ADDENDUM

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

WYETH LLC,	)	
	)	
Plaintiff,	)	
	)	
vs.	)	**Case No. 21-cv-1338-MFK**
	)	
ASTRAZENECA PHARMACEUTICALS LP and ASTRAZENECA AB,	)	
	)	
Defendants.	)	

FINAL JUDGMENT

Whereas, the Court having held a jury trial and the jury having rendered a verdict on May 17, 2024; the Court having held a bench trial regarding certain defenses and having issued findings of fact and conclusions of law on August 6, 2024; and the Court having ruled on the parties' post-trial motions on August 14, 2024,

Pursuant to Federal Rule of Civil Procedure 58(b), IT IS HEREBY ORDERED that:

1. Judgment is entered in favor of Defendants AstraZeneca Pharmaceuticals LP and AstraZeneca AB (collectively "AstraZeneca") on Counts 1 and 2 of the complaint by Plaintiff Wyeth LLC ("Wyeth") alleging infringement of United States Patent Nos. 10,603,314 (the '314 patent) and 10,596,162 (the '162 patent), based on a determination that AstraZeneca induced infringement of claims 1, 3, and 9 of the '314 patent and claim 1 of the '162 patent but that those claims are invalid under 35 U.S.C. § 112 for lack of enablement and lack of written description.

2. Judgment is entered in favor of Wyeth LLC on Counts 1, 3, and 5 of

AstraZeneca's Counterclaim.

3. Judgment is entered in favor of AstraZeneca on Counts 2 and 4 of its counterclaim based on a determination that claims 1, 3, and 9 of the '314 patent and claim 1 of the '162 patent are invalid under 35 U.S.C. § 112 for lack of enablement and lack of written description.

4. The non-final judgment entered on May 28, 2024 (dkt. 463) is vacated.

This is a final and appealable judgment.

Date: August 14, 2024

MATTHEW F. KENNELLY
United States District Judge

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

WYETH LLC,	)	
	)	
Plaintiff,	)	
	)	
vs.	)	**Case No. 21 C 1338**
	)	
ASTRAZENECA PHARMACEUTICALS LP and ASTRAZENECA AB,	)	
	)	
Defendants.	)	

MEMORANDUM OPINION AND ORDER

MATTHEW F. KENNELLY, District Judge:

Wyeth LLC has sued AstraZeneca Pharmaceuticals LP and AstraZeneca AB (collectively AstraZeneca) for infringement of two patents: United States Patent Nos. 10,603,314 (the '314 patent) and 10,596,162 (the '162 patent).[1] Wyeth contends that AstraZeneca, through the promotion and sale of its drug Tagrisso (osimertinib), induced infringement of claims 1, 3, and 9 of the '314 patent and claim 1 of the '162 patent. After a five-day trial, a jury found that the patents were not invalid and that AstraZeneca induced infringement of the patents. AstraZeneca has moved for judgment of a matter of law, arguing that no reasonable jury could have found that (1) AstraZeneca induced infringement of the patents-in-suit; (2) the patents were valid; or (3) that Wyeth suffered damages. In the alternative, AstraZeneca has moved for a new trial on invalidity. For the following reasons, the Court grants AstraZeneca's motion for judgment as a matter

[1] The Court granted the defendants' motion to dismiss Wyeth LLC's co-plaintiff, Puma Biotechnology, Inc., for lack of Article III standing. *See Puma Biotech., Inc. v. AstraZeneca Pharms. LP*, No. 21 C 1338, 2024 WL 1157120 (D. Del. Mar. 18, 2024).

of law on the question of invalidity.

Background

The parties to this suit are pharmaceutical companies that commercialize drugs to treat cancer and other illnesses. The patents-in-suit claim a method of treating a form of non-small cell lung cancer (NSCLC). NSCLC is associated with overactivity of the epidermal growth factor receptor (EGFR), an enzyme that is involved in cell division and growth. Drugs that treat this condition are known as EGFR tyrosine kinase inhibitors (TKIs or inhibitors), and these TKIs bind to certain parts of the EGFR to prevent the enzyme from triggering cancerous cell growth.

Two TKIs, gefitinib and erlotinib (referred to collectively as g/e), showed some promise in treating NSCLC. Gefitinib and erlotinib are classified as "reversible" inhibitors; they form non-covalent bonds with EGFR that dissociate over time. There are two principal limitations to g/e treatment. First, only patients with certain EGFR mutations are sensitive to g/e therapy; the parties refer to these mutations as "sensitizing mutations." In other words, to be a candidate for g/e treatment, a patient needs to have EGFR with the requisite sensitizing mutation(s). Second, "[a] significant limitation in using [reversible inhibitors such as g/e] is that recipients thereof may develop a resistance to their therapeutic effects after they initially respond to therapy, or they may not respond to EGFR-TKIs to any measurable degree at all." '314 Patent at 3:19–23.

The patents-in-suit claim a method for treating "g/e resistant NSCLC." The inventors claim that g/e resistance can be overcome by using "irreversible" EGFR inhibitors that covalently bind to a specific amino acid at a specific location of EGFR.

Specifically, the asserted claims of the '314 patent recite:

> 1. A method for treating gefitinib and/or erlotinib resistant non-small cell lung cancer in a patient in need thereof, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer a pharmaceutical composition comprising a unit dosage of an irreversible epidermal growth factor receptor (EGFR) inhibitor that covalently binds to cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2.
>
> [. . .]
>
> 3. The method of claim 1, wherein the irreversible EGFR inhibitor covalently binds to cysteine 773 residue of EGFR.
>
> [. . .]
>
> 9. The method of claim 1, wherein the route of administration is oral.

'314 Patent at 35:52–36:65.

The claims of the '162 patent are directed at EGFR with a specific mutation, the "T790M mutation," which is associated with g/e resistance. The asserted claim of '162 patent recites:

> 1. A method of treating gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 in a patient, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 a pharmaceutical composition comprising a unit dosage of 2-500 mg of an irreversible EGFR inhibitor that covalently binds to cysteine 773 of the catalytic domain within the SEQ ID NO: 1 having a T790M mutation; wherein the irreversible EGFR inhibitor is not CL-387,785.

'162 Patent at 35:48–36:48.

In September 2021, Wyeth sued AstraZeneca, alleging that AstraZeneca's irreversible EGFR inhibitor Tagrisso (osimertinib) infringes both patents-in-suit. After a claim construction hearing, the Court resolved numerous disputes regarding the meaning of the asserted claims. *See Puma Biotech., Inc. v. AstraZeneca Pharms. LP*,

21 C 1338, 2023 WL 2683559 (D. Del. Mar. 29, 2023). AstraZeneca then moved for summary judgment, arguing in relevant part that (1) the patents were invalid because they failed to meet the enablement and written description requirements of 35 U.S.C. § 112; and (2) the use of Tagrisso did not infringe the asserted claims. The Court concluded that there were genuine disputes of material fact regarding invalidity and infringement and therefore denied summary judgment on those points. *See Puma Biotech., Inc. v. AstraZeneca Pharms. LP*, No. 21 C 1338, 2024 WL 1157120 (D. Del. Mar. 18, 2024).

After a five-day trial, a jury concluded that the patents were not invalid and that AstraZeneca induced infringement of the patents. A key dispute at trial was whether, and to what extent, each of Tagrisso's indications (i.e., FDA-approved uses) infringed. Tagrisso currently has three FDA-approved indications listed on its product label. Originally, Tagrisso was FDA-approved only "for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy." JTX-5 (2015 Tagrisso Label).[2] The parties refer to this as the "second-line" or "2L" indication, in reference to the fact that the drug would only be prescribed after some other primary or "first-line" treatment had failed.

Subsequently, Tagrisso was FDA-approved for "the first-line treatment of adult patients with metastatic NSCLC whose tumors have [g/e sensitizing mutations], as indicated by an FDA-approved test." JTX-14 (2023 Tagrisso Label). The parties refer

[2] For simplicity, the Court refers to the parties' exhibits in the same format as the parties: "DTX" indicates the defendants' trial exhibit, "PTX" indicates the plaintiff's trial exhibit, and "JTX" indicates a joint trial exhibit.

to this as the "first-line" or "1L" indication, in reference to the fact that the drug would be prescribed as the primary or first treatment for the patient. Tagrisso was also FDA-approved "as adjuvant therapy after tumor resection in adult patients with non-small cell lung cancer (NSCLC) whose tumors have [g/e sensitizing mutations], as detected by an FDA-approved test." JTX-14 (2023 Tagrisso Label). The parties refer to this as the "adjuvant" indication, in reference to the fact that the drug would be prescribed as a follow-up to a primary treatment (in this case, surgery to remove a tumor) to target cancer cells that the primary treatment did not eliminate.

Wyeth argued that AstraZeneca induced infringement of the patents-in-suit with respect to all three indications of Tagrisso. Although the first-line and adjuvant indications are not directed at patients who have received and failed on g/e treatment, Wyeth asserted that prescribing doctors nevertheless infringe the patents-in-suit with respect to these "pretreatment" indications because they sometimes intend to proactively treat g/e resistance when prescribing under these indications. The jury concluded that AstraZeneca induced infringement with respect to all three indications and awarded Wyeth $107,500,000 in damages. The Court later held a two-day bench trial on AstraZeneca's equitable defenses and its counterclaim that the patents were invalid due to indefiniteness. The Court found that AstraZeneca did not show by clear and convincing evidence that the patents were unenforceable or that they were invalid for indefiniteness. *See* August 6, 2024 Findings of Fact & Conclusions of L, dkt. no. 515.

Discussion

The Court should grant judgment as a matter of law "only if, viewing the evidence

in the light most favorable to the nonmovant and giving it the advantage of every fair and reasonable inference, there is insufficient evidence from which a jury reasonably could find' for the nonmovant." *TransWeb, LLC v. 3M Innovative Properties Co.*, 812 F.3d 1295, 1301 (Fed. Cir. 2016) (quoting *Lightning Lube, Inc. v. Witco Corp.*, 4 F.3d 1153, 1166 (3d Cir. 1993)).

A. Infringement

AstraZeneca first argues that it is entitled to judgment as a matter of law on Wyeth's induced infringement claim because the evidence was insufficient to support a finding of direct infringement or induced infringement regarding any of Tagrisso's three indications. Although Wyeth sued AstraZeneca only under a theory of induced infringement, *see* 35 U.S.C. § 271(b), "direct infringement is a necessary predicate for a finding of induced infringement in the usual patent infringement case." *Vanda Pharms. Inc. v. W.-Ward Pharms. Int'l Ltd.*, 887 F.3d 1117, 1129 (Fed. Cir. 2018). In addition to establishing that some direct infringement occurred, the plaintiff must also establish "that the defendant possessed specific intent to encourage another's infringement and not merely that the defendant had knowledge of the acts alleged to constitute inducement." *Id.* (quoting *DSU Med. Corp. v. JMS Co.*, 471 F.3d 1293, 1306 (Fed. Cir. 2006)). "Circumstantial evidence can support a finding of specific intent to induce infringement." *Id.* (quoting *AstraZeneca LP v. Apotex, Inc.*, 633 F.3d 1042, 1060 (Fed. Cir. 2010)); *see also DSU Med. Corp.*, 471 F.3d at 1306 ("While proof of intent is necessary, direct evidence is not required; rather, circumstantial evidence may suffice."). "Infringement is a question of fact." *Godo Kaisha IP Bridge 1 v. TCL Commc'n Tech. Holdings Ltd.*, 967 F.3d 1380, 1383 (Fed. Cir. 2020).

1. Direct infringement

AstraZeneca argues that Wyeth failed to provide evidence of direct infringement to the jury because it did not show that "prescribers intend to treat g/e resistant NSCLC when prescribing Tagrisso" or that prescribers "actually treat g/e resistant NSCLC, that is, administer Tagrisso to a patient who has g/e resistant NSCLC." Defs.' Post-Trial Mot. at 2 (emphasis omitted). Wyeth asserts that it produced sufficient evidence on both points.

a. Intent

Regarding prescribers' intent to treat g/e resistant NSCLC, the Court concludes that Wyeth produced sufficient evidence for a reasonable jury to conclude that at least some doctors intended to treat g/e resistant NSCLC when prescribing Tagrisso in all three indications. As an initial matter, the Court notes that AstraZeneca does not offer any colorable argument that Wyeth failed to carry its burden on direct infringement with respect to Tagrisso's second-line indication. The Court agrees that the second-line indication "is specifically directed to patients who have already failed on a previous TKI therapy like gefitinib and who have the T790M mutation." Pl.'s Resp. at 2; *see also* JTX-5 (2015 Tagrisso Label); JTX-15 (2023 Tagrisso Label). It was therefore reasonable for the jury to conclude that at least some physicians prescribed Tagrisso for the express purpose stated in the indication, i.e., treating g/e resistant NSCLC.

With respect to the first-line and adjuvant indications, AstraZeneca's central argument is that Wyeth was required to either call prescribing physicians to testify regarding their intent when prescribing Tagrisso or to present survey evidence from physicians on their prescribing intentions. The Federal Circuit, however, has stated that

"[t]o support the verdict, the record does not need to contain direct evidence" of direct infringement because "[i]t is hornbook law that direct evidence of a fact is not necessary." *Metabolite Lab'ys, Inc. v. Lab'y Corp. of Am. Holdings*, 370 F.3d 1354, 1364–65 (Fed. Cir. 2004). "Circumstantial evidence is not only sufficient, but may also be more certain, satisfying and persuasive than direct evidence." *Id.* (quoting *Moleculon Rsch. Corp. v. CBS Inc.*, 793 F.2d 1261, 1272 (Fed. Cir. 1986)). It is therefore sufficient if "the record contains sufficient circumstantial evidence to permit the jury to imply that physicians directly infringe." *Id.*; *see also Lucent Techs., Inc. v. Gateway, Inc.*, 580 F.3d 1301, 1318 (Fed. Cir. 2009) (concluding that "circumstantial evidence was just adequate to permit a jury to find that at least one . . . person within the United States during the relevant time period . . . had performed the claimed method").

The Court concludes that Wyeth carried its burden. The jury was entitled to credit the testimony of Wyeth's expert, Dr. Glen Weiss, a physician specializing in the treatment of lung cancer, who explained that physicians prescribe Tagrisso in all three indications for the purpose of treating g/e resistance. *See, e.g.*, Jury Trial Tr. at 183:9–21 (Weiss testimony) (explaining that prescribing the second-line indication of Tagrisso infringes that patents-in-suit because "[i]n order for patients to be eligible in this second-line setting, they had to have disease that progressed on or after EGFR tyrosine kinase inhibitor therapy like gefitinib, so that would be gefitinib-resistant non-small cell lung cancer, and they also have a T790M mutation"); *id.* at 188:2–10 ("Well, about 35 percent of these patients that have pretreatment, never been exposed to an EGFR directed therapy, will have a T790M mutation in their tumor. And doctors are well aware that resistance to gefitinib or drugs like gefitinib are a big problem. And in order to

decrease that risk of exposure and having these patients on a brief time frame with those drugs, it's better to give Tagrisso up front to delay or to prevent the T790M resistance from being a problem"); *id.* at 188:24–189:15 (Q: In your experience, are oncologists who treat non-small cell lung cancer generally aware of the problems related to T790M and resistance who are in the pretreatment population? A: Yeah, I believe they're aware of this problem. Q: And does that knowledge play a role with respect to how they treat these patients? A: Yes. Having awareness that T790M mutation is a problem for patients, approximately 35 percent, giving a patient a reversible inhibitor which does not overcome the T790M resistance can be a problem for these patients. They often have a shorter duration of time before they progress and some of them—before they're able to get any other therapy like Tagrisso, and some of these patients may not live long enough if you tried something else first before giving Tagrisso because their cancer may continue to spread or cause them significant problems where they're not eligible for additional treatment."); *id.* at 190:1–2 (explaining that the possibility of a T790M mutation is "a big problem for clinicians in making treatment decisions").

AstraZeneca argues that Dr. Weiss's testimony provides no support for a finding that an act of direct infringement occurred. It argues that "[g]enerally, expert witnesses are not permitted to testify regarding intent, motive, or state of mind, or evidence by which such state of mind may be inferred." Defs.' Post-Trial Mot. at 3. This is not a case, however, where Dr. Weiss was speculating on the specific state of mind of a specific physician. Rather, Dr. Weiss testified regarding general practices, standards of care, and the different variables that underly treatment decisions in his specialty field of

lung cancer treatment. He was well-qualified to testify on these points, and the jury could reasonably infer from his testimony that at least some doctors acted in accordance with these practices. *See, e.g.*, *Packet Intel. LLC v. NetScout Sys., Inc.*, 965 F.3d 1299, 1316 (Fed. Cir. 2020) (jury was permitted to draw a reasonable inference regarding infringement from evidence presented at trial).

AstraZeneca also argues that Dr. Weiss testimony at most goes to treating doctors' "knowledge or awareness" and not whether they acted with an "intentional purpose." Defs.' Post-Trial Mot. at 4. Again, however, the jury was permitted to draw the straightforward inference that when prescribing Tagrisso, doctors would have applied their knowledge regarding what would extend their patient's lifespan.

AstraZeneca next argues that Dr. Weiss testified only that doctors prescribe Tagrisso to "prevent[ ]" or "delay[ ]" g/e resistance, not to "treat" g/e resistance. Defs.' Post-Trial Mot. at 4. In fact, however, Dr. Weiss testified that doctors prescribe Tagrisso to "prevent[ ]" or "delay[ ]" *problems* associated with g/e resistance. Jury Trial Tr. at 188:6–10; *see also id.* at 189:6–15 ("[G]iving a patient a reversible inhibitor which does not overcome T790M resistance can be a problem for these patients . [. . .] [S]ome of these patients may not live long enough if you tried something else first before giving Tagrisso because their cancer may continue to spread or cause them significant problems where they're not eligible for additional treatment."). Viewing the testimony in the light most favorable to Wyeth, as the Court must do when reviewing the jury's verdict in Wyeth's favor, this testimony supports Wyeth's position that prescribing physicians intend to treat g/e resistance (and thus avoid the "problems" that g/e resistance will cause patients to suffer).

Moreover, Dr. Weiss's testimony was not Wyeth's only evidence regarding direct infringement. Wyeth also presented evidence that AstraZeneca designed Tagrisso with the goal of targeting g/e resistance and marketed Tagrisso to physicians as "designed to inhibit EGFR sensitizing and resistance mutations." *See* PTX-447 at 2. This marketing was not limited to Tagrisso's second-line indication. *See id.* ("Don't miss a patient who could be eligible for first-line TAGRISSO."). Again, the jury was entitled to draw the straightforward inference that doctors used Tagrisso in accordance with how the drug was marketed to them. *See Lucent Techs., Inc.*, 580 F.3d at 1318 (holding that the plaintiff produced sufficient circumstantial evidence of direct infringement to support the jury's verdict where "[the defendant] not only designed the accused products to practice the claimed invention, but also instructed its customers to use the accused products in an infringing way").

Finally, AstraZeneca briefly argues that the evidence is insufficient to support the verdict with respect to the adjuvant indication because, it contends, gefitinib and erlotinib are not suitable for adjuvant treatment. AstraZeneca thus argues that doctors "would [not] have any plausible reason to be thinking or concerned about resistance to drugs they would not use." Defs.' Post-Trial Mot. at 4. But AstraZeneca never presented any evidence or argument along these lines to the jury. In fact, AstraZeneca's closing arguments suggested the opposite of what it now argues. *See* Jury Trial Tr. at 1105:9–15 (stating that, with respect to "adjuvant and first-line . . . Tagrisso is being used here where gefitinib would have otherwise been used because Tagrisso is better"); Jury Trial Tr. at 1126:21–25 ("Second, first-line adjuvant, those are sensitive patients. . . . Those are patients who would have received gefitinib,

but AstraZeneca developed something better."). The Court cannot grant judgment as a matter of law to AstraZeneca based on facts or evidence that it did not present at trial. Moreover, to the extent that AstraZeneca argues that it was Wyeth's burden to show that gefitinib and/or erlotinib are regularly used in the adjuvant context, the Court disagrees. Although Wyeth had to prove infringement by preponderance of the evidence, that does not mean it was required to anticipate and rebut every possible counterpoint that AstraZeneca might have raised. As discussed, Wyeth presented sufficient evidence that at least some physicians intend to treat g/e resistance in the adjuvant context.

b. Treatment of patients having g/e resistant NSCLC

AstraZeneca argues that Wyeth "presented no evidence of patients treated according to Tagrisso's 1L or Adjuvant indications who would not respond if given g/e" and therefore did not establish that patients "having g/e resistant" NSCLC were treated, as required by the asserted claims. Defs.' Post-Trial Mot. at 5. But Wyeth presented ample evidence that the T790M mutation confers resistance to g/e and that this mutation is present in some patients even before treatment with g/e. *See, e.g.*, '314 Patent at 4:37–38 ("The T790M mutation confers resistance to gefitinib and/or erlotinib treatment."); Jury Trial Tr. at 166:18–23 (Weiss testimony) ("So it's been reported by many that the T790M mutation is a resistance mutation, it can explain resistance to gefitinib."); Jury Trial Tr. at 224:23–225:6 (Berger testimony) ("Q: What did you determine the prevalence of T790M to be in this pretreatment population [of first-line and adjuvant patients]? A: So I found the prevalence to be at least 35 percent."). The Court therefore sees no basis to find that the jury could not credit this evidence and

conclude that at least some patients receiving Tagrisso in the first-line and adjuvant indications had g/e resistant NSCLC.

2. Induced infringement

AstraZeneca also argues that Wyeth failed to present sufficient evidence that AstraZeneca induced infringement with respect to all three indications of Tagrisso. With respect to the second-line indication, AstraZeneca argues that it "deliberately stopped promoting Tagrisso's 2L use in 2018—before the patents issued." But the second-line indication has remained on Tagrisso's label, and Wyeth presented evidence that second-line prescriptions continued to generate sales for AstraZeneca after the patents issued. *See* Jury Trial Tr. at 350:17–20 (Rao testimony). The jury was permitted to conclude from these facts that AstraZeneca "possessed specific intent to encourage another's infringement." *Vanda Pharms. Inc.*, 887 F.3d at 1129.

With respect to the first-line and adjuvant indications, Wyeth presented evidence that AstraZeneca consistently emphasized Tagrisso's ability to combat the T790M resistance mutation as a key feature of its new drug and that this marketing strategy was not limited to the second-line indication (which AstraZeneca itself alleges it stopped promoting in 2018). *See, e.g.*, PTX-448 (TAGRISSO is a third-generation, irreversible EGFR TKI designed to . . . inhibit mutated EGFR with the T790M resistance mutation."); PTX-114 ("TAGRISSO is a *better EGFR-TKI* that addresses significant areas of unmet need in EGFRm NSCLC" including "overcome[ing] T790M resistance"); PTX-114 (stating that AstraZeneca's 2018–2021 "strategy" included "advocacy for 1L TAGRISSO use (vs. waiting until 2L)"). This is sufficient to support the jury's determination that AstraZeneca induced infringement with respect to Tagrisso's first-line and adjuvant

indications. *See Lucent Techs., Inc.*, 580 F.3d at 1322 (stating that "advertising an infringing use[ ] can support a finding of an intention for the product to be used in an infringing manner"). Although AstraZeneca presented contrary evidence regarding its intent to promote treatment of g/e resistant NSCLC with respect to the pretreatment indications, the jury was not required to credit this evidence.

In sum, the Court concludes that AstraZeneca is not entitled to judgment as a matter of law on infringement.

B. Invalidity

AstraZeneca argues that it is entitled to judgment as a matter of law on its counterclaim that the patents-in-suit are invalid due to anticipation, obviousness, lack of enablement, and lack of written description.

1. Anticipation

AstraZeneca argues that the asserted claims of the '314 patent are invalid due to anticipation. "Under 35 U.S.C. § 102, a prior art reference will anticipate a patent claim if it discloses all of the limitations of the claim 'arranged or combined in the same way as in the claim.'" *Incept LLC v. Palette Life Scis., Inc.*, 77 F.4th 1366, 1371 (Fed. Cir. 2023) (quoting *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369–70 (Fed. Cir. 2008)). Anticipation can be express or inherent. *Arbutus Biopharma Corp. v. ModernaTX, Inc.*, 65 F.4th 656, 662 (Fed. Cir. 2023). "Anticipation is a question of fact." *Incept LLC*, 77 F.4th at 1371.

AstraZeneca cites to a 2003 article entitled "CI-1033, an Irreversible pan erbB Receptor Inhibitor and its Potential Application for the Treatment of Breast Cancer," which the parties refer to as "Allen 2003" in reference to its first author. *See* DTX-73.

The article states that the compound CI-1033, which it describes as "an irreversible, pan-erbB inhibitor, has the potential to have an important role in the future treatment of breast and other cancers." *Id.* at 2. Although the article focuses on breast cancer, it also mentions NSCLC. *See, e.g., id.* at 4. The article discusses orally administering a daily dosage of 50 to 650 milligrams of CI-1033 per day. AstraZeneca therefore argues that Allen 2003 "disclosed each and every element of the Asserted Claims of the '314 patent." Defs.' Post-Trial Mot. at 25.

The Court disagrees that AstraZeneca has shown by clear and convincing evidence that Allen 2003 anticipates the asserted claims of the '314 patent such that no reasonable jury could conclude otherwise. Allen 2003 is focused primarily on breast cancer, not NSCLC. Although Allen 2003 briefly discusses NSCLC, it is undisputed that it does not mention or discuss g/e resistance, which is a key component of the claimed invention. AstraZeneca relies on the fact that Allen 2003 mentions NSCLC with "EGFRvIII," which AstraZeneca asserts a POSA would have known is a g/e resistant variant of NSCLC. As evidence of a POSA's knowledge, AstraZeneca points to a 2004 article entitled "Resistance to Tyrosine Kinase Inhibition by Mutant Epidermal Growth Factor Receptor Variant III Contributes to the Neoplastic Phenotype of Glioblastoma Multiforme," which the parties refer to as "Learn 2004" in reference to its first author. *See* DTX-116. Because anticipation is limited to a single reference, AstraZeneca cannot argue that the combined teachings of Allen 2003 and Learn 2004 render the patent invalid as anticipated.[3] *See Arbutus Biopharma Corp.*, 65 F.4th at 662.

[3] AstraZeneca does not raise a defense of obviousness based on the combination of Allen 2003 and Learn 2004.

AstraZeneca instead asserts that Learn 2004 is evidence of what a POSA would know with respect to EGFRvIII when reading Allen 2003.

Even so, Allen 2003 does not cite or discuss any studies involving the treatment of EGFRvIII with CI-1033. In addition, Wyeth's expert, Dr. Frederick Hausheer, testified that Allen 2003 speculates that EGFRvIII would respond to any EGFR inhibitor (which would include g/e). *See* Jury Trial Tr. at 919:8–15 ("Allen has a mention of this EGFR variant III here, and you can see what he says at the very end. He says small molecule RTK inhibitors. He's saying that—he's not making any distinction with respect to reversible or irreversible. He says anything should work, and there's absolutely no experimental evidence in this article. This is pure speculation. There's no evidence."). Finally, Allen 2003 focuses only on a specific compound, CI-1033. Although it highlights the fact that it is an irreversible EGFR inhibitor, it does not draw the conclusion that *any* irreversible EGFR inhibitors that covalently binds to cysteine 773 will be effective at treating g/e resistant NSCLC. The jury therefore was not required to find that Allen 2003 disclosed all elements of the claim "arranged or combined in the same way as in the claim[s]" of the '314 patent or that the '314 patent was "the natural result flowing from the operation as taught in the prior art." *Incept LLC*, 77 F.4th at 1371 (quoting *Net MoneyIN, Inc.*, 545 F.3d at 1369–70 (Fed. Cir. 2008)); *Arbutus Biopharma Cor.*, 65 F.3d at 662.

