synergistic Action of TNF and IFN-γ in the Differentiation of Protective Granulomas, Eur. J. Immunol., 27:3182-3190 (1997).
Gascoigne et al., Secretion of a Chimeric T-Cell Receptor-Immunoglobulin Protein, Proc. Natl. Acad. Sci USA, 84:2936-2940 (1987).
Gehr et al., Both Tumor Necrosis Factor Receptor Types Mediate Proliferative Signals in Human MononuClear Cell Activation, J. Immunol., 149:911-917 (1992).
Gillies et al., Targeting Human Cytotoxic T Lymphocytes to Kill Heterologous Epidermal Growth Factor Receptor-Bearing Tumor Cells, J. Immunol., 144:1067-1071 (1991).
Goodman, Identification of Antigenic Determinants, in Basic & Clinical Immunol., 24-25 (1982).
Goodman, Immunogenicity & Antigenic Specificity, in Basic & Clinical Immunol., 101-108 (1991).
Goodwin et al., Molecular cloning and Expression of the Type 1 and Type 2 Murine Receptors for Tumor Necrosis Factor, Molecular and Cellular Biology, 11:3020-3026 (1991).
Gray et al, Cloning and Expression of cDNA for Human Lymphotoxin, a Lymphokine With Tumour Necrosis Activity, Nature, 312:721-724 (1984).
Gray et al., Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein, Proc. Natl. ACad. Sci. 87: 7380-84 (1990).
Grundmann et al., Characterization of cDNA Coding for Human Factor XIIIa, Proc. Natl. Acad. Sci. USA, 83:8024-8028 (1986).
Haak-Frendscho et al., Inhibition of TNF by a TNF Receptor Immunoadhesin, J. Immunol., 152:1347-1353 (1994).
Hannum et al., Interleukin-1 Receptor Antagonist Activity of a Human Interleukin-1 Inhibitor, Nature, 343:336-340 (1990).
Heflin et al., Prevention by Granulocyte Depletion of Increased Vascular Permeability of Sheep Lung Following Endotoxemia, J. Clin. Invest., 68:1253-1260 (1981).
Heller et al., Amplified Expression of Tumor Necrosis Factor Receptor in Cells Transfected With Epstein-Barr Virus Shuttle Vector cDNA Libraries, J. Biol. Chem., 265:5708-5717 (1990).
Heller et al., Amplified Expression of. The Turrior Necrosis Factor Receptor in Lymphoblastoid Cells Transfected with HeLa Cell-CDNA Expression Abstract WA 142, Napa Valley Conference 1989.
Himmler et al., Molecular Cloning and Expression of Human and Rat Tumor Necrosis Factor Receptor Chain (p60) and Its Soluble Derivative, Tumor Necrosis Factor-Binding Protein, DNA and Cell Biology, 9:705-715 (1990).
Hobart, The Immune System: A Course on the Molecular and Cellular Basis of immunity, Blackwell Scientific Pubs, p. 7 (1975).
Holtmann et al., Down Regulation of the Receptors for Tumor Necrosis Factor by Intereukin 1 and 4β-Phorbol-12-Myristate-13-Acetate, J. Immunol., 139:1161-1167 (1987).
Hsu et al., Differential Expression and Ligand Binding Properties of Tumor Necrosis Factor Receptor Chimeric Mutants, J. Biol. Chem., 268:16430-16436(1992).
Idzerda et al., Human Interleukin 4 Receptor Confers Biological Responsiveness and Defines a Novel Receptor Superfamily, J. Exp. Med., 171:861-873 (1990).
Imamura et al., Expression of Tumor Necrosis Factor Receptors on Human Monocytes and Internalization of Receptor Bound Ligand, J. Immunol., 139:2989-2992 (1987).
Ishikura et al., Differential Biologic Effects Resulting From Bimodal Binding of Recombinant Human Tumor Necrosis Factor to Myeloid Leukemia Cells, Blood, 73:419-424 (1989).
Israel et al., Binding of Human TNF-α to High-Affinity Cell Surface Receptors: Effect of IFN, Immunology Letters, 12:217-224(1986).
Jacobs et al., Pharmacokinetic Parameters and Biodistribution of Soluble Cytokine Receptors, International Review of Expermental Pathology, 34B:123-135 (1993).
Jones et al, Structure of Tumour Necrosis Factor, Nature, 338:225-228 (1989).
Kaczmarski et al., The Cytokine Receptor Superfamily, Blood Reviews, 5:193-203 (1991).
Kaushansky, Structure-Function Relationships of the Hematopoietic Growth Factors, Proteins: Structure, Function & Genetics, 12:1-9 (1992).
Keegan et al., The Interleukin-4 Receptor: Signal Transduction by a Hematopoietin Receptor, Journal of Leukocyte Biology, 55:272-279 (1994).
Keegan et al., Interleukin 4 Receptor: Signaling Mechanisms, Immunology Today, 15:423-432 (1994).
Kleinau et al., Importance of CD23 for Collagen-Induced Arthritis: Delayed Onset and Reduced Severity in CD23-Deficient Mice, J. Immunol., 162:4266-4270 (1999).

Klinkert et al., TNF-α Receptor Fusion Protein Prevents Experimental Auto-Immune Encephalomyelitis and Demyelination in Lewis Rats: an Overview, The Journal of Neuroimmunology, 72:163-168 (1997).
Kohno et al., A Second Tumor Necrosis Factor Receptor Gene Product Can Shed a Naturally Occurring Tumor Necrosis Factor Inhibitor, Proc. Natl. Acad. Sci. USA, 87:8331-8335 (1990).
Kruse et al., Conversion of Human Interleukin-4 Into a High Affinity Antagonist by a Single Amino Acid Replacement, The EMBO Journal, 11:3237-3244 (1992).
Kull et al., Cellular Receptor for ^{125}I-Labeled Tumor Necrosis Factor: Specific Binding, Affinity Labeling, and Relationship to Sensitivity, Proc. Natl. Acad. Sci. USA, 82:5756-5760 (1985).
Landolfi, A Chimeric IL-2/Ig Molecule Possesses the Functional Activity of Both Proteins, J. Immunol., 146:915-919 (1991).
Langner et al., Structural and Functional Analysis of a TNF Receptor-Immunoglobulin Fusion Protein, New Advances on Cytokines, 349-354 (1992).
Leberthon et al., Enhanced Tumor Uptake of Macromolecules Induced by a Novel Vasoactive Interleukin 2 Immunoconjugate, Cancer Research, 51:2694-2698 (1991).
Lesslauer et al., Recombinant Soluble Tumor Necrosis Factor Receptor Proteins Protect Mice From Lipopolysaccharide-Induced Lethality, Eur. J. Immunol., 21:2883-2886 (1991).
Liabakk et al., A Rapid and Sensitive. Immunoassay for Tumor Necrosis Factor Using Magnetic Monodisperse Polymer Particles, Journal of Immunological Methods, 134:253-259 (1990).
Loetscher et al., Efficacy of a Chimeric TNFR-IgG Fusion Protein to Inhibit TNF Activity in Animal Models of Septic Shock, Endotoxin Research Series, 2:455-462 (1993).
Loetscher et al., Two distinct human TNF receptors: purification, molecular cloning and expression, in Tumor Necrosis Factor: Structure-Function Relationship and Clinical Application, (3rd International Conference.
Maliszewski et al., Cytokine Receptors and B Cell Functions: Recombinant Soluble Receptors Specifically Inhibit IL-1 and IL-4 Induced. Cell Activities in Vitro, J. Immunol., 144:3028-3033(1990).
Mohler et al., Soluble Tumor Necrosis Factor (TNF) Receptors are Effective Therapeutic Agents in Lethal Endotoxemia and Function Simultaneously as Both TNF Carriers and TNF Antagonists, J. Immunol., 151:1548-1561 (1993).
Mori et al., Attenuation of Collagen-Induced Arthritis in 55-kDa TNF Receptor Type 1 (TNFR1)-IgG1-Treated and TNFR1-Deficient Mice, J. Immunol., 157:3178-3182 (1996).
Morrissey et al., Molecular Cloning of the cDNA for Tissue Factor, the Cellular Receptor for the Initiation of the Coagulation Protease Cascade, Cell: 50:129-135 (1987).
Morrison, in Vitro Antibodies: Strategies for Production and Application, Annu. Rev. Immunol., 10:239-265 (1992).
Mosley et al., The Murine Interleukin-4 Receptor: Molecular Cloning and Characterization of Secreted and Membrane Bound Forms, Cell: 59:335-348 (1989).
Nophar et al., Soluble Forms of Tumor Necrosis Factor Receptors (TNF-Rs). The cDNA for the Type I TNF-R, Cloned Using Amino Acid Sequence Data of its Soluble Form, Encodes Both the Cell Surface and a Soluble Form of the Receptor, The EMBO Journal, 9:3269-3278 (1990).
Novotny et al., A Soluble, Single-Chain T-Cell Receptor Fragment Endowed With Antigen-Combining Properties, Proc. Natl. Acad. Sci. USA, 88:8646-8650 (1991).
Okayama et al., High-Efficiency Cloning of Full-Length cDNA, Molecular and Cellular Biology, 2:161-170 (1982).
Okayama et al., A cDNA Cloning Vector That Permits Expression of cDNA Inserts in Mammalian Cells, Molecular and Cellular Biology,..3:280-289 (1983).
Old, Tumor Necrosis Factor, 2nd Intl Conference on Tumor Necrosis Factor & Related Cytokines, Napa, CA, 1-30 (1989).
Paborsky et al., Purification of Recombinant Human Tissue Factor, Biochemistry, 28:8072-8077 (1989).
Parrillo, Pathogenetic Mechanisms of Septic Shock, New Eng. J. Med., 328:1471-1477 (1993).
Peetre et al., A Tumor Necrosis Factor Binding Protein is Present in Human Biological Fluids, Eur. J. Haematol. 41:414-419 (1988).
Pennica et al., Human Tumour Necrosis Factor: Precursor Structure, Expression and Homology to Lymphotoxin. Nature, 312:724-729 (1984).
Peppel et al., Chimaeric TNF-Receptor—IgG Molecule Acts As Soluble Inhibitor of TNF Mediated Cytotoxicity, J. Cell. Biochem., Supp. 15F:439 (1991).
Piguet et al., Evolution of Collagen Arthritis in Mice is Arrested by Treatment With Anti-Tumor Necrosis (TNF) Antibody or a Recombinant Soluble TNF Receptor, Immunology, 77 (4):510-514 (1992).
Redfield et al., Secondary Structure and Topology of Human Interleukin 4 in Solution, Biochemistry, 30:11029-11035 (1991).
Rubin, Binding Receptor Characters Zako and Expression, and IntracellularEvents. 2nd Intl Conference on Tumor Necrosis Factor & Related Cytokines, Napa, CA, 94-96 (1989).
Ruddle et al., An Antibody to Lymphotoxin and Tumor Necrosis Factor Prevents Transfer of Experimental Allergic Encephalomyelitis, J. Exp. Med., 172:1193-1200 (1990).
Rutka et al., The Effects of Human Recombinant Tumor Necrosis Factor on Glioma-Derived Cell Lines: Cellular Proliferation, Cytotoxicity, Morphological and Radioreceptor Studies, Int. J. Cancer., 41:573-582 (1988).
Saxne et al.; Detection of Tumor Necrosis Factor α But Not Tumor Necrosis Factor β in Rheumatoid Arthritis Synovial Fluid and Serum, Arthritis & Rheumatism, 31:1041-1045 (1988).
Scallon of al., Functional Comparisons of Different Tumour Necrosis Factor Receptor/IgG Fusion Proteins, Cytokine, 7:759-770 (1995).
Scarpati et al., Human Tissue Factor, cDNA Sequence and Chromosome Localization of the Gene, Biochemistry, 26:5234-5238 (1987).
Schleiffenbaum et al., The Tumor Necrosis Factor Receptor and Human Neutrophil Function, J. Clin. Invest., 86:184-195 (1990).
Schnee et al., Construction and Expression of a Recombinant Antibody-Targeted Plasminogen Activator, Proc. Natl. Acad. Sci. USA, 84:6904-6908 (1987).
Seckinger et al., A Human Inhibitor of Tumor Necrosis Factor α, J. Exp. Med. 167:1511-1516 (1988).
Shalaby et al., Receptor Binding and Activation of Polmorphonuclear Neutrophils by Tumor Necrosis Factor-Alpha, Journal of Leukocyte Biology, 41:196-204 (1987).
Shalaby et al., Binding and Regulation of Cellular Function by Monoclonal antibodies Against Human Tumor Necrosis Factor Receptors, J. Exp. Med. 172: 1517-1520 (1990).
Sheehan et al., Generation and Characterization of Hamster Monoclonal Antibodies That Neutralize Murine Tumor Necrosis Factors, Journal of Immunology, 142:3884-3893 (1989).
Shin et al., Expression and Characterization of an Antibody Binding Specificity Joined to Insulin-Like Growth Factor 1: Potential Applications for Cellular Targeting, Proc. Natl. Acad. Sci., 87:5322-5326 (1990).
Sims et al., cDNA Expression Cloning of the IL-1 Receptor, a Member of the Immunoglobulin Superfamily, Science, 241:585-589 (1988).
Sims et al., Cloning the Interleukin 1 Receptor From Human T Cells, Proc. Natl. Acad. Sci., 86:8946-8950 (1989).
Smith et al.; The Active Form of Tumor Necrosis Factor Is a Trimer, J. Biol. Chem., 262:6951-6954 (1987).
Smith et al., Blocking of HIV-1 Infectivity by a Soluble, Secreted Form of the CD4 Antigen, Science, 238:1704-1707 (1987).
Smith et al., Multimeric Structure of the Tumor Necrosis Factor Receptor of HeLa Cells, J. Biol. Chem., 264:14646-14652 (1989).
Spicer et al., Isolation of cDNA Clones Coding for Human Tissue Factor: Primary Structure of the Protein and cDNA, Proc. Natl. Acad. Sci., 84:5148-5152 (1987).
Staines et al., Collagen Arthritis-What Can It Teach Us?, British Journal of Rheumatology, 33:798-807 (1994).
Strader et al., Structural Basis of β-Adrenergic Receptor Function, The FASEB Journal, 3:1825-1832 (1989).
Suggs et al., Use of Synthetic Oligonucleotides as Hybridization Probes: Isolation of Cloned cDNA Sequences for Human β_2-Microglobulin, Proc. Natl. Acad. Sci. U.S.A., 78:6613-6617 (1981).
Tauber et al:, Toxicity in Neuronal Cells Caused by Cererospinal Fluid fFom Pneumococcal and Gram-Negative Meningitis, The Journal of Infectious Diseases, 166:1045-1050 (1992).

Thoma et al., Identification of a 60-kD Tumor Necosis Factor (TNF) Receptor as the Major Signal Transducing Component in TNF Responses, J. Exp. Med. 172: 1019-23 (1990).
Tsujimoto et al., Characterization and Affinity Crosslinking of Receptors for Tumor Necrosis Factor on Human Cells, Archives of Biochemistry and Biophysics, 249:563-568 (1986).
Tsujimoto et al, Interferon-γEnhances Expression of Cellular Receptors for Tumor Necrosis Factor, J. Immunol., 136:2441-2444 (1986).
Tsujimoto et al, Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells, Proc. Natl. Acad. Sci. 82: 7626-30 (1985).
Ulich et al., Intratracheal Administration of Endotoxin and Cytokines, Clinical Immunology & Immunopathology., 72:137-140 (1994).
Unglaub et al., Downregulation of Tumor Necrosis Factor (TNF) Sensitivity Via Modulation of TNF Binding Capacity by Protein Kinase C Activators, J. Exp. Med. 166:1788-1797 (1987).
Van Der Poll et al., Pretreatment with a 55-kDa Tumor Necrosis Factor Receptor—Immunoglobulin Fusion Protein Attenuates Activation of Coagulation, but not of Fibrinolysis, during Lethal Bacteremia in Baboons, The Journal of Infectious Diseases., 176:296-299 (1997).
Van Zee et al., Protection Against Lethal *Escherichia coil* Bacteremia in Baboons (*Papio anubis*) by Pretreatment With a 55-kDa TNF Receptor (CD120a)-Ig Fusion Protein, Ro 45-2081, J. Immunol., 156:2221-2230 (1996).
Wallach et al., Soluble and Cell Surface Receptors for Tumor Necrosis Factor, Progress, Inflammation Research & Therapy, 51-57 (1991).
Wallach et al., Cell surface and soluble TNF receptors, in Tumor Necrosis Factor: Structure-Function Relationship and Clinical Application, (3rd International Conference on Tumor Necrosis Factor and Related Cytokines, Makuhari, Chiba, Nov. 21-25, 1990), Osawaand Bonavida, eds., Basel, Karger, pp. 47-57 (1992).
Wilks, The CD4 Receptor: Post Binding Events, Conformational Change and the Second Site, Molec. Aspects Med., 12:255-265 (1991).
Yamasaki et al., Cloning and Expression of the Human Interleukin-6 (BSF-2/IFNβ 2) Receptor, Science, 241:825-282 (1988).
Yonehara et al., A Cell-Killing Monoclonal Antibody (Anti-Fas) to a Cell Surface Antigen Co-Downregulated With the Receptor of Tumor Necrosis Factor, J. Exp. Med., 169:1747-1765 (1989).
Yoshie et al., Binding and Crosslinking of ^{125}I-Labeled Recombinant Human Tumor Necrosis Factor to Cell Surface Receptors, J. Biochem., 100: 531-541(1986).
Brower et al. Roche's RA Drug Crippled, Nature Biotechnology, 15:1325 (1997).
Nesbitt, et al., "Mechanism of Action of Certolizumab Pegol (CDP870): In Vitro Comparison with Other Anti-tumor Necrosis Factor a Agents", *Inflamm Bowel Dis*, 13: 1323-1332 (Nov. 2007).
Barone et al., Comparative Analysis of the Ability of Etanercept and Infliximab to Lyse TNF-Expressing Cells in a Complement Dependent Fashion. Arthritis Rheum., 42(9) supplement, Sep. 1999 (S90).
Bringman et al., Monoclonal antibodies to human tumor necrosis factors alpha and beta: application for affinity purification, immunoassays, and as structural probes. *Hybridoma*, 6(5):489-507 (1987).
Byrn et al., Biological properties of a CD4 immunoadhesin. *Nature*, 344:667-70 (1990).
Capon et al., Designing CD4 immunoadhesins for AIDS therapy. *Nature*, 337:525-31 (1989).
Cosman et al., A new cytokine receptor superfamily. *Trends Biochem. Sci.* 15:265-70 (1990).
Deen et al. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature*, 331(6151): 82-4 (1988).
Dembic et al., Two Human TNF receptors have similar extracellular, but distinct intracellular, domain sequences. *Cytokine* 2: 231-237, 1990).
Berke, Functions and mechanisms of lysis induced by cytotoxic T lymphocytes and natural killer cells. Fundamental Immunology, 2nd Edition, Paul, ed., Raven Press, New York, pp. 735-64 (1989).
Heller et al., Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor. *Proc. Natl. Acad. Sci USA*, 87:6151-5 (1990).
Irwin et al, Affinity precipitation methods, Chapter 22, Methods in Molecular Biology, 59: 217-38 (1996).
Khare et al, Mechanisms of cell death induced by tumor necrosis factor antagonists. Poster 715 presented at the Annual Meeting of the Society for Investigative Dermatology (SID), May 3-5, 2006, Philadelphia, PA.
Kohno et al., Adalimumab and Infliximab bind to Fc-receptor and C1q and generate immunoprecipitation: A different mechanism from Etanercept. Presentation 1495, Poster 271, presented at the American College of Rheumatology Annual Meeting, Nov. 13-17, 2005, San Diego, CA.
Larsson et al., Affinity precipitation of enzymes. *FEBS Lett.* 98(2):333-8 (1979).
Mohler et al., Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J. Immunol.*, 151:1548-61 (1993).
Sell, Immunology, Immunopathology and Immunity, 4th Edition, Elsevier Science Publishing Co., New York, 1987, at pp. 85-91.
Smith et al., Multimeric structure of the tumor necrosis factor receptor of HeLa cells. *J. Biol. Chem.* 262:6951-4 (1987).
Smith et al., A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science*, 248:1019-23 (1990).
Traunecker et al., Highly efficient neutralization of HIV with recombinant CD4-immunoglobulin molecules. *Nature*, 339:68-70 (1989).
Williams et al., Identification of a ligand for the c-kit proto-oncogene. *Cell*, 63: 167-74 (1990).
Wingfield et al., Tumour necorsis factor is a compact trimer. *FEBS Lett.* 211: 179-84 (1987).
Evans et al., Protective effect of 55- but not 75-kD soluble tumor necrosis factor receptor-immunoglobulin G fusion proteins in an animal model of gram-negative sepsis. *J. Exp. Med.* 180: 2173-9 (1994).
Exhibit A: Memorandum by D. Urdal to S. Gillis, M. Kranda, and P. Grassam, dated Oct. 27, 1989.
Exhibit B: Correspondence from D. Urdal to L. Lauffer dated Feb. 26, 1990.
Exhibit C: Lab Notebook of E. Jeffrey, pages dated May 1990 through Jan. 1991.
Exhibit D: Correspondence from L. Lauffer to D. Urdal, dated May 21, 1990.
Exhibit E: Meeting minutes, Immunex employee (author unknown) to file, dated Jun. 25, 1990.
Exhibit F: Lab notebook of Terri Davis, pages dated Jul. 11, 1990.
Exhibit G: Letter from M. Deeley to L. Lauffer, dated Jul. 20, 1990.
Exhibit H: Meeting minutes, Immunex employee (author unknown) to file, dated Jul. 23, 1990.
Exhibit I: Correspondence from Drs. Seiler and Zeittmeissl to D. Gillis, dated Aug. 8, 1990.
Exhibit J (J1-J21): Declaration of Bruce A. Beutler, Karsten Peppel, and David F. Crawford submitted to the USPTO on Jul. 16, 1993 during the prosecution of U.S. Appl. No. 07/862,495, filed Apr. 2, 1992 (issued as US 5,447,851 naming inventors B. Beutler, K. Peppel, and D. Crawford), including exhibits J-1-J21, which were submitted with the declaration.
Exhibit K: Confirmation page from D. Urdal to P. Oquendo, dated Oct. 4, 1990.
Letter from J. Thomas to L. Lauffer dated Dec. 10, 1990.
Memo from J. Thomas to P. Baum, D. Cosman, M. Deeley, R. Goodwin, S. Gillis, H. Sassenfeld, and D. Urdal, dated Dec. 17, 1990, conveying attached facsimile received Dec. 13, 1990 from L. Lauffer to J. Thomas.
Declaration of Taruna Arora under 37 C.F.R. § 1.132 plus Exhibits A-D dated Dec. 16, 2010, filed in sister case U.S. Appl. No. 08/444,790 (which was filed on May 19, 1995, inventors M. Brockhaus, Z. Dembic, R. Gentz, W. Lesslauer, H. Loetscher, E. Schlaeger, hereinafter "U.S. Appl. No. 08/444,790").
Arora et al., "Differences in Binding and Effector Functions Between Classes of TNF Antagonists," Cytokine 45: 124-131 (2009).

Arruffo et al., "CD44 Is the Principal Cell Surface Receptor for Hyaluronate," Cell 61: 1303-1313 (1990).
Brennan et al., "Inhibitory Effect of TNFα Antibodies on Synovial Cell Interleukin-1 Production in Rheumatoid Arthritis," Lancet 2(8657): 244-247 (1989).
Chan et al., "A Domain in TNF Receptors That Mediates Ligand-Independent Receptor Assembly and Signaling," Science 288: 2351-2354 (2000).
Engelmann et al., "Two Tumor Necrosis Factor-binding Proteins Purified from Human Urine," J. Biol. Chem. 265(3): 1531-36 (1990).
Mitoma et al., "Mechanisms for Cytotoxic Effects of Anti-Tumor Necrosis Factor Agents on Transmembrane Tumor Necrosis Factor a-Expressing Cells," Arthr. & Rheum. 58(5): 1248-1257 (2008).
Novick et al., "Soluble Cytokine Receptors Are Present in Normal Human Urine," J. Exp. Med., 170: 1409-1414 (1989).
Shalaby et al., The Involvement of Human Tumor Necrosis Factors-α and -β in the Mixed Lymphocyte Reaction, J. Immunol. 141: 499-503 (1988).
Smith and Baglioni, "Multimeric Structure of the Tumor Necrosis Factor Receptor of HeLa Cells," J. Biol. Chem. 264: 14646-14652 (1989).
Strand et al., "Biologic Therapies in Rheumatology: Lessons Learned, Future Directions," Nature Rev. 6: 75-92 (2007).
Strangfeld et al., "Risk of Herpes Zoster in Patients with Rheumatoid Arthritis Treated with AntiTNF-α Agents," JAMA 301(7): 737-744 (2009).
Wallis et al., "Reactivation of Latent Granulomatous Infections by Infliximab," Clin. Inf. Dis. 41(Suppl 2): S1-S5 (2005).
Wallis et al., "Granulomatous Infectious Diseases Associated with Tumor Necrosis Factor Antagonists," Clin. Inf. Dis. 38: 1261-1265 (2004).
Watson et al., "A Homing Receptor—IgG Chimera as a Probe for Adhesive Ligands of Lymph Node High Endothelial Venules," J. Cell Biol. 110: 2221-2229 (1990).
Winzor et al., "Evaluation of Equilibrium Constants from Precipitin Curves: Interaction of α-Crystallin with an Elicited Monoclonal Antibody," Arch. Biochem. Biophys. 268(1): 221-226 (1989).
Furst et al. "Tumor Necrosis Factor Antagonists: Different Kinetics and/or Mechanisms of Action may Explain Differences in the Risk for Developing Granulomatous Infection," Semin. Arthritis Rheum. 36(3):159-67. (2006).
United States Adopted Names (USAN) Council Report, *Clin. Pharm. & Ther.*, vol. 66, No. 2, Aug. 1999, p. 209.
Feldmann et al., "Cytokine production in the rheumatoid joint: implications for treatment," Ann. Rheum. Dis. 49: 480-486 (1990).
Hoogenboom et al., "Construction and Expression of Antibody-Tumor Necrosis Factor Fusion Proteins," Molecular Immunol. 28(9): 1027-1037 (1991).
US 6,224,867, 05/2001, Smith et al. (withdrawn)

* cited by examiner

FIGURE 1A

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-185 GAATTCGGGGGGGTTCAAGATCACTGGGACCAGGCCGTGATCTCTATGCCCGAGTCTCAA
-125 CCCTCAACTGTCACCCCAAGGCACTTGGGACGTCCTGGACAGACCGAGTCCCGGGAAGCC
 -65 CCAGCACTGCCGCTGCCACACTGCCCTGAGCCCAAATGGGGGAGTGAGAGGCCATAGCTG
          -28.          .          .          .          .          .
 -30      MetGlyLeuSerThrValProAspLeuLeuLeuProLeuValLeuLeuGluLeu
  -5 TCTGGCATGGGCCTCTCCACCGTGCCTGACCTGCTGCTGCCGCTGGTGCTCCTGGAGCTG
                .          .          .    +1    .          .          .
 -10 LeuValGlyIleTyrProSerGlyValIleGlyLeuValProHisLeuGlyAspArgGlu
  55 TTGGTGGGAATATACCCCTCAGGGGTTATTGGACTGGTCCCTCACCTAGGGGACAGGGAG
                .          .          .          .     ***  .          .
  10 LysArgAspSerValCysProGlnGlyLysTyrIleHisProGlnAsnAsnSerIleCys
 115 AAGAGAGATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAAATAATTCGATTTGC
                .          .          .          .          .          .
  30 CysThrLysCysHisLysGlyThrTyrLeuTyrAsnAspCysProGlyProGlyGlnAsp
 175 TGTACCAAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGAT
                .          .          .          .          .          .
  50 ThrAspCysArgGluCysGluSerGlySerPheThrAlaSerGluAsnHisLeuArgHis
 235 ACGGACTGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACAC
                .          .          .          .          .          .
  70 CysLeuSerCysSerLysCysArgLysGluMetGlyGlnValGluIleSerSerCysThr
 295 TGCCTCAGCTGCTCCAAATGCCGAAAGGAAATGGGTCAGGTGGAGATCTCTTCTTGCACA
                .          .          .          .          .          .
  90 ValAspArgAspThrValCysGlyCysArgLysAsnGlnTyrArgHisTyrTrpSerGlu
 355 GTGGACCGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAA
```

FIGURE 1B

```
                  .          ***          .          ***.          .          .
110 AsnLeuPheGlnCysPheAsnCysSerLeuCysLeuAsnGlyThrValHisLeuSerCys
415 AACCTTTTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGC
                  .          .          .          .          .          .
130 GlnGluLysGlnAsnThrValCysThrCysHisAlaGlyPhePheLeuArgGluAsnGlu
475 CAGGAGAAACAGAACACCGTGTGCACCTGCCATGCAGGTTTCTTTCTAAGAGAAAACGAG
                  .          .          .          .          .          .
150 CysValSerCysSerAsnCysLysLysSerLeuGluCysThrLysLeuCysLeuProGln
535 TGTGTCTCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAG
                  .          .          .          .          .          .
170 IleGluAsnValLysGlyThrGluAspSerGlyThrThrValLeuLeuProLeuValIle
595 ATTGAGAATGTTAAGGGCACTGAGGACTCAGGCACCACAGTGCTGTTGCCCCTGGTCATT
                  .          .          .          .          .          .
190 PhePheGlyLeuCysLeuLeuSerLeuLeuPheIleGlyLeuMetTyrArgTyrGlnArg
655 TTCTTTGGTCTTTGCCTTTTATCCCTCCTCTTCATTGGTTTAATGTATCGCTACCAACGG
                  .          .          .          .          .          .
210 TrpLysSerLysLeuTyrSerIleValCysGlyLysSerThrProGluLysGluGlyGlu
715 TGGAAGTCCAAGCTCTACTCCATTGTTTGTGGGAAATCGACACCTGAAAAAGACGGGGAG
                  .          .          .     ***  .          .          .
230 LeuGluGlyThrThrThrLysProLeuAlaProAsnProSerPheSerProThrProGly
775 CTTGAAGGAACTACTACTAAGCCCCTGGCCCCAAACCCAAGCTTCAGTCCCACTCCAGGC
```

FIGURE 1C

```
                .         .         .         .         .         .
 250 PheThrProThrLeuGlyPheSerProValProSerSerThrPheThrSerSerSerThr
 835 TTCACCCCCACCCTGGGCTTCAGTCCCGTGCCCAGTTCCACCTTCACCTCCAGCTCCACC
                .         .         .         .         .         .
 270 TyrThrProGlyAspCysProAsnPheAlaAlaProArgArgGluValAlaProProTyr
 895 TATACCCCCGGTGACTGTCCCAACTTTGCGGCTCCCCGCAGAGAGGTGGCACCACCCTAT
                .         .         .         .         .         .
 290 GlnGlyAlaAspProIleLeuAlaThrAlaLeuAlaSerAspProIleProAsnProLeu
 955 CAGGGGGCTGACCCCATCCTTGCGACAGCCCTCGCCTCCGACCCCATCCCCAACCCCCTT
                .         .         .         .         .         .
 310 GlnLysTrpGluAspSerAlaHisLysProGlnSerLeuAspThrAspAspProAlaThr
1015 CAGAAGTGGGAGGACAGCGCCCACAAGCCACAGAGCCTAGACACTGATGACCCCGCGACG
                .         .         .         .         .         .
 330 LeuTyrAlaValValGluAsnValProProLeuArgTrpLysGluPheValArgArgLeu
1075 CTGTACGCCGTGGTGGAGAACGTGCCCCCGTTGCGCTGGAAGGAATTCGTGCGGCGCCTA
                .         .         .         .         .         .
 350 GlyLeuSerAspHisGluIleAspArgLeuGluLeuGlnAsnGlyArgCysLeuArgGlu
1135 GGGCTGAGCGACCACGAGATCGATCGGCTGGAGCTGCAGAACGGGCGCTGCCTGCGCGAG
                .         .         .         .         .         .
 370 AlaGlnTyrSerMetLeuAlaThrTrpArgArgArgThrProArgArgGluAlaThrLeu
1195 GCGCAATACAGCATGCTGGCGACCTGGAGGCGGCGCACGCCGCGGCGCGAGGCCACGCTG
                .         .         .         .         .         .
 390 GluLeuLeuGlyArgValLeuArgAspMetAspLeuLeuGlyCysLeuGluAspIleGlu
1255 GAGCTGCTGGGACGCGTGCTCCGCGACATGGACCTGCTGGGCTGCCTGGAGGACATCGAG
```

FIGURE 1D

```
                .         .         .         .         .         .
 410 GluAlaLeuCysGlyProAlaAlaLeuProProAlaProSerLeuLeuArg
1315 GAGGCGCTTTGCGGCCCCGCCGCCCTCCCGCCCGCGCCCAGTCTTCTCAGATGAGGCTGC
1375 GCCCCTGCGGGCAGCTCTAAGGACCGTCCTGCGAGATCGCCTTCCAACCCCACTTTTTTC
1435 TGGAAAGGAGGGGTCCTGCAGGGGCAAGCAGGAGCTAGCAGCCGCCTACTTGGTGCTAAC
1495 CCCTCGATGTACATAGCTTTTCTCAGCTGCCTGCGCGCCGCCGACAGTCAGCGCTGTGCG
1555 CGCGGAGAGAGGTGCGCCGTGGGCTCAAGAGCCTGAGTGGGTGGTTTGCGAGGATGAGGG
1615 ACGCTATGCCTCATGCCCGTTTTGGGTGTCCTCACCAGCAAGGCTGCTCGGGGGCCCCTG
1675 GTTCGTCCCTGAGCCTTTTTCACAGTGCATAAGCAGTTTTTTTTGTTTTTGTTTTGTTTT
1735 GTTTTGTTTTTAAATCAATCATGTTACACTAATAGAAACTTGGCACTCCTGTGCCCTCTG
1795 CCTGGACAAGCACATAGCAAGCTGAACTGTCCTAAGGCAGGGGCGAGCACGGAACAATGG
1855 GGCCTTCAGCTGGAGCTGTGGACTTTTGTACATACACTAAAATTCTGAAGTTAAAAAAAA
1915 AACCCGAATTC
```

Figure 2A

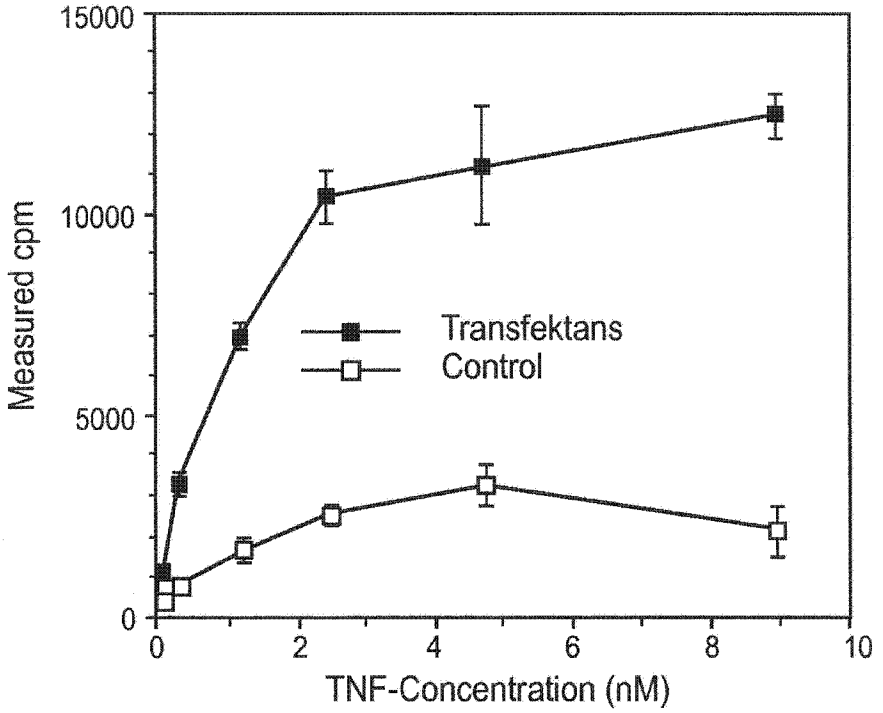

Figure 2B

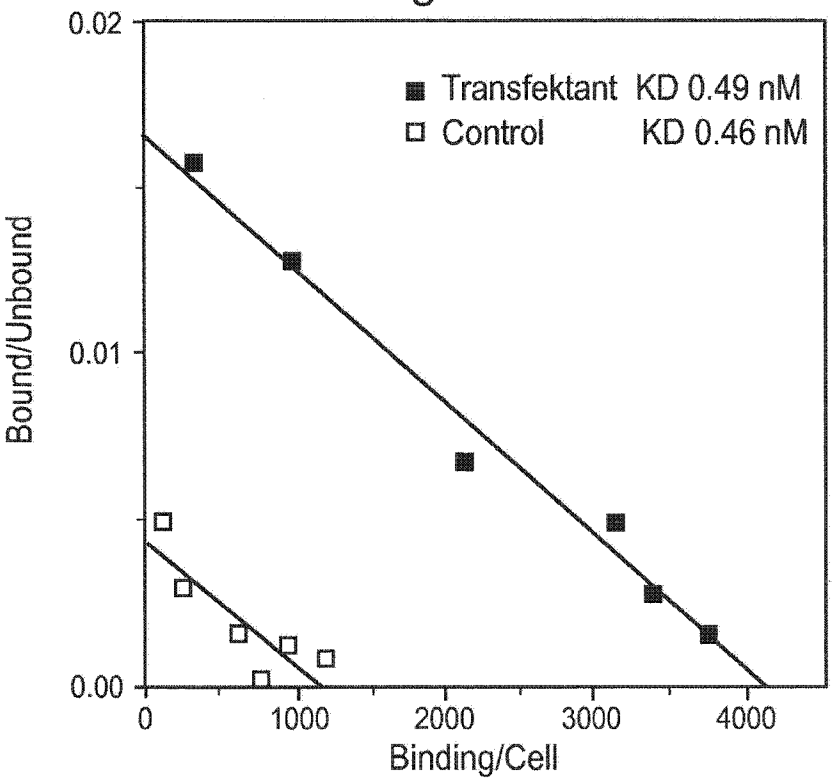

Figure 3

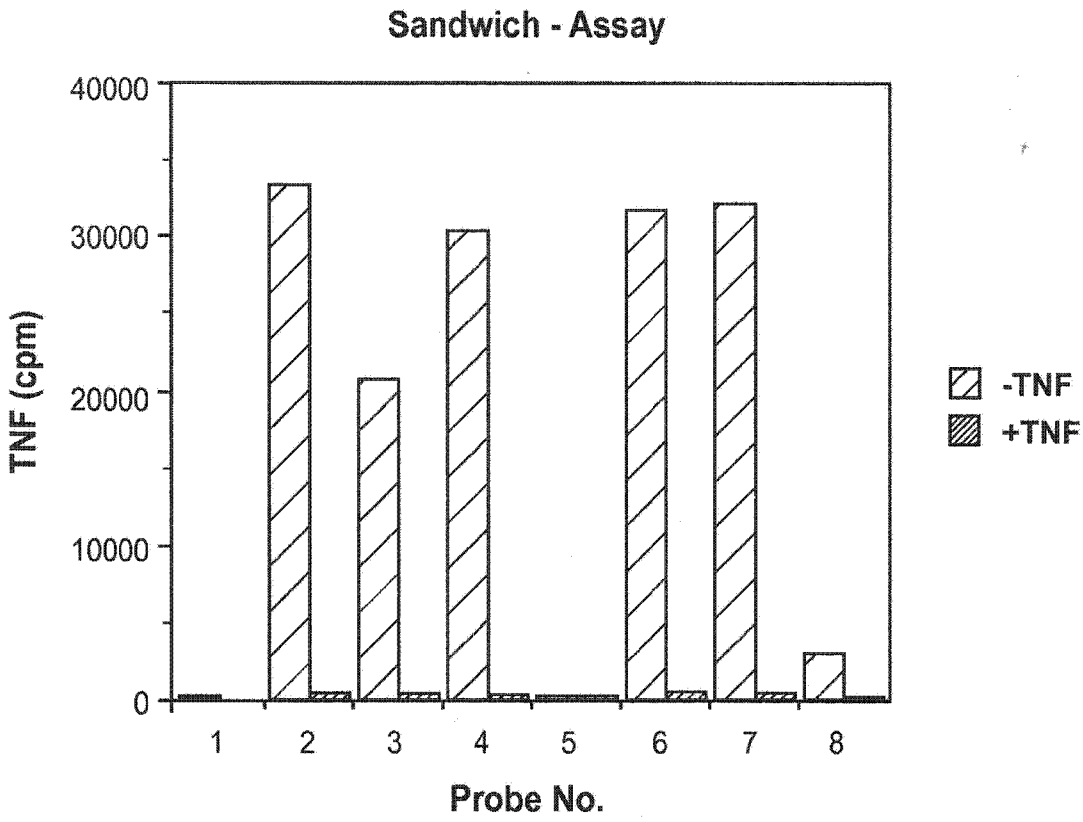
Sandwich - Assay
40000
30000
20000
10000
0
TNF (cpm)
1
2
3
4
5
6
7
8
Probe No.
-TNF
+TNF

FIGURE 4A