2. Obviousness

AstraZeneca next argues that the asserted claims of the '314 patent and the '162 patent are invalid for obviousness. "Obviousness is a question of law based on underlying factual determinations." *Incept LLC*, 77 F.4th at 1371. "Those underlying

factual determinations include: (1) the scope and content of the prior art; (2) differences between the prior art and the claims at issue; (3) the level of ordinary skill in the pertinent art; and (4) secondary considerations such as commercial success, long felt but unsolved needs, and failure of others." *Id.* In contrast to the defense of anticipation, the defense of obviousness can be based on a combination of prior art references. But "[a] determination of obviousness 'requires finding that a person of ordinary skill in the art would have been motivated to combine or modify the teachings in the prior art and would have had a reasonable expectation of success in doing so.'" *Adapt Pharma Operations Ltd. v. Teva Pharms. USA, Inc.*, 25 F.4th 1354, 1365 (Fed. Cir. 2022) (quoting *OSI Pharms., LLC v. Apotex Inc.*, 939 F.3d 1375, 1382 (Fed. Cir. 2019)). The jury may also consider whether the reference "teach[es] away from a claimed combination," which weighs against a finding of obviousness. *Arctic Cat Inc. v. Bombardier Recreational Prods. Inc.*, 876 F.3d 1350, 1360 (Fed. Cir. 2017).

a. '314 Patent

AstraZeneca asserts that the asserted claims of the '314 patent are obvious considering the combination of Allen 2003 and an additional prior art reference that the parties call "Agus 2003." Agus 2003 is a patent application entitled "Method of Treating Cancer Using Kinase Inhibitors." *See* DTX-71. Although Agus 2003 discusses the problem of g/e resistance in a broad sense, the jury was not required to find that a POSA would be motivated to combine Allen 2003 and Agus 2003 such that the asserted claims would be obvious. As Dr. Hausheer testified, the '314 patent teaches a completely different approach to treating g/e resistant NSCLC than Agus 2003. The method of treatment in the '314 patent involves a daily dosage of an irreversible EGFR

inhibitor that covalently binds to cysteine 773. In other words, the inventors' central discovery is that irreversible EGFR inhibitors can get the job done where reversible inhibitors like g/e cannot. Agus's approach, in contrast, is to *overdose* patients with increasing amounts of *any* inhibitor without regard to whether it is reversible or irreversible. *See, e.g.*, DTX-71 at 3:10–12 ("The method includes administering to patients a resistance surmounting quantity of a TKI which may be administered with less frequency than conventional TKI treatments."). In fact, Agus 2003 teaches, for example, administering an increased weekly dosage of *gefitinib*, whereas the patents-in-suit are directed at using a different class of compound (irreversible EGFR inhibitors) to treat g/e resistant NSCLC. *See, e.g.*, *id.* at 6:7–10 ("The inventor also surprisingly demonstrated that weekly IRESSA [gefitinib] dosages at an amount significantly greater than the recommended daily dosing was well tolerated and can inhibit tumor growth effectively . . . even in tumors that demonstrated a resistance to conventional TKI therapy.").

Even setting aside the fact that Agus 2003 teaches away from the claimed invention, the jury was not required to find that Agus 2003 makes up for all of the shortcomings the Court discussed with respect to Allen 2003. For example, Wyeth's expert, Dr. Hausheer, testified that Agus 2003 does not distinguish between mutated and non-mutated forms of NSCLC. *See* Jury Trial Tr. at 923:14–18 ("Agus is teaching to overdose once or twice a week as a treatment for non-mutated—he's not—there's no resistance; there's no T790M. You just treat any type of tumor, and he's got prostate cancer as his main example."). In addition, neither Allen 2003 nor Agus 2003 posit that g/e resistant NSCLC can be treated using the entire class of irreversible EGFR

inhibitors that covalently bind to cysteine 773. For these reasons, the jury was not required to find that the asserted claims were obvious in light of the combination of Allen 2003 and Agus 2003.

b. '162 Patent

AstraZeneca also argues that the '162 patent was obvious considering the combination of Allen 2003 and Kobayashi 2005. As a preliminary matter, the parties vigorously dispute whether Kobayashi 2005 is prior art. Specifically, the parties dispute whether the inventors of the '162 patent conceived of their invention before or after Kobayashi was published in February 2005. The Court need not resolve this dispute, however, because it concludes that even if Kobayashi 2005 is considered prior art, there was sufficient evidence for the jury to conclude that the combination of Allen 2003 and Kobayashi 2005 did not render the asserted claim of the '162 Patent obvious. In particular, Dr. Hausheer testified that a POSA would not have a motive to combine Allen 2003 and Kobayashi 2005, given that the references discuss different diseases and different mutations. *See, e.g.*, Jury Trial Tr. at 925:16–23. AstraZeneca did not provide clear and convincing evidence to the contrary that would leave a reasonable jury no choice but to credit AstraZeneca's expert witness over Wyeth's expert witness. In fact, AstraZeneca introduced hardly any evidence on this point beyond its expert's unelaborated testimony. *See* Jury Trial Tr. at 581:25–582:9 (Reider testimony) ("I have been instructed that in obviousness you can combine two or more references if a person of skill in the art would be motivated to look at them. And in this case, the Kobayashi paper, which came out in 2005, combined with a reference called Allen, which is from 2003, two years earlier, disclose all the elements of the asserted claims.

So if you look at—a POSA would have reason to combine them, and if you take the information in those two prior art references, they have everything that's in these patents with regard to the claimed invention."); *id.* at 585:17–22 ("Q: And would a POSA in 2005 be motivated to follow Kobayashi's description of the effectiveness of an irreversible EGFR inhibitor on T790M mutant-resistant non-small cell lung cancer by using the irreversible inhibitor described in Allen? A: Yes."). Indeed, Kobayashi 2005 does not cite to Allen 2003, which supports Wyeth's argument that a POSA would not have been motivated to combine the two because they addressed different diseases and mutations. The Court therefore cannot say that AstraZeneca presented clear and convincing evidence that the '162 patent was invalid due to obviousness.

3. Enablement

The Court next addresses AstraZeneca's argument that the patents-in-suit are invalid because they are not enabled. A patent must include a specification which contains "a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention." 35 U.S.C. § 112(a). The Federal Circuit has interpreted section 112(a) as containing both a "written description" requirement and an "enablement" requirement. *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1344 (Fed. Cir. 2010).

"Enablement is a legal question based on underlying factual determinations." *Vasudevan Software, Inc. v. MicroStrategy, Inc.*, 782 F.3d 671, 684 (Fed. Cir. 2015).

"Because patents are presumed valid, lack of enablement must be proven by clear and convincing evidence." *Baxalta Inc. v. Genentech, Inc.*, 81 F.4th 1362, 1365 (Fed. Cir. 2023). The enablement requirement is satisfied if the specification contains sufficient information to permit "a person of skill in the art to make and use the claimed invention." *Vasudevan Software, Inc.*, 782 F.3d at 684. "[T]he specification must enable the full scope of the invention as defined by its claims." *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610 (2023). Thus, "[i]f a patent claims an entire class of processes, machines, manufactures, or compositions of matter, the patent's specification must enable a person skilled in the art to make and use the entire class." *Id.* This does not mean, however, that "a specification necessarily [is] inadequate just because it leaves the skilled artist to engage in some measure of adaptation or testing." *Id.* at 611. "[A] specification may call for a reasonable amount of experimentation to make and use a patented invention." *Id*. at 612. "In other words, 'the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation.'"[4] *Baxalta Inc.*, 81 F.4th at 1365 (quoting *MagSil Corp. v. Hitachi Glob. Storage Techs.*, Inc., 687 F.3d 1377, 1380 (Fed. Cir. 2012)). Factors that may be considered to determine whether a claimed invention requires undue experimentation 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.

[4] After the Supreme Court's decision in *Amgen*, the Federal Circuit has used the terms "undue experimentation" and "unreasonable experimentation" interchangeably. *See Baxalta Inc.*, 81 F.4th at 1365–66, 1367 n. 4.

Amgen Inc., 987 F.3d at 1084 (quoting *In re Wands*, 858 F.2d 731, 736–37 (Fed. Cir. 1988)); *see also Baxalta Inc.*, 81 F.4th at 1367 ("We do not interpret [the Supreme Court's decision in] *Amgen* to have disturbed our prior enablement case law, including *Wands* and its factors.").

AstraZeneca challenges three aspects of the asserted claims as not enabled. First, it argues that the patents-in-suit claim but do not enable the use of "***any*** compound that functions to covalently bind to cysteine 773 and irreversibly inhibit EGFR." Defs.' Post-Trial Mot. at 9–10. Second, it argues that the patents claim but do not enable treatment of "the full sweep of g/e resistant NSCLC." *Id.* Third, it argues that the patents claim but do not enable treatment via a "unit dosage—i.e., a predetermined quantity of active material calculated to produce the desired therapeutic effect." *Id.* (internal quotation marks omitted) (emphasis omitted).

a. Any compound

AstraZeneca first argues that the patents-in-suit do not enable a POSA to practice the claimed method of treatment with the full scope of compounds that covalently bind to cysteine 773 and irreversibly inhibit EGFR. According to AstraZeneca, the asserted claims cover "hundreds of billions" of chemical compounds and "leave it to a POSA to undertake the extraordinarily onerous and unpredictable task" of determining which compounds in fact treat g/e resistant NSCLC. Defs.' Post-Trial Mot. at 11 (emphasis omitted).

The Court addressed AstraZeneca's arguments in detail at the summary judgment stage and concluded that "there [was] a genuine factual dispute regarding whether the specification would enable a POSA to practice the claims with *all*

'compound[s] that irreversibly inhibit[ ] EGFR and covalently bind[ ] to [cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2 / cysteine 773 of the catalytic domain within the SEQ ID NO: 1 having a T790M mutation]' without undue experimentation." *Puma Biotech.*, 2024 WL 1157120, at *7. The evidence presented to the jury at trial was very similar to the evidence submitted to the Court at summary judgment, and AstraZeneca provides no reason for the Court to reconsider at this stage its conclusion that this was appropriately left to the jury's determination.

First, although AstraZeneca argues that a POSA would be required to unduly experiment with billions of compounds to determine whether they treat g/e resistant NSCLC, Wyeth's expert, Dr. Jorgensen, testified that the universe of possible compounds was not in the billions, but rather far smaller. *See* Jury Trial Tr. at 962:9. Dr. Jorgensen further testified that a POSA would understand that the universe of possible compounds would be limited by certain features that would be required for the compound to covalently bind to cysteine 773. *See, e.g.*, Jury Trial Tr. at 977:5–21 ("Q: [AstraZeneca's expert] Dr. Reider suggested that basically any kind of core could be used to make an inhibitor that would be able to covalently bind to cysteine 773. Do you agree with that? A: Well, one can envision a world of cores, and from looking at that structure that I illustrated before of the slot in the protein and having to have a slot-like molecule, you're going to have to have cores that let the molecule be quite flat. So that's going to limit the cores. Also, there are other limitations. You have to have a nitrogen atom in the core that's capable of hydrogen bonding to that methionine hinge region to pin -- help pin the inhibitors, and we see that in these EGFR inhibitors. Plus you're

going to have to have -- the core has to have 19 substituents placed so that you can have the covalent bond form to cys 773. So there are a lot of restrictions on the size."). In addition, Dr. Jorgensen critiqued AstraZeneca's evidence, stating that it amounted to an "exaggeration" of the number of possible compound structures based on trivial substitutions. *See* Jury Trial Tr. at 979:11–25 ("I find [AstraZeneca's expert's slide] and a lot of the AstraZeneca presentation to involve exaggeration and also sort of attempts to confuse. I can just point out some things here. So I'll point immediately to the warhead in their highlighting in purple, what he's now calling dimethylaminobutenamide warhead. If you show that to any medicinal chemist and say what's the warhead, they're going to say it's an acrylamide. It just has a substituent on it that's a minor variation. It's like adding an extra mirror on your automobile. So that's just trying to say, oh, different warhead."); *see id.* at 980:20–24 ("And medicinal chemists would look at these structures and see the similarities, the core, the heteroaryl group or the aryl and the acrylamide, and there's no big difference here at all. Same intellectual entities."); *see id.* at 993:22–994:3 ("You're misrepresenting a POSA. A POSA is focused. He or she, making kinase inhibitors, focuses on a core of crystal structures that very much restricts the substituents that you can put on the core. It's not an isolated molecule that you can infinitely substitute as you take one of these structures from a composition-of-matter patent.").

Second, the parties' experts disagreed regarding the amount of guidance provided by the specification with respect to the required structure for irreversible EGFR inhibitors and the representativeness of the three examples provided in the specification. As the Court discussed at the summary judgment stage, the key question

is not whether the specification "describe[s] with particularity how to make and use every single embodiment within a claimed class," but rather "whether the specification describes some 'general quality' or 'rule' that 'may reliably enable a person skilled in the art to make and use all of what is claimed, not merely a subset' without having to engage in an '[un]reasonable amount of experimentation.'" *Puma Biotech., Inc*, 2024 WL 1157120, at *7 (quoting *Amgen Inc.*, 598 U.S. at 611–12). Dr. Jorgensen testified that the examples, information in the specification, the references disclosed in the patent, and the knowledge and experience of a POSA would be sufficient to enable a POSA to practice the claimed method-of-treatment. *See* Jury Trial Tr. at 962:17–981:7. The jury was not required to credit AstraZeneca's expert over Wyeth's with respect to these issues.

Third, AstraZeneca cites to the fact that the specification states that the chosen irreversible EGFR inhibitor "may also be a larger compound," which the parties agree is, in fact, not suitable for carrying out the claimed method-of-treatment. Defs.' Post-Trial Mot. at 15. But as the Court discussed in its decision on AstraZeneca's motion for summary judgment, the mention of "larger compound[s]" in the specification does not necessarily invalidate the patents:

> Although it is true that an inventor must enable the full scope of the claim, here, the asserted claims covers only those irreversible EGFR inhibitors that 'covalently bind[ ]' to the specified part of EGFR. The relevant question, therefore, is whether the patent enables a POSA to identify which irreversible EGFR inhibitors will covalently bind to the specified part of EGFR without "undue experimentation." *Baxalta Inc.*, 81 F.4th 1362 at 1365.

Puma Biotech., 2024 WL 1157120, at *6.

Wyeth presented evidence, which the jury was entitled to credit, that a POSA

would not be misled by this statement because a POSA would know that large compounds cannot covalently bind to cysteine 773 of EGFR. *See* Jury Trial Tr. at 977:21–24 (Jorgensen testimony) ("So there are a lot of restrictions on the size. You couldn't have a super large core. That wouldn't fit. It might also not be flexible enough to adjust to the lock. So there are a lot of limitations on reasonable cores. [. . .] A POSA in 2005 is focusing on small molecules [. . .] That was what dominated the literature, and it's consistent with having to get inside the cell.").

In sum, Dr. Jorgensen's conclusion was that identifying irreversible EGFR inhibitors that covalently bind to cysteine 773 would be "a slam dunk, no problem," for a POSA in 2005. Jury Trial Tr. at 983:4; *see also id.* at 982:23–983:1 (stating that "testing for them being irreversible inhibitors is straightforward, and this is in 2005. There are all sorts of things you can do easily in a day. [. . .] And a competent POSA would be able easily to design irreversible inhibitors of EGFR just as I did for two other proteins."). Thus, as the Court concluded at the summary judgment stage, the question of whether the patents-in-suit enabled a POSA to practice the claims with the full scope of the specified class of compounds turned on multiple factual disputes. The jury was entitled to conclude that AstraZeneca did not show by clear and convincing evidence that the patents were not enabled on this basis.

b. g/e resistance

AstraZeneca next argues that the patents do not meet the enablement requirement because the patents-in-suit "fail[ ] to teach how to treat broad categories of g/e resistant NSCLC." Defs.' Post-Trial Mot. at 20. Specifically, AstraZeneca argues that the method of treatment taught by the patents is not effective for patients with

NSCLC that lacks sensitizing mutations, NSCLC with MET amplification, or NSCLC with a KRAS mutation. AstraZeneca raised the same argument at the summary judgment stage. Again, the Court declined to grant summary judgment on this basis because it concluded that there was a genuine dispute regarding whether a POSA would consider these types of NSCLC to be g/e resistant. *See Puma Biotech., Inc.*, 2024 WL 1157120, at *7. AstraZeneca provides no reason for the Court to revisit its conclusion on this issue now.

Wyeth presented ample evidence to the jury that a POSA would not understand the disputed categories of NSCLC to be "g/e resistant." For example, Wyeth's experts persuasively testified that the method of treatment in the patents-in-suit was directed at NSCLC with sensitizing mutations. *See, e.g.*, Jury Trial Tr. at 181:15–22 (Weiss testimony) ("Q: Does the definition [of g/e resistant NSCLC] that you applied include people who do not have sensitizing mutations? A: No. It requires that all of these patients would have a sensitizing mutation. Q: And how do you know that? A: Because in order to have received or to be eligible for gefitinib and/or erlotinib, one needs to have a sensitizing mutation."); Jury Trial Tr. at 815:11–20 (Hausheer testimony) ("Q: You mentioned sensitizing mutations. And what's your opinion whether—as to whether gefitinib/erlotinib resistance means the patient has to have sensitizing mutations or not? A: They have to have sensitizing mutations. Q: Why is that? A: Because this is the basis for the gefitinib/erlotinib to be administered to such a patient. These are called sensitizing mutations. So reversible inhibitors will be used, and they're much more effective."); *id.* at 818:7–10. ("Non-small cell lung cancer patients that do not have sensitizing mutations are out of the scope of the claims, and

the specification does not need to describe or enable patients lacking, or any example, lacking sensitizing mutations."). This interpretation is also consistent with the testimony of Dr. Haber, one of the named inventors of the patents-in-suit. *See* Jury Trial Tr. at 296:18–22 (Haber testimony) (explaining that sensitizing mutations "predict[ ] who's going to respond, who's not going to respond. So that's very important because you're not giving drugs to people who won't benefit from them. You can actually be smart about that.").

Wyeth's experts further testified that a POSA would not consider NSCLC with a KRAS mutation or MET amplification to be g/e resistant. *See, e.g.*, Jury Trial Tr. at 181:23–182:1 (Weiss testimony) ("Q: Does the plain and ordinary meaning of gefitinib or erlotinib resistance depend on a specific kind of signaling pathway? A: It depends on the EGFR signaling pathway."); Jury Trial Tr. at 818:16–819:25 (Hausheer testimony) ("KRAS is a separate protein system. The mutations in KRAS are well known. It is independent of the EGFR pathway. And it's just not—it's not part of this. [. . .] KRAS is not a EGFR-mediated pathway. It's bypassing EGFR. It's a different protein system. You know, this is all well-known."); Haber Dep. at 58:05–13 (Q: [P]atients with gefitinib or erlotinib-resistant non-small cell lung cancer that exhibit MET amplification will not obtain a therapeutic benefit from an EGFR inhibitor; correct? A: [. . .] Based on lab experiments, you would predict not, because MET bypasses the entire pathway.").[5] This evidence was more than sufficient for a reasonable jury to conclude that a POSA would not understand "g/e resistant" NSCLC to cover NSCLC without sensitizing mutations, with a KRAS mutation, or with MET amplification. The

[5] This excerpt from Dr. Haber's deposition was presented to the jury at trial.

Court therefore declines to grant judgment as a matter of law for AstraZeneca on this basis.

c. Unit dosage

Lastly, AstraZeneca argues that the patents are not enabled because "an extraordinary and undue amount of experimentation . . . would be required to identify the 'unit dosage' for each of the myriad irreversible EGFR inhibitors encompassed within the scope of the Asserted Claims." Defs.' Post-Trial Mot. at 15. In its view, "[i]dentifying the 'unit dosage' for even just ***a single compound*** is highly unpredictable and involves tremendous amount of work and experimentation, including *in vitro* and *in vivo* tests, tests analyzing a given compound's pharmacokinetics, safety and toxicity studies, and formulation work, all before necessary clinical studies in human patients." *Id.* at 16. Although the patents-in-suit identify ranges for a "unit dosage" of between 1 to 1,000 milligrams for the '314 patent and between 2 to 500 milligrams for the '162 patent, AstraZeneca argues that these ranges are too broad and lacking in guidance for a POSA to determine the "unit dosage" for a given compound. This is particularly problematic, AstraZeneca says, because "the record establishes that for many compounds, including the specification's HKI-272 and EKB-569, many doses—or even the vast majority of doses—within these ranges are toxic." *Id.* at 18–19.

Wyeth responds that "a POSA would know that the desired therapeutic effect is to interfere with the EGFR pathway and 'kill cancer cells'" and that AstraZeneca did not present evidence that the claimed irreversible EGFR inhibitors do not achieve that effect. Pl.'s Resp. at 19. Wyeth argues that AstraZeneca's arguments regarding toxicity, safety, and clinical efficacy seek to add limitations that are not recited in the

asserted claims and that the Court rejected at claim construction. Wyeth further argues that the patents provide sufficient disclosure of "unit dosages" that would achieve the desire therapeutic effect of interfering with the EGFR pathway and killing cancer cells and that determining the dosage for any given irreversible EGFR inhibitor would not require undue experimentation.

Some background on the Court's previous rulings in this case is necessary to navigate the parties' arguments at this stage. At claim construction, the Court stated that the patents define the term "unit dosage" as "physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluents; i.e., carrier, or vehicle." *See Puma Biotech.*, 2023 WL 2683559, at *9 (quoting '314 Patent at 9:33–38). The Court therefore declined to adopt AstraZeneca's proposed construction that would add the words "effective amount" to the definition. *Id.* In ruling on the parties' motions in limine before the jury trial, the Court reiterated that the term "unit dosage" was defined by the specification and that the asserted claims therefore require neither FDA approval nor clinical effectiveness. *See* May 7, 2024 Order on Remaining Motions in Limine at 3–6 [dkt. no. 419]. In other words, the method of treatment described in the patent need not enable a POSA to carry out a successful clinical trial or to gain FDA approval for an irreversible EGFR inhibitor that practices the asserted claims. That is because the scope of the invention, and therefore the scope of what must be enabled, is determined by the patents' claims. In this case, the claims require only that the "unit dosage" "produce[s] the desired therapeutic effect" in a patient. '314 Patent at 9:33–38.

The Court does not agree with Wyeth, however, that the patents are enabled as long as the method of treatment "interfere[s] with the EGFR pathway and 'kill[s] cancer cells.'" Pl.'s Resp. at 19. That is because the patents do not claim "a method for killing cancer cells," or "a method for treating g/e resistant NSCLC," full stop. Rather, the patents claim "[a] method for treating gefitinib and/or erlotinib resistant non-small cell lung cancer *in a patient* in need thereof, comprising administering daily *to the patient* . . . a unit dosage . . . ." '319 Patent at 35:53–57 (emphasis added). The patents therefore must not only enable a unit dosage that produces the desired therapeutic effect, but also a unit dosage that can be *administered daily to the patient*. As Wyeth's expert agreed at trial, there is some level of toxicity—at the extreme, a fatal dose—that could not be administered to a patient. *See* Jury Trial Tr. at 939:24–9:40–5 (Hausheer testimony) ("Q: When you calculate a unit dose, do you make sure that dose is not so high it's going to be toxic to the patient? A: Yeah, you have several unit doses that you're going to use. This is very standard. Q: And so you'll want to avoid administering a toxic dose in order to achieve the desired therapeutic effect, correct? A: Yeah.").

Again, there is no requirement that the patents enable a "unit dosage" that is acceptable in terms of lacking side effects, meeting the FDA's safety criteria, having a particular level of effectiveness against a patient's cancer progression or other clinical symptoms, or meeting any other criteria that might make a drug an attractive option for a practicing clinician. Thus, the fact that an extensive amount of experimentation may be necessary to find an *ideal or optimal* dose is not relevant to the enablement inquiry. But if a POSA would have to undertake undue experimentation to find a "unit dosage" that would not fatally poison a patient, then the patents-in-suit do not enable a POSA to

treat g/e resistant NSCLC in a patient by administering daily a unit dosage to a patient.

Turning to the evidence presented at trial, the specification of the patents indicates that "satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.5 to about 1000 mg/kg of body weight, optionally given in divided doses two to four times a day, or in sustained release form. The total daily dosage is projected to be from about 1 to 1,000 mg, preferably from about 2 to 500 mg." '314 Patent at 8:60–66. AstraZeneca's expert emphasized that even the narrower range represented a "250-fold range of possible doses." Jury Trial Tr. at 711:2 (Taft testimony). AstraZeneca argues that this broad range of possible doses, standing alone, establishes that the claims are not enabled because a POSA would be required to conduct "substantial additional experimentation," including clinical trials, to determine the correct unit dosage for any given compound. Defs.' Post-Trial Motion at 17. The Court is not persuaded that the mere fact that the specification provides a range of possible dosages renders the asserted claims invalid for lack of enablement. This is particularly so because, as Wyeth points out, AstraZeneca did not provide clear and convincing evidence that "the claimed irreversible EGFR inhibitors do not achieve the desired therapeutic effect—inhibiting EGFR and killing cancer cells." Pl.'s Resp. at 19.

Nevertheless, the Court concludes that AstraZeneca presented clear and convincing evidence such that no reasonable jury could find that the patents-in-suit enabled a POSA to administer a unit dosage of any irreversible EGFR inhibitor covered by the claims *to a patient* without undue experimentation. First, it is undisputed that the specification did not disclose any working examples of unit dosages administered *to*

patients. Although this is not dispositive, it is a relevant factor to consider in the enablement inquiry. *See In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Second, AstraZeneca presented unrebutted evidence that some dosages of compounds within the ranges specified by the patents-in-suit would be toxic to patients, and, more specifically, that the dosage level required for the compounds to be therapeutically effective could be unduly toxic to a patient. *See* Jury Trial Tr. at 650:16–19 (Jänne testimony) (testifying that the dosage ranges described in the patents-in-suit and prior art could "be ineffective and not produce the desired therapeutic effect. It can be too toxic."); *id.* at 681:5–8 (Taft testimony) ("[I]f your toxicity threshold is here and you have to give a much higher dose in order to get activity, you don't have a therapeutic window, and that means you will not achieve a therapeutic effect."); *id.* at 711:6–9 (Taft testimony) ("And it's highly likely, not only if there was a unit dose within that range, there may also be a toxic dose within that range too. You just don't know. The patents really don't provide any guidance on that."). This included evidence that dosages within the specified ranges for two of the compounds specifically identified in the patents, HKI-272 and EKB-569, would be unduly toxic to patients. *See id.* at 707:22–708:18 (Taft testimony) ("Q: What are the named inventors and their co-authors describing about their preclinical research of HKI-272 in this article? A: Well, what they're describing is that those in vitro, like, test-tube-type experiments were done using a concentration of one micromole per liter. But they go on to say when they actually put it into people the highest concentration that they could achieve without causing toxicity was .2. So you're looking at least a five-fold difference between those values. And . . . I was discussing this concept of therapeutic range it's important that whatever the threshold is above

which you're going to have toxicity, you have to make sure your active concentration is in that—below that window and below that threshold. This is the opposite. In this case, what you're talking about is that the concentrations where you're going to have toxicity is .2, but they were saying that what they found is that five-fold higher is where you need activity. What that means is there is no therapeutic range, at least based on this statement for HKI-272, which also means there's not a unit dose for that compound."); *id.* at 943:2–944:1 (Hausheer testimony) ("What the named inventors said in 2008 is using the maximum tolerated dose [of HKI-272], you couldn't effectively inhibit T790M. That's what they said, correct? A: That's what they said." [. . .] Q: Now the maximum tolerated dose of EKB-569 is even lower, is it not? A: I believe so. Q: Okay. So the problem is—so—and we don't even know for the third [compound disclosed in the specification], HKI-357, what the maximum tolerated dose is, correct? A: No."); Jury Trial Tr. at 336:1–337:1 (Haber testimony) ("Q: So at this time in April of 2005, after you filed your patent application in February, you don't know how much of HKI-272 can be therapeutically administered to patients, correct? You don't know? […] A: Yes. [. . .] Q: And, in fact, you later found out, correct, that the concentrations that you were studying and reporting in your patent were five times higher than the maximum tolerated dose for HKI-272, correct? A: Yes.").

Wyeth did not provide any evidence to rebut AstraZeneca's evidence that some dosages of irreversible EGFR inhibitors that fall within the claims could be toxic if administered to patients. Thus, a POSA would be required to test different possible irreversible EGFR inhibitors to identify whether the dosage levels necessary to produce the desired therapeutic effect would be toxic if administered. *See* Jury Trial Tr. 711:6–9

(Taft testimony) ("[I]t's highly likely, not only if there was a unit dose within that range, there may also be a toxic dose within that range too. You just don't know. The patents really don't provide any guidance on that."). In scenarios where there is no non-toxic therapeutic range for a given compound, a POSA would not be able to practice the claimed method of treatment in the patient.