```
                .         .         .         .         .         .
  1 SerAspSerValCysAspSerCysGluAspSerThrTyrThrGlnLeuTrpAsnTrpVal
  1 TCGGACTCCGTGTGTGACTCCTGTGAGGACAGCACATACACCCAGCTCTGGAACTGGGTT
                .         .         .         .         .         .
 21 ProGluCysLeuSerCysGlySerArgCysSerSerAspGlnValGluThrGlnAlaCys
 61 CCCGAGTGCTTGAGCTGTGGCTCCCGCTGTAGCTCTGACCAGGTGGAAACTCAAGCCTGC
                .         .         .         .         .         .
 41 ThrArgGluGlnAsnArgIleCysThrCysArgProGlyTrpTyrCysAlaLeuSerLys
121 ACTCGGGAACAGAACCGCATCTGCACCTGCAGGCCCGGCTGGTACTGCGCGCTGAGCAAG
                .         .         .         .         .         .
 61 GlnGluGlyCysArgLeuCysAlaProLeuProLysCysArgProGlyPheGlyValAla
181 CAGGAGGGGTGCCGGCTGTGCGCGCCGCTGCCGAAGTGCCGCCCGGGCTTCGGCGTGGCC
                .         .         .         .         .         .
 81 ArgProGlyThrGluThrSerAspValValCysLysProCysAlaProGlyThrPheSer
241 AGACCAGGAACTGAAACATCAGACGTGGTGTGCAAGCCCTGTGCCCCGGGGACGTTCTCC
                .         .         .         .         .         .
101 AsnThrThrSerSerThrAspIleCysArgProHisGlnIleCysAsnValValAlaIle
301 AACACGACTTCATCCACGGATATTTGCAGGCCCCACCAGATCTGTAACGTGGTGGCCATC
                .         .         .         .         .         .
121 ProGlyAsnAlaSerArgAspAlaValCysThrSerThrSerProThrArgSerMetAla
361 CCTGGGAATGCAAGCAGGGATGCAGTCTGCACGTCCACGTCCCCCACCCGGAGTATGGCC
                .         .         .         .         .         .
141 ProGlyAlaValHisLeuProGlnProValSerThrArgSerGlnHisThrGlnProSer
421 CCAGGGGCAGTACACTTACCCCAGCCAGTGTCCACACGATCCCAACACACGCAGCCAAGT
```

FIGURE 4B

```
                .         .         .         .         .         .
161 ProGluProSerThrAlaProSerThrSerPheLeuLeuProMetGlyProSerProPro
481 CCAGAACCCAGCACTGCTCCAAGCACCTCCTTCCTGCTCCCAATGGGCCCCAGCCCCCCA

                .         .         .         .         .         .
181 AlaGluGlySerThrGlyAspPheAlaLeuProValGlyLeuIleValGlyValThrAla
541 GCTGAAGGGAGCACTGGCGACTTCGCTCTTCCAGTTGGACTGATTGTGGGTGTGACAGCC

                .         .         .         .         .         .
201 LeuGlyLeuLeuIleIleGlyValValAsnCysValIleMetThrGlnValLysLysLys
601 TTGGGTCTACTAATAATAGGAGTGGTGAACTGTGTCATCATGACCCAGGTGAAAAAGAAG

                .         .         .         .         .         .
221 ProLeuCysLeuGlnArgGluAlaLysValProHisLeuProAlaAspLysAlaArgGly
661 CCCTTGTGCCTGCAGAGAGAAGCCAAGGTGCCTCACTTGCCTGCCGATAAGGCCCGGGGT

                .         .         .         .         .         .
241 ThrGlnGlyProGluGlnGlnHisLeuLeuIleThrAlaProSerSerSerSerSerSer
721 ACACAGGGCCCCGAGCAGCAGCACCTGCTGATCACAGCGCCGAGCTCCAGCAGCAGCTCC

                .         .         .         .         .         .
261 LeuGluSerSerAlaSerAlaLeuAspArgArgAlaProThrArgAsnGlnProGlnAla
781 CTGGAGAGCTCGGCCAGTGCGTTGGACAGAAGGGCGCCCACTCGGAACCAGCCACAGGCA

                .         .         .         .         .         .
281 ProGlyValGluAlaSerGlyAlaGlyGluAlaArgAlaSerThrGlySerSerAlaAsp
841 CCAGGCGTGGAGGCCAGTGGGGCCGGGGAGGCCCGGGCCAGCACCGGGAGCTCAGCAGAT

                .         .         .         .         .         .
301 SerSerProGlyGlyHisGlyThrGlnValAsnValThrCysIleValAsnValCysSer
901 TCTTCCCCTGGTGGCCATGGGACCCAGGTCAATGTCACCTGCATCGTGAACGTCTGTAGC
```

FIGURE 4C

```
                .         .         .         .         .         .
 321 SerSerAspHisSerSerGlnCysSerSerGlnAlaSerSerThrMetGlyAspThrAsp
 961 AGCTCTGACCACAGCTCACAGTGCTCCTCCCAAGCCAGCTCCACAATGGGAGACACAGAT
                .         .         .         .         .         .
 341 SerSerProSerGluSerProLysAspGluGlnValProPheSerLysGluGluCysAla
1021 TCCAGCCCCTCGGAGTCCCCGAAGGACGAGCAGGTCCCCTTCTCCAAGGAGGAATGTGCC
                .         .         .         .         .         .
 361 PheArgSerGlnLeuGluThrProGluThrLeuLeuGlySerThrGluGluLysProLeu
1081 TTTCGGTCACAGCTGGAGACGCCAGAGACCCTGCTGGGGAGCACCGAAGAGAAGCCCCTG
                .         .         .         .         .         .
 381 ProLeuGlyValProAspAlaGlyMetLysProSer
1141 CCCCTTGGAGTGCCTGATGCTGGGATGAAGCCCAGTTAACCAGGCCGGTGTGGGCTGTGT
1201 CGTAGCCAAGGTGGCTGAGCCCTGGCAGGATGACCCTGCGAAGGGGCCCTGGTCCTTCCA
1261 GGCCCCCACCACTAGGACTCTGAGGCTCTTTCTGGGCCAAGTTCCTCTAGTGCCCTCCAC
1321 AGCCGCAGCCTCCCTCTGACCTGCAGGCCAAGAGCAGAGGCAGCGAGTTGTGGAAAGCCT
1381 CTGCTGCCATGGCGTGTCCCTCTCGGAAGGCTGGCTGGGCATGGACGTTCGGGGCATGCT
1441 GGGGCAAGTCCCTGAGTCTCTGTGACCTGCCCCGCCCAGCTGCACCTGCCAGCCTGGCTT
1501 CTGGAGCCCTTGGGTTTTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTCTCCCCCTGGGC
1561 TCTGCCCAGCTCTGGCTTCCAGAAAACCCCAGCATCCTTTTCTGCAGAGGGGCTTTCTGG
1621 AGAGGAGGGATGCTGCCTGAGTCACCCATGAAGACAGGACAGTGCTTCAGCCTGAGGCTG
1681 AGACTGCGGGATGGTCCTGGGGCTCTGTGCAGGGAGGAGGTGGCAGCCCTGTAGGGAACG
1741 GGGTCCTTCAAGTTAGCTCAGGAGGCTTGGAAAGCATCACCTCAGGCCAGGTGCAGTGGC
1801 TCACGCCTATGATCCCAGCACTTTGGGAGGCTGAGGCGGGTGGATCACCTGAGGTTAGGA
1861 GTTCGAGACCAGCCTGGCCAACATGGTAAAACCCCATCTCTACTAAAAATACAGAAATTA
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FIGURE 4D

```
1921 GCCGGGCGTGGTGGCGGGCACCTATAGTCCCAGCTACTCAGAAGCCTGAGGCTGGGAAAT
1981 CGTTTGAACCCGGGAAGCGGAGGTTGCAGGGAGCCGAGATCACGCCACTGCACTCCAGCC
2041 TGGGCGACAGAGCGAGAGTCTGTCTCAAAAGAAAAAAAAAAAGCACCGCCTCCAAATGCT
2101 AACTTGTCCTTTTGTACCATGGTGTGAAAGTCAGATGCCCAGAGGGCCCAGGCAGGCCAC
2161 CATATTCAGTGCTGTGGCCTGGGCAAGATAACGCACTTCTAACTAGAAATCTGCCAATTT
2221 TTTAAAAAAGTAAGTACCACTCAGGCCAACAAGCCAACGACAAAGCCAAACTCTGCCAGC
2281 CACATCCAACCCCCCACCTGCCATTTGCACCCTCCGCCTTCACTCCGGTGTGCCTGCAG
```

```
  1  MAPVAVWAAL AVGLELWAAA HALPAQVAFT PYAPEPGSTC RLREYYDQTA
 51  QMCCSKCSPG QHAKVFCTKT SDTVCDSCED STYTQLWNWV PECLSCGSRC
101  SSDQVETQAC TREQNRICTC RPGWYCALSK QEGCRLCAPL RKCRPGFGVA
151  RPGTETSDVV CKPCAPGTFS NTTSSTDICR PHQICNVVAI PGNASMDAVC
201  TSTSPTRSMA PGAVHLPQPV STRSQHTQPT PEPSTAPSTS FLLPMGPSPP
251  AEGSTGDFAL PVGLIVGVTA LGLLIIGVVN CVIMTQVKKK PLCLQREAKV
301  PHLPADKARG TQGPEQQHLL ITAPSSSSSS LESSASALDR RAPTRNQPQA
351  PGVEASGAGE ARASTGSSDS SPGGHGTQVN VTCIVNVCSS SDHSSQCSSQ
401  ASSTMGDTDS SPSESPKDEQ VPFSKEECAF RSQLETPETL LGSTEEKPLP
451  LGVPDAGMKP S
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FIGURE 5

FIGURE 6A

```
  1 S  D  T  V  C  D  S  C  E  D  S  T  Y  T  Q  L  W  N  W  V
  1 tcggacaccgtgtgtgactcctgtgaggacagcacatacacccagctctggaactgggtt
  1          10         20         30         40         50

 21 P  E  C  L  S  C  G  S  R  C  S  S  D  Q  V  E  T  Q  A  C
 61 cccgagtgcttgagctgtggctcccgctgtagctctgaccaggtggaaactcaagcctgc
 61          70         80         90         100        110

 41 T  R  E  Q  N  R  I  C  T  C  R  P  G  W  Y  C  A  L  S  K
121 actcgggaacagaaccgcatctgcacctgcaggcccggctggtactgcgcgctgagcaag
121          130        140        150        160        170

 61 Q  E  G  C  R  L  C  A  P  L  P  K  C  R  P  G  F  G  V  A
181 caggaggggtgccggctgtgcgcgccgctgccgaagtgccgcccgggcttcggcgtggcc
181          190        200        210        220        230

 81 R  P  G  T  E  T  S  D  V  V  C  K  P  C  A  P  G  T  F  S
241 agaccaggaactgaaacatcagacgtggtgtgcaagccctgtgccccggggacgttctcc
241          250        260        270        280        290

101 N  T  T  S  S  T  D  I  C  R  P  H  Q  I  C  N  V  V  A  I
301 aacacgacttcatccacggatatttgcaggccccaccagatctgtaacgtggtggccatc
301          310        320        330        340        350
```

FIGURE 6B

```
121 P  G  N  A  S  R  D  A  V  C  T  S  T  S  P  T  R  S  M  A
361 cctgggaatgcaagcagggatgcagtctgcacgtccacgtcccccacccggagtatggcc
361          370        380        390        400        410

141 P  G  A  V  H  L  P  Q  P  V  S  T  R  S  Q  H  T  Q  P  S
421 ccaggggcagtacacttaccccagccagtgtccacacgatcccaacacacgcagccaagt
421          430        440        450        460        470

161 P  E  P  S  T  A  P  S  T  S  F  L  L  P  M  G  P  S  P  P
481 ccagaacccagcactgctccaagcacctccttcctgctcccaatgggccccagcccccca
481          490        500        510        520        530

181 A  E  G  S  T  G  D  F  A  L  P  V  G  L  I  V  G  V  T  A
541 gctgaagggagcactggcgacttcgctcttccagttggactgattgtgggtgtgacagcc
541          550        560        570        580        590

201 L  G  L  L  I  I  G  V  V  N  C  V  I  M  T  Q  V  K  K  K
601 ttgggtctactaataataggagtggtgaactgtgtcatcatgacccaggtgaaaaagaag
601          610        620        630        640        650

221 P  L  C  L  Q  R  E  A  K  V  P  H  L  P  A  D  K  A  R  G
661 cccttgtgcctgcagagagaagccaaggtgcctcacttgcctgccgataaggccccggggt
661          670        680        690        700        710
```

FIGURE 6C

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241 T  Q  G  P  E  Q  Q  H  L  L  I  T  A  P  S  S  S  S  S  S
721 acacagggccccgagcagcagcacctgctgatcacagcgccgagctccagcagcagctcc
721          730        740        750        760        770

261 L  E  S  S  A  S  A  L  D  R  R  A  P  T  R  N  Q  P  Q  A
781 ctggagagctcggccagtgcgttggacagaagggcgcccactcggaaccagccacaggca
781          790        800        810        820        830

281 P  G  V  E  A  S  G  A  G  E  A  R  A  S  T  G  S  S  A  D
841 ccaggcgtggaggccagtggggccggggaggcccgggccagcaccgggagctcagcagat
841          850        860        870        880        890

301 S  S  P  G  G  H  G  T  Q  V  N  V  T  C  I  V  N  V  C  S
901 tcttcccctggtggccatgggacccaggtcaatgtcacctgcatcgtgaacgtctgtagc
901          910        920        930        940        950

321 S  S  D  H  S  S  Q  C  S  S  Q  A  S  S  T  M  G  D  T  D
961 agctctgaccacagctcacagtgctcctcccaagccagctccacaatgggagacacagat
961          970        980        990        1000       1010

341 S  S  P  S  E  S  P  K  D  E  Q  V  P  F  S  K  E  E  C  A
1021 tccagcccctcggagtccccgaaggacgagcaggtcccttctccaaggaggaatgtgcc
1021         1030       1040       1050       1060       1070
```

FIGURE 6D

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 361 F  R  S  Q  L  E  T  P  E  T  L  L  G  S  T  E  E  K  P  L
1081 tttcggtcacagctggagacgccagagaccctgctggggagcaccgaagagaagcccctg
1081          1090       1100       1110       1120       1130

 381 P  L  G  V  P  D  A  G  M  K  P  S
1141 ccccttggagtgcctgatgctgggatgaagcccagttaaccaggccggtgtgggctgtgt
1141          1150       1160       1170       1180       1190
1201 cgtagccaaggtggctgagccctggcaggatgaccctgcgaaggggccctggtccttcca
1201          1210       1220       1230       1240       1250
1261 ggcccccaccactaggactctgaggctctttctgggccaagttcctctagtgccctccac
1261          1270       1280       1290       1300       1310
1321 agccgcagcctccctctgacctgcaggccaagagcagaggcagcgagttgtggaaagcct
1321          1330       1340       1350       1360       1370
1381 ctgctgccatggcgtgtccctctcggaaggctggctgggcatggacgttcggggcatgct
1381          1390       1400       1410       1420       1430
1441 ggggcaagtccctgagtctctgtgacctgccccgcccagctgcacctgccagcctggctt
1441          1450       1460       1470       1480       1490
1501 ctggagcccttgggtttttgtttgtttgtttgtttgtttgtttgtttctccccctgggc
1501          1510       1520       1530       1540       1550
1561 tctgcccagctctggcttccagaaaaccccagcatcctttctgcagaggggctttctgg
1561          1570       1580       1590       1600       1610
1621 agaggagggatgctgcctgagtcacccatgaagacaggacagtgcttcagcctgaggctg
1621          1630       1640       1650       1660       1670
```

FIGURE 6E

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1681 agactgcgggatggtcctggggctctgtgcagggaggaggtggcagccctgtagggaacg
1681          1690      1700      1710      1720      1730
1741 gggtccttcaagttagctcaggaggcttggaaagcatcacctcaggccaggtgcagtggc
1741          1750      1760      1770      1780      1790
1801 tcacgcctatgatcccagcactttgggaggctgaggcgggtggatcacctgaggttagga
1801          1810      1820      1830      1840      1850
1861 gttcgagaccagcctggccaacatggtaaaaccccatctctactaaaaatacagaaatta
1861          1870      1880      1890      1900      1910
1921 gccgggcgtggtggcgggcacctatagtcccagctactcagaagcctgaggctgggaaat
1921          1930      1940      1950      1960      1970
1981 cgtttgaacccgggaagcggaggttgcagggagccgagatcacgccactgcactccagcc
1981          1990      2000      2010      2020      2030
2041 tgggcgacagagcgagagtctgtctcaaaagaaaaaaaaaaagcaccgcctccaaatgct
2041          2050      2060      2070      2080      2090
2101 aacttgtccttttgtaccatggtgtgaaagtcagatgcccagagggcccaggcaggccac
2101          2110      2120      2130      2140      2150
2161 catattcagtgctgtggcctgggcaagataacgcacttctaactagaaatctgccaattt
2161          2170      2180      2190      2200      2210
2221 tttaaaaaagtaagtaccactcaggccaacaagccaacgacaaagccaaactctgccagc
2221          2230      2240      2250      2260      2270
2281 cacatccaacccccccacctgccatttgcaccctccgccttcactccggtgtgcctgcag
2281          2290      2300      2310      2320      2330
```

HUMAN TNF RECEPTOR

This is a division of application Ser. No. 08/095,640, filed Jul. 21, 1993; now U.S. Pat. No. 5,610,279, which is a continuation application of Ser. No. 07/580,013, filed Sep. 10, 1990, now abandoned. This application claims priority under 35 U.S.C. §119 to application Ser. Nos. 3319/89, 746/90 and 1347/90, filed on Sep. 12, 1989, Mar. 8, 1990 and Apr. 20, 1990, respectively, all in Switzerland. This application also claims priority under 35 U.S.C. §119 to European Patent Application Number 90116707.2 (now Patent Number EP 0417563), filed Aug. 31, 1990.

BACKGROUND OF THE INVENTION

Tumor necrosis factor α (TNFα, also cachectin), discovered as a result of its haemorragic-necrotizing activity on certain tumors, and lymphotoxin (TNFβ) are two closely related peptide factors [3] from the class of lymphokines/cytokines which are both referred to hereinafter as TNF [see references 2 and 3]. TNF possesses a broad cellular spectrum of activity. For example, TNF has inhibitory or cytotoxic activity on a series of tumor cell lines [2, 3], stimulates the proliferation of fibroblasts and the phagocytic/cytotoxic activity of myeloic cells [4, 5, 6], induces adhesion molecules in endothelial cells or exerts an inhibitory activity on the endothelium [7, 8, 9, 10], inhibits the synthesis of specific enzymes in adipocytes [11] and induces the expression of histocompatibility antigens [12]. Many of these TNF activities are produced via induction of other factors or by synergistic effects with other factors such as interferons or interleukins [13-16].

TNF is involved in pathological conditions such as shock states in meningococcal sepsis [17], the development of autoimmune glomerulonephritis in mice [18] and cerebral malaria in mice [19] and human beings [41]. The toxic effects of endotoxin appear to be mediated by TNF [20]. Furthermore, TNF can trigger interleukin-1 fever [39]. On the basis of its pleiotropic functional properties, TNF in interaction with other cytokines is involved in additional pathological conditions as a mediator of immune response, inflammation, and other processes.

These biological effects are mediated by TNF via specific receptors. According to present knowledge not only TNFα, but also TNFβ bind to the same receptors [21]. Different cell types differ in their number of TNF receptors [22, 23, 24]. Generally known TNF-binding proteins (TNF-BP) have been detected by covalent bonding to radioactively labelled TNF [24-29], and the following apparent molecular weights of the TNF/TNF-BP complexes obtained have been determined to be: 95/100 kD and 75 kD [24], 95 kD and 75 kD [25], 138 kD, 90 kD, 75 kD and 54 kD [26], 100±5 kD [27], 97 kD and 70 kD [28] and 145 kD [29]. One such TNF/TNF-BP complex was isolated by anti-TNF-antibody immune affinity chromatography and preparative SDS-polyacrylamide gel electrophoreses (SDS-PAGE) [27]. The reductive cleavage of this complex and subsequent SDS-PAGE analysis gave several bands which were not tested for TNF-binding activity. Since the specific conditions which must be used for the cleavage of the complex lead to inactivation of the binding protein [31], the latter has also not been possible. The separation of soluble TNF-BP from human serum or urine by ion exchange chromatography and gel filtration (molecular weight in the region of 50 kD) was described by Olsson et al. [30].

Brockhaus et al. [32] obtained an enriched TNF-BP preparation from membrane extracts of HL_{60} cells by TNFα-ligand affinity chromatography and HPLC which, in turn, was used as an antigen preparation for the production of monoclonal antibodies against TNF-BP. Using such an immobilized antibody (immune affinity chromatography) Loetscher and Brockhaus obtained an enriched preparation of TNF-BP [31] from an extract of human placenta using TNFα-ligand affinity chromatography and HPLC, which gave a strong broad band at 35 kD, a weak band at about 40 kD and a very weak band in the region between 55 kD and 60 kD on SDS-PAGE analysis. Moreover, the gel showed a protein background smear in the region of 33 kD to 40 kD. The significance of these protein bands was, however, not clear due to the heterogenicity of the starting material which was used (placenta tissue; combined material from several placentas). In the state of the art TNF-BP have already been characterized by a N-terminal partial sequence [European Patent Application, Publication No. 308 378], whereby this sequence differs from the N-terminal partial sequence according to formula (IA) in accordance with the invention. Moreover, the TNF-binding proteins described in the state of the art are soluble, i.e. non-membrane bound, TNF-BP and not membrane-bound, i.e. insoluble, TNF-BP isolated from urine.

SUMMARY OF THE INVENTION

This invention comprises insoluble, homogenous proteins or soluble or insoluble fragments thereof, capable of binding tumor necrosis factor-(TNF).

This invention also comprises TNF-binding proteins containing amino acid sequences of FIG. 1 or FIG. 4, proteins containing fragments of these sequences, and proteins analagous to the sequences of FIG. 1 or FIG. 4 or to fragments thereof.

This invention further comprises DNA sequences encoding the proteins described above, proteins encoded by these sequences, and antibodies to any of these proteins.

This invention comprises DNA sequences which combine two partial DNA sequences, one sequence encoding soluble fragments of TNF binding proteins and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE, and the recombinant proteins encoded by these sequences.

This invention additionally comprises vectors containing the above DNA sequences, and host systems transfected with such vectors.

This invention finally comprises a process for the isolation of an insoluble homogenous protein capable of binding TNF.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A-1D. Nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) for cDNA clone derived from 55 kD TNF-BP. The 19 amino acid transmembrane region is underlined. Hypothetical glycosylation sites are identified by asterisks.

FIG. 2. Binding analysis of COS cells transfected with plasmid pN123. Panel 2A—binding of transfected cells to ^{125}I-TNFα. Panel 2B—Scatchard plot of binding data.

FIG. 3. Sandwich assays of cells transfected with plasmid pK19. Culture supernatants of cells transfected with pK19 were incubated with anti-55 kD TNF-BP antibody followed by ^{125}I-TNFα. Columns 1, 5, and 8 are controls. Columns 2, 3, 4, 5, and 6 are five parallel transfections.

FIG. 4A-4D. Nucleotide sequence (SEQ ID NO: 28) and deduced amino acid sequence (SEQ ID NO: 29) for cDNA clones derived from 75/65 kD TNF-BP.

FIG. 5. Deduced amino acid sequence (SEQ ID NO: 27) for a 75/65 kD TNF-BP cDNA clone described in Smith et al., Science 248, 1019-1023, (1990). The leader region is singly underlined, the transmembrane domain is shown boxed, and potential N-linked glycosylation sites are doubly underlined.

FIGS. 6A-6E: Corrected nucleotide sequence (SEQ ID NO: 3) and deduced amino acid sequence (SEQ ID NO: 4) of FIG. 4 after repeated sequencing, showing a threonine coded by "ACC" at position 3 instead of a serine coded by "TCC".

DETAILED DESCRIPTION OF THE INVENTION

The TNF-binding proteins of the present invention are homogenous, insoluble proteins and soluble or insoluble fragments of such proteins which are capable of binding TNF. These proteins have the ability to bind TNF as measured by standard assays.

The TNF-binding proteins of the present invention include homogenous proteins containing the amino acid sequence depicted in FIG. 1 (SEQ ID NO: 2) or in FIG. 4 (SEQ ID NO: 4), proteins containing fragments of either sequence, and analogues of any such proteins for example proteins containing amino acid sequences analogous to the amino acid sequences of FIG. 1 (SEQ ID NO: 2) or FIG. 4 (SEQ ID NO: 4) or to fragments thereof. An analogue is a protein in which one or more amino acids of the sequences depicted in FIG. 1 (SEQ ID NO: 2) or in FIG. 4 (SEQ ID NO: 4) have had their side-groups chemically modified in a known manner, or those in which one or more amino acids have been replaced or deleted, without thereby eliminating TNF-binding ability. Such analogues may be produced by known methods of peptide chemistry, or by known methods of recombinant DNA technology, such as planned mutagenesis.

The TNF binding activity of the proteins of the present invention may be determined using the assay described in Example 1.

TNF-binding proteins of this invention are obtained as follows:

TNF binding proteins may be isolated from tissues and purified to homogeneity, or isolated from cells which contain membrane-bound TNF binding protein, and purified to homogeneity. One possible method for growing cells and isolating cell extract is described in Example 2, however, other cells types and other growth and isolation methods are well known in the art. Purification of TNF-binding proteins from cell extracts may be performed using the methods described in Examples 4, 5, and 6 in combination with the assay described in Example 1. TNF-binding proteins isolated and purified by these methods were sequenced by well-known methods, as described in Example 7. From these amino acid sequences, DNA probes were produced and used to obtain mRNA encoding TNF binding proteins from which cDNA was made, all by known methods described in Examples 8 and 11. Other well-known methods for producing cDNA are known in the art and may effectively be used. In general, any TNF-binding protein can be isolated from any cell or tissue expressing such proteins using a cDNA probe such as the probe described above, isolating mRNA and transcribing the mRNA into cDNA. Thereafter, the protein can be produced by inserting the cDNA into an expression vector as described in Example 9, such as a virus, plasmid, cosmid, or other vector, inserting the expression vector into a cell, such as the COS cell described in Example 9 or the insect cell described in Example 10, proliferating the resulting cells, and isolating the expressed TNF-binding protein from the medium or from cell extract as described above. Alternatively, TNF-binding proteins may be chemically synthesized

using the sequence described above and an amino acid synthesizer, or manual synthesis using chemical conditions well known to form peptide bonds between selected amino acids. Analogues and fragments of TNF-binding proteins may be produced by the above methods. In the case of analogues, the proteins may be chemically modified, or modified by genetic engineering as described above. These fragments and analogues may then be tested for TNF-binding activity using methods such as the assay of Example 1.

Finally, monoclonal antibodies directed against TNF-binding proteins, such as the antibodies described in Example 3, may be produced by known techniques, and used to isolate TNF-binding proteins.

In more detail, the proteins of the present invention are non-soluble proteins, i.e. for example membrane proteins or so-called receptors, and soluble or non-soluble fragments thereof, which bind TNF (TNF-BP), in homogeneous form, as well as their physiologically compatible salts. Preferred proteins are those which according to SDS-PAGE under non-reducing conditions are characterized by apparent molecular weights of about 55 kD, 51 kD, 38 kD, 36 kD and 34 kD or 75 kD and 65 kD, especially those with about 55 kD and 75 kD. Furthermore, there are preferred those proteins which are characterized by containing at least one of the following amino acid partial sequences:

(IA) Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5)

(IB) Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6)

(IIA) Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7)

(IIB) Val-Phe-Cys-Thr (SEQ ID NO: 8)

(IIC) Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9)

(IID) Leu-pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys (SEQ ID NO: 9)

(IIE) Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

(IIF) Leu-Cys-Ala-Pro (SEQ ID NO: 12)

(IIG) Val-Pro-His-Leu-Pro-Ala-Asp (SEQ ID NO: 13)

(IIH) Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14)

in which X stands for an amino acid residue which could not be unequivocally determined.

A process for the isolation of the TNF-BP in accordance with the invention is also an object of the present invention. This process comprises carrying out essentially the following purification steps in sequence: production of a cell or tissue extract, immune affinity chromatography and/or single or multiple ligand affinity chromatography, high resolution liquid chromatography (HPLC) and preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The combination of the individual purification steps, which are known from the state of the art, is essential to the success of the process in accordance with the invention, whereby individual steps have been modified and improved having regard to the problem to be solved. Thus, for example, the original combined immune affinity chromatography/TNFα-ligand affinity chromatography step originally used for the enrichment of TNF-BP from human placenta [31] has been altered by using a BSA-Sepharose 4B pre-column. For the application of the cell or membrane extract, this pre-column was connected in series with the immune affinity column followed by the ligand affinity column. After the application of the extract the two aforementioned columns were coupled, each eluted and the TNF-BP-active fractions were purified again via a ligand affinity

column. The use of a detergent-containing solvent mixture for the performance of the reversed-phase HPLC step is essential to the invention.

Further, an industrial process for the production of high cell densities of mammalian cells from which TNF-BP can be isolated is also an object of the present invention. Such a process comprises using a medium, which has been developed for the specific growth requirements of the cell line used, in combination with a perfusion apparatus as described e.g. in detail in Example 2. By means of such a process there can be produced, for example, in the case of HL-60 cells up to more than 20-fold higher cell densities than usual.

In addition thereto, the present invention is also concerned with DNA sequences coding for proteins and soluble or non-soluble fragments thereof, which bind TNF. Thereunder there are to be understood, for example, DNA sequences coding for non-soluble proteins or soluble as well as non-soluble fragments thereof, which bind TNF, such DNA sequences being selected from the following:

(a) DNA sequences as given FIG. **1** or FIG. **4** as well as their complementary strands, or those which include these sequences;

(b) DNA sequences which hybridize with sequences defined under (a) or fragments thereof;

(c) DNA sequences which, because of the degeneracy of the genetic code, do not hybridize with sequences as defined under (a) and (b), but which code for polypeptides having exactly the same amino acid sequence.

That is to say, the present invention embraces not only allelic variants, but also those DNA sequences which result from deletions, substitutions and additions from one or more nucleotides of the sequences given in FIG. **1** or FIG. **4**, whereby in the case of the proteins coded thereby there come into consideration, just as before, TNF-BP. One sequence which results from such a deletion is described, for example, in Smith et al., Science 248, 1019-1023, (1990), which is incorporated by reference herein. FIG. **5** (a reproduction of FIG. **3B** of Smith et al.) shows the deduced amino acid sequence (SEQ ID NO: 27) of the cDNA coding region of a human TNF receptor cDNA clone. The leader region is singly underlined, the transmembrane domain is shown boxed, and potential N-linked glycosylation sites are doubly underlined. The entire nucleotide sequence is available upon request and has been deposited at Genbank under Accession Number M32315.

There are preferred first of all those DNA sequences which code for such a protein having an apparent molecular weight of about 55 kD, whereby the sequence given in FIG. **1** is especially preferred, and sequences which code for non-soluble as well as soluble fragments of such proteins. A DNA sequence which codes, for example, for such a non-soluble protein fragment extends from nucleotide −185 to 1122 of the sequence given in FIG. **1**. DNA sequences which code for soluble protein fragments are, for example, those which extend from nucleotide −185 to 633 or from nucleotide −14 to 633 of the sequence given in FIG. **1**. There are also preferred DNA sequences which code for a protein of about 75/65 kD, whereby those which contain the partial cDNA sequences shown in FIG. **4** are preferred. Especially preferred DNA sequences in this case are the sequences of the open reading frame of nucleotide 2 to 1,177. The peptides IIA, IIC, IIE, IIF, IIG and IIH are coded by the partial cDNA sequence in FIG. **4**, whereby the insignificant deviations in the experimentally determined amino acid sequences are based on the cDNA-derived sequence with highest probability from the limited resolution of the gas phase sequencing. DNA sequences which code for insoluble (deposited on Oct. 17, 2006 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, under Accession No. PTA 7942) as well as soluble fractions of TNF-binding proteins having an apparent molecular weight of 65 kD/75 kD are also preferred. DNA sequences for such soluble fragments can be determined on the basis of the amino acid sequences derived from the nucleic acid sequences coding for such non-soluble TNF-BP.

The invention is also concerned with DNA sequences which comprise a combination of two partial DNA sequences, with one of the partial sequences coding for those soluble fragments of non-soluble proteins which bind TNF (see above) and the other partial sequence coding for all domains other than the first domain of the constant region of the heavy chain of human immunoglobulins such as IgG, IgA, IgM or IgE, in particular IgG_1 or IgG_3 subtypes.

The present invention is also concerned with the recombinant proteins coded by any of DNA sequences described above. Of course, there are thereby also included such proteins in whose amino acid sequences amino acids have been exchanged, for example by planned mutagenesis, so that the activity of the TNF-BP or fragments thereof, namely the binding of TNF or the interaction with other membrane components participating in the signal transfer, have been altered or maintained in a desirable manner. Amino acid exchanges in proteins and peptides which do not generally alter the activity of such molecules are known in the state of the art and are described, for example, by H. Neurath and R. L. Hill in "The Proteins" (Academic Press, New York, 1979, see especially FIG. **6**, page 14). The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse. The present invention is also concerned with vectors which contain any of the DNA sequences described above in accordance with the invention and which are suitable for the transformation of suitable pro- and eukaryotic host systems, whereby there are preferred those vectors whose use leads to the expression of the proteins which are coded by any of the DNA sequences described above in accordance with the invention. Finally, the present invention is also concerned with pro- and eukaryotic host systems transformed with such vectors, as well as a process for the production of recombinant compounds in accordance with the invention by cultivating such host systems and subsequently isolating these compounds from the host systems themselves or their culture supernatants.