Although the existence of some inoperative embodiments does not necessarily render a patent invalid, *see Crown Operations Int'l, Ltd. v. Solutia Inc.*, 289 F.3d 1367, 1380 (Fed. Cir. 2002), that is only so if a POSA need not engage in undue experimentation to cull out inoperative embodiments. *See Atlas Powder Co. v. E.I. du Pont De Nemours & Co.,* 750 F.2d 1569, 1576 (Fed. Cir. 1984). The Supreme Court's decision in *Amgen* makes clear that although "a specification may call for a reasonable amount of experimentation to make and use a patented invention," courts "in allowing that much tolerance . . . cannot detract from the basic statutory requirement that a patent's specification describe the invention 'in such full, clear, concise, and exact terms as to enable any person skilled in the art' to 'make and use' the invention." *Amgen Inc.*, 598 U.S. at 612 (quoting 35 U.S.C. § 112(a)). Although the Supreme Court declined to establish bright-line rules regarding what a specification must disclose in order to enable patents that claim "an entire class of processes, machines, manufactures, or compositions of matter," the Court suggested that the patent must provide *some* guidance that will "reliably enable a person skilled in the art to make and use all of what is claimed, not merely a subset." *Id.* at 611. For example, the Supreme Court cited to *Wood v. Underhill*, 46 U.S. 1 (1847), in which it concluded that a patent that "claimed a process for making bricks" was enabled because it "included 'a general rule' about the

proportion of dust and clay to use and offered two alternative proportions 'where the clay has some peculiarity.'" *Amgen Inc.*, 598 U.S. at 611 (quoting *Wood*, 46 U.S. at 5). In contrast, the Supreme Court cited to *The Incandescent Lamp Patent*, 159 U.S. 465 (1895), in which it held that a patent for an "electric lamp with an incandescing conductor made of carbonized fibrous or textile material" was not enabled because "the record showed that most fibrous and textile materials failed to work" and the patent did not "disclose[ ] a quality common to fibrous and textile substances that made them peculiarly adapted to incandescent lighting." *Amgen Inc.*, 598 U.S. at 608 (quoting *Incandescent Lamp*, 159 U.S. at 608–09 (internal quotation marks omitted)). Similarly, the Court held that the patent at issue in *Amgen*, which claimed a class of functionally-defined antibodies, was not enabled because it "call[ed] on scientists to create a wide range of candidate antibodies and then screen each to see which happen to [exhibit the desired functional effect]" without "identif[ying] a quality common to every functional embodiment." *Id.* at 614.

Here, the patents-in-suit do not teach which unit dosages of compounds covered by the claims could be administered daily to a patient and which could not. Thus, the patents-in-suit provide "only a starting point, a direction for future research" that places the burden on a POSA to conduct "an iterative, trial-and-error approach to practice the claimed invention." *ALZA Corp. v. Andrx Pharms., LLC*, 603 F.3d 935, 939–41 (Fed Cir. 2010) (quoting *Automotive Techs. Int'l, Inc. v. BMW of N.A., Inc.*, 501 F.3d 1274, 1284 (Fed. Cir. 2007)). Nor can this experimentation be said to be merely routine where the patents-in-suit provide no guidance that would help a POSA *reliably* screen between compounds that would have the desired therapeutic effects at toxic versus non-toxic

dosage ranges. *See Amgen Inc.*, 598 U.S. at 614 (finding the patent-in-suit invalid for lack of enablement where specification "called on scientists to create a wide range of candidate antibodies and then screen each to see which happen to [exhibit the desired functional effect]"). Instead, a POSA would have to conduct further experimentation unassisted by the patents-in-suit. This renders the claims insufficient to meet the enablement requirement. *See ALZA Corp.*, 603 F.3d at 943 ("Patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable."). Stated differently, this case is more like *Incandescent Lamp* and *Amgen* than *Wood.* For these reasons, the Court concludes that AstraZeneca presented clear and convincing evidence such that no reasonable jury could find that the patents-in-suit enabled a POSA to administer a unit dosage of any irreversible EGFR inhibitor covered by the claims to a patient without undue experimentation. The Court therefore concludes that the asserted claims of the '314 and '162 patents are invalid for failure to meet the enablement requirement of 35 U.S.C. § 112(a).

4. Written description

Although the Court has concluded that AstraZeneca is entitled to judgment as a matter of law on the issue of enablement and therefore that the patents-in-suit are invalid, the Court will nevertheless address the remainder of the parties' arguments for the sake of completeness.

AstraZeneca further argues that judgment as a matter of law should be entered in its favor because the asserted claims are invalid for lack of written description. A patent specification must contain a written description that "clearly allow[s] persons of

ordinary skill in the art to recognize that [the inventor] invented what is claimed." *Ariad Pharms., Inc.*, 598 F.3d at 1351 (quoting *Vas–Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991)). "The test for the sufficiency of the written description 'is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date.'" *Vasudevan Software, Inc.*, 782 F.3d at 682 (quoting *Ariad Pharms., Inc.*, 598 F.3d at 1351). This is a question of fact. *Id.* "A party must prove invalidity for lack of written description by clear and convincing evidence." *Id.* (quoting *Laryngeal Mask Co. Ltd. v. Ambu*, 618 F.3d 1367, 1373–74 (Fed. Cir. 2010)).

As the Court discussed in its decision on AstraZeneca's motion for summary judgment, "the asserted claims encompass not just a method of treatment with *specific* irreversible EGFR inhibitors defined by their structure but rather a method of treatment with the entire *class* of irreversible EGFR inhibitors that are capable of performing a specific *function*." *Puma Biotech.*, 2024 WL 1157120, at *8. The Federal Circuit has explained in the analogous (though not identical) context of patents claiming a functionally-defined genus of compounds that "a sufficient description of a genus . . . requires the disclosure of either a representative number of species falling within the scope of the genus or structural features common to the members of the genus so that one of skill in the art can 'visualize or recognize' the members of the genus." *AbbVie Deutschland GmbH & Co., KG v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1299 (Fed. Cir. 2014) (quoting *Ariad Pharms., Inc.*, 598 F.3d at 1350).

AstraZeneca's arguments with respect to written description mirror its arguments with respect to enablement. It argues that the patents-in-suit fail to provide adequate

description of (1) every irreversible EGFR inhibitor claimed to practice the claimed method of treatment; (2) the treatment of all types of g/e resistant NSCLC; and (3) every unit dosage of the claimed inhibitors.

a. Any compound

As the Court discussed with respect to AstraZeneca's enablement argument, the parties presented conflicting evidence regarding the representativeness of the three compounds disclosed in the specification and whether the specification, viewed in light of the state of the art at the time the patent was filed, would allow a POSA to "visualize or recognize" the compounds that can be used to practice the claims. *Id*. For these same reasons, AstraZeneca is not entitled to judgment as a matter of law on its written description defense. AstraZeneca attempts to distinguish "pyrimidine-based irreversible EGFR inhibitors (containing a pyrimidine core) that are characteristic of the so-called 'third generation' inhibitors like osimertinib [Tagrisso] and others," which it argues are not represented by the "second generation" inhibitors described by the patents-in-suit. Defs.' Post-Trial Mot. at 22. But Wyeth's expert testified that Tagrisso and other third-generation inhibitors did not have meaningfully different structural features compared to the example compounds and structures disclosed in the specification. *See* Jury Trial Tr. at 979:5–981:1 (Jorgensen testimony). The jury was not obligated to credit AstraZeneca's evidence over Wyeth's. AstraZeneca also argues that the specification "does not describe the wild-type sparing, 'mutant selective' profile of third generation EGFR inhibitors" such as Tagrisso and therefore it is "functionally different than what the patents-in-suit describe." Defs.' Post-Trial Mot. at 22–23 (emphasis omitted). But the fact that Tagrisso may have some additional characteristic that makes it particularly

effective does not mean that it does not share the basic features of the so-called second-generation irreversible EGFR inhibitors disclosed in the patents-in-suit. Further, there is simply no requirement that the specification describe each and every compound that might be used to practice the claimed method of treatment. *See Amgen Inc.*, 598 U.S. at 610–11 (stating that a specification need not always "describe with particularity how to make and use every single embodiment within a claimed class").

b. g/e resistant NSCLC

Similarly, AstraZeneca argues that the specification does not adequately describe a method of treating NSCLC lacking sensitizing mutations, NSCLC with a KRAS mutation, and NSCLC with MET amplification. As discussed, however, Wyeth presented ample and persuasive evidence that a POSA would not consider these types of NSCLC to be g/e resistant. AstraZeneca therefore is not entitled to judgment as a matter of law on this basis.

c. Unit dosage

Lastly, AstraZeneca asserts that the patents-in-suit "fail to demonstrate possession of the 'unit dosage' of even a single irreversible EGFR inhibitor, much less the full scope of claimed 'unit dosages.'" Defs.' Post-Trial Mot. at 24 (emphasis omitted). The Court agrees that the patents-in-suit do not contain a written description that "clearly allow[s] persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed," i.e. a unit dosage of an irreversible EGFR inhibitor that can be administered daily to patient. *Ariad Pharms., Inc.*, 598 F.3d at 1351 (quoting *Vas–Cath Inc.*, 935 F.2d at 1563). Although the specification discusses a suggested range for a unit dosage between 2 and 500 milligrams per day, nothing in the specification

suggests that the inventors in fact had identified a unit dosage of the specified compounds that could be administered daily to a patient at levels high enough to show the desired therapeutic effect of interfering with the EGFR pathway and killing cancer cells. Instead, as the Court discussed with respect to enablement, a POSA would be required to engage in significant experimentation to determine an administrable unit dosage—if any—for different compounds covered by the asserted claims. Again, the patents-in-suit perhaps contain a sufficient written description of a process for disrupting EGFR pathways and eliminating cancer cells, but that is not the extent of what the patents claim. With respect to a method of treatment involving administering daily a unit dosage to a patient, the specification describes an unfinished project, not a completed invention. For these reasons, the Court concludes that AstraZeneca is entitled to judgment as a matter of law that the patents-in-suit are invalid for lack of written description.

C. Damages

Finally, AstraZeneca argues that it is entitled to judgment as a matter of law that Wyeth did not suffer any damages. It asserts that the jury's award of $107.5 million in damages was "premised on a legally insufficient basis" for two reasons. Defs.' Post-Trial Mot. at 30. First, it asserts Wyeth did not present sufficient evidence regarding the "royalty base." *Id.* AstraZeneca does not expand on this argument but rather refers back to the portion of its brief discussing infringement. The Court therefore assumes that AstraZeneca is referring to its argument that it did not induce infringement as to all three indications of Tagrisso. As the Court discussed above, there was sufficient evidence for the jury to conclude that AstraZeneca induced infringement with respect to

all three indications of Tagrisso. Because AstraZeneca does not articulate any other argument with respect to the "royalty base," the Court declines to grant it judgment as a matter of law on this basis.

Second, AstraZeneca argues that "Wyeth's evidence on royalty rate is also insufficient" because Wyeth's expert, Dr. Mohan Rao, "based his opinion as to royalty rate on his analysis of six BioSci database licenses" but "did not perform the baseline comparability analysis required to provide a legally sufficient basis for his opinion based on these licenses." *Id.* AstraZeneca raised this precise argument, however, in support of its motion to exclude Dr. Rao's testimony. The Court previously concluded "that Dr. Rao has established the 'baseline comparability' of the six licenses at issue and the patents-in-suit." *See Puma Biotech., Inc.*, 2024 WL 1157120, at *22. And his testimony at trial was likewise sufficient. AstraZeneca does not articulate any reason why the Court should reconsider its conclusion with respect to baseline comparability, nor does it provide additional arguments regarding the insufficiency of the evidence beyond its renewed baseline-comparability argument. Furthermore, at trial, Dr. Rao's testimony was not based exclusively or even primarily on the disputed BioSci database licenses. To the contrary, he testified that he used a license agreement involving the patents-in-suit between Pfizer and Puma Biotechnologies as the "starting point" for his analysis. *See* Jury Trial Tr. at 357:13–361:16 (discussing his analysis of the Pfizer-Puma license and the adjustments needed to account for differences between that license and the hypothetical license at issue in this case). In addition, AstraZeneca's own damages expert, Carla Mulhern, testified that a reasonable royalty rate, after correcting for errors in Dr. Rao's analysis, would be three percent. *See* Jury Trial Tr. at 768:10–13 (Mulhern

testimony) ("And so based on my analysis, if we adjusted for those flaws, some of those flaws, that would result in a revised or adjusted royalty rate of 3 percent rather than the 7.1 percent that Dr. Rao testified about."). This is quite close to the implied royalty rate of 3.5 percent that would result in the $107.5 million in damages awarded by the jury. AstraZeneca therefore is not entitled judgment as a matter of law on this basis.

Conclusion

For the reasons discussed above, the Court grants AstraZeneca's motion for judgment as a matter of law [dkt. no. 483] that the patents-in-suit are invalid due to lack of enablement and lack of written description of the claimed invention but otherwise overrules AstraZeneca's motion. The Court will cause entry of an amended judgment accordingly. The Court denies as moot Wyeth's motion under Federal Rule of Civil Procedure 59(e) for supplemental damages, interest, and ongoing royalties [dkt. no. 494].

MATTHEW F. KENNELLY
United States District Judge

Date: August 14, 2024

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

PUMA BIOTECHNOLOGY, INC. and WYETH LLC,	)	
Plaintiffs,	)	
vs.	)	**Case No. 21 C 1338**
ASTRAZENECA PHARMACEUTICALS LP and ASTRAZENECA AB,	)	
Defendants.	)	

MEMORANDUM OPINION AND ORDER

MATTHEW F. KENNELLY, District Judge:

Puma Biotechnology, Inc. and Wyeth LLC have sued AstraZeneca Pharmaceuticals LP and AstraZeneca AB (collectively AstraZeneca) for infringement of two patents: United States Patent Nos. 10,603,314 (the '314 patent) and 10,596,162 (the '162 patent). The plaintiffs contend that AstraZeneca's drug Tagrisso (osimertinib) infringes claims 1, 3, and 9 of the '314 patent and claim 1 of the '162 patent. AstraZeneca has moved to dismiss the case for lack of Article III standing. In the alternative, AstraZeneca has moved for summary judgment, arguing that the patents are invalid, that Tagrisso does not infringe the patents, and that the plaintiffs are not entitled to pre-issuance damages. The plaintiffs have moved for partial summary judgment on AstraZeneca's advice-of-counsel defense. Both parties have filed motions to exclude certain expert testimony related to damages. For the reasons set forth below, the Court (1) grants AstraZeneca's motion to dismiss with respect to Puma but denies the motion to dismiss with respect Wyeth; (2) grants AstraZeneca's motion for

summary judgment on the issue of pre-issuance damages but otherwise denies AstraZeneca's motions for summary judgment; (3) denies the plaintiffs' motion for summary judgment on AstraZeneca's advice-of-counsel defense; and (4) denies both parties' motions to exclude.

Background

The Court explained the background of this case in its March 29, 2023 claim construction order. The Court will briefly review that background and summarize additional facts relevant to the pending motions.

The parties to this suit are pharmaceutical companies that commercialize drugs to treat cancer and other illnesses. The patents-in-suit claim a method of treating a certain form of non-small cell lung cancer (NSCLC). NSCLC is associated with overactivity of the epidermal growth factor receptor (EGFR), an enzyme that is involved in cell division and growth. Drugs that treat this condition are known as EGFR tyrosine kinase inhibitors (TKIs or inhibitors), and these TKIs bind to certain parts of the EGFR to prevent the enzyme from triggering cancerous cell growth.

Two TKIs, gefitinib and erlotinib (referred to collectively as g/e), showed some promise in treating NSCLC. Gefitinib and erlotinib are classified as "reversible" inhibitors; they form non-covalent bonds with EGFR that dissociate over time. There are two principal limitations to g/e treatment. First, only patients with certain EGFR mutations are sensitive to g/e therapy; the parties refer to these mutations as "sensitizing mutations." In other words, to be a candidate for g/e treatment, a patient needs to have EGFR with the requisite sensitizing mutation(s). Second, "[a] significant limitation in using [reversible inhibitors such as g/e] is that recipients thereof may

develop a resistance to their therapeutic effects after they initially respond to therapy, or they may not respond to EGFR-TKIs to any measurable degree at all." '314 Patent at 3:19–23.

The patents-in-suit claim a method for treating "g/e resistant NSCLC." The inventors claim that g/e resistance can be overcome by using "irreversible" EGFR inhibitors that covalently bind to a specific amino acid at a specific location of EGFR. Specifically, the asserted claims of the '314 patent recite:

> 1. A method for treating gefitinib and/or erlotinib resistant non-small cell lung cancer in a patient in need thereof, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer a pharmaceutical composition comprising a unit dosage of an irreversible epidermal growth factor receptor (EGFR) inhibitor that covalently binds to cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2.
>
> [. . .]
>
> 3. The method of claim 1, wherein the irreversible EGFR inhibitor covalently binds to cysteine 773 residue of EGFR.
>
> [. . .]
>
> 9. The method of claim 1, wherein the route of administration is oral.

'314 Patent at 35:52–36:65.

In addition, the claims of the '162 patent are directed at EGFR with a specific mutation, the "T790M mutation," which is associated with g/e resistance. The asserted claim of '162 patent recites:

> 1. A method of treating gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 in a patient, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 a pharmaceutical composition comprising a unit dosage of 2-500 mg of an irreversible EGFR inhibitor that covalently binds to cysteine 773 of the catalytic domain within the SEQ ID NO: 1 having a T790M mutation; wherein the

irreversible EGFR inhibitor is not CL-387,785.

'162 Patent at 35:48–36:48.

The patents-in-suit were originally issued to Wyeth and the non-party General Hospital Corporation. In 2006, General Hospital Corporation assigned its rights in the patents-in-suit to Wyeth. Wyeth was acquired by Pfizer in 2009 and remains a wholly owned subsidiary of Pfizer. In 2011, Puma signed an agreement with Pfizer to exclusively license the patents-in-suit with respect to a compound known as neratinib and certain other compounds. It is undisputed that the scope of Puma's license does not cover the use of the patents-in-suit with respect to osimertinib, the compound in AstraZeneca's Tagrisso drug. In 2020, Puma and Pfizer signed an amendment to the license agreement that gave Puma the right to control the enforcement of the patents-in-suit, subject to Pfizer's approval of any settlement agreement. In July 2021, Puma and Wyeth signed a "Confirmatory License" in order to "confirm (i) that the rights controlled by Wyeth in and to [certain intellectual property, including the patents-in-suit] are licensed from Wyeth to Puma in accordance with the Puma License and (ii) that Pfizer had the authority to grant Puma such rights on behalf of Wyeth." Winkler Decl., Ex. 6 at PUMA-TAG00000054 (2021 Confirmatory License).

In September 2021, the plaintiffs sued AstraZeneca, alleging that AstraZeneca's irreversible EGFR inhibitor Tagrisso (osimertinib) infringes both patents-in-suit. The parties disputed the meaning of four claim terms. After briefing and a hearing, the Court issued a March 29, 2023 order construing the disputed terms [dkt. 121]. Now before the Court are AstraZeneca's motions to dismiss and for summary judgment, the plaintiffs' motion for partial summary judgment, and both parties' motions to exclude expert

testimony.

A. Article III standing

AstraZeneca first argues that the case must be dismissed because neither Puma nor Wyeth can satisfy the constitutional requirements for Article III standing. "As the party invoking federal jurisdiction, the plaintiffs bear the burden of demonstrating that they have standing." *TransUnion LLC v. Ramirez*, 594 U.S. 413, 430–31 (2021). "To establish standing, the party invoking federal jurisdiction must demonstrate (1) an 'injury in fact' that is (2) 'fairly traceable' to the defendant's challenged conduct and is (3) 'likely to be redressed by a favorable judicial decision.'" *Apple Inc. v. Qualcomm Inc.*, 17 F.4th 1131, 1135 (Fed. Cir. 2021) (quoting *Spokeo, Inc. v. Robins*, 578 U.S. 330, 338 (2016)). With respect to the injury-in-fact requirement, the Federal Circuit has explained that "the touchstone of constitutional standing in a patent infringement suit is whether a party can establish that it has an exclusionary right in a patent that, if violated by another, would cause the party holding the exclusionary right to suffer legal injury." *Univ. of S. Fla. Rsch. Found., Inc. v. Fujifilm Med. Sys. U.S.A., Inc.*, 19 F.4th 1315, 1323 (Fed. Cir. 2021) (quoting *WiAV Sols. LLC v. Motorola, Inc.*, 631 F.3d 1257, 1266 (Fed. Cir. 2010)).

1. Wyeth

Wyeth is the patentee of the patents-in-suit.[1] Typically, that is all that is needed to satisfy Article III standing in a patent infringement suit. *See Morrow v. Microsoft Corp.*, 499 F.3d 1332, 1340 (Fed. Cir. 2007). But AstraZeneca argues that Wyeth lacks

[1] The patents-in-suit were originally issued to Wyeth and the General Hospital Corporation. The parties do not dispute that General Hospital Corporation assigned all of its rights in the patents-to-suit to Wyeth, leaving Wyeth as the only patentee for purposes of the present litigation.

standing because "it has apparently given away significant and necessary rights" to the patents-in-suit to its parent company Pfizer. Defs.' Mot. to Dismiss at 7. In AstraZeneca's view, "the fact that the original 2011 License Agreement was between Pfizer and Puma, rather than Wyeth and Puma, demonstrates Pfizer holds a substantial, unfettered right to sublicense the patents-in-suit." *Id.* AstraZeneca also points out that the Pfizer-Puma License Agreement states that "as between Pfizer and Wyeth, Pfizer dictates settlement authorization and receives the applicable portion of the recoveries in connection with any T790M Enforcement." *Id.* (quoting 2021 Confirmatory License § 1.5(b)). AstraZeneca argues that these provisions "support[ ] a finding that Wyeth lacks constitutional standing." *Id.*

The Court disagrees. First, the Court disagrees that the agreements between Pfizer and Puma suggest that Wyeth assigned its rights in the patents-in-suit to Pfizer. It is undisputed that Wyeth is a wholly owned subsidiary of Pfizer. The 2011 License Agreement clearly states that it is an agreement between Puma and "Pfizer Inc., . . . on its own behalf *and on behalf of its affiliates*." 2011 License Agreement at 1 (emphasis added). The agreement further states that "PFIZER controls, directly *or through its affiliates*, certain technology relating to a compound known as neratinib . . . ." *Id.* (emphasis added). The agreement further states that the "patent rights" involved in the Pfizer-Puma deal "are controlled by PFIZER *or its Affiliates*." *Id.* at 7 § 1.41 (emphasis added). The fact that Pfizer, as Wyeth's parent company, may conduct deals on behalf of Wyeth does not mean that Wyeth has assigned all of its patent rights to Pfizer. To hold otherwise would imply that no subsidiary has standing to enforce its own patents. In addition, the 2021 Confirmatory License signed by Wyeth, Pfizer, and Puma confirms

that Wyeth *authorized* Pfizer to act on its behalf with respect to certain "rights owned, licensed, or otherwise controlled by Wyeth" for purposes of the Puma deal. If Wyeth had in fact assigned its rights to the patents-in-suit to Pfizer, it would be unnecessary to clarify Pfizer's authority to act on Wyeth's behalf.

Second, the fact that Wyeth must receive approval from Pfizer before entering into a settlement agreement does not mean that Wyeth has not suffered an injury-in-fact for Article III purposes. Even if Wyeth has given Pfizer veto power over settlements in litigation related to the patents-in-suit, the bottom line is that Wyeth, as patentee, maintains the right to grant or refuse a license to AstraZeneca for the alleged infringing conduct. The Federal Circuit has said that these exclusionary rights lie at the heart of the Article III injury-in-fact inquiry. *See Lone Star Silicon Innovations LLC v. Nanya Tech. Corp.*, 925 F.3d 1225, 1234 (Fed. Cir. 2019) ("We have recognized that those who possess 'exclusionary rights' in a patent suffer an injury when their rights are infringed." (quoting *WiAV Sols. LLC*, 631 F.3d at 1264); *Morrow*, 499 F.3d at 1340 (explaining that parties that "hold exclusionary rights and interests created by the patent statutes, but not all substantial rights to the patent" nevertheless have Article III standing). Although Wyeth may have contracted away some sticks in its bundle of patent rights to Puma and Pfizer, this is not a case where the patentee has given up its core exclusionary rights vis-à-vis the alleged infringing conduct. The Court therefore finds that Wyeth has suffered an injury-in-fact for Article III purposes.[2]

[2] The parties do not dispute that the remaining two requirements, traceability and redressability, are satisfied. But because the Court has an independent obligation to evaluate subject-matter jurisdiction, the Court notes for completeness that it finds that the injury is traceable to AstraZeneca's alleged conduct and that it would be redressable

2. Puma

It is undisputed that Puma's exclusive license to the patents-in-suit is "compound-specific"—i.e., Puma holds rights to practice the patents only with respect to certain compounds. Pls.' Opp. to Mot. to Dismiss at 6. Importantly, Puma's exclusive license does *not* permit it to practice the patents-in-suit with respect to osimertinib, the compound in Tagrisso. This means that AstraZeneca's allegedly infringing activity falls outside of the scope of Puma's license. Because Puma has no right to use or exclude others from practicing the patents-in-suit with respect to osimertinib, it has no standing to sue AstraZeneca for infringement. *See WiAV Sols. LLC*, 631 F.3d at 1266 ("Because an exclusive licensee derives its standing from the exclusionary rights it holds, it follows that its standing will ordinarily be coterminous with those rights. Depending on the scope of its exclusionary rights, an exclusive licensee may have standing to sue some parties and not others."); *Flow Devices & Sys., Inc. v. Pivotal Sys. Corp.,* 666 F. Supp. 3d 1024, 1029 (N.D. Cal. 2023) ("[E]xclusionary rights against the defendant are a necessary and sufficient condition for Article III standing" in a patent infringement suit.).

Puma advances various arguments for why it has suffered an injury-in-fact despite its lack of any exclusionary rights with respect to AstraZeneca's conduct. This Court, however, is bound by Federal Circuit precedent on this issue. The Federal Circuit has recognized that it would be "contrary to [its] precedent" to hold that a party with no exclusionary rights has Article III standing. *See In re Cirba Inc.*, No. 2021-154, 2021 WL 4302979, at *3 (Fed. Cir. Sept. 22, 2021) (explaining that to hold that "exclusionary

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by a ruling by this Court. *See Lone Star Silicon Innovations LLC*, 925 F.3d at 1234 ("[I]t is clear that a court could redress an injury caused by [the defendant's infringement].")

rights are not necessary for a concrete injury, and [that a plaintiff] has been sufficiently injured for Article III standing by virtue of [a] competitive injury" would be "contrary to our precedent"). Puma may pursue its arguments on appeal, but its admission that its exclusionary rights to the patents do not overlap with AstraZeneca's alleged infringement ends the inquiry before this Court.

Puma argues that it can proceed as a plaintiff in this case regardless of whether it has Article III standing because its co-plaintiff, Wyeth, has standing. The Supreme Court, however, has emphasized that "Article III does not give federal courts the power to order relief to any uninjured plaintiff." *TransUnion LLC*, 594 U.S. at 430. Puma cannot seek relief in this suit unless it independently satisfies the requirements of Article III. *See id.* ("[S]tanding is not dispensed in gross; rather, plaintiffs must demonstrate standing for each claim that they press and for each form of relief that they seek (for example, injunctive relief and damages).").

Lastly, Puma argues that Federal Rule of Civil Procedure 19 secures its standing in this suit. But procedural rules cannot alter the requirements of Article III standing. *See id.* at 429 ("A regime where Congress could freely authorize *unharmed* plaintiffs to sue defendants who violate federal law [. . .] would violate Article III."). The Court therefore concludes that Puma lacks standing and must be dismissed as a plaintiff in this case.

B. Invalidity

AstraZeneca argues that it is entitled to summary judgment on all of the Wyeth's claims because the patents-in-suit fail to meet the enablement and written description requirements of 35 U.S.C. § 112. At the summary judgment stage, "the court must view

the facts in the light most favorable to the nonmoving party and draw all inferences in that party's favor." *ArcelorMittal Atlantique et Lorraine v. AK Steel Corp.*, 908 F.3d 1267, 1273 (Fed. Cir. 2018) (quoting *Gonzalez v. Sec'y of Dep't of Homeland Sec.*, 678 F.3d 254, 257 (3d Cir. 2012)). "Because patents are presumed valid, 'a moving party seeking to invalidate a patent at summary judgment must submit such clear and convincing evidence of facts underlying invalidity that no reasonable jury could find otherwise.'" *TriMed, Inc. v. Stryker Corp.*, 608 F.3d 1333, 1340 (Fed. Cir. 2010) (quoting *SRAM Corp. v. AD-II Eng'g, Inc.*, 465 F.3d 1351, 1357 (Fed. Cir. 2006)).