An object of the present invention are also pharmaceutical preparations which contain at least one of these TNF-BPs or fragments thereof, if desired in combination with other pharmaceutically active substances and/or non-toxic, inert, therapeutically compatible carrier materials.

Finally, the present invention is concerned with the use of such a TNF-BP on the one hand for the production of pharmaceutical preparations and on the other hand for the treatment of illnesses, preferably those in which TNF is involved in their course.

Starting materials for the TNF-BP in accordance with the invention are quite generally cells which contain such TNF-BP in membrane-bound form and which are generally accessible without restrictions to a person skilled in the art, such as, for example, HL60 [ATCC No. CCL 240], U 937 [ATCC No. CRL 1593], SW 480 [ATCC No. CCL 228] and HEp2 cells [ATCC No. CCL 23]. These cells can be cultivated according to known methods of the state of the art [40] or, in order to produce high cell densities, according to the procedure already described generally and described in detail in

Example 2 for HL60 cells. TNF-BP can then be extracted from the cells, which are centrifuged-off from the medium and washed, according to known methods of the state of the art using suitable detergents, for example Triton X-114, 1-0-n-octyl-β-D-glucopyranoside (octylglucoside) or 3-[(3-cholylamido-propyl)-dimethylammonio]-1-propane sulphonate (CHAPS), especially using Triton X-100. For the detection of such TNF-BP there can be used the usually used detection methods for TNF-BP, for example a polyethylene glycol-induced precipitation of the ^{125}I-TNF/TNF-BP complex [27], especially filter-binding tests with radioactively labelled TNF according to Example 1. In order to produce the TNF-BP in accordance with the invention, the general methods of the state of the art used for the purification of proteins, especially of membrane proteins, such as, for example, ion exchange chromatography, gel filtration, affinity chromatography, HPLC and SDS-PAGE can be used. Especially preferred methods for the production of TNF-BP in accordance with the invention are affinity chromatography, especially with TNF-α as the ligand bound to the solid phase, and immune affinity chromatography, HPLC and SDS-PAGE. The elution of TNF-BP bands which are separated using SDS-PAGE can be effected according to known methods of protein chemistry, for example using electroelution according to Hunkapiller et al. [34], whereby according to present knowledge the electro-dialysis times given there generally have to be doubled. Thereafter, traces of SDS which still remain can then be removed in accordance with Bosserhoff et al. [50].

The thus-purified TNF-BP can be characterized by methods of peptide chemistry which are known in the state of the art, such as, for example, N-terminal amino acid sequencing or enzymatic as well as chemical peptide cleavage. Fragments obtained by enzymatic or chemical cleavage can be separated according to usual methods such as, for example, HPLC and can themselves be subjected to further N-terminal sequencing. Such fragments which themselves bind TNF can be identified using the aforementioned detection methods for TNF-BP and are likewise objects of the present invention.

Starting from the thus-obtained amino acid sequence information or the DNA and amino acid sequences given in FIG. **1** as well as in FIG. **4** there can be produced, taking into consideration the degeneracy of the genetic code, according to methods known in the state of the art suitable oligonucleotides [51]. By means of these, again according to known methods of molecular biology [42, 43], cDNA or genomic DNA banks can be searched for clones which contain nucleic acid sequences coding for TNF-BP. Moreover, using the polymerase chain reaction (PCR) [49] cDNA fragments can be cloned by completely degenerating the amino acid sequence of two spaced apart relatively short segments while taking into consideration the genetic code and introducing into their complementarity suitable oligo-nucleotides as a "primer", whereby the fragment lying between these two sequences can be amplified and identified. The determination of the nucleotide sequence of a such a fragment permits an independent determination of the amino acid sequence of the protein fragment for which it codes. The cDNA fragments obtainable by PCR can also, as already described for the oligonucleotides themselves, be used according to known methods to search for clones containing nucleic acid sequences coding for TNF-BP from cDNA or genomic DNA banks. Such nucleic acid sequences can then be sequenced according to known methods [42]. On the basis of the thus-determined sequences and of the already known sequences for certain receptors, those

partial sequences which code for soluble TNF-BP fragments can be determined and cut out from the complete sequence using known methods [42].

The complete sequence or such partial sequences can then be integrated using known methods into vectors described in the state of the art for their multiplication and expression in prokaryotes [42]. Suitable prokaryotic host organisms are, for example, gram-negative and gram-positive bacteria such as, for example, *E. coli* strains such as *E. coli* HB101 [ATCC No. 33 694] or *E. coli* W3110 [ATCC No. 27 325] or *B. subtilis* strains.

Furthermore, nucleic acid sequences in accordance with the invention which code for TNF-BP as well as for TNF-BP fragments can be integrated using known methods into suitable vectors for reproduction and expression in eukaryotic host cells, such as, for example, yeast, insect cells and mammalian cells. Expression of such sequences is preferably effected in mammalian and insect cells.

A typical expression vector for mammalian cells contains an efficient promoter element in order to produce a good transcription rate, the DNA sequence to be expressed and signals for an efficient termination and polyadenylation of the transcript. Additional elements which can be used are "enhancers" which lead to again intensified transcription and sequences which e.g. can bring about a longer half life of the mRNA. For the expression of nucleic acid sequences in which the endogenous sequence fragment coding for a signal peptide is missing, there can be used vectors which contain such suitable sequences which code for signal peptides of other known proteins. See, for example, the vector pLJ268 described by Cullen, B. R. in Cell 46, 973-982 (1986) as well as Sharma, S. et al. in "Current Communications in Molecular Biology", edt. by Gething, M. J., Cold Spring Harbor Lab. (1985), pages 73-78.

Most of these vectors which are used for a transient expression of a particular DNA sequence in mammalian cells contain the replication source of the SV40 virus. In cells which express the T-antigen of the virus (e.g. COS cells), these vectors are reproduced abundantly. A transient expression is, however, not limited to COS cells. In principle any transfectable mammalian cell line can be used for this purpose. Signals which can bring about a strong transcription are e.g. the early and late promoters of SV40, the promoter and enhancer of the "major immediate-early" gene of HCMV (human cytomegalovirus), the LTR's ("long terminal repeats") of retroviruses such as, for example, RSV, HIV and MMTV. There can, however, also be used signals of cellular genes such as e.g. the promoters of the actin and collagenase genes.

Alternatively, however, stable cell lines which have the specific DNA sequence integrated into the genome (chromosome) can also be obtained. For this, the DNA sequence is cotransfected together with a selectable marker, e.g. neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthin guanine phosphoribosyl transferase (hgpt). The DNA sequence stably incorporated in the chromosome can also be reproduced abundantly. A suitable selection marker for this is, for example, dihydrofolate reductase (dhfr). Mammalian cells (e.g. CHO cells), which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transinfection has been effected. In this manner cell lines which contain more than a thousand copies of the desired DNA sequence can be obtained.

Mammalian cells which can be used for the expression are e.g. cells of the human cell lines Hela [ATCC CCL2] and 293 [ATCC CRL 1573] as well as 3T3 [ATCC CCL 163] and L cells, e.g. [ATCC CCL 149], (CHO) cells [ATCC CCL 61],

BHK [ATCC CCL 10] cells as well as the CV 1 [ATCC CCL 70] and the COS cell lines [ATCC CRL 1650, CRL 1651].

Suitable expression vectors include, for example, vectors such as pBC12MI [ATCC 67 109], pSV2dhfr [ATCC 37 146], pSVL [Pharmacia, Uppsala, Sweden], pRSVcat [ATCC 37 152] and pMSG [Pharmacia, Uppsala, Sweden]. The vectors "pK19" and "pN123" used in Example 9 are especially preferred vectors. These can be isolated according to known methods from *E. coli* strains HB101(pK19) and HB101 (pN123) transformed with them [42]. These *E. coli* strains have been deposited on the 26 Jan. 1990 at the Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG, under DSM 5761 for HB101 (pK19) and DMS 5764 for HB101(pN123). For the expression of proteins which consist of a soluble fragment of non-soluble TNF-BP and an immunoglobulin fragment, i.e. all domains except the first of the constant region of the heavy chain, there are especially suitable pSV2-derived vectors as described, for example, by German, C. in "DNA Cloning" [Vol. II., edt. by Glover, D. M., IRL Press, Oxford, 1985]. The vectors pCD4-Hμ (DSM 5315), pDC4-Hγ1 (DSM 5314) and pCD4-Hγ3 (DSM 5523) which have been deposited at the Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) in Braunschweig, FRG, and which are described in detail in European Patent Application No. 90107393.2 are especially preferred vectors. This European Patent Specification and the equivalent Applications referred to in Example 11 also contain data with respect to the further use of these vectors for the expression of chimeric proteins (see also Example 11) and for the construction of vectors for the expression of such chimeric proteins with other immunoglobulin fragments.

The manner in which these cells are transfected depends on the chosen expression system and vector system. An overview of these methods is to be found e.g. in Pollard et al., "DNA Transformation of Mammalian Cells" in "Methods in Molecular Biology" [Nucleic Acids Vol. 2, 1984, Walker, J. M., ed, Humana, Clifton, N. J.]. Further methods are to be found in Chen and Okayama ["High-Efficiency Transformation of Mammalian Cells by Plasmid DNA", Molecular and Cell Biology 7, 2745-2752, 1987] and in Feigner [Feigner et al., "Lipofectin: A highly efficient, lipid-mediated DNA-transfection procedure", Proc. Nat. Acad. Sci. USA 84, 7413-7417, 1987].

The baculovirus expression system, which has already been used successfully for the expression of a series of proteins (for an overview see Luckow and Summers, Bio/Technology 47-55, 1988), can be used for the expression in insect cells. Recombinant proteins can be produced in authentic form or as fusion proteins. The thus-produced proteins can also be modified such as, for example, glycosylated (Smith et al., Proc. Nat. Acad. Sci. USA 82, 8404-8408, 1987). For the production of a recombinant baculovirus which expresses the desired protein there is used a so-called "transfer vector". Under this there is to be understood a plasmid which contains the heterologous DNA sequence under the control of a strong promoter, e.g. that of the polyhedron gene, whereby this is surrounded on both sides by viral sequences. The vectors "pN113", "pN119" and "pN124" used in Example 10 are especially preferred vectors. These can be isolated according to known methods from *E. coli* strains HB101(pN113), HB101(pN119) and HB101(pN124) transformed with them. These *E. coli* strains have been deposited on the 26 Jan. 1990 at the Deutschen Sammlung von Mikroorganismen and Zellkulturen GmbH (DSM) in Braunschweig, FRG, under DSM 5762 for HB101(pN113), DSM 5763 for HB101 (pN119) and DSM 5765 for HB101(pN124). The transfer vector is then transfected into the insect cells together with DNA of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. An overview of the baculovirus expression system and the methods used therein is to be found in Luckow and Summers [52].

Expressed TNF-BP as well as its non-soluble or soluble fractions can then be purified from the cell mass or the culture supernatants according to methods of protein chemistry which are known in the state of the art, such as, for example, the procedure already described on pages 5-6.

The TNF-BP obtained in accordance with the invention can also be used as antigens to produce polyclonal and monoclonal antibodies according to known techniques [44, 45] or according to the procedure described in Example 3. Such antibodies, especially monoclonal antibodies against the 75 kD TNF-BP species, are also an object of the present invention. Those antibodies which are directed against the 75 kD TNF-BP can be used for the isolation of TNF-BP by modifications of the purification procedure described in detail in Examples 4-6 which are familiar to a person skilled in the art.

On the basis of the high binding affinity of TNF-BP in accordance with the invention for TNF (K_d value in the order of 10^{-9}-10^{-10}M), these or fragments thereof can be used as diagnostics for the detection of TNF in serum or other body fluids according to methods known in the state of the art, for example in solid phase binding tests or in combination with anti-TNF-BP antibodies in so-called "sandwich" tests.

Moreover, TNF-BP in accordance with the invention can be used on the one hand for the purification of TNF and on the other hand for the detection of TNF agonists and TNF antagonists according to procedures which are known in the state of the art.

The TNF-BP in accordance with the invention as well as their physiologically compatible salts, which can be manufactured according to methods which are known in the state of the art, can also be used for the production of pharmaceutical preparations, primarily those for the treatment of illnesses in which TNF is involved in their course. For this purpose, one or more of the said compounds, where desired or required in combination with other pharmaceutically active substances, can be processed in a known manner with the usually used solid or liquid carrier materials. The dosage of such preparations can be effected having regard to the usual criteria in analogy to already used preparations of similar activity and structure.

Since the invention has been described hereinbefore in general terms, the following Examples are intended to illustrate details of the invention, but they are not intended to limit its scope in any manner.

Example 1

Detection of TNF-Binding Proteins

The TNF-BP were detected in a filter test with human radioiodinated ^{125}I-TNF. TNF (46, 47) was radioactively labelled with Na^{125} I (IMS40, Amersham, Amersham, England) and iodo gene (#28600, Pierce Eurochemie, Oud-Beijerland, Netherlands) according to Fraker and Speck [48]. For the detection of the TNF-BP, isolated membranes of the cells or their solubilized, enriched and purified fractions were applied to moist nitrocellulose filter (0.45μ, BioRad, Richmond, Calif., USA). The filters were then blocked in buffer solution with 1% skimmed milk powder and subsequently incubated with $5 \cdot 10^5$ cpm/ml of ^{125}I-TNFα ($0.3\text{-}1.0 \cdot 10^8$ cpm/

μg) in two batches with and without the addition of 5 μg/ml of non-labelled TNFα, washed and dried in the air. The bound radioactivity was detected semiquantitatively by autoradiography or counted in a gamma-counter. The specific ^{125}I-TNF-α binding was determined after correction for unspecific binding in the presence of unlabelled TNF-α in excess. The specific TNF-binding in the filter test was measured at various TNF concentrations and analyzed according to Scatchard, whereby a K_d value of $\cdot 10^{-9}$-10^{-10}M was determined.

Example 2

Cell Extracts of HL-60 Cells

HL60 cells [ATCC No. CCL 240] were cultivated on an experimental laboratory scale in a RPMI 1640 medium [GIBCO catalogue No. 074-01800], which contained 2 g/l $NaHCO_3$ and 5% foetal calf serum, in a 5% CO_2 atmosphere and subsequently centrifuged.

The following procedure was used to produce high cell densities on an industrial scale. The cultivation was carried out in a 75 l Airlift fermenter (Fa. Chemap, Switzerland) with a working volume of 58 l. For this there was used the cassette membrane system "PROSTAK" (Millipore, Switzerland) with a membrane surface of 0.32 m^2 (1 cassette) integrated into the external circulation circuit. The culture medium (see Table 1) was pumped around with a Watson-Marlow pump, Type 603U, with 5 l/min. After a steam sterilization of the installation, whereby the "PROSTAK" system was sterilized separately in autoclaves, the fermentation was started with growing HL-60 cells from a 20 l Airlift fermenter (Chemap). The cell cultivation in the inoculation fermenter was effected in a conventional batch process in the medium according to Table 1 and an initial cell titre of 2×10^5 cells/ml. After 4 days the HL60 batch was transferred with a titre of 4.9×10^6 cells/ml into the 75 l fermenter. The pH value was held at 7.1 and the pO_2 value was held at 25% saturation, whereby the oxygen introduction was effected through a microporous frit. After initial batch fermentation, on the 2nd day the perfusion at a cell titre of 4×10^6 cells/ml was started with 30 l of medium exchange per day. On the filtrate side of the medium the conditioned medium was removed and replaced by the addition of fresh medium. The added medium was fortified as follows: Primatone from 0.25% to 0.35%, glutamine from 5 mM to 6 mM and glucose from 4 g/l to 6 g/l. The perfusion rate was then increased on the 3rd and 4th day to 72 l of medium/day and on the 5th day to 100 l of medium/day. The fermentation had finished after 120 hours of continuous cultivation. Exponential cell growth up to 40×10^6 cells/ml took place under the given fermentation conditions. The duplication time of the cell population was 20-22 hours to 10×10^6 cells/ml and then increased to 30-36 hours with increasing cell density. The proportion of living cells lay at 90-95% during the entire fermentation period. The HL-60 batch was then cooled down in the fermenter to about 12° C. and the cells were harvested by centrifugation (Beckman centrifuge [Model J-6B, Rotor JS], 3000 rpm, 10 min., 4° C.).

TABLE 1

HL-60 medium

Components	Concentrations mg/l
$CaCl_2$ (anhydrous)	112.644
$Ca(NO_3)_2 \cdot 4H_2O$	20

TABLE 1-continued

HL-60 medium

Components	Concentrations mg/l
$CuSO_4 \cdot 5H_2O$	$0.498\cdot10^{-3}$
$Fe(NO_3)_3 \cdot 9H_2O$	0.02
$FeSO_4 \cdot 7H_2O$	0.1668
KCl	336.72
KNO_3	0.0309
$MgCl_2$ (anhydrous)	11.444
$MgSO_4$ (anhydrous)	68.37
NaCl	5801.8
Na_2HPO_4 (anhydrous)	188.408
$NaH_2PO_4 \cdot H_2O$	75
$Na_2SeO_3 \cdot 5H_2O$	$9.6\cdot10^{-3}$
$ZnSO_4 \cdot 7H_2O$	0.1726
D-Glucose	4000
Glutathion (red.)	0.2
Hepes buffer	2383.2
Hypoxanthin	0.954
Linoleic acid	0.0168
Lipoic acid	0.042
Phenol Red	10.24
Putrescine 2HCl	0.0322
Na pyruvate	88
Thymidine	0.146
Biotin	0.04666
D-Ca pantothenate	2.546
Choline chloride	5.792
Folic acid	2.86
i-Inositol	11.32
Niacinamide	2.6
Nicotinamide	0.0074
para-Aminobenzoic acid	0.2
Pyridoxal HCl	2.4124
Pyridoxin HCl	0.2
Riboflavin	0.2876
Thiamin HCl	2.668
Vitamin B_{12}	0.2782
L-Alanine	11.78
L-Aspartic acid	10
L-Asparagine H_2O	14.362
L-Arginine	40
L-Arginine HCl	92.6
L-Aspartate	33.32
L-Cystine 2HCl	62.04
L-Cysteine HCl$\cdot H_2O$	7.024
L-Glutamic acid	36.94
L-Glutamine	730
L-Glycine	21.5
L-Histidine	3
L-Histidine HCl$\cdot H_2O$	27.392
L-Hydroxypyroline	4
L-Isoleucine	73.788
L-Leucine	75.62
L-Lysine HCl	102.9
L-Methionine	21.896
L-Phenylalanine	43.592
L-Proline	26.9
L-Serine	31.3
L-Threonine	53
L-Tryptophan	11.008
L-Tyrosine$\cdot$2Na	69.76
L-Valine	62.74
Penicillin/streptomycin	100 U/ml
Insulin (human)	5 μg/ml
Tranferrin (human)	15 pg/ml
Bovine serum albumin	67 pg/ml
Primatone RL (Sheffield Products, Norwich NY, USA)	0.25%
Pluronic F68 (Serva, Heidelberg, FRG)	0.01%
Foetal calf serum	0.3-3%

The centrifugate was washed with isotonic phosphate buffer (PBS; 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 8.0 g/l NaCl, 2.16 g/l $Na_2HPO_4.7H_2O$), which had been treated with 5% dim-

ethylformamide, 10 mM benzamidine, 100 U/ml aprotinin, 10 μM leupeptin, 1 μM pepstatin, 1 mM o-phenanthroline, 5 mM iodoacetamide, 1 mM phenyl-methylsulphonyl fluoride (referred to hereinafter as PBS-M). The washed cells were extracted at a density of $2.5 \cdot 10^8$ cells/ml in PBS-M with Triton X-100 (final concentration 1.0%). The cell extract was clarified by centrifugation (15,000×g, 1 hour; 100,000×g, 1 hour).