A patent must include a specification which contains "a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention." 35 U.S.C. § 112(a). The Federal Circuit has interpreted section 112(a) as containing both a "written description" requirement and an "enablement" requirement. *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1344 (Fed. Cir. 2010). AstraZeneca argues that the patents-in-suit fail both requirements.

1. Enablement

"Enablement is a legal question based on underlying factual determinations." *Vasudevan Software, Inc. v. MicroStrategy, Inc.*, 782 F.3d 671, 684 (Fed. Cir. 2015). "Because patents are presumed valid, lack of enablement must be proven by clear and convincing evidence." *Baxalta Inc. v. Genentech, Inc.*, 81 F.4th 1362, 1365 (Fed. Cir. 2023). The enablement requirement is satisfied if the specification contains sufficient

information to permit "a person of skill in the art to make and use the claimed invention." *Vasudevan Software, Inc.*, 782 F.3d at 684. "[T]he specification must enable the full scope of the invention as defined by its claims." *Amgen Inc. v. Sanofi*, 598 U.S. 594, 610 (2023). Thus, "[i]f a patent claims an entire class of processes, machines, manufactures, or compositions of matter, the patent's specification must enable a person skilled in the art to make and use the entire class." *Id.* This does not mean, however, that "a specification necessarily [is] inadequate just because it leaves the skilled artist to engage in some measure of adaptation or testing." *Id.* at 611. "[A] specification may call for a reasonable amount of experimentation to make and use a patented invention." *Id*. at 612. "In other words, 'the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation.'"[3] *Baxalta Inc.*, 81 F.4th at 1365 (quoting *MagSil Corp. v. Hitachi Glob. Storage Techs.*, Inc., 687 F.3d 1377, 1380 (Fed. Cir. 2012)).

a. Whether the claims enable use of full scope of irreversible EGFR inhibitors

AstraZeneca first argues that the patents-in-suit do not enable a person of ordinary skill in the art (POSA) to practice the claimed method of treatment with the full scope of irreversible EGFR inhibitors described in the specification. Specifically, AstraZeneca argues that the specification fails to enable a POSA to practice the method of treatment using "larger compounds" or with "epoxides or other non-Michael

[3] After the Supreme Court's decision in *Amgen*, the Federal Circuit has used the terms "undue experimentation" and "unreasonable experimentation" interchangeably. *See Baxalta Inc.*, 81 F.4th at 1365–66, 1367 n. 4.

acceptors." Defs.' Mot. for Summ. Judgment on Invalidity at 6. The Court concludes that, drawing all inferences in favor of Wyeth as the non-movant, there are genuine disputes of material fact regarding both issues.

i. "Larger compounds"

At claim construction, the Court interpreted the term "irreversible EGFR inhibitor" in the asserted claims of both patents to mean "[a] compound that irreversibly inhibits EGFR." Claim Constr. Op. at 14. The patent specification, in turn, defines a "compound" as:

> a chemical entity or biological product, or combination of chemical entities or biological products, administered to a person to treat or prevent or control a disease or condition. The chemical entity or biological product is preferably, but not necessarily a low molecular weight compound, but may also be a larger compound, for example, an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNA-zymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof.

'314 Patent at 13:3–13; '162 Patent at 13:4–14. AstraZeneca therefore asserts that the specification must enable a POSA to practice the claims with not only a "low molecular weight" irreversible EGFR inhibitor, but also any irreversible EGFR inhibitor that is "a larger compound." Because it is undisputed that the method of treatment claimed in the patents-in-suit cannot be carried out with these so-called "larger compounds," AstraZeneca argues that the claims are invalid for lack of enablement as a matter of law.

The Court disagrees that the mention of "larger compound[s]" in the specification necessarily invalidates the patents. Although it is true that an inventor must enable the full scope of the claim, here, the asserted claims covers only those irreversible EGFR inhibitors that "covalently bind[ ]" to the specified part of EGFR. The relevant question,

therefore, is whether the patent enables a POSA to identify which irreversible EGFR inhibitors will covalently bind to the specified part of EGFR without "undue experimentation." *Baxalta Inc*, 81 F.4th 1362 at 1365.

Certainly, the specification's suggestion that the chosen irreversible EGFR inhibitor "may also be a larger compound"—which both parties agree is incorrect—is evidence that the specification does *not* provide sufficient guidance to a POSA regarding what types of compounds are or are not suitable for practicing the claims. But Wyeth has offered evidence that this arguable red herring in the specification would not impede a POSA from identifying which irreversible EGFR inhibitors are useful for practicing the claims or require a POSA to engage in undue experimentation to do so. Specifically, Wyeth's expert, Dr. Jorgensen, asserts that a POSA would know that "larger compounds" cannot covalently bind to EGFR. *See* Winchester Decl., Ex. 10 at 69:21–70:16 (Jorgensen Dep.) (explaining that "for something to be an inhibitor of a EGFR kinase, because the kinase is in the intracellular area, compounds do have to get into the cell," and that only "small molecules can get into cells," which "precludes other types of molecules as potential EGFR inhibitors, very large molecules, very polar molecules, proteins, nucleic acids, compounds that would have poor or negligible cell permeability"); *id.* at 80:10–19 (Q: And in your opinion, a POSA in 2005 would not consider irreversible EGFR inhibitors that were those larger compounds described [in the specification] as irreversible EGFR inhibitors that could be made to form a covalent bond to a cysteine in the intracellular domain of EGFR, correct? A: A POSA would not be considering molecules like that. He or she would be focusing on small molecules."); *id.* at 89:3–89:5 ("So there is an attempt here to complicate things, and I think in an

unnecessary way, because of the literature at the time on kinase inhibitors is all about small molecules. A POSA knows that.). The Court therefore concludes that there is a genuine dispute regarding whether a POSA would understand that "large molecules cannot bind the EGFR kinase domain and cannot covalently bind to cys773 as the claims require" or whether a POSA would need to experiment in order to rule out such compounds as potential candidates for the claimed method of treatment. Pls.' Stmt. of Material Facts on Invalidity ¶ 9 (emphasis omitted).

ii. Epoxides and other non-Michael acceptors

Next, AstraZeneca argues that the asserted claims of the patents-in-suit "over-reach even with regard to small molecules." Defs.' Mot. for Summ. Judgment on Invalidity at 6. It argues that, although the patents claim a method of treating NSCLC with *any* irreversible EGFR inhibitor that covalently bonds to EGFR, the "examples in the specification are limited to compounds with a specific type of reactive agent (a Michael acceptor) that forms the requisite covalent bond." *Id.* AstraZeneca argues that because "the patents-in-suit fail to describe or enable compounds with other reactive agents, such as epoxides," they are invalid as a matter of law. *Id.*

The Supreme Court's recent decision in *Amgen* makes clear that "the specification must enable the *full* scope of the invention as defined by its claims." *Amgen Inc.*, 598 U.S. at 610 (emphasis added). The Supreme Court clarified, however, that this does not mean that "a specification always must describe with particularity how to make and use every single embodiment within a claimed class." *Id.* at 610–11. "[I]t may suffice to give an example (or a few examples) if the specification also discloses 'some general quality . . . running through' the class that gives it 'a peculiar fitness for

the particular purpose.'" *Id.* at 611 (quoting *The Incandescent Lamp Patent*, 159 U.S. 465, 475 (1895)). Thus, the mere fact that the specification does not expressly describe epoxides or other non-Michael acceptor reactive agents as examples does not necessarily mean that the asserted claims are not enabled. Rather, the key question is whether the specification describes some "general quality" or "rule" that "may reliably enable a person skilled in the art to make and use all of what is claimed, not merely a subset" without having to engage in an "[un]reasonable amount of experimentation." *Id.* at 611–12.

There is a genuine factual dispute regarding whether the specification would enable a POSA to practice the claims with *all* "compound[s] that irreversibly inhibit[ ] EGFR and covalently bind[ ] to [cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2 / cysteine 773 of the catalytic domain within the SEQ ID NO: 1 having a T790M mutation]" without undue experimentation. For example, AstraZeneca's expert characterizes the three concrete examples provided in the specification as "very similar," while Wyeth's expert characterizes the examples as "quite different" and "showing some variety in the structures." Pls.' Opp. to Mot. for Summ. Judgment at 13 (quoting Pls.' Stmt. of Material Facts on Invalidity ¶ 13). In addition, AstraZeneca's experts state that "potentially . . . many trillions of compounds" fall within the claims, Reider Decl., Ex. A ¶ 337, while Wyeth's expert represents that "the list of . . . likely cores is not big and one could go to the kinase literature and start tabulating the cores. . . . It's not a large list. I don't know how many it would be, but tens, maybe tens of cores. It's not hundreds of different cores." Winchester Decl., Ex. 10 at 167:3–10 (Jorgensen Dep.). Wyeth's experts

further assert that irreversible EGFR inhibitors were already well-studied and that "[a] POSA knew the precise 3-D structure of EGFR and, as a result, the common structural features for an inhibitor to covalently bind to cys773 in the kinase domain of EGFR." Pls.' Opp. to Mot. for Summ. Judgment on Invalidity at 6. The parties also dispute whether epoxides are truly distinct from the basic structures outlined in the specification or whether they are easily derived from those structures. In brief, Wyeth's expert says that a POSA "would readily be able to test" compounds for the relevant qualities identified in the specification and that "the task would be reasonably predictable, unburdensome, and nothing more than a routine process for a medicinal chemist of ordinary skill." Pls.' Stmt. of Material Facts on Invalidity, Ex. A ¶¶ 170–71 (Jorgensen Rep.). Wyeth's evidence is sufficient to carry its burden at the summary judgment stage.

b. Whether the claims enable the treatment of all types of "g/e resistant NSCLC"

AstraZeneca next argues that the patents-in-suit do not enable a POSA to practice the claims for all types of "g/e resistant NSCLC." Defs.' Mot. for Summ. Judgment on Invalidity at 6–7. Specifically, AstraZeneca asserts that the patents-in-suit do not enable a POSA to treat NSCLC that lacks "sensitizing mutations," NSCLC with a "KRAS mutation," or NSCLC with "MET amplification." *Id.* at 7–8.

Wyeth does not dispute that the claimed method does not treat NSCLC that lacks sensitizing mutations, NSCLC with a KRAS mutation, or NSCLC with MET amplification. Instead, it argues that these types of NSCLC are not g/e resistant. Specifically, Wyeth's experts assert that a POSA would not regard these types of NSCLC as g/e resistant.

See Hausheer Decl., Ex. B. ¶ 747 ("[A] POSA would understand that TKI therapy is beneficial for NSCLC patient having EGFR-sensitizing mutations."); Weiss Decl., Ex. B ¶ 9 ("[B]ased on the claim language, and the teachings of the specification, including that EGFR TKIs are beneficial for NSCLC patients having sensitizing mutations, a POSA would understand that the claimed methods of treating are directed to patients with EGFR-sensitizing mutations."); Hausheer Decl., Ex. B ¶¶832–833 (explaining that "A POSA would understand that non-EGFR/ERBB2 dependent pathways [such as MET amplification], would be treatable by other drugs, not EGFR inhibitors."); Winchester Decl., Ex. 34 at 243:13–16 (Hausheer Dep.) (KRAS mutation is "not the type of resistance that the patents – the methods of the patents embody in the claim"); Winchester Decl., Ex. 13 at 75:24–76:8 (Jänne Dep.) ("KRAS mutations . . . are mutually exclusive with EGFR mutations"). The Court concludes that these conflicting expert opinions establish a genuine dispute regarding whether NSCLC that lacks sensitizing mutations, NSCLC with a KRAS mutation, and NSCLC with MET amplification are g/e resistant.

AstraZeneca points out that the specification states that "[i]n one embodiment, the subject's tumor does not harbor mutations indicative of gefitinib and/or erlotinib sensitivity and does harbor mutations indicative of gefitinib resistance" as evidence that NSCLC without sensitizing mutations is g/e resistant NSCLC. Although this is evidence in AstraZeneca's favor, it is not dispositive. *See Crown Operations Intern., Ltd. v. Solutia Inc.*, 289 F.3d 1367, 1380 (Fed. Cir. 2002) (stating that "inoperative embodiments do not necessarily invalidate the claim" but "support [the party seeking invalidation's] assertion that there is a genuine issue of material fact with respect to

enablement"); *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.,* 750 F.2d 1569, 1576 (Fed. Cir. 1984) (holding that, where "patent disclosure list[ed] numerous salts, fuels, and emulsifiers that could form thousands of" claimed combinations, some of which would be inoperable, "the claims [were] not necessarily invalid" for lack of enablement unless a POSA needed to "experiment unduly in order to practice the claimed invention").

2. Written description

AstraZeneca next argues that the patents-in-suit fail to satisfy the written description requirement. A patent specification must contain a written description that "clearly allow[s] persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed." *Ariad Pharms.*, 598 F.3d at 1351 (quoting *Vas–Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991)). "The test for the sufficiency of the written description 'is whether the disclosure of the application relied upon reasonably conveys to those skilled in the art that the inventor had possession of the claimed subject matter as of the filing date.'" *Vasudevan Software, Inc.*, 782 F.3d at 682 (quoting Ariad Pharm., Inc., 598 F.3d at 1351). This is a question of fact. *Id.* "A party must prove invalidity for lack of written description by clear and convincing evidence." *Id.* (quoting *Laryngeal Mask Co. Ltd. v. Ambu*, 618 F.3d 1367, 1373–74 (Fed. Cir. 2010)).

Because the asserted claims encompass not just a method of treatment with *specific* irreversible EGFR inhibitors defined by their structure but rather a method of treatment with the entire *class* of irreversible EGFR inhibitors that are capable of performing a specific *function*, the Court agrees with AstraZeneca that the Federal

Circuit's precedent regarding the requirements for the written description of generic and functionally defined claims applies. In particular, the Federal Circuit has explained that "[w]hen a patent claims a genus using functional language to define a desired result, 'the specification must demonstrate that the applicant has made a generic invention that achieves the claimed result and do so by showing that the applicant has invented species sufficient to support a claim to the functionally-defined genus.'" *AbbVie Deutschland GmbH & Co., KG v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1299 (Fed. Cir. 2014) (quoting *Ariad Pharms.*, 598 F.3d at 1351). "A sufficient description of a genus . . . requires the disclosure of either a representative number of species falling within the scope of the genus or structural features common to the members of the genus so that one of skill in the art can 'visualize or recognize' the members of the genus." *Id.* (quoting *Ariad Pharms.*, 598 F.3d at 1350).

As the Court has explained with respect to AstraZeneca's enablement invalidity argument, there are genuine factual disputes regarding the representativeness of the species disclosed in the specification and regarding whether the specification, combined with the state of the art at the time the patent was filed, would allow a POSA to "visualize or recognize" the compounds that can be used to practice the claims. These same unresolved factual disputes preclude summary judgment in AstraZeneca's favor on the written description defense.

Next, AstraZeneca asserts that the patents-in-suit fail to show possession of the full scope of the claimed methods of treating g/e resistance NSCLC. Again, however, the Court already has concluded that there is a genuine factual dispute regarding whether a POSA would consider the types of NSCLC that AstraZeneca highlights to be

g/e resistant.

Lastly, AstraZeneca argues that the asserted claim of the '162 patent is invalid because there is no written description support for its exclusion of the irreversible EGFR inhibitor CL-387,785. "For negative claim limitations . . . there is adequate written description when, for example, 'the specification describes a reason to exclude the relevant [compound].'" *Novartis Pharms. Corp. v. Accord Healthcare, Inc.*, 38 F.4th 1013, 1016 (Fed. Cir. 2022) (quoting *Santarus, Inc. v. Par Pharm., Inc.*, 694 F.3d 1344, 1351 (Fed. Cir. 2012)). Here, the specification is not silent regarding the exclusion of Cl-387,785. Rather, it discloses that "[w]hile this work was in progress, another irreversible inhibitor of EGFR [CL-387,785] was shown to inhibit the kinase activity of the T790M EGFR mutant" but "[t]he effectiveness of CL-387,785 in the context of T790M was proposed to result from the absence of a chloride at position 3 of the aniline group" rather than its ability "to bind irreversibly to EGFR." '314 Patent at 18:33–45. This is sufficient to meet the written description requirement for negative claim limitations. AstraZeneca may argue that the exclusion of CL-387,785 casts doubt on whether the specification *enables* a POSA to identify the compounds with which the claimed method of treatment can be practiced without undue experimentation, but as the Court has discussed, genuine disputes of material fact preclude summary judgment on enablement.

C. Non-infringement

AstraZeneca has moved for summary judgment on the ground that Tagrisso does not infringe the asserted claims of the patents-in-suit. "[S]ummary judgment of non-infringement can only be granted if, after viewing the alleged facts in the light most

favorable to the non-movant, there is no genuine issue whether the accused device is encompassed by the claims" as construed by the court. *Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1304 (Fed. Cir. 1999)).

1. '314 patent

AstraZeneca asserts that there is no genuine dispute that Tagrisso "does not bind in the 'ligand binding pocket' of EGFR (or erbB-2) as required by each of the Asserted Claims of the '314 patent" and therefore it is entitled to judgment as a matter of law. Defs.' Mot. for Summ. Judgment on Non-Infringement at 2. The parties agree that "EGFR is a transmembrane protein that contains three regions, or domains": (1) "the extracellular domain (outside the cell)"; (2) the "transmembrane domain (spans the cell membrane)"; and (3) the "intracellular domain (inside the cell and is also known as the tyrosine kinase domain)." Defs.' Stmt. of Material Facts on Non-infringement ¶ 6. It is further undisputed that "Tagrisso binds to the *intracellular* domain of EGFR, and not the *extracellular* domains of either EGFR or erbB-2." *Id.* ¶ 7 (emphasis added).

The parties dispute whether a POSA would understand the phrase "cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2" in the asserted claims to refer to the ligand-binding pocket located in the extracellular domain, or whether a POSA would understand the phrase to identify a different ligand-binding pocket located in the intracellular domain.[4]

Because the parties' dispute hinges on the meaning of the claim language rather

[4] For simplicity, the Court will, like the parties, focus its analysis on the portion of the claim that reads "cysteine 773 residue in the ligand-binding pocket of EGFR." The parties do not raise any arguments that are unique to the latter half of the phrase ("cysteine 805 residue in the ligand-binding pocket of erb-B2"), so the Court assumes that part of the claim is not at issue.

than the manner in which Tagrisso functions, the Court agrees with AstraZeneca that this is an issue of claim construction. The question therefore must be resolved by the Court, not the jury. *See O2 Micro Int'l Ltd. v. Beyond Innovation Tech. Co.*, 521 F.3d 1351, 1362 (Fed. Cir. 2008) ("When the parties present a fundamental dispute regarding the scope of a claim term, it is the court's duty to resolve it.").

A court should construe the words of a claim in accordance with their "ordinary and customary meaning," namely "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–13 (Fed. Cir. 2005). Sometimes, the meaning of a term is not immediately apparent, and a court will need to look to other sources to determine "what a person of skill in the art would have understood disputed claim language to mean." *Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc.*, 381 F.3d 1111, 1116 (Fed. Cir. 2004). These sources include "the words of the claims themselves, the remainder of the specification, the prosecution history, and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." *Id.* Courts are permitted to consider expert testimony "to ensure that the court's understanding of technical aspects of the patent is consistent with that of a person of skill in the art, or to establish that a particular term in the patent or the prior art has a particular meaning in the pertinent field." *Phillips*, 415 F.3d at 1318. However, courts must approach expert testimony with caution and ensure that it is "considered in the context of the intrinsic evidence," i.e. "the patent and its prosecution history." *Id.* at 1318–19.

There are two exceptions to the general rule that claim terms are given their ordinary meaning: "1) when a patentee sets out a definition and acts as his own

lexicographer, or 2) when the patentee disavows the full scope of a claim term either in the specification or during prosecution." *Starhome GmbH v. AT&T Mobility LLC*, 743 F.3d 849, 856 (Fed. Cir. 2014). "To disavow claim scope, the specification must contain expressions of manifest exclusion or restriction, representing a clear disavowal of claim scope." *See Cont'l Circuits LLC v. Intel Corp.*, 915 F.3d 788, 797 (Fed. Cir. 2019) (internal quotation marks omitted); *see also, Home Diagnostics, Inc. v. LifeScan, Inc.*, 381 F.3d 1352, 1358 (Fed. Cir. 2004) ("Absent a clear disavowal or contrary definition in the specification or the prosecution history, the patentee is entitled to the full scope of its claim language.").

AstraZeneca argues that the intrinsic evidence—namely, the plain meaning, patent specification, and prosecution history—supports its interpretation of the claim language as referring to an extracellular ligand-binding pocket. AstraZeneca points out that the specification states that:

> EGFR is composed of three principal domains, namely, the ***extracellular domain (ECD), which*** is glycosylated and ***contains the ligand-binding pocket*** with two cysteine-rich regions; a short transmembrane domain, and an intracellular domain that has intrinsic tyrosine kinase activity. ***The transmembrane region joins the ligand-binding domain to the intracellular domain***.

Defs.' Mot. for Summ. Judgment on Non-infringement at 3 (quoting '314 Patent at 2:13–19) (emphasis added by AstraZeneca). AstraZeneca also argues that the prosecution history supports its interpretation because "the patent applicants deliberately added the requirement of binding 'in the ligand binding pocket' during prosecution, proposing the following amended claim":

> 29. A method for treating gefitinib and/or erlotinib resistant non-small cell lung cancer in a patient in need thereof, comprising administering to the patient a pharmaceutical composition comprising an irreversible epidermal growth factor

> receptor (EGFR) inhibitor that covalently binds to a cysteine 773 residue in the ligand-binding pocket of EGFR or a cysteine 805 residue in the ligand-binding pocket of erb-B2.

Id. at 4–5 (quoting Defs.' Stmt. of Material Facts on Non-infringement ¶ 14) (underlining reflecting claim amendments). AstraZeneca further asserts that, when the applicants submitted this amendment, they directed the patent officer to "paragraph 0005" of the provisional application, which stated that "the ***extracellular* domain** (ECD) . . . ***contains the ligand-binding pocket*** . . ." *Id.* at 5.

Wyeth does not dispute that EGFR has a "ligand-binding pocket" in the extracellular region or that this extracellular ligand-binding pocket is described in the specification's explanation of EGFR's general structure. It argues, however, that a POSA would understand that the claims refer to a *different* ligand-binding pocket located in the intracellular domain. That is because "EGFR consists of a chain of amino acids bound together, and the 773rd position in the EGFR chain is a cysteine ('cysteine 773')." Pls.' Opp. to Summ. Judgment on Non-Infringement at 2. "Cysteine 773 exists in the intracellular kinase domain, always." *Id.* Wyeth argues that there is an additional ligand, ATP, that "binds inside the cell, where cysteine 773 resides." *Id.* Wyeth asserts that a POSA would "know that the 'ligand-binding pocket' referred to in the claims of the '314 patent is inside the cell where ATP binds and where cysteine 773 resides, not outside the cell where it does not." *Id.* at 5.

The Court construes the term "ligand-binding pocket" to refer to the intracellular ligand-binding pocket of ATP. First, the Court recognizes that the "background" section of the specification includes a reference to "the ligand-binding pocket" in "the extracellular domain." '314 Patent at 2:14–15. But no one disputes that this description

of EGFR's general structure is correct; rather, the question before the court is whether a POSA would understand the "ligand-binding pocket" in the asserted claims to refer to *another* ligand-binding pocket in the intracellular domain. The specification's background reference to the extracellular "ligand-binding pocket" is therefore not dispositive.

Second, the Court disagrees with AstraZeneca that the cited prosecution history sheds light on the issue. The amendment—which added *both* the specific "773" identification and the "ligand-binding pocket" language—came in response to the patent officer's concern that the claims were preempted by prior art (Agus). In response, the applicants explained that the prior art at issue suggested that the "solution to the problem of gefitinib and/or erlotinib resistance in a patient is to simply overwhelm the patient by dosing any TKI at significantly higher doses than the recommended daily dosage or conventional dosage amounts." Winkler Decl., Ex. 30 at 7 ('314 Prosecution Hist., 12/27/2016 Am. and Resp.). In addition, the applicants explained that the prior art failed to "recognize—much less suggest—that the mechanism of action of the selected inhibitor is even a relevant factor in treating gefitinib and/or erlotinib resistant non-small cell lung cancer, nor that the specifically selected inhibitor achieve irreversibility by covalently binding to either the cysteine 773 residue or cysteine 805 residue in the ligand-binding pocket of EGFR or erb-B2, respectively." *Id.* at 8. This reveals only that the applicants sought to provide a more specific identification of how and where the irreversible EGFR inhibitors bind; it does not suggest anything about whether the ligand-binding pocket is extracellular or intracellular.

AstraZeneca is correct that the applicants stated that support for the amendment

could be found in "at least" paragraph 0005, which contains a general description of the structure of EGFR that refers to "the ligand-binding pocket" in the extracellular domain. But that paragraph also contains an explanation that EGFR is made up of "1186 amino acid residues" and a citation to an Ulrich 1984 study, which discloses the complete amino acid sequence of EGFR, including cysteine 773, and explains that the intracellular "cytoplasmic" domain contains "the stretch of the amino acid sequence (approximately residues 690–940)." Winchester Decl., Ex. 18 at PUMA-TAG00000547 (Ulrich 1984). This could just as plausibly be the "support" for the amendment's identification of cysteine 773's location that the applicants were referring to in their response and amendment. The Court therefore does not see how the prosecution history reduces any ambiguity in the claim language.

Finally, both parties have proffered expert testimony on whether a POSA would understand the claim language to refer to an extracellular or intracellular binding site. *Compare, e.g.*, Hausheer Decl., Ex. C ¶¶ 27, 51 ("Within the catalytic domain, EGFR has a binding site for ATP, also known as the ATP-binding pocket. The ATP binding pocket is also sometimes referred to as the ligand-binding pocket because ATP is a ligand that binds to the ATP-binding pocket in the catalytic domain" and "a POSA with a basic understanding of pharmacology, chemistry, and/or biochemistry readily knows that a ligand (Latin; *ligare* meaning 'to bind') means the binding action of the pharmacophore to its intended biologic target, and in this case the irreversible EGFR kinase inhibitor forms a ligand in the kinase domain in EGFR (not somewhere else on EGFR) and covalently binds to the cysteine 773 sulfur atom in the kinase domain of EGFR, thereby inhibiting the aberrant EGFR kinase activity.") *and* Weiss Decl., Ex. C

¶¶ 149, 151 ("[A] POSA would understand that the claim term is *not* referring to the ligand-binding pocket in the extracellular domain. The identification, by number, of the specific cysteine residue in the claim language, would leave no doubt in the POSA's mind that the claim term is referring to a cysteine that is present in the intracellular domain of EGFR" and "would also know that 'ligand binding pocket' in the context of these claims refers to the well-known ATP-binding pocket of EGFR. A 'ligand' for EGFR, in this context is ATP, *i.e.*, ATP, when bound to the ATP-binding pocket, is referred to as a ligand of EGFR.") *with* Reider Decl., Ex. B ¶¶ 136–37 ("The POSA would understand that the 'ligand-binding pocket' referred to in claim 1 of the '314 patent is in the extracellular domain of EGFR . . . . The specification cites to publications that further confirm that EGF is the 'ligand' for EGFR and the 'ligand-binding pocket' is in the extracellular domain of the EGR and is distinct from the intracellular kinase domain.") *and* Jänne Decl., Ex. A ¶¶ 46, 49 ("[T]he '314 Patent itself is clear that the ligand-binding pocket of EGFR is different from the tyrosine kinase domain (which contains the ATP-binding pocket) . . . . A [POSA] reading the patent would understand that the ligand-binding pocket referenced in the [claims] is part of the EGFR enzyme located outside the cell where the EGFR ligand . . . binds, and is not the tyrosine kinase domain inside the cell to which ATP binds.").