Example 3

Production of Monoclonal (TNF-BP) Antibodies

A centrifugation supernatant from the cultivation of HL60 cells on an experimental laboratory scale, obtained according to Example 2, was diluted with PBS in the ratio 1:10. The diluted supernatant was applied at 4° C. (flow rate: 0.2 ml/min.) to a column which contained 2 ml of Affigel 10 (Bio Rad Catalogue No. 153-6099) to which had been coupled 20 mg of recombinant human TNF-α [Pennica, D. et al. (1984) Nature 312, 724; Shirai, T. et al. (1985) Nature 313, 803; Wang, A. M. et al. (1985) Science 228, 149] according to the recommendations of the manufacturer. The column was washed at 4° C. and a throughflow rate of 1 ml/min firstly with 20 ml of PBS which contained 0.1% Triton X 114 and thereafter with 20 ml of PBS. Thus-enriched TNF-BP was eluted at 22° C. and a flow rate of 2 ml/min with 4 ml of 100 mM glycine, pH 2.8, 0.1% decylmaltoside. The eluate was concentrated to 10 μl in a Centricon 30 unit [Amicon].

10 μl of this eluate were mixed with 20 μl of complete Freund's adjuvant to give an emulsion. 10 μl of the emulsion were injected according to the procedure described by Holmdahl, R. et al. [(1985), J. Immunol. Methods 83, 379] on each of days 0, 7 and 12 into a hind paw of a narcotized Balb/c mouse.

The immunized mice were sacrificed on day 14, the popliteal lymph nodes were removed, minced and suspended by repeated pipetting in Iscove's medium (IMEM, GIBCO Catalogue No. 074-2200) which contained 2 g/l $NaHCO_3$. According to a modified procedure of De St. Groth and Scheidegger [J. Immunol. Methods (1980), 35, 1] 5×10^7 cells of the lymph nodes were fused with 5×10^7 PAI mouse myeloma cells (J. W. Stocker et al. Research Disclosure, 217, May 1982, 155-157) which were in logarithmic growth. The cells were mixed, collected by centrifugation and resuspended in 2 ml of 50% (v/v) polyethylene glycol in IMEM at room temperature by slight shaking and diluted by the slow addition of 10 ml of IMEM during careful shaking for 10 minutes. The cells were collected by centrifugation and resuspended in 200 ml of complete medium [IMEM+20% foetal calf serum, glutamine (2.0 mM), 2-mercaptoethanol (100 μM), 100 μM hypoxanthine, 0.4 μM aminopterine and 16 μM thymidine (HAT)]. The suspension was distributed on 10 tissue culture dishes each containing 96 wells and incubated at 37° C. for 11 days without changing the medium in an atmosphere of 5% CO_2 and a relative humidity of 98%.

The antibodies are distinguished by their inhibitory action on the binding of TNF to HL60 cells or by their binding to antigens in the filter test according to Example 1. The following procedure was used to detect the biological activity of anti(TNF-BP) antibodies: 5×10^6 HL60 or U937 cells were incubated in complete RPMI 1640 medium together with affinity-purified monoclonal anti-(TNF-BP) antibodies or control antibodies (i.e. those which are not directed against TNF-BP) in a concentration range of 1 ng/ml to 10 μg/ml. After incubation at 37° C. for one hour the cells were collected by centrifugation and washed with 4.5 ml of PBS at 0° C. They were resuspended in 1 ml of complete RPMI 1640 medium (Example 2) which additionally contained 0.1% sodium azide and ^{125}I-TNFα (10^6 cpm/ml) with or without the addition of unlabelled TNFα (see above). The specific radioactivity of the ^{125}I-TNFα amounted to 700 Ci/mmol. The cells were incubated at 4° C. for 2 hours, collected and washed 4 times at 0° C. with 4.5 ml of PBS which contained 1% BSA and 0.001% Triton X 100 (Fluke). The radioactivity bound to the cells was measured in a γ-scintillation counter. The cell-bound radioactivity of cells which had not been treated with anti-(TNF-BP) antibodies was determined in a comparative experiment (approximately 10 000 cpm/5×10^6 cells).

Example 4

Affinity Chromatography

For the further purification, a monoclonal anti-(55 kD TNF-BP) antibody (2.8 mg/ml gel), obtained according to Example 3, TNFα (3.9 mg/ml gel) and bovine serum albumin (BSA, 8.5 mg/ml gel) were each covalently coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the directions of the manufacturer. The cell extract obtained according to Example 2 was passed through the thus-prepared columns which were connected in series in the following sequence: BSA-Sepharose pre-column, immune affinity column [anti-(55 kD-TNF-BP) antibody], TNFα-ligand affinity column. After completion of the application the two last-mentioned columns were separated and washed individually with in each case 100 ml of the following buffer solutions: (1) PBS, 1.0% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin; (2) PBS, 0.1% Triton X-100, 0.5M NaCl, 10 mM ATP, 10 mM benzamidine, 100 U/ml aprotinin; and (3) PBS, 0.1% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin. Not only the immune affinity column, but also the TNFα-ligand affinity column were then each eluted with 100 mM glycine pH 2.5, 100 mM NaCl, 0.2% decylmaltoside, 10 mM benzamidine, 100 U/ml aprotinin. The fractions of each column which were active in the filter test according to Example 1 were thereafter combined and neutralized with 1M Tris pH 8.0.

The thus-combined TNF-BP active fractions of the immune affinity chromatography on the one hand and of the TNFα-ligand affinity chromatography on the other hand were, for further purification, again applied to in each case one small TNFα-ligand affinity column. Thereafter, these two columns were washed with in each case 40 ml of (1) PBS, 1.0% Triton X-100, 10 mM benzamidine, 100 U/ml aprotinin, (2) PBS, 0.1% Triton X-100, 0.5M NaCl, 10 mM ATP, 10 mM benzamidine, 100 U/ml aprotinin, (3) PBS, 0.1% Triton X-100, (4) 50 mM Tris pH 7.5, 150 mM NaCl, 1.0% NP-40, 1.0% desoxycholate, 0.1% SDS, (5) PBS, 0.2% decylmaltoside. Subsequently, the columns were eluted with 100 mM glycine pH 2.5, 100 mM NaCl, 0.2% decylmaltoside. Fractions of 0.5 ml from each column were collected and the fractions from each column which were active according to the filter test (Example 1) were combined and concentrated in a Centricon unit (Amicon, molecular weight exclusion 10,000).

Example 5

Separation by Means of HPLC

The active fractions obtained according to Example 4 were each applied according to their different source (immune or

ligand affinity chromatography) to C1/C8 reversed phase HPLC-columns (ProRPC, Pharmacia, 5×20 mm) which had been equilibrated with 0.1% trifluoroacetic acid, 0.1% octylglucoside. The columns were then eluted with a linear acetonitrile gradient (0-80%) in the same buffer at a flow of 0.5 ml/min. Fractions of 1.0 ml were collected from each column and the active fractions from each column were combined (detection according to Example 1).

Example 6

Separation by Means of SDS-PAGE

The fractions which were obtained according to Example 5 and which were active according to the filter test (Example 1) were further separated by SDS-PAGE according to [34]. For this purpose, the samples were heated to 95° C. for 3 minutes in SDS sample buffer and subsequently separated electrophoretically on a 12% acrylamide separation gel with a 5% collection gel. The following standard proteins were used as a reference for the determination of the apparent molecular weights on the SDS-PAGE gel: phosphorylase B (97.4 kD), BSA (66.2 kD), ovalbumin (42.7 kD), carboanhydrase (31.0 kD), soya trypsin inhibitor (21.5 kD) and lysozyme (14.4 kD).

Under the mentioned conditions there were obtained for samples which has been obtained according to Example 4 by TNF-α-ligand affinity chromatography of immune affinity chromatography eluates and which had been further separated by HPLC according to Example 5 two bands of 55 kD and 51 kD as well as three weaker bands of 38 kD, 36 kD and 34 kD. These bands were transferred electrophoretically during 1 hour at 100 V in 25 mM Tris, 192 mM glycine, 20% methanol on to a PVDF membrane (Immobilon, Millipore, Bedford, Mass. USA) in a Mini Trans Blot System (BioRad, Richmond, Calif., USA). Thereafter, the PVDF membrane was either protein-stained with 0.15% Serva-Blue (Serva, Heidelberg, FRG) in methanol/water/glacial acetic acid (50/40/10 parts by volume) or blocked with skimmed milk powder and subsequently incubated with ^{125}I-TNFα according to the filter test conditions described in Example 1 in order to detect bands having TNF-BP activity. This showed that all bands produced in the protein staining bonded TNFα specifically. In the Western blot according to Towbin et al. [38] all of these bands also bonded the monoclonal anti-55 kD-TNF-BP antibody produced according to Example 3. In this case, a procedure according to that described in Example 1 with Na^{125}I radioactively-labelled, affinity-purified (mouse immunoglobulin-Sepharose-4B affinity column) rabbit-anti-mouse-immunoglobulin antibody was used for the autoradiographic detection of this antibody.

Samples which had been obtained according to Example 4 by two-fold TNF-α-ligand affinity chromatography of the throughput of the immune affinity chromatography and which had been further separated by HPLC according to Example 5 showed under the above-specified SDS-PAGE and blot transfer conditions two additional bands of 75 kD and 65 kD, both of which bonded TNF specifically in the filter test (Example 1). In the Western blot according to Towbin et al. (see above) the proteins of these two bands did not react with the anti-(55 kD TNF-BP) antibody produced according to Example 3. They reacted, however, with a monoclonal antibody which had been produced starting from the 75 kD band (anti-75 kD TNF-BP antibody) according to Example 3.

Example 7

Amino Acid Sequence Analysis

For the amino acid sequence analysis, the fractions which had been obtained according to Example 5 and which were active according to the filter test (Example 1) were separated using the SDS-PAGE conditions described in Example 6, but now reducing (SDS sample buffer with 125 mM dithiothreitol). The same bands as in Example 6 were found, but because of the reducing conditions of the SDS-PAGE in comparison to Example 6 all showed an about 1-2 kD higher molecular weight. These bands were then transferred according to Example 6 on to PVDF membranes and stained with 0.15% Serva-Blue in methanol/water/glacial acetic acid (50/400/10 parts by volume) for 1 minute, decolorized with methanol/water/glacial acetic acid (45/48/7 parts by volume), rinsed with water, dried in air and thereafter cut out. The conditions given by Hunkapiller [34] were adhered to in all steps in order to avoid N-terminal blocking. Initially, the purified TNF-BP were used unaltered for the amino acid sequencing. In order to obtain additional sequence information, the TNF-BP after reduction and S-carboxymethylation [Jones, B. N. (1986) in "Methods of Protein Micro-characterisation", J. E. Shively, ed., Humana Press, Clifton N. J., 124-125] were cleaved with cyanogen bromide (Tarr, G. E. in "Methods of Protein Micro-characterisation", 165-166, loc. cit.), trypsin and/or proteinase K and the peptides were separated by HPLC according to known methods of protein chemistry. Thus-prepared samples were then sequenced in an automatic gas phase microsequencing apparatus (Applied Biosystems Model 470A, ABI, Foster City, Calif., USA) with an on-line automatic HPLC PTH amino acid analyzer (Applied Biosystems Model 120, ABI see above) connected to the outlet, whereby the following amino acid sequences were determined:

1. For the 55 kD band (according to non-reducing SDS-PAGE):

Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile (SEQ ID NO: 5),

and

Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys (SEQ ID NO: 6) in which X stands for an amino acid residue which could not be determined,

2. for the 5110 and 38 kD bands (according to non-reducing SDS-PAGE):

Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu (SEQ ID NO: 15)

3. for the 65 kD band (according to non-reducing SDS-PAGE)

In the N-terminal sequencing of the 65 kD band two parallel sequences were determined up to the 15th residue without interruption. Since one of the two sequences corresponded to a partial sequence of ubiquitin [36, 37], the following sequence was derived for the 65 kD band:

Leu-Pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr-Cys. (SEQ ID NO: 16)

in which X stands for an amino acid residue which could not be determined.

Additional peptide sequences for 75(65) kDa-TNF-BP were determined:

Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu (SEQ ID NO: 11)

and

Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-Glu-Lys-Pro-Leu (SEQ ID NO: 7) and Val-Phe-Cys-Thr (SEQ ID NO: 8)

and

Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-Glu-Ala (SEQ ID NO: 9) and Leu-Cys-Ala-Pro (SEQ ID NO: 12)

and

Val-Pro-His-Leu-Pro-Ala-Asp SEQ ID NO: 13)

and

Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro (SEQ ID NO: 14),

in which X stands for an amino acid residue which could not be determined.

Example 8

Determination of Base Sequences of Complementary DNA (cDNA)

Starting from the amino acid sequence according to formula IA there were synthesized having regard to the genetic code for the amino acid residues 2-7 and 17-23 corresponding completely degenerated oligonucleotides in suitable complementarity ("sense" and "antisense" oligonucleotides). Total cellular RNA was isolated from HL60 cells [42, 43] and the first cDNA strand was synthesized by oligo-dT priming or by priming with the "antisense" oligonucleotide using a cDNA synthesis kit (RPN 1256, Amersham, Amersham, England) according to the instructions of the manufacturer. This cDNA strand and the two synthesized degenerate "sense" and "antisense" oligonucleotides were used in a polymerase chain reaction (PCR, Perkin Elmer Cetus, Norwalk, Conn., USA according to the instructions of the manufacturer) to synthesize as a cDNA fragment the base sequence coding for the amino acid residues 8-16 (formula IA). The base sequence of this cDNA fragment accorded to: 5'-AGGGAGAA-GAGAGATAGTGTGTGTCCC-3' (SEQ ID NO: 16). This cDNA fragment was used as a probe in order to identify according to a known procedure a cDNA clone coding for the 55 kD TNF-BP in a λgt11-cDNA gene bank from human placenta (42, 43). This clone was then cut according to usual methods from the λ-vector and cloned in the plasmids pUC18 (Pharmacia, Uppsala, Sweden) and pUC19 (Pharmacia, Uppsala, Sweden) and in the M13 mp18/M13 mp19 bacteriophage (Pharmacia, Uppsala, Sweden) (42, 43). The nucleotide sequence of this cDNA clone was determined using a Sequenase kit (U.S. Biochemical, Cleveland, Ohio, USA) according to the details of the manufacturer. The nucleotide sequence and the amino acid sequence derived therefrom for the 55 kD TNF-BP and its signal peptide (amino acid "−28" to amino acid "O") is given in FIG. 1 using the abbreviations for bases such as amino acids usual in the state of the art. From sequence comparisons with other already known receptor protein sequences there can be determined a N-terminal domain containing approximately 180 amino acids and a C-terminal domain containing 220 amino acids which are separated from one another by a transmembrane region of 19 amino acids (underlined in FIG. 1) which is typical according to the sequence comparisons. Hypothetical glycosylation sites are characterized in FIG. 1 by asterisks above the corresponding amino acid.

Essentially analogous techniques were used to identify 75/65 kD TNF-BP-coding partial cDNA sequences, whereby, however, in this case genomic human DNA and completely degenerated 14-mer and 15-mer "sense" and "antisense" oligonucleotides derived from peptide IIA were used in order to produce a primary 26 by cDNA probe in a polymerase chain reaction. This cDNA probe was then used in a HL-60 cDNA library to identify cDNA clones of different lengths. This cDNA library was produced using isolated HL60 RNA and a cDNA cloning kit (Amersham) according to the details of the manufacturer. The sequence of such a cDNA clone is given in FIG. 4 (SEQ ID NO: 28), whereby repeated sequencing lead to the following correction as depicted in FIG. 6 (SEQ ID NO: 3). A threonine coded by "ACC" not "TCC", has to be at position 3 instead of the serine.

Example 9

Expression in COS1 Cells

Vectors starting from the plasmid "pN11" were constructed for the expression in COS cells. The plasmid "pN11" contains the efficient promotor and enhancer of the "major immediate-early" gene of human cytomegalovirus ("HCMV"; Boshart et al., Cell 41, 521-530, 1985). After the promotor there is situated a short DNA sequence which contains several restriction cleavage sites, which are present only once in the plasmid ("polylinker"), inter alia the cleavage sites for HindIII, BalI, BamHI and PvuII (see sequence).

PvuII

5'-AAGCTTGGCCAGGATCCAGCTGACT-GACTGATCGCGAGATC-3' (SEQ ID NO: 17)

3'-TTCGAACCGGTCCTAGGTCGACTGACT-GACTAGCGCTCTAG-5' (SEQ ID NO: 18)

After these cleavage sites there are situated three translation stop codons in all three reading frames. After the polylinker-sequence there is situated the 2nd intron and the polyadenylation signal of the preproinsulin gene of the rat (Lomedico et al., Cell 18, 545-558, 1979). The plasmid also contains the replication origin of the SV40 virus and a fragment from pBR322 which confers *E. coli*-bacteria ampicillin resistance and permits the replication of the plasmid in *E. coli*.

For the construction of the expression vector "pN123", this plasmid "pN11" was cleaved the restriction endonuclease PvuII and subsequently treated with alkaline phosphatase. The dephosphorylated vector was thereafter isolated from an agarose gel (V1). The 5'-projecting nucleotides of the EcoRI-cleaved 1.3 kb fragment of the 55 kD TNF-BP-cDNA (see Example 8) were filled in using Klenow enzyme. Subsequently, this fragment was isolated from an agarose gel (F1). Thereafter, V1 and F1 were joined together using T4-ligase. *E. coli* HB101 cells were then transformed with this ligation batch according to known methods [42]. By means of restriction analyses and DNA sequencing according to known methods [42] there were identified transformants which had been transformed with a plasmid and which contained the 1.3 kb EcoRI fragment of the 55 kD TNF-BP-cDNA in the correct orientation for expression via the HCMV-promoter. This vector received the designation "pN123".

The following procedure was used for the construction of the vector "pK19". A DNA fragment which contained only the cDNA coding for the extracellular part of the 55 kD TNF-BP (amino acids −28 to 182 according to FIG. 1) was obtained by PCR technology (Saiki et al., Science 230, 1350-1354, 1985, see also Example 8). The following oligonucleotides were used in order to amplify the cDNA from "pN123" coding for the extracellular part of the 55 kD TNF-BP:

BAMHI

5'-CACAGGGATCCATAGCTGTCTG-GCATGGGCCTCTCCAC-3' (SEQ ID NO: 19)

ASP718

3'-CGTGACTCCTGAGTCCGTGGTGTAT-TATCTCTAGACCA TGGCCC-5' (SEQ ID NO: 20)

By means of these oligonucleotides there were also introduced two stop codons of the translation after amino acid 182. The thus-amplified DNA fragment was cleaved with BamHI and Asp718, the thereby resulting projecting ends were filled in using Klenow enzyme and this fragment was subsequently isolated from an agarose gel (F2). F2 was then ligated with V1 and the entire batch was used for the transformation of *E. coli* HB101, as already described. Transformants which had been transformed with a plasmid containing the DNA fragment in the correct orientation for the expression via the HCMV-

promoter were identified by DNA sequencing (see above). The plasmid isolated therefrom received the designation "pK19".

Transfection of the COS cells with the plasmids "pN123" or "pK19" was carried out according to the lipofection method published by Felgner et al. (Proc. Natl. Acad. Sci. USA 84, 7413-7417, 1987). 72 hours after the transfection had been effected the cells transfected with "pN123" were analyzed for binding with ^{125}I-TNFα according to known methods. The results of the Scatchard analysis [Scatchard, G., Ann. N.Y. Acad. Sci. 51, 660, 1949] of the thus-obtained binding data (FIG. 2A) is given in FIG. 2B. The culture supernatants of the cells transfected with "pK19" were investigated in a "sandwich" test. For this purpose, PVC microtitre plates (Dynatech, Arlington, Va., USA) were sensitized with 100 µl/well of a rabbit-anti-mouse immunoglobulin (10 µg/ml PBS). Subsequently, the plates were washed and incubated (3 hours, 20° C.) with an anti-55 kD TNF-BP antibody which had been detected by its antigen binding and isolated according to Example 3, but which did not inhibit the TNF-binding to cells. The plates were then again washed and incubated overnight at 4° C. with 100 µl/well of the culture supernatant (diluted 1:4 with buffer A containing 1% skimmed milk powder: 50 mM Tris/HCl pH 7.4, 140 mM NaCl, 5 mM EDTA, 0.02% Na azide). The plates were emptied and incubated at 4° C. for 2 hours with buffer A containing ^{125}I-TNFα (10^6 cpm/ml, 100 µl/well) with or without the addition of 2 µg/ml of unlabelled TNF. Thereafter, the plates were washed 4 times with PBS, the individual wells were cut out and measured in a λ-counter. The results of 5 parallel transfections (columns #2, 3, 4, 6 and 7), of two control transfections with the pN11 vector (columns #1, 5) and of a control with HL60 cell lysate (column #8) are given in FIG. 3.

Example 10

Expression in Insect Cells

The plasmid "pVL941" (Luckow and Summers, 1989, "High Level Expression of Nonfused Foreign Genes with *Autographa californa* Nuclear Polyhedrosis virus Expression Vectors", Virology 170, 31-39) was used for the expression in a baculovirus expression system and was modified as follows. The single EcoRI restriction cleavage site in "pVL941" was removed by cleaving the plasmid with EcoRI and the projecting 5'-end was filled in with Klenow enzyme. The plasmid pVL941/E obtained therefrom was digested with BamHI and Asp718 and the vector trunk was subsequently isolated from an agarose gel. This fragment was ligated with a synthetic oligonucleotide of the following sequence:

BamHI EcoRI Asp718

5'-GATCCAGAATTCATAATAG-3' (SEQ ID NO: 21)

3'-GTCTTAAGTATTATCCATG-5' (SEQ ID NO: 22)

E. coli HB101 was transformed with the ligation batch and transformants containing a plasmid in which the oligonucleotide had been incorporated correctly were identified by restriction analysis and DNA sequencing according to known methods (see above); this plasmid was named "pNR704". For the construction of the transfer vector "pN113", this plasmid "pNR704" was cleaved with EcoRI, treated with alkaline phosphatase and the thus-produced vector trunk (V2) was subsequently isolated from an agarose gel. The 1.3 kb fragment of the 55 kD TNF-BP-cDNA cleaved with EcoRI as above was ligated with fragment V2. Transformants obtained with this ligation batch, which contained a plasmid containing the cDNA insert in the correct orientation for the expression via the polyhedron promoter, were identified (see above). The vector isolated therefrom received the designation "pN113".

The following procedure was used for the construction of the transfer vector "pN119". The 1.3 kb EcoRI/EcoRI fragment of the 55 kD TNF-BP cDNA in the "pUC19" plasmid (see Example 8) was digested with BanI and ligated with the following synthetic oligonucleotide:

BanI Asp718

5'-GCACCACATAATAGAGATCTGGTACCGGGAA-3' (SEQ ID NO: 23)

3'-GTGTATTATCTCTAGACCATGGCCC-5' (SEQ ID NO: 24)

Two stop codons of the translation after amino acid 182 and a cleavage site for the restriction endonuclease Asp718 are incorporated with the above adaptor. After carrying out ligation the batch was digested with EcoRI and Asp718 and the partial 55 kD TNF-BP fragment (F3) was isolated. Furthermore, the plasmid "pNR704", likewise cleaved with Asp718 and EcoRI, was ligated with F3 and the ligation batch was transformed into *E. coli* HB101. The identification of the transformants which contained a plasmid in which the partial 55 kD TNF-BP cDNA had been correctly integrated for the expression was effected as already described. The plasmid isolated from these transformants received the name "pN119".

The following procedure was used for the construction of the transfer vector "pN124". The cDNA fragment coding for the extracellular part of the 55 kD TNF-BP, described in Example 9, was amplified with the specified oligonucleotides with the aid of PCR technology as described in Example 9. This fragment was cleaved with BamHI and Asp718 and isolated from an agarose gel (F4). The plasmid "pNR704" was also cleaved with BamHI and Asp718 and the vector trunk (V4) was isolated (see above). The fragments V4 and F4 were ligated, *E. coli* HB101 was transformed therewith and the recombinant transfer vector "pN124" was identified and isolated as described.

The following procedure was used for the transfection of the insect cells. 3 µg of the transfer vector "pN113" were transfected with 1 µg of DNA of the *Autographa californica* nuclear polyhedrosisvirus (AcMNPV) (EP 127839) in Sf9 cells (ATCC CRL 1711). Polyhedron-negative viruses were identified and purified from "plaques" [52]. Sf9 cells were again infected with these recombinant viruses as described in [52]. After 3 days in the culture the infected cells were investigated for TNF-binding using ^{125}I-TNFα. For this purpose, the transfected cells were washed from the cell culture dish with a Pasteur pipette and resuspended at a cell density of 5×10^6 cells/ml of culture medium [52] which contained 10 ng/ml of ^{125}I-TNF-α, not only in the presence of, but also in the absence of 5 µg/ml of non-labelled TNF-α and incubated on ice for 2 hours. Thereafter, the cells were washed with pure culture medium and the cell-bound radioactivity was counted in a γ-counter (see Table 2).

TABLE 2

Cells	Cell-bound radioactivity per 10^6 cells
Non-infected cells (control)	60 cpm
Infected cells	1600 ± 330 cpm [1]

[1] Average and standard deviation from 4 experiments

Example 11

Analogously to the procedure described in Example 9, the cDNA fragment coding for the extracellular region of the 55

kDa TNF-BP was amplified in a polymerase chain reaction, but now using the following oligonucleotides as the primer:
Oligonucleotide 1:
Sst I
5'-TAC GAG CTC GGC CAT AGC TGT CTG GCA TG-3' (SEQ ID NO: 25)
Oligonucleotide 2:
Sst I
5'-ATA GAG CTC TGT GGT GCC TGA GTC CTC AG-3' (SEQ ID NO: 26)

This cDNA fragment was ligated in the pCD4-Hγ3 vector [DSM 5523; European Patent Application No. 90107393.2; Japanese Patent Application No. 108967/90; U.S. patent application Ser. No. 51/077,390] from which the CD4-cDNA had been removed via the SstI restriction cleavage sites. SstI cleavage sites are situated in vector pCD4-Hγ3 not only in front of, but also behind the CD4-partial sequence fragment. The construction was transfixed in J558 myeloma cells (ATCC No. TIB6) by means of protoplast fusion according to Oi et al. (Procd. Natl. Acad. Sci. USA 80, 825-829, 1983). Transfectants were selected by adding 5 μg/ml of mycophenolic acid and 250 μg/ml of xanthin (Traunecker et al., Eur. J. Immunol. 16, 851-854 [1986]) in basic medium (Dulbecco's modified Eagle's Medium, 10% foetal calf serum, 5×10^{-5}M 2-mercaptoethanol). The expression product secreted by the transfixed cells could be purified using usual methods of protein chemistry, e.g. TNF-BP-antibody affinity chromatography. Unless not already specifically indicated, standard procedures as described e.g. by Freshney, R. I. in "Culture of Animal Cells", Alan R. Liss, Inc., New York (1983) were used for the cultivation of the cell lines employed, for the cloning, for the selection or for the expansion of the cloned cells.

REFERENCES

1. G. E. Nedwin, S. L. Naylor, A. Y. Sakaguchi, D. Smith, J. Jarrett-Nedwin, D. Pennica, D. V. Goeddel and P. W. Gray: Nucl. Acids Res. 13, 6361, 1985
2. B. Beutler and A. Cerami: New England J. Med. 316, 379, 1987
3. L. J. Old: Science 230, 630, 1985
4. G. Trinchieri, M. Kobayashi, M. Rosen, R. Loudon, M. Murphy and B. Perussia: J. exp. Med. 164, 1206, 1986
5. J. Vilcek, V. J. Palombella, D. Henriksen-de Stefano, C. Swenson, R. Feinman, M. Hirai and M. Tsujimoto: J. exp. Med. 163, 632, 1986
6. B. J. Sugarman, B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino and H. M. Shepard: Science 230, 943, 1985
7. J. R. Gamble, J. M. Harlan, S. J. Klebanoff and M. A. Vadas: Proc. Natl. Acad. Sci. USA 82, 8667, 1985
8. N. Sato, T. Goto, K. Haranaka, N. Satomi, H. Nariuchi, Y. Mano and Y. Sawasaki: J. Natl. Cancer Inst. 76, 1113, 1986
9. A. H. Stolpen, E. C. Guinan, W. Fiers and J. S. Pober: Am. J. Pathol. 123, 16, 1986
10. J. S. Pober, L. A. Lapierre, A. H. Stolpen, T. A. Brock, T. A. Springer, W. Fiers, M. P. Bevilacqua, D. L. Mendrick and M. A Gimbrone: J. Immunol. 138, 3319, 1987
11. M. Kawakami, P. Pekala, M. Lane and A. Cerami: Proc. Natl. Acad. Sci. USA 79, 912, 1982
12. T. Collins, L. A. Lapierre, W. Fiers, J. L. Strominger and J. S Pober: Proc. Natl. Acad. Sci. USA 83, 446, 1986
13. G. H. W. Wong and D. V. Goeddel: Nature 323, 819, 1986
14. J. W. Lowenthal, D. W. Ballard, E. Böhnlein and W. C. Greene: Proc. Natl. Acad. Sci. USA 86, 2331, 1989
15. M. J. Lenardo, C. M. Fan, T. Maniatis and D. Baltimore: Cell 57, 287, 1989
16. A. E. Goldfeld and T. Maniatis: Proc. Natl. Acad. Sci. USA 86, 1490, 1989
17. A. Waage, A. Halsteuren and T. Espevik: Lancet, Febr. 14, 1987, 355,
18. C. O. Jacob and H. O. McDevitt: Nature 331, 356, 1988
19. G. E. Grau, L. F. Fajardo, P. Piquet, B. Allet, P. Lambert and P. Vassalli: Science 237, 1210, 1987
20. B. Beutler, I. W. Milsark and A. C. Cerami: Science 229, 869, 1985
21. B. B. Aggarwal, T. E. Eessalu and P. E. Hass: Nature 318, 665, 1985
22. M. Tsujimoto, Y. K. Yip and J. Vilcek: Proc. Natl. Acad. Sci. USA 82, 7626, 1985
23. C. Baglioni, S. McCandless, J. Tavernier and W. Fiers: J. Biol. Chem. 260, 13395, 1985
24. P. Hohmann, R. Remy, M. Brockhaus and A. P. G. M. van Loon: J. Biol. Chem., im Druck
25. F. C. Kull, S. Jacobs and P. Cuatrecasas: Proc. Natl. Acad. Sci. USA 82, 5756, 1985
26. A. A. Creasy, R. Yamamoto and Ch. R. Vitt: Proc. Natl. Acad. Sci. USA 84, 3293, 1987
27. G. B. Stauber, R. A. Aiyer and B. B. Aggarwal: J. Biol. Chem. 263, 19098, 1988
28. K. Hirano, K. Yamamoto, Y. Kobayashi and T. Osawa: J. Biochem. 105, 120, 1989
29. Y. Niitsu, N. Watanabe, H. Sone, H. Neda, N. Yamauchi, M. Maeda and I. Urushizaki: J. Biol. Resp. Modifiers 7, 276, 1988
30. I. Olsson, A. Grubb, U. Gullberg, M. Lantz, E. Nilsson, C. Peetre and H. Thysell: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. Januar 1989
31. H. R. Loetscher and M. Brockhaus: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. Januar 1989
32. M. Brockhaus, H. Loetscher, H.-P. Hohmann and W. Hunziker: Abstract, 2nd Intern. Conference on Tumor Necrosis Factor and Related Cytokines, Napa, Calif., 15.-20. Januar 1989
33. C. R. Cantor and P. R. Schimmel, in Biophysical Chemistry, W.H. Freeman, ed., San Francisco, 1980, P. 850
34. M. W. Hunkapiller, E. Lujan, F. Ostrander, L. E. Hood: Methods Enzymol. 91, 227, 1983
35. U. K. Lämmli: Nature 227, 680, 1970
36. T. St. John, W. M. Gallatin, M. Siegelman. H. T. Smith, V. A. Fried and I. L. Weissman: Science 231, 845, 1986
37. M. Siegelman, M. W. Bond, W. M. Gallatin, T. St. John, H. T. Smith, V. A. Fried and I. L. Weissman: Science 231, 823, 1986
38. H. Towbin, T. Staehelin and J. Gordon: Proc. Natl. Acad. Sci. USA 76, 4350, 1979
39. Dinarello, Ch. A., in Lymphokines, Vol. 14, E. Pick, ed., p. 1, Academic Press, London, 1987
40. D. J. Merchant, R. H. Kahn and W. H. Murphy: Handbook of Cell and Organ Culture, Burgess Publ. Co., Minneapolis, 1969
41. G. E. Grau, T. E. Taylor, M. E. Molyneux, J. J. Wirima, P. Vassalli, M. Hommel and P. Lambert: New Engl. J. Med. 320, 1586, 1989
42. J. Sambrook, E. F. Fritsch and T. Maniatis: Molecular. Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989
43. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman and K. Struhl: Current Protocols in Molecular Biology 1987-1988, S. Wiley and Sons, New York, 1987

44. E. Harlow and D. Lane: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Publications, New York, 1988
45. S. Fazekas de St. Groth and D. Scheidegger: J. Immunol. Methods 35, 1, 1980
46. D. Pennica and D. V. Goeddel, in Lymphokines, Vol. 13, D. R. Webb and D. V. Goeddel, eds. p. 163, Academic Press, London, 1987
47. J. Tavernier, L. Franzen, A. Marmenout, J. van der Heyden, R. Muller, M. Ruysschaert, A. van Vliet, R. Banden and W. Fiers, in Lymphokines, Vol. 13, D. R. Webb and D. V. Goeddel, eds., p. 181, Academic Press, London
48. P. J. Fraker and J. C. Speck: Biochem. Biophys. Res. Commun. 80, 849, 1987
49. D. H. Erlich, D. H. Gelfand, R. K. Saiki: Nature 331, 61, 1988
50. Bosserhoff, J. Wallach and R. W. Frank: J. Chromatogr. 473, 71, 1989
51. R. Lathe: J. Mol. Biol. 183, 1, 1985
52. Luckow and Summers, "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures", Texas Agricultural Experimental Station, Texas A & M University, Bulletin No. 1555, 2nd edition, 1988

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ccctcgatgt acatagcttt tctcagctgc ctgcgcgccg ccgacagtca gcgctgtgcg 1740

cgcggagaga ggtgcgccgt gggctcaaga gcctgagtgg gtggtttgcg aggatgaggg 1800

acgctatgcc tcatgcccgt tttgggtgtc ctcaccagca aggctgctcg ggggcccctg 1860

gttcgtcccc gagccttttt cacagtgcat aagcagtttt ttttgttttt gttttgtttt 1920

gttttgtttt taaatcaatc atgttacact aatagaaact tggcactcct gtgccctctg 1980

cctggacaag cacatagcaa gctgaactgt cctaaggcag gggcgagcac ggaacaatgg 2040

ggccttcagc tggagctgtg gacttttgta catacactaa aattctgaag ttaaaaaaaa 2100

aacccgaatt c 2111

<210> SEQ ID NO 2
<211> LENGTH: 455
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
210 215 220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225 230 235 240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
245 250 255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
260 265 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val

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        275                 280                 285

Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys
    290                 295                 300

Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
305                 310                 315                 320

Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
                325                 330                 335

Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
            340                 345                 350

Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
        355                 360                 365

Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
    370                 375                 380

Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
385                 390                 395                 400

Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala
                405                 410                 415

Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
            420                 425                 430

Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro
        435                 440                 445