The Court notes that there is a key fact that appears to be undisputed by the parties: the asserted claims identify a specific amino acid—cysteine 773—that is *always* found in the intracellular region. Despite the centrality of this fact to Wyeth's arguments, AstraZeneca does not dispute in its brief that cysteine 773 is always located in the intracellular domain or that this is readily known by a POSA. AstraZeneca's

proposed construction would therefore require binding to cysteine 773 outside of the cell, which "is impossible" and would mean that "no compound could practice the invention." Pls.' Opp. to Mot. for Summ. Judgment on Non-Infringement at 4. The Federal Circuit has instructed that, with respect to ambiguous claim language, "where claim language permits an operable construction, the inoperable construction is wrong." *Power Integrations, Inc. v. Fairchild Semiconductor Int'l, Inc.*, 904 F.3d 965, 972 (Fed. Cir. 2018) (citing *Ecolab, Inc. v. FMC Corp.*, 569 F.3d 1335, 1345 (Fed. Cir. 2009)). In *Power Integrations*, for example, the Federal Circuit found that where the claim term was ambiguous but expert testimony demonstrated that one construction would be inoperable, the construction that rendered the claims operable was correct. *Id.* Here, where the location of cysteine 773 was apparently well-established before the patent application was filed and where experts have explained that an additional, intracellular ligand-binding pocket exists (the ATP-binding pocket), the Court is disinclined to adopt a construction that would require a POSA to ignore this existing knowledge and would render the invention inoperable.

The Court concludes that a POSA would understand the ligand-binding pocket referred to in the claims to be the ligand-binding pocket in the intracellular domain (namely, the ATP-binding pocket), where cysteine 773 is located, and not the ligand-binding pocket in the extracellular domain. The Court therefore construes the phrase "cysteine 773 residue in the ligand-binding pocket of EGFR" in the asserted claims as follows: cysteine 773 residue in the ATP-binding pocket of EGFR.

Because AstraZeneca's motion for summary judgment on non-infringement of the '314 patent is premised on a claim construction which the Court has declined to adopt,

the Court denies the motion. Any remaining questions regarding whether Tagrisso infringes the asserted claims of the '314 patent as constructed by the Court must be resolved by the jury.

2. '162 patent

AstraZeneca next asserts that there is no genuine dispute of fact that Tagrisso does not infringe the asserted claim of the '162 patent because Tagrisso does not bind "within the SEQ ID NO: 1 having a T790M mutation." The term "SEQ ID NO:1" refers to "the sequence of 1,210 amino acids making up EGFR ***without any mutations***," which is also known as "wild type" EGFR. Defs.' Mot. for Summ. Judgment on Non-Infringement at 8. AstraZeneca argues that the asserted claim only covers treatment with compounds that bind to wild type EGFR with a single mutation (the T790M mutation) and *with no other mutations*. It is undisputed, however, that "each of Tagrisso's approved indications requires that the patient's cancer have mutations in EGFR different from—or in the case of the second line indication, in addition to—the T790M mutation." *Id.* at 8. Specifically, Tagrisso requires that patients have sensitizing mutations. Because "[t]he Asserted Claim of the '162 patent, by contrast, plainly requires EGFR to have *only* a T790M mutation," AstraZeneca argues that it does not infringe the patent. *Id.*

In response, Wyeth argues that the claim's reference to "SEQ ID NO: 1 having a T790M mutation" "provides information about the *location* of particular mutation (T790M) in the chain; it does not preclude the existence of any other mutation." Pls.' Opp. to Mot. for Summ. Judgment on Non-infringement at 8. In brief, Wyeth argues that a POSA would understand that the entire method of treatment described in the patents

is aimed at patients with sensitizing mutations, and therefore would not read the claim in a manner which would exclude EGFR with sensitizing mutations.

Like AstraZeneca's previous non-infringement argument, the Court concludes that the parties' dispute on this issue hinges on the scope of the claim language (does it cover additional mutations?) and therefore necessitates further claim construction by the Court.

AstraZeneca argues that one of the embodiments in the specification is consistent with its interpretation of the claim. The specification states that "[i]n one embodiment, the subject's tumor ***does not harbor mutations indicative of gefitinib and/or erlotinib sensitivity*** and ***does harbor*** mutations indicative of gefitinib and/or erlotinib resistance, e.g., ***the T790M mutation*** in EGFR, e.g., increased EGFR internalization." Defs.' Mot. for Summ. Judgment on Non-infringement at 9 (quoting '162 Patent at 8:42–47) (emphasis by AstraZeneca). In addition, AstraZeneca argues that the prosecution history supports its construction of the claim because "[t]he applicants intentionally narrowed the claim of the '162 patent during prosecution to require the specific wild-type + T790M sequence." *Id*. at 9. Specifically, AstraZeneca asserts that the claim originally recited the phrase "having a T790M mutation in EGFR (SEQ ID NO: 1)" which the examiner rejected for indefiniteness noting that it suggests that "the ***broad recitation*** *is EGFR* and the claim also recites ***SEQ ID NO: 1 which is the narrower*** statement of the range/limitation." *Id.* (quoting Winkler Decl., Ex. 36 at 7 ('162 Pros. Hist., 06/27/2019 Non-Final Rejection)) (emphasis by AstraZeneca). The applicants then amended the claim "to clarify that the T790M mutation is with respect to SEQ ID NO: 1." Winkler Decl., Ex. 37 ('162 Pros. Hist., 10/28/2019 Am. & Resp.). AstraZeneca

thus argues that "the applicants fully considered whether to elect the broader term, 'EGFR' having a T790M mutation . . . but deliberately declined to do so." Defs.' Mot. for Summ. Judgment on Non-infringement at 10.

In response, Wyeth argues that the plain language of the claim "does not expressly exclude patients with sensitizing mutations, nor does it use the 'closed' language that a patentee might employ to signal that T790M must exist to the exclusion of all other mutations." Pls.' Opp. to Mot. for Summ. Judgment on Non-infringement at 8. Rather, "[a] POSA would understand that the reference sequence provides information about the location of a particular mutation (T790M) in the chain; it does not preclude the existence of any other mutation." *Id.*

Wyeth argues that the plain language of the claim would be reinforced by a POSA's knowledge of the art at the relevant time. Wyeth explains that prior scientific research cited in the patent and familiar to a POSA established that g/e should only be given to patients with at least one sensitizing mutation. Therefore, "a POSA would understand that the '162 patent claims a method of treating patients who are or have become resistant to treatment with gefitinib or erlotinib—two drugs only given to patients with at least one sensitizing mutation." *Id.* at 9. Therefore, a POSA would not understand the phrase "within the SEQ ID NO: 1 having a T790M mutation" as referring exclusively to wild-type EGFR with a single T790M mutation (and no other mutations).

Wyeth also emphasizes that the patent specification "includes numerous examples describing the use of irreversible EGFR inhibitors with NSCLC samples containing sensitizing mutations, including the H1975 cell line, which had both sensitizing mutations and the T790M resistance mutation." *Id.* In addition, Wyeth

points out that AstraZeneca focuses on a single embodiment in the claim, while ignoring two additional embodiments *with* sensitizing mutations.

Finally, Wyeth argues that AstraZeneca misstates the prosecution history of the amendment changing EGFR (SEQ ID NO: 1) to SEQ ID NO: 1. At the time of the examiner's rejection and the subsequent amendment, the specification incorrectly identified SEQ ID NO: 1 as the *nucleic acid* sequence, rather than the *amino acid sequence*. Wyeth's expert explains that a protein, such as EGFR, "can be encoded for by different nucleic acid sequences." Weiss Decl., Ex. C ¶ 37. Therefore, at the time of the examiner's objection, the claim recited "both EGFR generally (the broader limitation) and *a specific nucleic acid sequence* (the misidentified SEQ ID NO: 1)." Weiss Decl., Ex. C ¶ 38. The examiner further noted that it was unclear where the T790M mutation was located in the nucleic acid sequence. The applicants amended the specification to clarify the SEQ ID NO: 1 refers to the amino acid sequence of EGFR and to identify where the mutation occurs by referring to SEQ ID NO: 1 (the amino acid sequence) in the claim. Thus, Wyeth argues that, when read in context, the prosecution history reflects an attempt to identify the location of the T790M mutation and does *not* reflect any intention to limit the claims to *only* wild-type EGFR having a T790M mutation and no other mutations.

The Court declines to adopt AstraZeneca's construction of the claim. First, AstraZeneca's construction would require the Court to add an additional limitation that the compound covalently bind "within the SEQ ID NO: 1 having *only* a T790M mutation *and no other mutations*." But courts cannot "redraft claims." *Process Control Corp. v. HydReclaim Corp.*, 190 F.3d 1350, 1357 (Fed. Cir. 1999)

Second, the Court agrees with Wyeth that "[a] POSA would understand that the reference sequence provides information about the location of a particular mutation (T790M) in the chain; it does not preclude the existence of any other mutation." Pls. Opp. to Mot. for Summ. Judgment on Non-infringement at 8. The patent specification highlights that "[t]he inventors of the present invention have surprisingly discovered that irreversible EGFR inhibitors are effective in the treatment of cancer in subjects who are no longer responding to gefitinib and/or erlotinib therapies." '162 Patent at 3:48–51. At the time the patent application was filed, the scientific literature taught that "only those NSCLC patients who had at least one EGFR sensitizing mutation would respond to gefitinib or erlitinib"; therefore those drugs were "given only to patients with at least one sensitizing mutation." Pls.' Opp. to Mot. for Summ. Judgment on Non-infringement at 9. The Court must consider how a POSA would "read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification." *Phillips*, 415 F.3d at 1313. AstraZeneca's construction would mean that, despite the specification's indication, the patented method of treatment could not be used on a key patient population—those who had initiated treatment with g/e but developed resistance to those drugs.

Finally, the Court disagrees with AstraZeneca that the prosecution history clearly evidences the applicants' intent to narrow the scope of the claim to include only wild-type EGFR with a single T790M mutation to the exclusion of all other mutations. At best, given the competing explanations of the prosecution history, the evidence is ambiguous. AstraZeneca points to no reason, for example, *why* the applicants would be inclined to specifically disclaim critical embodiments and contradict the purpose of

the invention described in the specification. The examiner's objection (as AstraZeneca frames it) could have been resolved just as easily by amending the claim to include "EGFR having a T790M mutation" and excluding the parenthetical "SEQ ID NO: 1." That would leave unresolved, however, the examiner's concern about the failure to identify the location of T790M. Thus, AstraZeneca's reading of the prosecution history seems to raise more questions than it answers. In sum, the Court does not believe that the prosecution history evidences any intentional choice to limit the plain meaning of the claim in the manner that AstraZeneca now asserts. *See Home Diagnostics, Inc. v. LifeScan, Inc.*, 381 F.3d 1352, 1358 (Fed. Cir. 2004) ("Absent a clear disavowal or contrary definition in the specification or the prosecution history, the patentee is entitled to the full scope of its claim language.").

Because AstraZeneca's motion for summary judgment on non-infringement of the '162 patent is premised on a claim construction which the Court has declined to adopt, the Court denies the motion. Any remaining questions regarding whether Tagrisso infringes the asserted claim of the '162 patent as constructed by the Court must be resolved by the jury.

D. Pre-issuance damages

AstraZeneca argues that it is entitled to summary judgment on Wyeth's request for pre-issuance damages because these damages are unavailable for induced infringement (the only type of infringing conduct of which AstraZeneca is accused). The Court agrees. As a general rule, "patent owners may only collect damages for patent infringement that takes place during the term of the patent." *Rosebud LMS Inc. v. Adobe Sys. Inc.*, 812 F.3d 1070, 1073 (Fed. Cir. 2016). There is "a narrow exception to

that rule," codified at 35 U.S.C. § 154(d), that provides some "provisional rights" to patent owners while the patent application is pending. These provisional rights include "the right to obtain a reasonable royalty from any person who, during the period beginning on the date of publication of the application for such patent . . . and ending on the date the patent is issued":

> (A)(i) makes, uses, offers for sale, or sells in the United States the invention as claimed in the published patent application or imports such an invention into the United States; or
>
> (ii) if the invention as claimed in the published patent application is a process, uses, offers for sale, or sells in the United States or imports into the United States products made by that process as claimed in the published patent application[.]
>
> [. . .]

35 U.S.C. § 154(d)(1). Notably, the language of subsections (A)(i) and (A)(ii) parallels the language of 35 U.S.C. §§ 271(a) and 271(g), which define certain acts constituting infringement during the term of the patent. But section 154(d) includes *no* provision incorporating the language of section 271(b), which defines induced infringement during the term of a patent. The clear takeaway is that the statute does not authorize pre-issuance damages for induced infringement.

Wyeth does not dispute that AstraZeneca's alleged conduct falls outside the scope of sections 154(d)(1)(A)(i) and (ii). Rather, it argues that because section 271(b) states that "[w]hoever actively induces infringement of a patent shall be liable as an infringer," there is no need for section 154(d) to "expressly mention *induced* infringement." Pls.' Opp. to Mot. for Summ. Judgment on Non-infringement at 11. The Court disagrees, for two reasons. First, Wyeth's interpretation is contrary to the plain

text of section 271(b), which covers induced infringement "*of a patent.*" (emphasis added). By definition, pre-issuance damages occur before any patent exists. Therefore, section 271(b) does not permit recovery. Second, Wyeth's interpretation is contrary to section 154(d). Section 154(d) does not extend *all* post-issuance patent rights to the pre-issuance period; rather, it is a "narrow exception" that permits recovery only for certain conduct that is specifically enumerated in the statute. *Rosebud LMS Inc.*, 812 F.3d at 1073. Because AstraZeneca is only accused of induced infringement, it cannot be liable for pre-issuance damages as a matter of law.

E. Advice-of-counsel defense

Wyeth has moved for partial summary judgment on AstraZeneca's advice-of-counsel defense to Wyeth's willful infringement claim. Wyeth asserts that AstraZeneca cannot argue that it relied on advice of its counsel regarding whether Tagrisso infringed the patents-in-suit because there is no evidence that such advice was communicated to "business decisionmakers with the authority to decide whether to go forward with potentially infringing acts." Pls.' Mot. for Summ. Judgment on Advice-of-Counsel Defense at 1. AstraZeneca argues that the Court is not authorized to resolve this issue at the summary judgment stage because "[a]n accused infringer's reliance on advice of counsel is not a standalone issue . . . [but] instead one consideration among the 'totality of circumstances' bearing on alleged 'culpability' and the ultimate question of willfulness." Defs.' Opp. to Mot. for Summ. Judgment on Advice-of-Counsel Defense at 1 (quoting *Acumed LLC v. Stryker Corp.*, 483 F.3d 800, 811 (Fed. Cir. 2007)). AstraZeneca argues in the alternative that the evidence supports its advice-of-counsel defense.

In *Halo Electronics, Inc. v. Pulse Electronics, Inc.*, 579 U.S. 93 (2016), the Supreme Court clarified that the standard for "willful" infringement is a subjective standard that depends on the infringer's state of mind at the time it infringed. *Id.* at 105. "[A]n accused infringer's reliance on an opinion of counsel regarding noninfringement or invalidity of the asserted patent" is "relevant to the infringer's state of mind." *Sunoco Partners Mktg. & Terminals L.P. v. U.S. Venture, Inc.*, 32 F.4th 1161, 1178 (Fed. Cir. 2022) (quoting *Omega Pats., LLC v. CalAmp Corp.*, 920 F.3d 1337, 1353 (Fed. Cir. 2019)). By presenting an advice-of-counsel defense, "an accused willful infringer aims to establish that due to reasonable reliance on advice from counsel, its continued accused activities were done in good faith." *In re Seagate Tech., LLC*, 497 F.3d 1360, 1369 (Fed. Cir. 2007), *abrogated on other grounds by Halo Elecs., Inc.*, 579 U.S. 93. "Typically, counsel's opinion concludes that the patent is invalid, unenforceable, and/or not infringed." *Id.* "Although an infringer's reliance on favorable advice of counsel, or conversely his failure to proffer any favorable advice, is not dispositive of the willfulness inquiry, it is crucial to the analysis." *Id.*

As an initial matter, the Court concludes that this issue is appropriate for summary judgment. Rule 56(a) permits parties to seek summary judgment on "part" of a "claim or defense." *See* Fed. R. Civ. P. 56(a) ("A party may move for summary judgment, identifying each claim or defense—*or the part of each claim or defense*—on which summary judgment is sought.") (emphasis added). If no reasonable jury could conclude based on the record that AstraZeneca relied on the advice of counsel with respect to its alleged infringement of the patents-in-suit, then the Court has the authority to grant summary judgment on that issue in favor of Wyeth. Although this would not

resolve whether AstraZeneca's infringement was willful, it would resolve "part" of AstraZeneca's defense to that claim. The Court concludes that Wyeth's motion is proper and thus will address its merits.

AstraZeneca asserts that it relied on opinions from outside counsel, Robert Armitage, concerning the validity of the patents-in-suit. The parties refer to these opinions as the Armitage Opinions. It is undisputed that "the only persons at AstraZeneca with whom [Armitage] discussed the Armitage Opinions were in-house attorneys," specifically, Dr. Scott Alban (Senior Vice President for Global Intellectual Property), Dr. Shannon Carroll (Assistant General Counsel, IP Oncology), and Christin Sullivan-Miller (Senior Counsel, Oncology). Pls.' Stmt. of Material Facts on Advice-of-Counsel Defense ¶ 12.[5]

AstraZeneca designated Dr. Alban as its Rule 30(b)(6) witness and "confirmed that Dr. Alban was prepared to testify about how any opinion of counsel . . . made its way to a decisionmaker at AstraZeneca." Pls.' Stmt. of Material Facts on Advice-of-Counsel Defense ¶ 32. During the deposition, the following exchange took place between the plaintiffs' counsel and Alban:

> Q: So with respect to the continued marketing of Tagrisso by AstraZeneca, who has the authority to – at AstraZeneca to say, you need to stop marketing Tagrisso, or will you just continue marketing Tagrisso?
>
> A: That would probably be a board level decision.

Winchester Decl., Ex. 12 at 76:22–77:4 (Alban Dep.). Dr. Alban also testified during the deposition that he discussed the Armitage Opinions with only two other people (besides

[5] There appears to be some confusion between the parties over the exact job titles of Dr. Alban, Dr. Carroll, and Sullivan-Miller, but any dispute is immaterial to the present motion.

Dr. Carroll and Sullivan-Miller): Jeffrey Pott, General Counsel at AstraZeneca, and Benjamin McDonald, in-house counsel at AstraZeneca. Dr. Alban stated that neither Pott nor McDonald were on AstraZeneca's Board of Directors.

After Dr. Alban's deposition, Wyeth served subpoenas for the depositions of Sir Pascal Soriot (the CEO of AstraZeneca), Dr. Alban, McDonald, Pott, and Sullivan-Miller. AstraZeneca filed a motion for a protective order to prevent the depositions. In granting the motion, the Court stated that AstraZeneca would not be able to rely on the testimony of the employees whose depositions it declined. *See* Pls.' Stmt. of Material Facts on Advice-of-Counsel Defense ¶ 37 ("That's the price you pay by preventing their depositions. They won't be able to be witnesses. That means an affidavit on a summary judgment motion, an affidavit in response to a summary judgment motion, a witness at a trial or other hearing, full stop.") (internal citation omitted).

Wyeth now argues that, because Dr. Alban stated that only the Board could decide whether to continue selling Tagrisso, and because there is no evidence that the Armitage Opinions or in-house counsel's conclusions from those opinions were communicated to members of the Board of Directors, AstraZeneca's advice-of-counsel defense fails as a matter of law. AstraZeneca responds that Alban, Pott, and McDonald—not the Board—were the relevant decisionmakers, and that there is evidence from Dr. Alban's 30(b)(6) deposition and from e-mail communications that Dr. Alban, Pott, and McDonald relied on the advice from the Armitage Opinions in deciding not to license the patents-in-suit. Wyeth counters that in-house lawyers categorically cannot be considered "business decisionmakers" for purposes of the advice-of-counsel defense, and that, at any rate, it is undisputed that Alban, Pott, and McDonald lacked

the authority to pull Tagrisso from the market.

Neither the law nor the evidence supports Wyeth's contention that only AstraZeneca's Board of Directors can be considered the relevant "decisionmaker" that must rely directly on the Armitage Opinions for purposes of the advice-of-counsel defense. First, Wyeth cites no support for its categorical rule that "[a]dvice of counsel cannot provide a defense to a charge of willful infringement if it starts and ends in the legal group." Pls.' Mot. for Summ. Judgment on Advice-of-Counsel Defense at 2. Although the Court agrees with the straightforward proposition that the legal advice at issue must have been "communicated to the defendant's decisionmakers," *Omega Pats., LLC*, 920 F.3d at 1353, there is nothing that prevents a business from delegating some decision-making authority to employees who happen to be lawyers (as AstraZeneca asserts that it does). Courts examining this issue have focused on the authority of the employees relying on (or ignoring) the advice of counsel, not on their job titles. *See, e.g.*, *Harris Corp. v. Ericsson Inc.*, 417 F.3d 1241, 1259 (Fed. Cir. 2005) (upholding a jury's finding of willfulness because the defendant "could not confirm that any executive with decision-making authority in [the defendant's] marketing or engineering departments had ever relied on the opinion" *or* "whether the known recipients of the opinion, *two in-house attorneys, had any product or marketing responsibility*" (emphasis added)); (overturning jury's finding of willfulness in part because the defendant presented evidence that it had commissioned an opinion from outside counsel and the defendant's "head of U.S. Patent Litigation" testified that "he relied on the opinion letter to conclude that [the defendant] had a legitimate invalidity defense"); *Chiron Corp. v. Genentech, Inc.*, 268 F. Supp. 2d 1117, 1121 (E.D. Cal.

2002) (noting that the actor "ultimately responsible for deciding not to license [the plaintiff's] patent" was the defendant's "Executive Committee," which included the defendant's "general counsel").

Second, Dr. Alban's testimony that only the Board of Directors held the power to decide whether to pull Tagrisso from the market does not establish that the Board is the only relevant decisionmaker for purposes of the willfulness inquiry. As AstraZeneca explains, pulling Tagrisso—a highly successful drug—from the market was likely not a plausible option. There is evidence that AstraZeneca considered but declined to license the patents-in-suit, which likely would have been a more realistic option for responding to the alleged infringement. Although Dr. Alban's 30(b)(6) testimony establishes that only the Board had the authority to pull Tagrisso from the market, it does not establish that only the Board had authority to make the kind of IP licensing decision at issue here.

The Court notes that, given AstraZeneca's refusal to permit Wyeth to depose Pott, McDonald, and Alban in his personal capacity, there may be lingering evidentiary and sufficiency issues with AstraZeneca's apparent intention to argue now that those actors were, in fact, the decisionmakers whose subjective reliance on the Armitage Opinions is the crux of its defense. But because Wyeth has not pressed this issue in its summary judgment motion, the Court will not consider whether summary judgment is warranted on these grounds.

F. Motions to exclude expert testimony

"Pursuant to *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 113 S. Ct. 2786, 125 L.Ed.2d 469 (1993), district courts perform a gatekeeping function to ensure that expert testimony meets the requirements of Federal Rule of Evidence 702."

Karlo v. Pittsburgh Glass Works, LLC, 849 F.3d 61, 80 (3d Cir. 2017). "Rule 702 embodies three distinct substantive restrictions on the admission of expert testimony: qualifications, reliability, and fit." *Id.* (quoting *Elcock v. Kmart Corp.*, 233 F.3d 734, 741 (3d Cir. 2000)).

First, to satisfy the "qualifications" requirement, the witness must "possess specialized expertise." *Pineda v. Ford Motor Co.*, 520 F.3d 237, 244 (3d Cir. 2008) (quoting *Scheider ex rel. Schneider v. Fried*, 320 F.3d 396, 404 (3d Cir. 2003)). The Third Circuit "interpret[s] Rule 702's qualification requirement liberally." *Id.* Accordingly, a "broad range of knowledge, skills, and training qualify an expert"; the expert need not be the "best qualified" or "have the specialization that the court considers most appropriate" to satisfy Rule 702. *Id.* (quoting *Holybrook v. Lykes Bros. S.S. Co.*, 80 F.3d 777, 782 (3d Cir. 1996)).

Second, to satisfy the "reliability" requirement, the expert testimony "be based on the methods and procedures of science, not on subjective belief and unsupported speculation." *In re TMI Litig.*, 193 F.3d 613, 663 (3d Cir. 1999). "The standard for reliability is 'not that high' [and] is 'lower that the merits standard of correctness.'" *Karlo*, 849 F.3d at 81 (quoting *In re TMI Litig.*, 193 F.3d at 665). The question is whether "the expert's testimony is supported by 'good grounds.'" *Id.* (quoting *In re TMI Litig.*, 193 F.3d at 665). "Where there is a logical basis for an expert's opinion testimony, the credibility and weight of that testimony is to be determined by the jury, not the trial judge." *Leonard v. Stemtech Int'l Inc.*, 834 F.3d 376, 391 (3d Cir. 2016) (quoting *Breidor v. Sears, Roebuck & Co.*, 772 F.2d 1134, 1138–39 (3d Cir. 1983)).

Finally, the "fit" requirement "goes primarily to relevance" and "ensures that the

evidence or testimony '[helps] the trier of fact to understand the evidence or to determine a fact in issue.'" *Karlo*, 849 F.3d at 81 (quoting *In re TMI Litig.*, 193 F.3d at 663).

1. Mulhern and Dr. Bivona

Wyeth first argues that the Court should exclude the opinions of AstraZeneca's damages expert, Carla Mulhern. Mulhern's damages opinion relies on her statistical analysis of patient health data compiled from Flatiron, a health technology company that "maintains a database of information abstracted from electronic medical records," including records from oncology patients. Defs.' Opp. to Mot. to Exclude Test. of Mulhern & Bivona at 2. Mulhern used this data to calculate the percentage of certain NSCLC patients with a T790M mutation. (Wyeth asserts that the prevalence of this mutation is relevant to distinguishing infringing from non-infringing uses of Tagrisso.) Mulhern calculated that the rate of pretreatment T790M recorded in the Flatiron data was approximately 3.8%. This is significantly lower than the 35% estimate offered by Wyeth's expert, Dr. Alice Berger.

Wyeth first argues that Mulhern's opinion must be excluded because she is "an economist with no scientific or medical training" and therefore is not qualified to opine about the prevalence of T790M. Pls.' Mot. to Exclude Test. of Mulhern & Bivona at 1. The Court disagrees. The scope of Mulhern's report and testimony with respect to T790M prevalence is clear: she conducted a statistical analysis of the Flatiron patient data. That is squarely in her wheelhouse as an experienced economist. Contrary to Wyeth's argument, Mulhern need not be a "clinician, genomics researcher, or an epidemiologist" merely because the data she is analyzing relates to genetic cancer

testing. *Id.* at 6. Mulhern is not personally testing patients for T790M, opining on the sensitivity or accuracy of such testing, or otherwise offering medical or scientific opinions about the substance of the data. The fact that Wyeth believes that Dr. Berger's expertise as a cancer researcher makes her opinion on T790M prevalence based on scientific studies more *persuasive* than Mulhern's statistical analysis of real-world patient data does not mean that Mulhern is not qualified to conduct such an analysis or that her opinion is unreliable. There is no requirement that an expert be the "best qualified," *Pineda*, 520 F.3d at 244, or that an expert's opinion be based on the "best foundation" or the "best methodology" to be admissible. *Karlo*, 849 F.3d at 81. Apart from questioning the reliability of the Flatiron data (which the Court addresses next), AstraZeneca offers no reason to doubt Mulhern's ability to reliably calculate the prevalence of T790M mutation in that data.

Wyeth next argues that Mulhern's testimony should be excluded because the Flatiron data is unreliable. Wyeth asserts that Mulhern does not know, for example, if AstraZeneca "processed or otherwise manipulated the data before it was given to Ms. Mulhern," "whether the Flatiron data is representative of the population of Tagrisso patients," or "whether the T790M mutation status was recorded in all of the underlying electronic health records that she used," among other potential flaws with the raw data. Pls.' Mot. to Exclude Test. of Mulhern & Bivona at 11. Along the same lines, Wyeth argues that Mulhern makes too many "unwarranted assumptions" regarding the Flatiron data because she assumes that the Flatiron data is representative of the relevant patient population, that all patients in the dataset were tested for T790M, and that the test results were reliably recorded in the dataset. *Id.* at 14.