Pro Ala Pro Ser Leu Leu Arg
    450                 455


<210> SEQ ID NO 3
<211> LENGTH: 2339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

tcggacaccg tgtgtgactc ctgtgaggac agcacataca cccagctctg gaactgggtt     60

cccgagtgct tgagctgtgg ctcccgctgt agctctgacc aggtggaaac tcaagcctgc    120

actcgggaac agaaccgcat ctgcacctgc aggcccggct ggtactgcgc gctgagcaag    180

caggaggggt gccggctgtg cgcgccgctg ccgaagtgcc gcccgggctt cggcgtggcc    240

agaccaggaa ctgaaacatc agacgtggtg tgcaagccct gtgccccggg gacgttctcc    300

aacacgactt catccacgga tatttgcagg ccccaccaga tctgtaacgt ggtggccatc    360

cctgggaatg caagcaggga tgcagtctgc acgtccacgt cccccacccg gagtatggcc    420

ccaggggcag tacacttacc ccagccagtg tccacacgat cccaacacac gcagccaagt    480

ccagaaccca gcactgctcc aagcacctcc ttcctgctcc caatgggccc cagccccccа    540

gctgaaggga gcactggcga cttcgctctt ccagttggac tgattgtggg tgtgacagcc    600

ttgggtctac taataatagg agtggtgaac tgtgtcatca tgacccaggt gaaaaagaag    660

cccttgtgcc tgcagagaga agccaaggtg cctcacttgc ctgccgataa ggccccgggt    720

acacagggcc ccgagcagca gcacctgctg atcacagcgc cgagctccag cagcagctcc    780

ctggagagct cggccagtgc gttggacaga agggcgccca ctcggaacca gccacaggca    840

ccaggcgtgg aggccagtgg ggccggggag gcccgggcca gcaccgggag ctcagcagat    900

tcttcccctg gtggccatgg gacccaggtc aatgtcacct gcatcgtgaa cgtctgtagc    960

agctctgacc acagctcaca gtgctcctcc caagccagct ccacaatggg agacacagat   1020

tccagccccт cggagtcccc gaaggacgag caggtcccct tctccaagga ggaatgtgcc   1080

tttcggtcac agctggagac gccagagacc ctgctgggga gcaccgaaga gaagcccctg   1140
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ccccttggag tgcctgatgc tgggatgaag cccagttaac caggccggtg tgggctgtgt 1200

cgtagccaag gtggctgagc cctggcagga tgaccctgcg aaggggccct ggtccttcca 1260

ggcccccacc actaggactc tgaggctctt tctgggccaa gttcctctag tgccctccac 1320

agccgcagcc tccctctgac ctgcaggcca agagcagagg cagcgagttg tggaaagcct 1380

ctgctgccat ggcgtgtccc tctcggaagg ctggctgggc atggacgttc ggggcatgct 1440

ggggcaagtc cctgagtctc tgtgacctgc cccgcccagc tgcacctgcc agcctggctt 1500

ctggagccct tgggtttttt gtttgtttgt ttgtttgttt gtttgtttct ccccctgggc 1560

tctgcccagc tctggcttcc agaaaacccc agcatccttt tctgcagagg ggctttctgg 1620

agaggaggga tgctgcctga gtcacccatg aagacaggac agtgcttcag cctgaggctg 1680

agactgcggg atggtcctgg ggctctgtgc agggaggagg tggcagccct gtagggaacg 1740

gggtccttca agttagctca ggaggcttgg aaagcatcac ctcaggccag gtgcagtggc 1800

tcacgcctat gatcccagca ctttgggagg ctgaggcggg tggatcacct gaggttagga 1860

gttcgagacc agcctggcca acatggtaaa accccatctc tactaaaaat acagaaatta 1920

gccgggcgtg gtggcgggca cctatagtcc cagctactca gaagcctgag gctgggaaat 1980

cgtttgaacc cgggaagcgg aggttgcagg gagccgagat cacgccactg cactccagcc 2040

tgggcgacag agcgagagtc tgtctcaaaa gaaaaaaaaa aagcaccgcc tccaaatgct 2100

aacttgtcct tttgtaccat ggtgtgaaag tcagatgccc agagggccca ggcaggccac 2160

catattcagt gctgtggcct gggcaagata acgcacttct aactagaaat ctgccaattt 2220

tttaaaaaag taagtaccac tcaggccaac aagccaacga caaagccaaa ctctgccagc 2280

cacatccaac cccccacctg ccatttgcac cctccgcctt cactccggtg tgcctgcag 2339

<210> SEQ ID NO 4
<211> LENGTH: 392
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
1 5 10 15

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
20 25 30

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
35 40 45

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
50 55 60

Arg Leu Cys Ala Pro Leu Pro Lys Cys Arg Pro Gly Phe Gly Val Ala
65 70 75 80

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
85 90 95

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
100 105 110

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
115 120 125

Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
130 135 140

His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Ser
145 150 155 160

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly

```
                165                 170                 175

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Phe Ala Leu Pro Val
            180                 185                 190

Gly Leu Ile Val Gly Val Thr Ala Leu Gly Leu Leu Ile Ile Gly Val
        195                 200                 205

Val Asn Cys Val Ile Met Thr Gln Val Lys Lys Lys Pro Leu Cys Leu
    210                 215                 220

Gln Arg Glu Ala Lys Val Pro His Leu Pro Ala Asp Lys Ala Arg Gly
225                 230                 235                 240

Thr Gln Gly Pro Glu Gln Gln His Leu Leu Ile Thr Ala Pro Ser Ser
                245                 250                 255

Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser Ala Leu Asp Arg Arg Ala
            260                 265                 270

Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala
        275                 280                 285

Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser Ala Asp Ser Ser Pro Gly
    290                 295                 300

Gly His Gly Thr Gln Val Asn Val Thr Cys Ile Val Asn Val Cys Ser
305                 310                 315                 320

Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln Ala Ser Ser Thr Met
                325                 330                 335

Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro Lys Asp Glu Gln Val
            340                 345                 350

Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser Gln Leu Glu Thr Pro
        355                 360                 365

Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro Leu Pro Leu Gly Val
    370                 375                 380

Pro Asp Ala Gly Met Lys Pro Ser
385                 390


<210> SEQ ID NO 5
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa = unknown amino acid

<400> SEQUENCE: 5

Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro
1               5                   10                  15

Gln Gly Lys Tyr Ile His Pro Gln Xaa Asn Ser Ile
            20                  25


<210> SEQ ID NO 6
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 6

Ser Thr Pro Glu Lys Glu Gly Glu Leu Glu Gly Thr Thr Thr Lys
1               5                   10                  15


<210> SEQ ID NO 7
<211> LENGTH: 18
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 7

Ser Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys
1 5 10 15

Pro Leu

<210> SEQ ID NO 8
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 8

Val Phe Cys Thr
1

<210> SEQ ID NO 9
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 9

Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala Gly Glu Ala
1 5 10 15

<210> SEQ ID NO 10
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa = unknown amino acid

<400> SEQUENCE: 10

Leu Pro Ala Gln Val Ala Phe Xaa Pro Tyr Ala Pro Glu Pro Gly Ser
1 5 10 15

Thr Cys

<210> SEQ ID NO 11
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa = unknown amino acid

<400> SEQUENCE: 11

Ile Xaa Pro Gly Phe Gly Val Ala Tyr Pro Ala Leu Glu
1 5 10

<210> SEQ ID NO 12
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 12

Leu Cys Ala Pro
1

<210> SEQ ID NO 13
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 13

Val Pro His Leu Pro Ala Asp
1 5

<210> SEQ ID NO 14
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (9)..(10)
<223> OTHER INFORMATION: Xaa = unknown amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa = unknown amino acid

<400> SEQUENCE: 14

Gly Ser Gln Gly Pro Glu Gln Gln Xaa Xaa Leu Ile Xaa Ala Pro
1 5 10 15

<210> SEQ ID NO 15
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 15

Leu Val Pro His Leu Gly Asp Arg Glu
1 5

<210> SEQ ID NO 16
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 16

agggagaaga gagatagtgt gtgtccc 27

<210> SEQ ID NO 17
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 17

aagcttggcc aggatccagc tgactgactg atcgcgagat c 41

<210> SEQ ID NO 18

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<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 18

gatctcgcga tcagtcagtc agctggatcc tggccaagct t 41

<210> SEQ ID NO 19
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 19

cacagggatc catagctgtc tggcatgggc ctctccac 38

<210> SEQ ID NO 20
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 20

cccggtacca gatctctatt atgtggtgcc tgagtcctca gtgc 44

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 21

gatccagaat tcataatag 19

<210> SEQ ID NO 22
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 22

gtacctatta tgaattctg 19

<210> SEQ ID NO 23
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 23

gcaccacata tagagatct ggtaccggga a 31

<210> SEQ ID NO 24
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense primer

<400> SEQUENCE: 24

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cccggtacca gatctctatt atgtg 25

<210> SEQ ID NO 25
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 25

tacgagctcg gccatagctg tctggcatg 29

<210> SEQ ID NO 26
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 26

atagagctct gtggtgcctg agtcctcag 29

<210> SEQ ID NO 27
<211> LENGTH: 461
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1 5 10 15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
50 55 60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65 70 75 80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
85 90 95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
100 105 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
115 120 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
130 135 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145 150 155 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
165 170 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
180 185 190

Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
195 200 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
210 215 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225 230 235 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly

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               245                 250                 255

Asp Phe Ala Leu Pro Val Gly Leu Ile Val Gly Val Thr Ala Leu Gly
            260                 265                 270

Leu Leu Ile Ile Gly Val Val Asn Cys Val Ile Met Thr Gln Val Lys
        275                 280                 285

Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro
    290                 295                 300

Ala Asp Lys Ala Arg Gly Thr Gln Gly Pro Glu Gln Gln His Leu Leu
305                 310                 315                 320

Ile Thr Ala Pro Ser Ser Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser
                325                 330                 335

Ala Leu Asp Arg Arg Ala Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly
            340                 345                 350

Val Glu Ala Ser Gly Ala Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser
        355                 360                 365

Asp Ser Ser Pro Gly Gly His Gly Thr Gln Val Asn Val Thr Cys Ile
    370                 375                 380

Val Asn Val Cys Ser Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln
385                 390                 395                 400

Ala Ser Ser Thr Met Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro
                405                 410                 415

Lys Asp Glu Gln Val Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser
            420                 425                 430

Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro
        435                 440                 445

Leu Pro Leu Gly Val Pro Asp Ala Gly Met Lys Pro Ser
    450                 455                 460
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<210> SEQ ID NO 28
<211> LENGTH: 2339
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

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tcggactccg tgtgtgactc ctgtgaggac agcacataca cccagctctg gaactgggtt     60

cccgagtgct tgagctgtgg ctcccgctgt agctctgacc aggtggaaac tcaagcctgc    120

actcgggaac agaaccgcat ctgcacctgc aggcccggct ggtactgcgc gctgagcaag    180

caggaggggt gccggctgtg cgcgccgctg ccgaagtgcc gcccgggctt cggcgtggcc    240

agaccaggaa ctgaaacatc agacgtggtg tgcaagccct gtgccccggg gacgttctcc    300

aacacgactt catccacgga tatttgcagg ccccaccaga tctgtaacgt ggtggccatc    360

cctgggaatg caagcaggga tgcagtctgc acgtccacgt cccccacccg gagtatggcc    420

ccaggggcag tacacttacc ccagccagtg tccacacgat cccaacacac gcagccaagt    480

ccagaaccca gcactgctcc aagcacctcc ttcctgctcc caatgggccc cagccccca     540

gctgaaggga gcactggcga cttcgctctt ccagttggac tgattgtggg tgtgacagcc    600

ttgggtctac taataatagg agtggtgaac tgtgtcatca tgacccaggt gaaaaagaag    660

cccttgtgcc tgcagagaga agccaaggtg cctcacttgc ctgccgataa ggcccggggt    720

acacagggcc ccgagcagca gcacctgctg atcacagcgc cgagctccag cagcagctcc    780

ctggagagct cggccagtgc gttggacaga agggcgccca ctcggaacca gccacaggca    840

ccaggcgtgg aggccagtgg ggccggggag gcccgggcca gcaccgggag ctcagcagat    900

tcttcccctg gtggccatgg gacccaggtc aatgtcacct gcatcgtgaa cgtctgtagc    960
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agctctgacc acagctcaca gtgctcctcc caagccagct ccacaatggg agacacagat 1020

tccagcccct cggagtcccc gaaggacgag caggtccect tctccaagga ggaatgtgcc 1080

tttcggtcac agctggagac gccagagacc ctgctgggga gcaccgaaga gaagcccctg 1140

cccettggag tgcctgatgc tgggatgaag cccagttaac caggccggtg tgggctgtgt 1200

cgtagccaag gtggctgagc cctggcagga tgaccctgcg aaggggccct ggtccttcca 1260

ggcccccacc actaggactc tgaggctctt tctgggccaa gttcctctag tgccctccac 1320

agccgcagcc tccctctgac ctgcaggcca agagcagagg cagcgagttg tggaaagcct 1380

ctgctgccat ggcgtgtccc tctcggaagg ctggctgggc atggacgttc ggggcatgct 1440

ggggcaagtc cctgagtctc tgtgacctgc cccgcccagc tgcacctgcc agcctggctt 1500

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tctgcccagc tctggcttcc agaaaacccc agcatccttt tctgcagagg ggctttctgg 1620

agaggaggga tgctgcctga gtcacccatg aagacaggac agtgcttcag cctgaggctg 1680

agactgcggg atggtcctgg ggctctgtgc agggaggagg tggcagccct gtagggaacg 1740

gggtccttca agttagctca ggaggcttgg aaagcatcac ctcaggccag gtgcagtggc 1800

tcacgcctat gatcccagca ctttgggagg ctgaggcggg tggatcacct gaggttagga 1860

gttcgagacc agcctggcca acatggtaaa accccatctc tactaaaaat acagaaatta 1920

gccgggcgtg gtggcgggca cctatagtcc cagctactca gaagcctgag gctgggaaat 1980

cgtttgaacc cgggaagcgg aggttgcagg gagccgagat cacgccactg cactccagcc 2040

tgggcgacag agcgagagtc tgtctcaaaa gaaaaaaaaa aagcaccgcc tccaaatgct 2100

aacttgtcct tttgtaccat ggtgtgaaag tcagatgccc agagggccca ggcaggccac 2160

catattcagt gctgtggcct gggcaagata acgcacttct aactagaaat ctgccaattt 2220

tttaaaaaag taagtaccac tcaggccaac aagccaacga caaagccaaa ctctgccagc 2280

cacatccaac cccccacctg ccatttgcac cctccgcctt cactccggtg tgcctgcag 2339

<210> SEQ ID NO 29
<211> LENGTH: 392
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

Ser Asp Ser Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
1 5 10 15

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
20 25 30

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
35 40 45

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
50 55 60

Arg Leu Cys Ala Pro Leu Pro Lys Cys Arg Pro Gly Phe Gly Val Ala
65 70 75 80

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
85 90 95

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
100 105 110

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
115 120 125

Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val

-continued

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    130                 135                 140

His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Ser
145                 150                 155                 160

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
                165                 170                 175

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Phe Ala Leu Pro Val
            180                 185                 190

Gly Leu Ile Val Gly Val Thr Ala Leu Gly Leu Leu Ile Ile Gly Val
        195                 200                 205

Val Asn Cys Val Ile Met Thr Gln Val Lys Lys Lys Pro Leu Cys Leu
    210                 215                 220

Gln Arg Glu Ala Lys Val Pro His Leu Pro Ala Asp Lys Ala Arg Gly
225                 230                 235                 240

Thr Gln Gly Pro Glu Gln Gln His Leu Leu Ile Thr Ala Pro Ser Ser
                245                 250                 255

Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser Ala Leu Asp Arg Arg Ala
            260                 265                 270

Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala
        275                 280                 285

Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser Ala Asp Ser Ser Pro Gly
    290                 295                 300

Gly His Gly Thr Gln Val Asn Val Thr Cys Ile Val Asn Val Cys Ser
305                 310                 315                 320

Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln Ala Ser Ser Thr Met
                325                 330                 335

Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro Lys Asp Glu Gln Val
            340                 345                 350

Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser Gln Leu Glu Thr Pro
        355                 360                 365

Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro Leu Pro Leu Gly Val
    370                 375                 380

Pro Asp Ala Gly Met Lys Pro Ser
385                 390
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The invention claimed is:

1. A method comprising the steps of:

(a) culturing a host cell comprising a polynucleotide, wherein the polynucleotide encodes a protein consisting of:

(i) the extracellular region of an insoluble human TNF receptor, wherein the insoluble human TNF receptor has an apparent molecular weight of about 75 kilodaltons as determined on a non-reducing SDS-polyacrylamide gel and comprises the amino acid sequence LPAQVAFX-PYAPEPGSTC (SEQ ID NO: 10), and

(ii) all of the domains of the constant region of a human IgG immunoglobulin heavy chain other than the first domain of said constant region, and

(b) purifying an expression product of the polynucleotide from the cell mass or the culture medium.

2. The method of claim **1**, wherein the host cell is a CHO cell.

3. The method of claim **1**, wherein the IgG heavy chain is an IgG_1 heavy chain.

4. A polynucleotide encoding a protein consisting of:

(a) the extracellular region of an insoluble human TNF receptor,

wherein the insoluble human TNF receptor (i) has an apparent molecular weight of about 75 kilodaltons as determined on a non-reducing SDS-polyacrylamide gel and (ii) comprises the amino acid sequence LPAQVAFXPYAPEPGSTC (SEQ ID NO: 10), and

(b) all of the domains of the constant region of a human IgG_1 immunoglobulin heavy chain other than the first domain of said constant region.

5. A vector comprising the polynucleotide of claim **4**.

6. A mammalian host cell comprising the polynucleotide of claim **4**.

7. A method comprising the steps of:

(a) culturing a host cell comprising a polynucleotide, wherein the polynucleotide encodes a protein consisting of:

(i) the extracellular region of an insoluble human TNF receptor, wherein the insoluble human TNF receptor comprises the amino acid sequence of SEQ ID NO:27 and

(ii) all of the domains of the constant region of a human IgG immunoglobulin heavy chain other than the first domain of said constant region, and

(b) purifying an expression product of the polynucleotide from the cell mass or the culture medium.

8. The method of claim **7**, wherein the human IgG immunoglobulin heavy chain is an IgG_1 heavy chain.

9. The method of claim **7**, wherein the host cell is a CHO cell.

10. The method of claim **8**, wherein the host cell is a CHO cell.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that on November 8, 2019, I electronically filed the foregoing with the Clerk of the Court for the United States Court of Appeals for the Federal Circuit using the Court's CM/ECF system. Counsel for all parties to the case are registered CM/ECF users and will be served by the CM/ECF system.

/s/ *William M. Jay*
William M. Jay

RULE 32(g) CERTIFICATE OF COMPLIANCE

Undersigned counsel certifies that this brief complies with the type-volume limitation of Fed. Cir. R. 32(a) because it contains 13,985 words, excluding the parts of the brief exempted by Fed. R. App. P. 32(f).

Undersigned counsel further certifies that this brief complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6) because this brief has been prepared in a proportionally spaced 14-point Times New Roman typeface using Microsoft Word 2010.

/s/ *William M. Jay*
William M. Jay