The Court disagrees that these potential flaws with the Flatiron data justify the exclusion of Mulhern's opinion as unreliable. Generally, when the data supporting an expert's opinion is called into question, courts need only "assess whether there are good grounds to rely on this data to draw the conclusion reached by the expert." *In re Paoli R.R. Yard PCB Litig.*, 35 F.3d 717, 749 (3d Cir. 1994); *see also In re Zoloft Prod. Liab. Litig.*, 858 F.3d 787, 792–93 (3d Cir. 2017) ("A court should not, however, usurp the role of the fact-finder; instead, an expert should only be excluded if the flaw is large enough that the expert lacks the good grounds for his or her conclusions." (internal quotations and citations omitted)). Here, there is evidence that the Flatiron data contains a large sample size from a demographically and geographically diverse population and that this data is used by pharmaceutical companies and researchers. In addition, there is evidence that the Flatiron data was "specifically designed to permit analysis of the biologic makeup—including the T790M status—of Tagrisso patients in a comprehensive way." Defs.' Opp. to Mot. to Exclude Test. of Mulhern & Bivona at 11. The Court concludes that this is sufficient to establish that Mulhern had "good grounds" to rely on the Flatiron data to estimate the prevalence of T790M mutations in Tagrisso patients. Wyeth's critiques of the limitations of the Flatiron data are more appropriately explored on cross-examination. *See Daubert*, 509 U.S. at 596 ("Vigorous cross-examination, presentation of contrary evidence, and careful instruction on the burden of proof are the traditional and appropriate means of attacking shaky but admissible evidence.").

Wyeth also suggests that AstraZeneca failed to timely produce the Flatiron data during discovery. According to Wyeth, "[a]t no point during the fact discovery period did

AstraZeneca produce Flatiron data or suggest that historical Flatiron data was appropriate for determining the prevalence of de novo T790M" and "[t]he first time Flatiron data was used in this case was in Ms. Mulhern's rebuttal report." *Id.* at 14. To the extent that Wyeth is requesting that the Court exclude Mulhern's testimony as a sanction under Rule 37(c) for a discovery violation, the Court declines to do so because Wyeth has not explained whether and how it was prejudiced by the late disclosure. As AstraZeneca points out, there is no dispute that Wyeth eventually received the raw Flatiron data and that Wyeth's expert examined that data following Mulhern's report and arrived at "nearly identical calculations" regarding the T790M rate in the patient records. Defs.' Opp. to Mot. to Exclude Test. of Mulhern & Bivona at 17. The Court therefore concludes that any violation of Rule was harmless. *See* Fed. R. Civ. P. 37(c)(1) ("If a party fails to provide information . . . as required by Rule 26(a) or (e), the party is not allowed to use that information . . . unless that failure was substantially justified or is harmless.").

Finally, Wyeth argues that Dr. Bivona's opinion should be excluded because he relies on Mulhern's data analysis. Because the Court has concluded that Mulhern's testimony is admissible and Wyeth offers no independent rationale for exclusion, the Court declines to exclude Dr. Bivona's testimony.

2. Dr. Jänne and Mulhern

Wyeth argues that the Court should exclude Dr. Jänne's testimony because he "has no experience or specialized knowledge regarding economics, marketing, or licensing and thus lacks the relevant expertise" to offer opinions regarding Tagrisso's commercial success, commercial acquiescence to the patents-in-suit, and market

competition between Pfizer's Vizimpro product and Tagrisso. Pls.' Mot. to Exclude Test. of Jänne & Mulhern at 1. Specifically, Wyeth takes issue with three of Dr. Jänne's conclusions: (1) Tagrisso's sales are not driven by the patented features, (2) the industry did not acquiesce that the patents-in-suit have value, and (3) Vizimpro does not compete with Tagrisso.

First, Wyeth asserts that Dr. Jänne is not qualified to opine that Tagrisso's sales are not driven by patented features because he is not an economics or marketing expert. Wyeth also argues that Dr. Jänne impermissibly relies on "his own personal prescribing practices" that are not generalizable to other physicians. *Id.* at 4–5.

The Court disagrees with this characterization of Dr. Jänne's opinion. Dr. Jänne's testimony is largely focused on explaining the differences between the method of treatment claimed by the patents-in-suit and the uses, effects, and advantages of Tagrisso. For example, he explains that Tagrisso is prescribed to "multiple patient populations (not just T790M+ patients)" because patients tend to tolerate it far better than other drugs. Defs.' Opp. to Mot. to Exclude Test. of Jänne & Mulhern at 8. As an expert in the clinical treatment of NSCLC and the development of drugs to treat NSCLC, Dr. Jänne clearly is qualified to explain the clinical attributes of Tagrisso that lead oncologists to prescribe the drug. This type of testimony requires precisely Dr. Jänne's expertise—not knowledge of economic or marketing strategies.

Second, Wyeth argues that Dr. Jänne should not be permitted to offer a rebuttal opinion that licensing activity demonstrates disregard for the patents-in-suit "because he is not an expert in patent licensing and he has never been involved in negotiation of a patent license." Pls.' Mot. to Exclude Test. of Jänne & Mulhern at 6. Again, however,

Dr. Jänne's rebuttal opinion is based on his extensive experience in the field of NSCLC treatment research and drug development. Wyeth's expert, Dr. Rao, asserts that there is evidence of commercial acquiescence to the non-obviousness of the patents-in-suit; Dr. Jänne counters that "the groups and companies developing [ ] irreversible inhibitors did not seek access to the technology within the patents-in-suit through a license," which "contradicts Dr. Rao's opinions that the licensing activity amongst the Plaintiffs demonstrates that the patents-in-suit are valuable or non-obvious." Defs.' Opp. to Mot. to Exclude Test. of Jänne & Mulhern at 13. Dr. Jänne's specialized knowledge regarding the research groups, companies, and institutions operating in this specific area qualifies him to opine on whether and how these groups' lack of licensing activity reflects on the industry's view of the value of the patents-in-suit. This is underscored by his identification and evaluation of specific entities and clinical trials that have undertaken relevant work in the area of irreversible EGFR inhibitors.

Wyeth emphasizes that Dr. Jänne fails to explain whether the entities and clinical trials that he identifies "needed" licenses to the patents-in-suit. But this argument begs the question. The Court understands the thrust of Dr. Jänne's testimony to be that, if the patents were as valuable as Wyeth claims, these are precisely the groups that one would expect to license them. In other words, whether the groups "needed" licenses would depend at least in part on whether the groups viewed the patents-in-suit as valuable. At any rate, the fact that Wyeth may be able to advance an alternative explanation for these actors' behavior for reasons unrelated to the value of (or the commercial acquiescence to) the patents-in-suit does not render inadmissible Dr. Jänne's testimony to the contrary.

Third, Wyeth argues that Dr. Jänne lacks the necessary economic/marketing expertise to opine whether Vizimpro is a "competitor" to Tagrisso. In addition, Wyeth argues that Dr. Jänne based his opinion only on his personal prescribing preferences. Pls.' Mot. to Exclude Test. of Jänne & Mulhern at 7. Again, however, the Court disagrees with Wyeth's characterization of Dr. Jänne's testimony. Dr. Jänne does not purport to offer an *economic* opinion on the issue. Nor does he rely solely on his personal prescribing preferences as a physician. Rather, his opinion compares Vizimpro and Tagrisso from a medical and scientific perspective. For example, Dr. Jänne compares the approved indications of each drug, the presence/absence of each drug in clinical practice guidelines, and the published research regarding the effectiveness, resistance, and other aspects of the drugs. Thus, Dr. Jänne properly relies on his technical expertise as an oncologist-researcher specializing in the treatment of NSCLC to rebut Wyeth's experts' testimony regarding the similarity of the drugs. In sum, the Court concludes that Dr. Jänne is qualified to offer his technical opinion on the nexus between the commercial success of Tagrisso and the patents-in-suit, commercial acquiescence to the patents-in-suit, and market competition between Tagrisso and Vizimpro.

With respect to Mulhern, Wyeth mainly argues that her opinions should be excluded because she relies on Dr. Jänne's opinions. Because the Court has concluded that Dr. Jänne's opinions are admissible, the Court will not exclude Mulhern's opinions for that reason. In addition, Wyeth argues that Mulhern (like Dr. Jänne) "opines that third-party development of irreversible EGFR inhibitors by others without a license undermines [Wyeth's] expert's opinions on commercial acquiescence," but "fails

to consider whether those third-parties even needed a license." Pls.' Mot. to Exclude Test. of Jänne & Mulhern at 2. The Court declines to exclude Mulhern's opinion on that basis for the same reasons already discussed.

3. Dr. Rao

AstraZeneca argues that the Court should exclude the opinion of Wyeth's damages expert, Dr. Mohan Rao, regarding a reasonable royalty rate because he relies on six licenses of patents that he has not shown are comparable to the patents-in-suit. In AstraZeneca's view, Dr. Rao "failed to offer any analysis . . . showing that the patents exchanged in any of those licenses are technically comparable to the patents at issue" in this case. Defs.' Mot. to Exclude Test. of Rao at 2. Wyeth responds that Dr. Rao has adequately established the comparability of the licenses and accounted for technological and economic differences between the comparator licenses and the hypothetical license of the patents-in-suit.

"The party proffering a license bears the burden of establishing it is sufficiently comparable to support a proposed damages award." *Adasa Inc. v. Avery Dennison Corp.*, 55 F.4th 900, 915 (Fed. Cir. 2022). This means that "the proponent 'must account for differences in the technologies and economic circumstances of the contracting parties.'" *Id.* (quoting *Finjan, Inc. v. Secure Computing Corp.*, 626 F.3d 1197, 1211 (Fed. Cir. 2010)). But the Federal Circuit has held "that the issue of comparability is often one of sufficiency of the evidence, not admissibility." *Bio-Rad Lab'ys, Inc. v. 10X Genomics Inc.*, 967 F.3d 1353, 1373 (Fed. Cir. 2020). "[T]he fact that a license is not perfectly analogous generally goes to the weight of the evidence, not its admissibility." *Id.* (quoting *Ericsson, Inc. v. D-Link Sys., Inc.*, 773 F.3d 1201,

1227 (Fed. Cir. 2014)). As a result, "the 'degree of comparability' of the license agreements is a 'factual issue[ ] best addressed by cross examination and not by exclusion.'" *Id.* (quoting *ActiveVideo Networks, Inc. v. Verizon Commc'ns, Inc.*, 694 F.3d 1312, 1333 (Fed. Cir. 2012)). Expert testimony on reasonable royalty rates based on allegedly comparable licenses generally is admissible so long as the expert has made "a showing of 'baseline comparability.'" *Id.* at 1374.

The Court concludes that Dr. Rao has established the "baseline comparability" of the six licenses at issue and the patents-in-suit. To identify comparable licenses, Dr. Rao relied on BioSciDB, "a comprehensive, peer-reviewed reference database on biopharma alliances consisting of more than 11,000 contracts." Pls.' Opp. to Mot. to Exclude Test. of Rao at 2. The licenses Dr. Rao analyzed (1) "all involved running royalties to sell products to treat lung cancer patients," (2) were entered into within ten years of the hypothetical negotiation between the parties, and (3) involved technology "at the same or similar stage of development as Tagrisso." Pls.' Opp. to Mot. to Exclude Test. of Rao at 2. Moreover, Dr. Rao explained in his report and deposition the various adjustments he made to account for the different circumstances of each license, such as whether the licenses were exclusive, entered into under threat of litigation, contained redacted terms, or included potentially more valuable patents such as compound patents. This is sufficient to establish "baseline comparability" between the licenses for purposes of the admissibility of Dr. Rao's testimony. Accordingly, AstraZeneca's critiques regarding the alleged differences between the BioSciDB licenses and patents-in-suit are more appropriate for cross-examination or to argue that the evidence is insufficient to support Wyeth's reasonable royalty calculation.

AstraZeneca also argues that the Court should exclude Dr. Rao's testimony because the BioSciDB licenses appeared for the first time in his opening expert report and were not disclosed during fact discovery. But the Court does not see, and AstraZeneca does not explain, how it has been prejudiced by the late disclosure (which, at any rate, was eight months before trial). AstraZeneca briefly states in its reply that the late disclosure "prevent[ed] discovery into those licenses." Defs.' Reply to Mot. to Exclude Test. of Rao at 1. It is unclear, however, and AstraZeneca does not explain, what additional discovery it needed given that the licenses were obtainable from a publicly available database. The Court therefore concludes that any late disclosure was harmless. *See* Fed. R. Civ. P. 37(c)(1) ("If a party fails to provide information . . . as required by Rule 26(a) or (e), the party is not allowed to use that information . . . unless that failure was substantially justified or is harmless.").

Conclusion

The Court grants AstraZeneca's motion to dismiss [dkt. 268] with respect to Puma Biotechnology but denies the motion to dismiss with respect to Wyeth. The Court denies AstraZeneca's motion for summary judgment on invalidity [dkt. 270]. The Court denies AstraZeneca's motion for summary judgment on non-infringement [dkt. 273], except with respect to the issue of pre-issuance damages, on which the Court rules in favor of AstraZeneca. The Court denies Wyeth's motion for summary judgment on AstraZeneca's advice-of-counsel defense [dkt. 288]. Finally, the Court denies Wyeth's motions to exclude the testimony of Mulhern, Dr. Bivona, and Dr. Jänne, [dkt. 277 & dkt. 280] and denies AstraZeneca's motion to exclude the testimony of Dr. Rao [dkt. 276]. The case is set for a telephonic status hearing on March 22, 2024 at 8:30

a.m. The following call-in number will be used: 888-684-8852, access code 746-1053. Trial counsel should participate in the hearing. Prior to that date, the parties are to confer regarding the motion to limit prior art defenses to attempt to agree upon appropriate limitations.

MATTHEW F. KENNELLY
United States District Judge

Date: March 18, 2024

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

WYETH LLC, Plaintiff, v. ASTRAZENECA PHARMACEUTICALS LP and ASTRAZENECA AB, Defendants.	)	C.A. No. 21-1338-MFK

VERDICT FORM

INSTRUCTIONS:

When answering the following questions and filling out this Verdict Form, please follow the directions provided throughout this Verdict Form. Your answer to each question must be unanimous. Some of the questions contain legal terms that are defined and explained in detail in the Jury Instructions. Please refer to the Jury Instructions if you are unsure about the meaning or usage of any legal term that appears in the questions below.

As used in this Verdict Form:

1. "Wyeth" refers to Plaintiff Wyeth LLC.
2. "AstraZeneca" refers collectively to Defendants AstraZeneca Pharmaceuticals LP and AstraZeneca AB.
3. The "'314 patent" refers to U.S. Patent No. 10,603,314.
4. The "'162 patent" refers to U.S. Patent No. 10,596,162.

Please proceed to the next page.

We, the jury, unanimously agree to the answers to the following questions and return them under the instructions of the Court as our verdict in this case.

Please proceed to Issue No. 1. (Please note that text in bold in this Jury Verdict form provides instructions to guide you through the Jury Verdict form.)

ISSUE NO. 1: INFRINGEMENT

Did Wyeth prove by a preponderance of the evidence that AstraZeneca induces infringement of any of the claims of the '314 patent and '162 patent for the indications of Tagrisso® listed below?

Please check "Yes" or "No" for each claim. "Yes" is in favor of Wyeth, and "No" is in favor of AstraZeneca.

Second-Line

	YES (for Wyeth)	NO (for AstraZeneca)
Claim 1 of the '314 patent	✓	
Claim 3 of the '314 patent	✓	
Claim 9 of the '314 patent	✓	
Claim 1 of the '162 patent	✓	

First-Line and Adjuvant

	YES (for Wyeth)	NO (for AstraZeneca)
Claim 1 of the '314 patent	✓	
Claim 3 of the '314 patent	✓	
Claim 9 of the '314 patent	✓	
Claim 1 of the '162 patent	✓	

If you answered "Yes" as to any Claim, please proceed to Issue No. 2. Otherwise, skip Issue No. 2 and proceed to Issue No. 3.

ISSUE NO. 2: WILLFULNESS

If you answered "Yes" that AstraZeneca has induced infringement of one or more of the listed claims in Issue No. 1, then did Wyeth prove by a preponderance of the evidence that AstraZeneca willfully infringed the '314 patent and/or '162 patent?

Yes: _____ (for Wyeth)

No: ✓ (for AstraZeneca)

Please proceed to Issue No. 3.

ISSUE NO. 3: INVALIDITY—LACK OF ENABLEMENT

Did AstraZeneca prove by clear and convincing evidence that any of the claims of the '314 and '162 patents below is invalid for lack of enablement?

Please check "Yes" or "No" for each claim. "Yes" is in favor of AstraZeneca, and "No" is in favor of Wyeth.

	Yes (for AstraZeneca)	No (for Wyeth)
Claim 1 of the '314 patent	_____	✓
Claim 3 of the '314 patent	_____	✓
Claim 9 of the '314 patent	_____	✓
Claim 1 of the '162 patent	_____	✓

Please proceed to Issue No. 4.

ISSUE NO. 4: INVALIDITY—LACK OF WRITTEN DESCRIPTION

Did AstraZeneca prove by clear and convincing evidence that any of the claims of the '314 and '162 patents listed below is invalid for a lack of written description?

Please check "Yes" or "No" for each claim. "Yes" is in favor of AstraZeneca, and "No" is in favor of Wyeth.

	Yes (for AstraZeneca)	No (for Wyeth)
Claim 1 of the '314 patent	_____	✓
Claim 3 of the '314 patent	_____	✓
Claim 9 of the '314 patent	_____	✓
Claim 1 of the '162 patent	_____	✓

Please proceed to Issue No. 5.

ISSUE NO. 5: INVALIDITY—ANTICIPATION

Did AstraZeneca prove by clear and convincing evidence that any of the claims of the '314 patent listed below is invalid as anticipated?

Please check "Yes" or "No" for each claim. "Yes" is in favor of AstraZeneca, and "No" is in favor of Wyeth.

	Yes (for AstraZeneca)	No (for Wyeth)
Claim 1 of the '314 patent	_____	✓
Claim 3 of the '314 patent	_____	✓
Claim 9 of the '314 patent	_____	✓

Please proceed to Issue No. 6.

ISSUE NO. 6: INVALIDITY—OBVIOUSNESS

Did AstraZeneca prove by clear and convincing evidence that any of the claims of the '314 and '162 patents listed below is invalid as obvious?

Please check "Yes" or "No" for each claim. "Yes" is in favor of AstraZeneca, and "No" is in favor of Wyeth.

	Yes (for AstraZeneca)	No (for Wyeth)
Claim 1 of the '314 patent	_____	✓
Claim 3 of the '314 patent	_____	✓
Claim 9 of the '314 patent	_____	✓
Claim 1 of the '162 patent	_____	✓

Please proceed to the next page.

If you checked "Yes" for any claims in Issue No. 1, and checked "No" for those same claims in Issues Nos. 3, 4, 5, and 6, then please proceed to Issue No. 7 on the next page.

In other words, please proceed to Issue No. 7 if you found that AstraZeneca infringed at least one claim in Issue No. 1 that you found is not invalid in Issue Nos. 3, 4, 5, and 6.

If you found that AstraZeneca did not infringe any valid claims, please proceed directly to the Final Page of the Verdict form and sign and date that page.

ISSUE NO. 7: DAMAGES—REASONABLE ROYALTY

What is the total amount of reasonable royalty damages that Wyeth has proven by a preponderance of the evidence would compensate Wyeth for AstraZeneca's past infringement through December 31, 2023?

Amount: $ 107,500,000

Please proceed to the Final Page of the Verdict Form and sign and date that page.

Final Page of the Jury Verdict

You have reached the end of the verdict form. Review the completed form to ensure that it accurately reflects your unanimous determinations. The Foreperson should then sign and date the verdict form in the space below and notify the Court Security Officer that you have reached a verdict.

The Foreperson should retain possession of the verdict form and bring it when the jury is brought back into the courtroom.

Dated: 5-17-2024

[redacted]
Foreperson

8203728

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

January 25, 2022

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF:

PATENT NUMBER: *10,603,314*
ISSUE DATE: *March 31, 2020*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

M. Tarver

Miguel Tarver
Certifying Officer

JTX-001
1:21-cv-01338-MFK


US010603314B2

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

(10) **Patent No.: US 10,603,314 B2**
(45) **Date of Patent: *Mar. 31, 2020**

(54) **METHOD FOR TREATING GEFITINIB RESISTANT CANCER**

(75) Inventors: **Daniel A. Haber**, Chestnut Hill, MA (US); **Daphne Winifred Bell**, Chevy Chase, MD (US); **Jeffrey E. Settleman**, Newton, MA (US); **Raffaella Sordella**, Cold Spring Harbor, NY (US); **Nadia G. Godin-Heymann**, Stanmore (GB); **Eunice L. Kwak**, Marlborough, MA (US); **Sridhar Krishna Rabindran**, Eagleville, PA (US)

(73) Assignees: **The General Hospital Corporation**, Boston, MA (US); **Wyeth LLC**, New York, NY (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.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **11/883,474**

(22) PCT Filed: **Feb. 2, 2006**

(86) PCT No.: **PCT/US2006/003717**

§ 371 (c)(1),
(2), (4) Date: **Aug. 5, 2008**

(87) PCT Pub. No.: **WO2006/084058**

PCT Pub. Date: **Aug. 10, 2006**

(65) **Prior Publication Data**

US 2010/0087482 A1 Apr. 8, 2010

Related U.S. Application Data

(60) Provisional application No. 60/649,483, filed on Feb. 3, 2005, provisional application No. 60/671,989, filed on Apr. 15, 2005.

(51) **Int. Cl.**

A61K 31/4709	(2006.01)
A61K 31/4706	(2006.01)
A61K 31/17	(2006.01)
A61K 38/17	(2006.01)

(52) **U.S. Cl.**
CPC ***A61K 31/4709*** (2013.01); ***A61K 31/4706*** (2013.01); ***A61K 38/17*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Peter J Reddig
(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

The present invention is directed to methods for the treatment of gefitinib and/or erlotinib resistant cancer. An individual with cancer is monitored for cancer progression following treatment with gefitinib and/or erlotinib. Progression of the cancer is indicative that the cancer is resistant to gefitinib and/or erlotinib. Once progression of cancer is noted, the subject is administered a pharmaceutical composition comprising an irreversible epidermal growth factor receptor (EGFR) inhibitor. In preferred embodiments, the irreversible EGFR inhibitor is EKB-569, HKI-272 and HKI-357.

9 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

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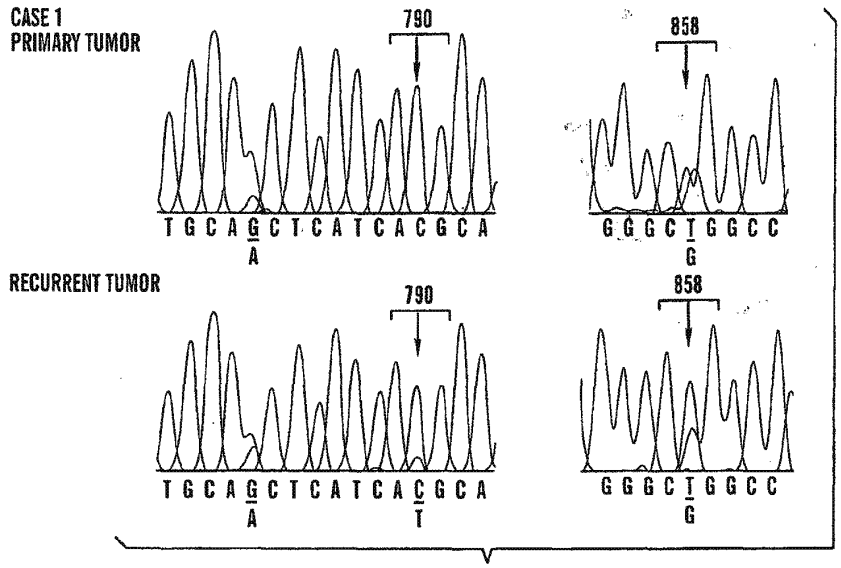

CASE 1
PRIMARY TUMOR
790
858
T G C A G C T C A T C A C G C A
A
G G G C T G G C C
G
RECURRENT TUMOR
790
858
T G C A G C T C A T C A C G C A
A
T
G G G C T G G C C
G

FIG. 1A

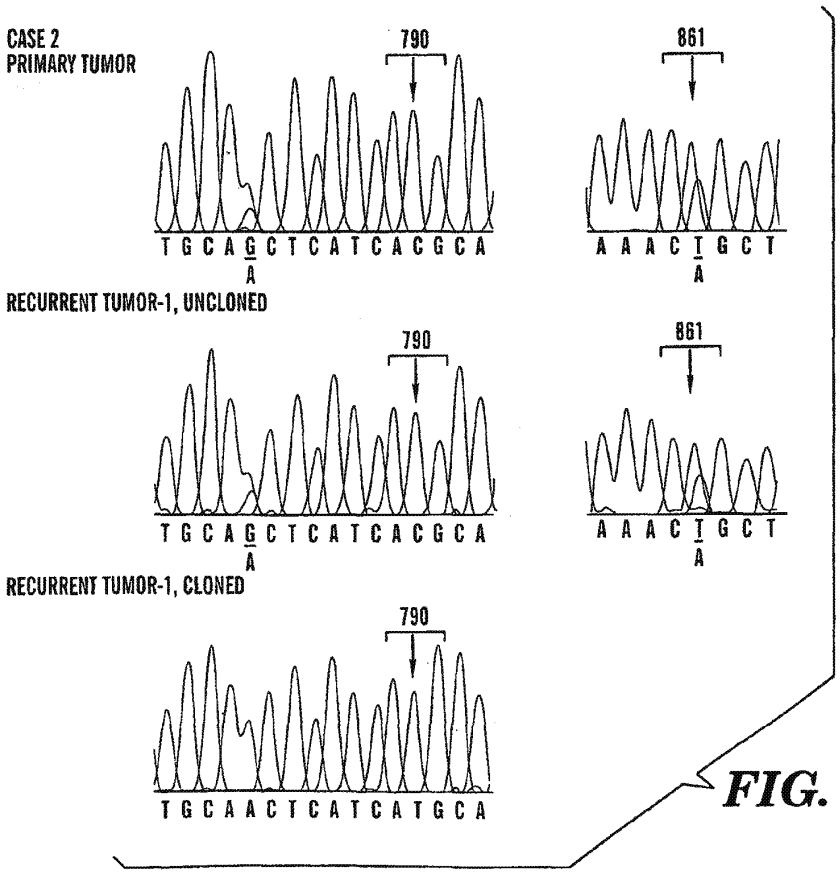
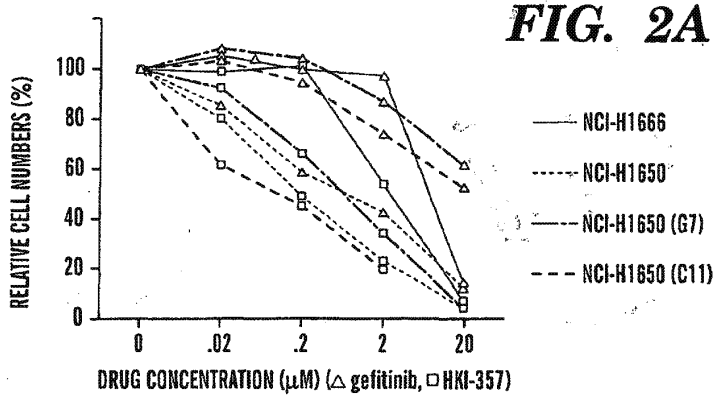
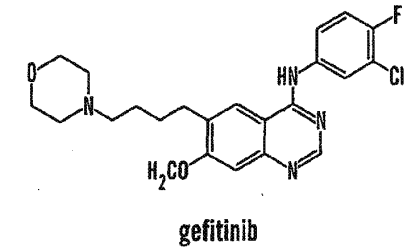
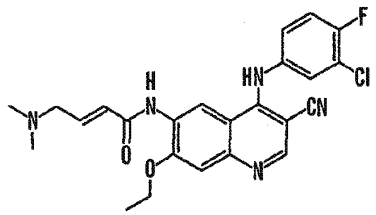

CASE 2
PRIMARY TUMOR
790
861
T G C A G C T C A T C A C G C A
A
A A A C T G C T
A
RECURRENT TUMOR-1, UNCLONED
790
861
T G C A G C T C A T C A C G C A
A
A A A C T G C T
A
RECURRENT TUMOR-1, CLONED
790
T G C A A C T C A T C A T G C A

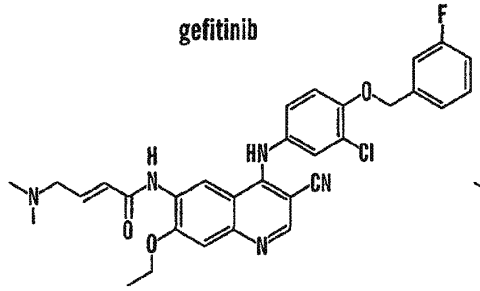
FIG. 1B

FIG. 2A









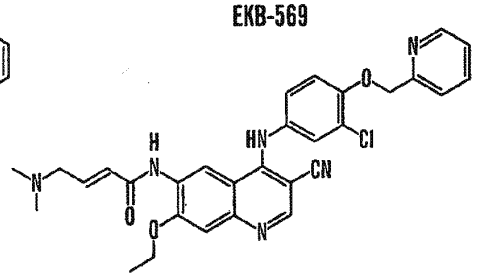


FIG. 2B

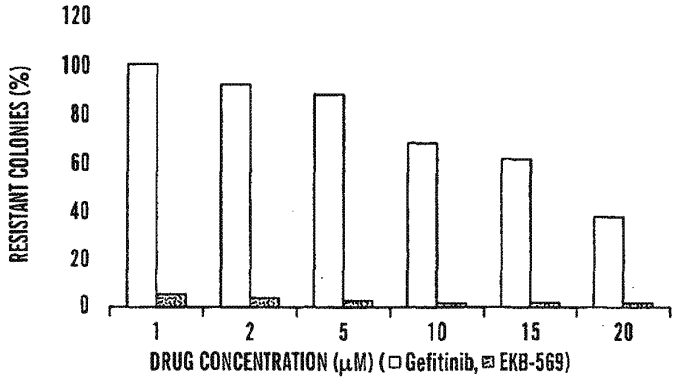


FIG. 2C

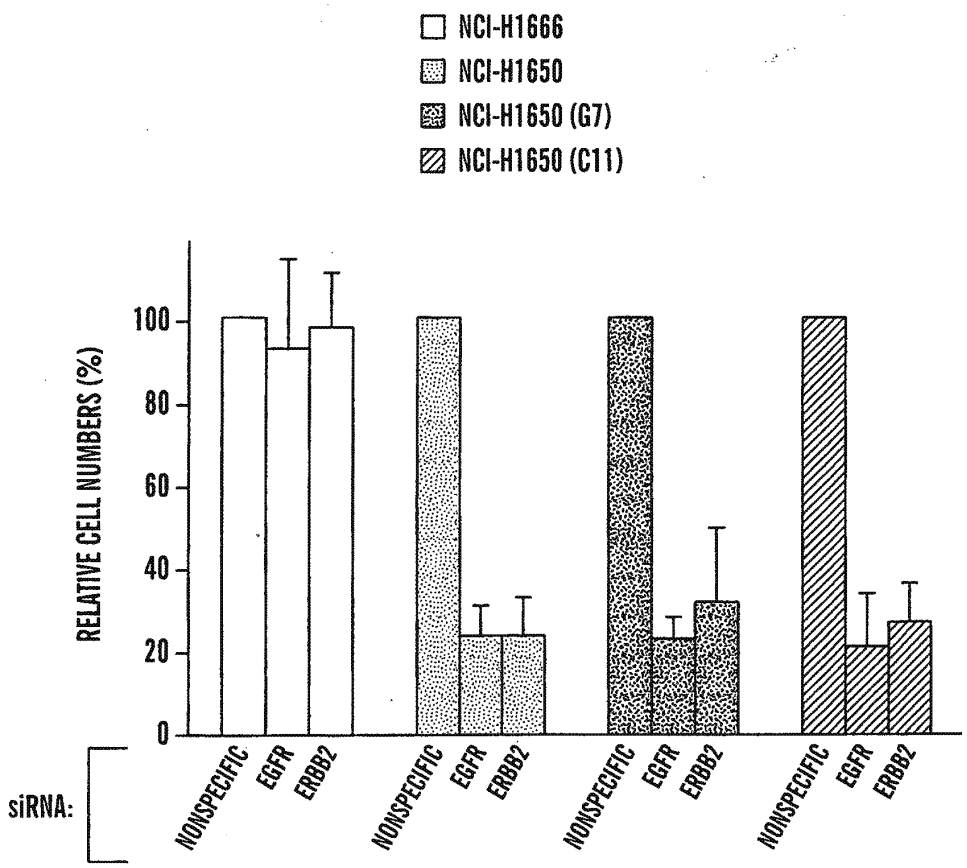

NCI-H1666
NCI-H1650
NCI-H1650 (G7)
NCI-H1650 (C11)
RELATIVE CELL NUMBERS (%)
100
80
60
40
20
0
siRNA:
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2

FIG. 3A

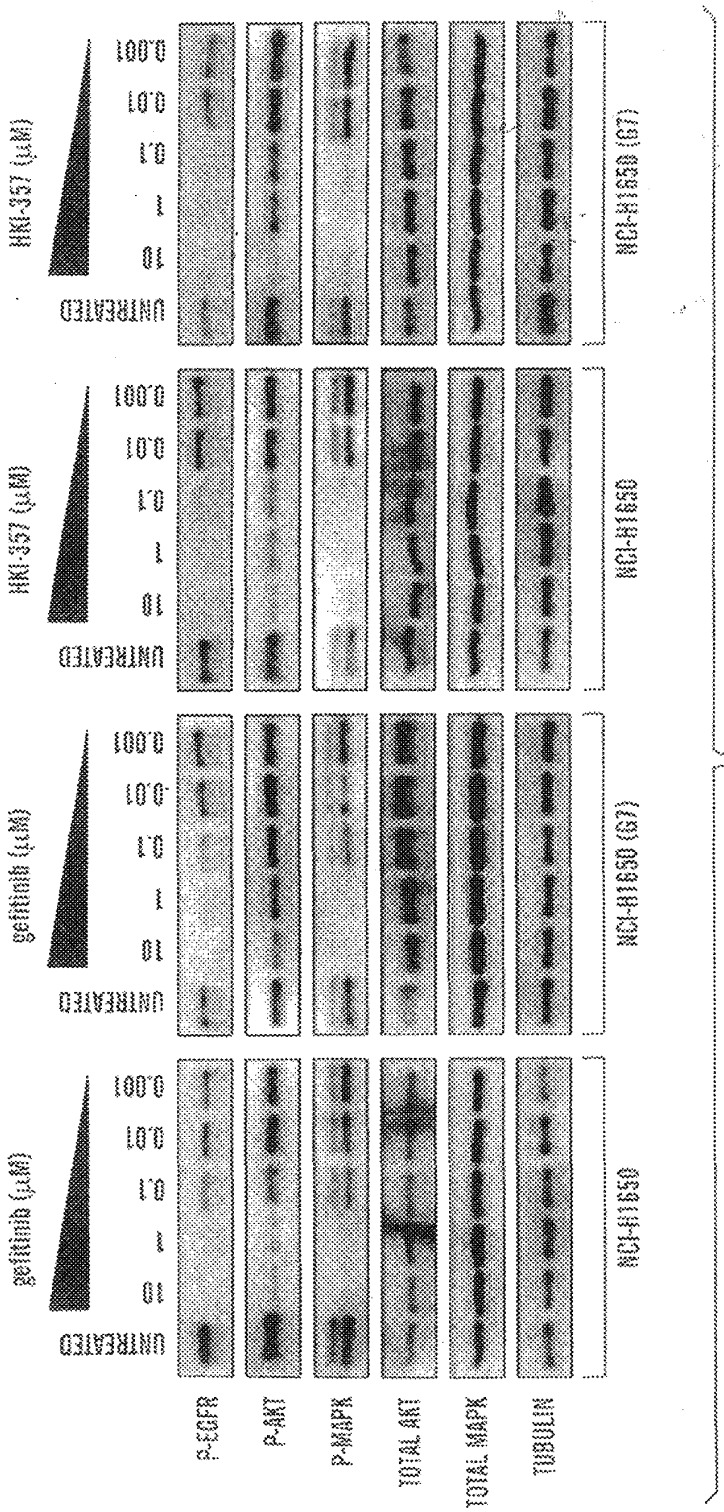

UNTREATED
10
1
0.1
0.01
0.001
HKI-357 (μM)
NCI-H1650 (G7)
NCI-H1650
gefitinib (μM)
P-EGFR
P-AKT
P-MAPK
TOTAL AKT
TOTAL MAPK
TUBULIN

FIG. 3B

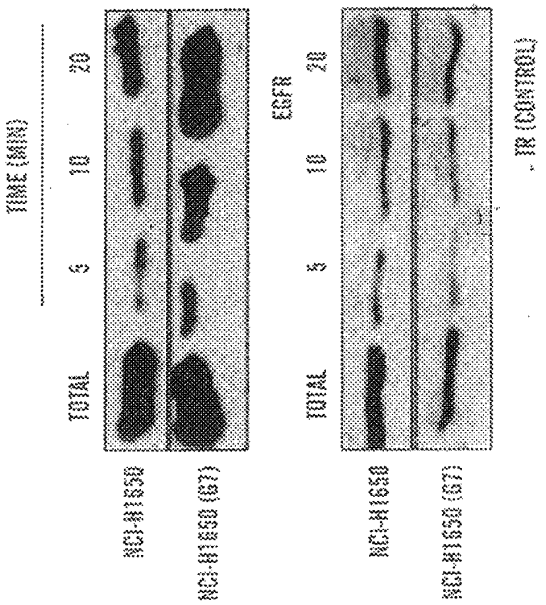

TIME (MIN)
TOTAL
5
10
20
NCI-H1650
NCI-H1650 (G7)
EGFR
TOTAL
5
10
20
IR (CONTROL)
NCI-H1650
NCI-H1650 (G7)

FIG. 3D

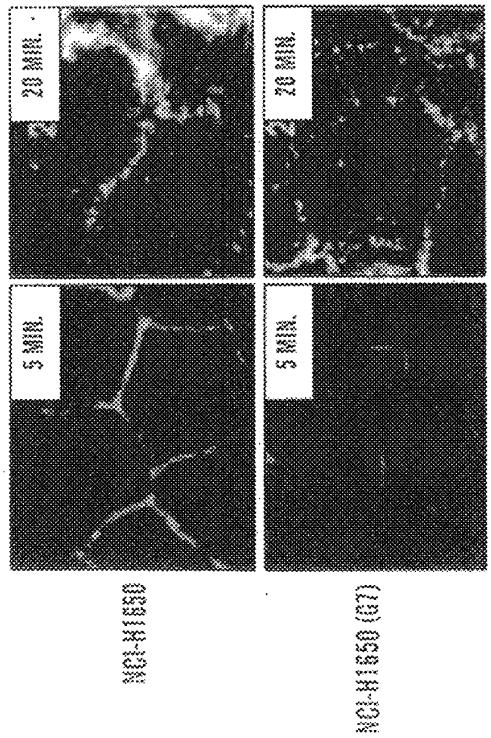

20 MIN.
5 MIN.
20 MIN.
5 MIN.
NCI-H1650
NCI-H1650 (G7)

FIG. 3C

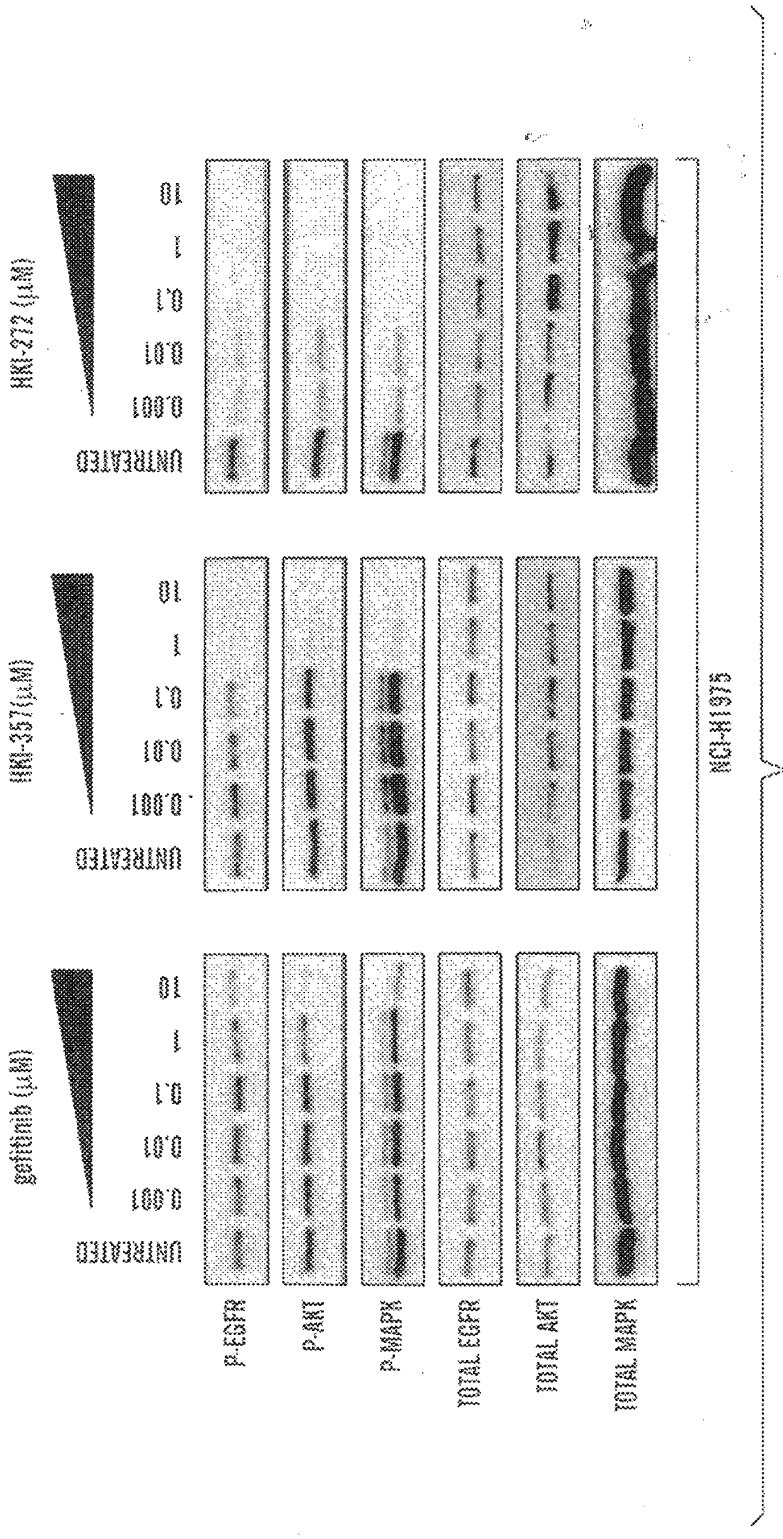

NCI-H1975
HKI-272 (μM)
HKI-357(μM)
gefitinib (μM)
UNTREATED
0.001
0.01
0.1
1
10
P-EGFR
P-AKT
P-MAPK
TOTAL EGFR
TOTAL AKT
TOTAL MAPK

FIG. 4A

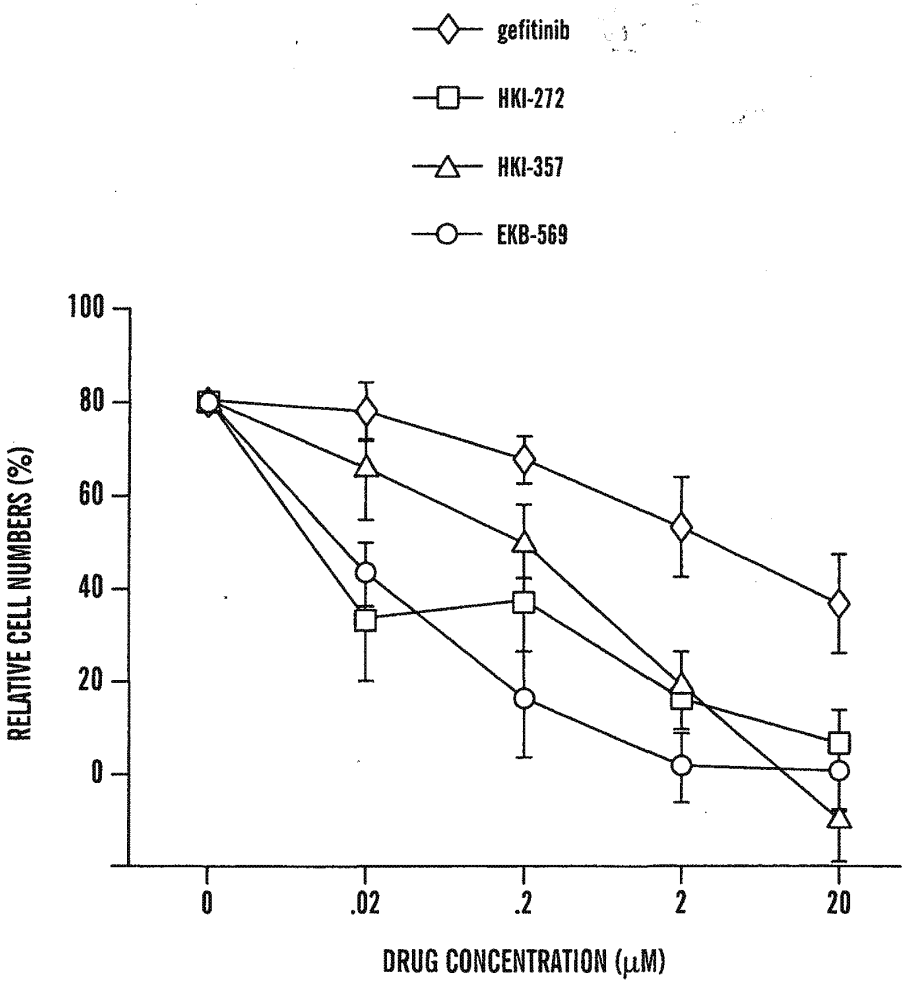

gefitinib
HKI-272
HKI-357
EKB-569
RELATIVE CELL NUMBERS (%)
100
80
60
40
20
0
0
.02
.2
2
20
DRUG CONCENTRATION (μM)

FIG. 4B

```
CCCGGCGCAGCGCGGCCGCAGCAGCCTCCGCCCCCCGCACGGTGTGAGCGCCCGACGCGG -185
.............................................................

CCGAGGCGGCCGGAGTCCCGAGCTAGCCCCGGCGGCCGCCGCCGCCCAGACCGGACGACA -125
.............................................................

GGCCACCTCGTCGGCGTCCGCCCGAGTCCCCGCCTCGCCGCCAACGCCACAACCACCGCG -65
.............................................................

CACGGCCCCCTGACTCCGTCCAGTATTGATCGGGAGAGCCGGAGCGAGCTCTTCGGGGAG -5
.............................................................

CAGCGATGCGACCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCT 55
.....-M--R--P--S--G--T--A--G--A--A--L--L--A--L--L--A--A--L-- 18

GCCCGGCGAGTCGGGCTCTGGAGGAAAAGAAAGTTTGCCAAGGCACGAGTAACAAGCTCA 115
C--P--A--S--R--A--L--E--E--K--K--V--C--Q--G--T--S--N--K--L-- 38

CGCAGTTGGGCACTTTTGAAGATCATTTTCTCAGCCTCCAGAGGATGTTCAATAACTGTG 175
T--Q--L--G--T--F--E--D--H--F--L--S--L--Q--R--M--F--N--N--C-- 58

AGGTGGTCCTTGGGAATTTGGAAATTACCTATGTGCAGAGGAATTATGATCTTTCCTTCT 235
E--V--V--L--G--N--L--E--I--T--Y--V--Q--R--N--Y--D--L--S--F-- 78

TAAAGACCATCCAGGAGGTGGCTGGTTATGTCCTCATTGCCCTCAACACAGTGGAGCGAA 295
L--K--T--I--Q--E--V--A--G--Y--V--L--I--A--L--N--T--V--E--R-- 98

TTCCTTTGGAAAACCTGCAGATCATCAGAGGAAATATGTACTACGAAAATTCCTATGCCT 355
I--P--L--E--N--L--Q--I--I--R--G--N--M--Y--Y--E--N--S--Y--A-- 118

TAGCAGTCTTATCTAACTATGATGCAAATAAAACCGGACTGAAGGAGCTGCCCATGAGAA 415
L--A--V--L--S--N--Y--D--A--N--K--T--G--L--K--E--L--P--M--R-- 138

ATTTACAGGAAATCCTGCATGGCGCCGTGCGGTTCAGCAACAACCCTGCCCTGTGCAACG 475
N--L--Q--E--I--L--H--G--A--V--R--F--S--N--N--P--A--L--C--N-- 158

TGGAGAGCATCCAGTGGCGGGACATAGTCAGCAGTGACTTTCTCAGCAACATGTCGATGG 535
V--E--S--I--Q--W--R--D--I--V--S--S--D--F--L--S--N--M--S--M-- 178

ACTTCCAGAACCACCTGGGCAGCTGCCAAAAGTGTGATCCAAGCTGTCCCAATGGGAGCT 595
D--F--Q--N--H--L--G--S--C--Q--K--C--D--P--S--C--P--N--G--S-- 198

GCTGGGGTGCAGGAGAGGAGAACTGCCAGAAACTGACCAAAATCATCTGTGCCCAGCAGT 655
C--W--G--A--G--E--E--N--C--Q--K--L--T--K--I--I--C--A--Q--Q-- 218

GCTCCGGGCGCTGCCGTGGCAAGTCCCCCAGTGACTGCTGCCACAACCAGTGTGCTGCAG 715
C--S--G--R--C--R--G--K--S--P--S--D--C--C--H--N--Q--C--A--A-- 238

GCTGCACAGGCCCCCGGGAGAGCGACTGCCTGGTCTGCCGCAAATTCCGAGACGAAGCCA 775
G--C--T--G--P--R--E--S--D--C--L--V--C--R--K--F--R--D--E--A-- 258

CGTGCAAGGACACCTGCCCCCCACTCATGCTCTACAACCCCACCACGTACCAGATGGATG 835
T--C--K--D--T--C--P--P--L--M--L--Y--N--P--T--T--Y--Q--M--D-- 278

TGAACCCCGAGGGCAAATACAGCTTTGGTGCCACCTGCGTGAAGAAGTGTCCCCGTAATT 895
V--N--P--E--G--K--Y--S--F--G--A--T--C--V--K--K--C--P--R--N-- 298
```

FIG. 5

```
ATGTGGTGACAGATCACGGCTCGTGCGTCCGAGCCTGTGGGGCCGACAGCTATGAGATGG 955
Y--V--V--T--D--H--G--S--C--V--R--A--C--G--A--D--S--Y--E--M-- 318

AGGAAGACGGCGTCCGCAAGTGTAAGAAGTGCGAAGGGCCTTGCCGCAAAGTGTGTAACG 1015
E--E--D--G--V--R--K--C--K--K--C--E--G--P--C--R--K--V--C--N-- 338

GAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAAACACT 1075
G--I--G--I--G--E--F--K--D--S--L--S--I--N--A--T--N--I--K--H-- 358

TCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGGCATTTAGGGGTG 1135
F--K--N--C--T--S--I--S--G--D--L--H--I--L--P--V--A--F--R--G-- 378

ACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCTGAAAACCGTAA 1195
D--S--F--T--H--T--P--P--L--D--P--Q--E--L--D--I--L--K--T--V-- 398

AGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATG 1255
K--E--I--T--G--F--L--L--I--Q--A--W--P--E--N--R--T--D--L--H-- 418

CCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTTG 1315
A--F--E--N--L--E--I--I--R--G--R--T--K--Q--H--G--Q--F--S--L-- 438

CAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATG 1375
A--V--V--S--L--N--I--T--S--L--G--L--R--S--L--K--E--I--S--D-- 458

GAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAA 1435
G--D--V--I--I--S--G--N--K--N--L--C--Y--A--N--T--I--N--W--K-- 478

AACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGCT 1495
K--L--F--G--T--S--G--Q--K--T--K--I--I--S--N--R--G--E--N--S-- 498

GCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGG 1555
C--K--A--T--G--Q--V--C--H--A--L--C--S--P--E--G--C--W--G--P-- 518

AGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGT 1615
E--P--R--D--C--V--S--C--R--N--V--S--R--G--R--E--C--V--D--K-- 538

GCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCC 1675
C--N--L--L--E--G--E--P--R--E--F--V--E--N--S--E--C--I--Q--C-- 558

ACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACT 1735
H--P--E--C--L--P--Q--A--M--N--I--T--C--T--G--R--G--P--D--N-- 578

GTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAG 1795
C--I--Q--C--A--H--Y--I--D--G--P--H--C--V--K--T--C--P--A--G-- 598

TCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACC 1855
V--M--G--E--N--N--T--L--V--W--K--Y--A--D--A--G--H--V--C--H-- 618

TGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGA 1915
L--C--H--P--N--C--T--Y--G--C--T--G--P--G--L--E--G--C--P--T-- 638

ATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGG 1975
N--G--P--K--I--P--S--I--A--T--G--M--V--G--A--L--L--L--L--L-- 658

TGGTGGCCCTGGGGATCGGCCTCTTCATGCGAAGGCGCCACATCGTTCGGAAGCGCACGC 2035
V--V--A--L--G--I--G--L--F--M--R--R--R--H--I--V--R--K--R--T-- 678
```

FIG. 5 (con'd.)

```
TGCGGAGGCTGCTGCAGGAGAGGGAGCTTGTGGAGCCTCTTACACCCAGTGGAGAAGCTC 2095
L--R--R--L--L--Q--E--R--E--L--V--E--P--L--T--P--S--G--E--A-- 698

CCAACCAAGCTCTCTTGAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGG 2155
P--N--Q--A--L--L--R--I--L--K--E--T--E--F--K--K--I--K--V--L-- 718

GCTCCGGTGCGTTCGGCACGGTGTATAAGGGACTCTGGATCCCAGAAGGTGAGAAAGTTA 2215
G--S--G--A--F--G--T--V--Y--K--G--L--W--I--P--E--G--E--K--V-- 738

AAATTCCCGTCGCTATCAAGGAATTAAGAGAAGCAACATCTCCGAAAGCCAACAAGGAAA 2275
K--I--P--V--A--I--K--E--L--R--E--A--T--S--P--K--A--N--K--E-- 758

TCCTCGATGAAGCCTACGTGATGGCCAGCGTGGACAACCCCCACGTGTGCCGCCTGCTGG 2335
I--L--D--E--A--Y--V--M--A--S--V--D--N--P--H--V--C--R--L--L-- 778

GCATCTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTCC 2395
G--I--C--L--T--S--T--V--Q--L--I--T--Q--L--M--P--F--G--C--L-- 798

TGGACTATGTCCGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAACTGGTGTG 2455
L--D--Y--V--R--E--H--K--D--N--I--G--S--Q--Y--L--L--N--W--C-- 818

TGCAGATCGCAAAGGGCATGAACTACTTGGAGGACCGTCGCTTGGTGCACCGCGACCTGG 2515
V--Q--I--A--K--G--M--N--Y--L--E--D--R--R--L--V--H--R--D--L-- 838

CAGCCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGG 2575
A--A--R--N--V--L--V--K--T--P--Q--H--V--K--I--T--D--F--G--L-- 858

CCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTGCCTATCA 2635
A--K--L--L--G--A--E--E--K--E--Y--H--A--E--G--G--K--V--P--I-- 878

AGTGGATGGCATTGGAATCAATTTTACACAGAATCTATACCCACCAGAGTGATGTCTGGA 2695
K--W--M--A--L--E--S--I--L--H--R--I--Y--T--H--Q--S--D--V--W-- 898

GCTACGGGGTGACTGTTTGGGAGTTGATGACCTTTGGATCCAAGCCATATGACGGAATCC 2755
S--Y--G--V--T--V--W--E--L--M--T--F--G--S--K--P--Y--D--G--I-- 918

CTGCCAGCGAGATCTCCTCCATCCTGGAGAAAGGAGAACGCCTCCCTCAGCCACCCATAT 2815
P--A--S--E--I--S--S--I--L--E--K--G--E--R--L--P--Q--P--P--I-- 938

GTACCATCGATGTCTACATGATCATGGTCAAGTGCTGGATGATAGACGCAGATAGTCGCC 2875
C--T--I--D--V--Y--M--I--M--V--K--C--W--M--I--D--A--D--S--R-- 958

CAAAGTTCCGTGAGTTGATCATCGAATTCTCCAAAATGGCCCGAGACCCCCAGCGCTACC 2935
P--K--F--R--E--L--I--I--E--F--S--K--M--A--R--D--P--Q--R--Y-- 978

TTGTCATTCAGGGGGATGAAAGAATGCATTTGCCAAGTCCTACAGACTCCAACTTCTACC 2995
L--V--I--Q--G--D--E--R--M--H--L--P--S--P--T--D--S--N--F--Y-- 998

GTGCCCTGATGGATGAAGAAGACATGGACGACGTGGTGGATGCCGACGAGTACCTCATCC 3055
R--A--L--M--D--E--E--D--M--D--D--V--V--D--A--D--E--Y--L--I-- 1018

CACAGCAGGGCTTCTTCAGCAGCCCCTCCACGTCACGGACTCCCCTCCTGAGCTCTCTGA 3115
P--Q--Q--G--F--F--S--S--P--S--T--S--R--T--P--L--L--S--S--L-- 1038

GTGCAACCAGCAACAATTCCACCGTGGCTTGCATTGATAGAAATGGGCTGCAAAGCTGTC 3175
S--A--T--S--N--N--S--T--V--A--C--I--D--R--N--G--L--Q--S--C-- 1058
```

FIG. 5 (con'd.)

```
CCATCAAGGAAGACAGCTTCTTGCAGCGATACAGCTCAGACCCCACAGGCGCCTTGACTG 3235
P--I--K--E--D--S--F--L--Q--R--Y--S--S--D--P--T--G--A--L--T-- 1078

AGGACAGCATAGACGACACCTTCCTCCCAGTGCCTGAATACATAAACCAGTCCGTTCCCA 3295
E--D--S--I--D--D--T--F--L--P--V--P--E--Y--I--N--Q--S--V--P-- 1098

AAAGGCCCGCTGGCTCTGTGCAGAATCCTGTCTATCACAATCAGCCTCTGAACCCCGCGC 3355
K--R--P--A--G--S--V--Q--N--P--V--Y--H--N--Q--P--L--N--P--A-- 1118

CCAGCAGAGACCCACACTACCAGGACCCCCACAGCACTGCAGTGGGCAACCCCGAGTATC 3415
P--S--R--D--P--H--Y--Q--D--P--H--S--T--A--V--G--N--P--E--Y-- 1138

TCAACACTGTCCAGCCCACCTGTGTCAACAGCACATTCGACAGCCCTGCCCACTGGGCCC 3475
L--N--T--V--Q--P--T--C--V--N--S--T--F--D--S--P--A--H--W--A-- 1158

AGAAAGGCAGCCACCAAATTAGCCTGGACAACCCTGACTACCAGCAGGACTTCTTTCCCA 3535
Q--K--G--S--H--Q--I--S--L--D--N--P--D--Y--Q--Q--D--F--F--P-- 1178

AGGAAGCCAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAAATGCAGAATACCTAA 3595
K--E--A--K--P--N--G--I--F--K--G--S--T--A--E--N--A--E--Y--L-- 1198

GGGTCGCGCCACAAAGCAGTGAATTTATTGGAGCATGA 3633 (SEQ ID NO 2)
R--V--A--P--Q--S--S--E--F--I--G--A--*- 1210 (SEQ ID NO 1)
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FIG. 5 (con'd.)

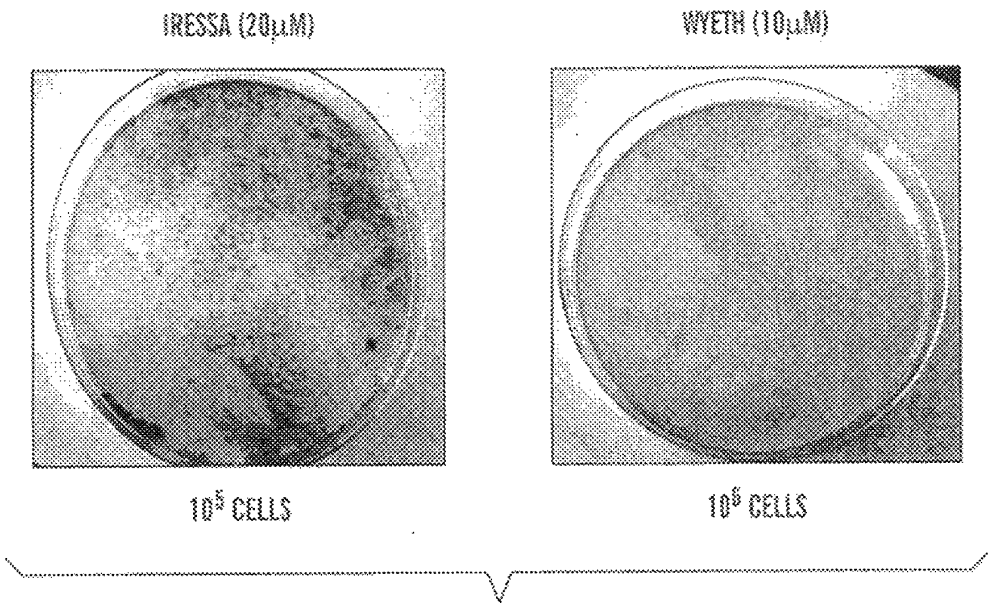

FIG. 6

METHOD FOR TREATING GEFITINIB RESISTANT CANCER

CROSS-REFERENCED APPLICATIONS

This application is a 371 National Phase Entry Application of co-pending International Application PCT/US2006/003717, filed Feb. 2, 2006, which designated the U.S. and claims the benefit under 35 U.S.C. §119(e) of U.S. provisional Patent Application No. 60/649,483, filed Feb. 3, 2005, and U.S. Provisional Application No. 60/671,989, Filed Apr. 15, 2005, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

This application incorporates by reference in its entirety the Computer Readable Form (CRF) of a Sequence Listing in ASCII text format submitted via EFS-Web. The Sequence Listing text file submitted via EFS-Web, entitled 13120-004-999 SUB SEQ LISTING.txt, was created on Dec. 16, 2019, and is 27,589 bytes in size.

BACKGROUND

Epithelial cell cancers, for example, prostate cancer, breast cancer, colon cancer, lung cancer, pancreatic cancer, ovarian cancer, cancer of the spleen, testicular cancer, cancer of the thymus, etc., are diseases characterized by abnormal, accelerated growth of epithelial cells. This accelerated growth initially causes a tumor to form. Eventually, metastasis to different organ sites can also occur. Although progress has been made in the diagnosis and treatment of various cancers, these diseases still result in significant mortality.

Lung cancer remains the leading cause of cancer death in industrialized countries. Cancers that begin in the lungs are divided into two major types, non-small cell lung cancer and small cell lung cancer, depending on how the cells appear under a microscope. Non-small cell lung cancer (squamous cell carcinoma, adenocarcinoma, and large cell carcinoma) generally spreads to other organs more slowly than does small cell lung cancer. About 75 percent of lung cancer cases are categorized as non-small cell lung cancer (e.g., adenocarcinomas), and the other 25 percent are small cell lung cancer. Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths in the United States, Japan and Western Europe. For patients with advanced disease, chemotherapy provides a modest benefit in survival, but at the cost of significant toxicity, underscoring the need for therapeutic agents that are specifically targeted to the critical genetic lesions that direct tumor growth (Schiller J H et al., N Engl J Med, 346: 92-98, 2002).

Epidermal growth factor receptor (EGFR) is a 170 kilodalton (kDa) membrane-bound protein expressed on the surface of epithelial cells. EGFR is a member of the growth factor receptor family of protein tyrosine kinases, a class of cell cycle regulatory molecules. (W. J. Gullick et al., 1986, Cancer Res., 46:285-292). EGFR is activated when its ligand (either EGF or TGF-α) binds to the extracellular domain, resulting in autophosphorylation of the receptor's intracellular tyrosine kinase domain (S. Cohen et al., 1980, J. Biol. Chem., 255:4834-4842; A. B. Schreiber et al., 1983, J. Biol. Chem., 258:846-853).

EGFR is the protein product of a growth promoting oncogene, erbB or ErbB1, that is but one member of a family, i.e., the ERBB family of protooncogenes, believed to play pivotal roles in the development and progression of

many human cancers. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. The ERBB family of oncogenes encodes four, structurally-related transmembrane receptors, namely, EGFR, HER-2/neu (erbB2), HER-3 (erbB3) and HER-4 (erbB4). Clinically, ERBB oncogene amplification and/or receptor overexpression in tumors have been reported to correlate with disease recurrence and poor patient prognosis, as well as with responsiveness in therapy. (L. Harris et al., 1999, Int. J. Biol. Markers, 14:8-15; and J. Mendelsohn and J. Baselga, 2000, Oncogene, 19:6550-6565).

EGFR is composed of three principal domains, namely, the extracellular domain (ECD), which is glycosylated and contains the ligand-binding pocket with two cysteine-rich regions; a short transmembrane domain, and an intracellular domain that has intrinsic tyrosine kinase activity. The transmembrane region joins the ligand-binding domain to the intracellular domain. Amino acid and DNA sequence analysis, as well as studies of nonglycosylated forms of EGFR, indicate that the protein backbone of EGFR has a mass of 132 kDa, with 1186 amino acid residues (A. L. Ullrich et al., 1984, Nature, 309:418-425; J. Downward et al., 1984, Nature, 307:521-527; C. R. Carlin et al., 1986, Mol. Cell. Biol., 6:257-264; and F. L. V. Mayes and M. D. Waterfield, 1984, The EMBO J., 3:531-537).

The binding of EGF or TGF-α to EGFR activates a signal transduction pathway and results in cell proliferation. The dimerization, conformational changes and internalization of EGFR molecules function to transmit intracellular signals leading to cell growth regulation (G. Carpenter and S. Cohen, 1979, Ann. Rev. Biochem., 48:193-216). Genetic alterations that affect the regulation of growth factor receptor function, or lead to overexpression of receptor and/or ligand, result in cell proliferation. In addition, EGFR has been determined to play a role in cell differentiation, enhancement of cell motility, protein secretion, neovascularization, invasion, metastasis and resistance of cancer cells to chemotherapeutic agents and radiation. (M.-J. Oh et al., 2000, Clin. Cancer Res., 6:4760-4763).

A variety of inhibitors of EGFR have been identified, including a number already undergoing clinical trials for treatment of various cancers. For a recent summary, see de Bono, J. S. and Rowinsky, E. K. (2002), "The ErbB Receptor Family: A Therapeutic Target For Cancer", *Trends in Molecular Medicine,* 8, S19-26.

A promising set of targets for therapeutic intervention in the treatment of cancer includes the members of the HER-kinase axis. They are frequently upregulated in solid epithelial tumors of, by way of example, the prostate, lung and breast, and are also upregulated in glioblastoma tumors. Epidermal growth factor receptor (EGFR) is a member of the HER-kinase axis, and has been the target of choice for the development of several different cancer therapies. EGFR tyrosine kinase inhibitors (EGFR-TKIs) are among these therapies, since the reversible phosphorylation of tyrosine residues is required for activation of the EGFR pathway. In other words, EGFR-TKIs block a cell surface receptor responsible for triggering and/or maintaining the cell signaling pathway that induces tumor cell growth and division. Specifically, it is believed that these inhibitors interfere with the EGFR kinase domain, referred to as HER-1. Among the more promising EGFR-TKIs are three series of compounds: quinazolines, pyridopyrimidines and pyrrolopyrimidines.

Two of the more advanced compounds in clinical development include Gefitinib (compound ZD1839 developed by AstraZeneca UK Ltd.; available under the tradename

IRESSA; hereinafter "IRESSA") and Erlotinib (compound OSI-774 developed by Genentech, Inc. and OSI Pharmaceuticals, Inc.; available under the tradename TARCEVA; hereinafter "TARCEVA"); both have generated encouraging clinical results. Conventional cancer treatment with both IRESSA and TARCEVA involves the daily, oral administration of no more than 500 mg of the respective compounds. In May, 2003, IRESSA became the first of these products to reach the United States market, when it was approved for the treatment of advanced non-small cell lung cancer patients.

IRESSA is an orally active quinazoline that functions by directly inhibiting tyrosine kinase phosphorylation on the EGFR molecule. It competes for the adenosine triphosphate (ATP) binding site, leading to suppression of the HER-kinase axis. The exact mechanism of the IRESSA response is not completely understood, however, studies suggest that the presence of EGFR is a necessary prerequisite for its action.

A significant limitation in using these compounds is that recipients thereof may develop a resistance to their therapeutic effects after they initially respond to therapy, or they may not respond to EGFR-TKIs to any measurable degree at all. The response rate to EGFR-TKIs varies between different ethnic groups. At the low end of EGFR-TKI responders, in some populations, only 10-15 percent of advanced non-small cell lung cancer patients respond to EGFR kinase inhibitors. Thus, a better understanding of the molecular mechanisms underlying sensitivity to IRESSA and TARCEVA would be extremely beneficial in targeting therapy to those individuals whom are most likely to benefit from such therapy.

There is a significant need in the art for a satisfactory treatment of cancer, and specifically epithelial cell cancers such as lung, ovarian, breast, brain, colon and prostate cancers, which incorporates the benefits of TKI therapy and overcoming the non-responsiveness exhibited by patients. Such a treatment could have a dramatic impact on the health of individuals, and especially older individuals, among whom cancer is especially common.

SUMMARY

The inventors of the present invention have surprisingly discovered that irreversible EGFR inhibitors are effective in the treatment of cancer in subjects who are no longer responding to gefitinib and/or erlotinib therapies. Thus, in one embodiment, the present invention provides a method for the treatment of gefitinib and/or erlotinib resistant cancer. In this embodiment, progression of cancer in a subject is monitored at a time point after the subject has initiated gefitinib and/or erlotinib treatment. Progression of the cancer is indicative of cancer that is resistant to gefitinib and/or erlotinib treatment and the subject is administered a pharmaceutical composition comprising an irreversible epidermal growth factor receptor (EGFR) inhibitor.

In preferred embodiments, the irreversible EGFR inhibitor EKB-569, HKI-272 or HKI-357. Alternatively, the irreversible EGFR inhibitor may be any compound which binds to cysteine 773 of EGFR (SEQ ID NO: 1).

The progression of cancer may be monitored by methods well known to those of skill in the art. For example, the progression may be monitored by way of visual inspection of the cancer, such as, by means of X-ray, CT scan or MRI. Alternatively, the progression may be monitored by way of tumor biomarker detection.

In one embodiment, the patient is monitored at various time points throughout the treatment of the cancer. For example, the progression of a cancer may be monitored by analyzing the progression of cancer at a second time point and comparing this analysis to an analysis at a first time point. The first time point may be before or after initiation of gefitinib and/or erlotinib treatment and the second time point is after the first. An increased growth of the cancer indicates progression of the cancer.

In one embodiment, the progression of cancer is monitored by analyzing the size of the cancer. In one embodiment, the size of the cancer is analyzed via visual inspection of the cancer by means of X-ray, CT scan or MRI. In one embodiment, the size of the cancer is monitored by way of tumor biomarker detection.

In one embodiment, the cancer is epithelial cell cancer. In one embodiment, the cancer is gastrointestinal cancer, prostate cancer, ovarian cancer, breast cancer, head and neck cancer, esophageal cancer, lung cancer, non-small cell lung cancer, cancer of the nervous system, kidney cancer, retina cancer, skin cancer, liver cancer, pancreatic cancer, genital-urinary cancer and bladder cancer.

In one embodiment, the size of the cancer is monitored at additional time points, and the additional time points are after the second time point.

In one embodiment, the later time point is at least 2 months after the preceding time point. In one embodiment, the later time point is at least 6 months after preceding time point. In one embodiment, the later time point is at least 10 months after preceding time point. In one embodiment, the later time point is at least one year after preceding time point.

In another embodiment, the present invention provides a method of treating cancer, comprising administering to a subject having a mutation in EGFR, namely, a substitution of a methionine for a threonine at position 790 (T790M) of SEQ ID. No. 1, a pharmaceutical composition comprising an irreversible EGFR inhibitor. The T790M mutation confers resistance to gefitinib and/or erlotinib treatment.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A-**1**B show EGFR sequence analysis in recurrent metastatic lesions from two NSCLC patients with acquired gefitinib resistance. FIG. **1**A shows sequence analysis for Case 1. The T790M mutation in EGFR is present in a recurrent liver lesion after the development of clinical gefitinib resistance. (Left) The mutation was not detected in the primary lung lesion at the time of diagnosis. (Right) Both the primary lung tumor and the recurrent liver lesion harbor the L858R gefitinib-sensitizing mutation. Of note, the L858R mutation is present in the expected ratio for a heterozygous mutation in both primary and recurrent lesions, whereas T790M is detectable at low levels compared with the wild-type allele. A polymorphism (G/A) is shown in the same tracing to demonstrate equivalent representation of the two alleles in the uncloned PCR product (SEQ ID NOS 3 & 4 disclosed respectively, in order of appearance). FIG. **1**B shows sequence analysis for Case 2. The T790M mutation is present within a small minority of gefitinib-resistant cells. (Left) The T790M mutation was undetectable either in the lung primary tumor or in eight recurrent liver lesions from this case by sequencing uncloned PCR products. Heterozygosity at an adjacent polymorphism (G/A) confirms amplification of both EGFR alleles from these specimens. The heterozygous gefitinib-sensitizing mutation, L861 Q, was detected at the expected ratio within the primary lung tumor as well as each of the

eight recurrent liver lesions (SEQ ID NOS 3 & 5 disclosed respectively, in order of appearance).

FIGS. **2**A-**2**C show acquired resistance to gefitinib in bronchoalveolar cancer cell lines and persistent sensitivity to irreversible ERBB family inhibitors. FIG. **2**A shows inhibition by tyrosine kinase inhibitors of proliferation of bronchoalveolar cancer cell lines with wild-type EGFR (NCI-H1666), the activating del E746-A750 mutation in EGFR (NCI-H1650), or two representative gefitinib-resistant subclones of NCI-H1650 (G7 and C11). The effect of the reversible inhibitor gefitinib is compared with that of the irreversible inhibitor HKI-357. Comparable results were observed with the other irreversible inhibitors. Cell numbers were measured by crystal violet staining, after culture in 5% FCS, with 100 ng/ml EGFR, at 72 h after exposure to indicated drug concentrations. Each data point represents the mean of four samples. FIG. **2**B shows the chemical structure of gefitinib, a reversible inhibitor of EGFR; EKB-569, an irreversible inhibitor of EGFR; and HKI-272 and HKI-357, two irreversible dual inhibitors of EGFR and ERBB2. FIG. **2**C shows generation of drug-resistant NCI-H1650 cells after treatment with varying concentrations of gefitinib or the irreversible ERBB inhibitor EKB-569. Colonies were stained after 12 days in culture in the presence of inhibitors.

FIGS. **3**A-**3**D show persistent dependence on EGFR and ERBB2 signaling in gefitinib-resistant cells, and altered receptor trafficking. FIG. **3**A shows cell viability after siRNA-mediated knockdown of EGFR and ERBB2 in bronchoalveolar cell lines with wild-type EGFR (NCI-H1666), compared with cells with the activating del E746-A750 mutation in EGFR (NCI-H1650) and two gefitinib-resistant derivatives (G7 and C11). Viable cells were counted 72 h after treatment with double-stranded RNA and are shown as a fraction relative to cells treated with nonspecific siRNA, with standard deviations based on triplicate samples. FIG. **3**B shows inhibition of EGFR autophosphorylation (Y1068) and phosphorylation of downstream effectors AKT and MAPK (ERK) in cells treated with increasing concentrations of gefitinib or the irreversible inhibitor HKI-357, followed by a 2-h pulse with EGF. The parental cell line NCI-H1650 is compared with a representative gefitinib-resistant line, G7. Total AKT and MAPK are shown as controls; tubulin is used as loading control for total EGFR levels, which are at the lower limit of detection in these cells. FIG. **3**C shows altered EGFR internalization in gefitinib-resistant NCI-H1650 (G7) cells, compared with the sensitive NCI-H1650 parental cell line. Rhodamine-tagged EGF is used to label EGFR at 5 and 20 min, after addition of ligand. The increased internalization of EGFR in NCI-H1650 (G7) cells is most evident at 20 min. (Zeiss microscope, ×63 magnification). FIG. **3**D shows immunoblotting of internalized EGFR from NCI-H1650 parental cells and the resistant derivative G7 after pulse labeling of cell surface proteins by biotinylation and chase over 20 min. The increased intracellular EGFR in NCI-H1650 (G7) cells is compared with the unaltered transferrin receptor (TR) internalization.

FIGS. **4**A-**4**B show Effectiveness of irreversible ERBB inhibitors in suppressing the T790M EGFR mutant. FIG. **4**A shows comparison of gefitinib and two irreversible inhibitors, HKI-357 and HKI-272, in their ability to suppress EGFR autophosphorylation (Y1068) and phosphorylation of downstream effectors AKT and MAPK (ERK) in the NCI-H1975 bronchoalveolar cell line, harboring both a sensitizing mutation (L858R) and the resistance-associated mutation (T790M). Total EGFR, AKT, and MAPK are shown as loading controls. FIG. **4**B shows suppression of proliferation in NCI-H1975 cells harboring the L858R and T790M mutations by the three irreversible ERBB family inhibitors, compared with gefitinib.

FIG. **5** shows the nucleotide sequence (SEQ ID NO: 2) and the amino acid sequence (SEQ ID NO: 1) of EGFR.

FIG. **6** shows that like gefitinib, HKI 357 and EKB 569 (labeled "Wyeth") demonstrated increased cell killing of NSCLC cells harboring an EGFR mutation, but unlike gefitinib, clones resistant to these drugs were not readily generated in vitro and they retained their effectiveness against gefitinib-resistant clones.

DETAILED DESCRIPTION

Gefitinib and Erlotinib Resistant Cancers

Gefitinib (compound ZD1839 developed by AstraZeneca UK Ltd.; available under the tradename IRESSA) and erlotinib (compound OSI-774 developed by Genentech, Inc. and OSI Pharmaceuticals, Inc.; available under the trade name TARCEVA) induce dramatic clinical responses in cases of non-small cell lung cancers (NSCLCs) harboring activating mutations in the EGF receptor (EGFR) (1-3), which is targeted by these competitive inhibitors of ATP binding (4, 5). The effectiveness of these tyrosine kinase inhibitors may result both from alterations in the ATP cleft associated with these mutations, which lead to enhanced inhibition of the mutant kinase by these drugs, and from biological dependence of these cancer cells on the increased survival signals transduced by the mutant receptors, a phenomenon described as "oncogene addiction" (6, 7).

Although therapeutic responses to both gefitinib and erlotinib can persist for as long as 2-3 years, the mean duration of response in most cases of NSCLC is only 6-8 months (8-10). The mechanisms underlying acquired drug resistance are not well understood. By analogy with imatinib (GLEEVEC), which inhibits the BCR-ABL kinase involved in chronic myeloid leukemias (CMLs), the C-KIT kinase implicated in gastrointestinal stromal tumors (GISTs), and the FIP1L1-PDGFR-α kinase in idiopathic hypereosinophilic syndrome (HES), secondary kinase domain mutations can potentially suppress drug binding (11-16). However, recurrent NSCLC is not readily biopsied; hence, only limited clinical specimens are available for analysis. Recently, a single secondary mutation, T790M, within the EGFR kinase domain has been reported in three of six cases with recurrent disease after gefitinib or erlotinib therapy (17, 18). Codon 315 of BCR-ABL, which is analogous to EGFR codon 790, is frequently mutated in imatinib-resistant CML (11, 12), and mutation of the corresponding residue in C-KIT (codon 670) and FIP1L1-PDGFR-α (codon 674) is associated with imatinib-resistant GIST and HES, respectively (15, 16). Early in vitro modeling of resistance to EGFR inhibitors indicated that mutation of codon 790 within the wild-type receptor would similarly suppress inhibition by an EGFR tyrosine kinase inhibitor (19). Recently, transfected EGFR proteins containing activating mutations together with the T790M substitution were shown to exhibit reduced inhibition by gefitinib and erlotinib (17, 18). Although the T790M mutation seems to contribute to acquired resistance in some cases of NSCLC, the mechanisms underlying treatment failure in cases lacking secondary EGFR mutations remain unexplained.

In contrast to the cytoplasmic kinase BCR-ABL, signaling by the membrane-bound EGFR involves a complex pathway of ligand binding, receptor homodimerization, and heterodimerization with ERBB2 and other family members, followed by internalization and recycling of the ligand-

bound receptor or ubiquitin-mediated receptor degradation (20). Significant EGF-dependent signaling is thought to occur during the process of internalization, which is also associated with the dissociation of EGFR complexes at the low pH of intracellular vesicles. As such, multiple factors modulate the strength and quality of the signal transduced by the receptor, and alterations in EGFR trafficking have been closely linked with the regulation of EGF-dependent cellular responses (20).

The present invention is based on the discovery that gefitinib resistant cancers can include those wherein the T790M EGFR mutation is only present in a subset of resistant tumor cells and those wherein the T790M mutation is not observed, but increased EGFR internalization is observed. The invention is further based on the discovery that irreversible EGFR inhibitors, which covalently cross-link the receptor, are effective in inhibiting cancers with the T790M mutation and in cancers with altered EGFR trafficking that can make such cancers resistance to treatment with gefitinib and/or erlotinib. Accordingly, the present invention provides a method of treating gefitinib and/or erlotinib resistant cancers comprising administering irreversible EGFR inhibitors.

Method of Treating a Patient

In one embodiment, the invention provides a method for treating gefitinib/erlotinib resistant cancer. The method comprises administering to a patient in need of such treatment an effective amount of certain irreversible EGFR inhibitors, including EKB-569 (4-anilinoquinoline-3-carbonitrile; Greenberger et al., 11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy, Amsterdam, Nov. 7-10, 2000, abstract 388; Wyeth), HKI-357 (a derivative of 4-anilinoquinoline-3-carbonitrile; Tsou et al. J. Med. Chem. 2005, 48: 1107-1131; Wyeth) and/or HKI-272 (a derivative of 4-anilinoquinoline-3-carbonitrile; Rabindran et al., Cancer Res. 2004, 64, 3958-3965; Wyeth). In one preferred embodiment, the invention provides a method comprising administering to a patient in need of such treatment an effective amount of EKB-569. In one preferred embodiment, the invention provides a method comprising administering to a patient in need of such treatment an effective amount of HKI-357.

The treatment may also involve a combination of treatments, including, but not limited to a tyrosine kinase inhibitor in combination with other tyrosine kinase inhibitors, chemotherapy, radiation, etc.

Cancers may initially be diagnosed as gefitinib/erlotinib sensitive or predicted to be gefitinib/erlotinib sensitive by means of the methods described in Lynch et al., 2004; 350:2129-2139. Gefitinib/erlotinib sensitivity may be predicted by the presence in the tumor of EGFR mutations including, for example, deletion of residues 747 (lysine) to 749 (glutamic acid) combined with a mutation in 750 (alanine), deletion of residues 747 (lysine) to 750 (alanine), substitution of arginine for leucine at residue 858, of substitution of glutamine for leucine at residue 861.

Cancers may be diagnosed as gefitinib and/or erlotinib resistant after treatment with gefitinib and/or erlotinib has commenced. Alternatively, cancers may be diagnosed as gefitinib and/or erlotinib resistant prior to initiation of treatment with such compounds. Gefitinib and/or erlotinib resistance in the tumor may occur after, e.g., 6 months or longer of gefitinib and/or erlotinib treatment. Alternatively, gefitinib and/or erlotinib resistance of the tumor may be diagnosed less than 6 months after gefitinib and/or erlotinib treatment has commenced. Diagnosis of gefitinib and/or erlotinib resistance may be accomplished by way of monitoring tumor progression during gefitinib and/or erlotinib treatment. Tumor progression may be determined by comparison of tumor status between time points after treatment has commenced or by comparison of tumor status between a time point after treatment has commenced to a time point prior to initiation of gefitinib and/or erlotinib treatment. Tumor progression may be monitored during gefitinib and/or erlotinib treatment visually, for example, by means of radiography, for example, X-ray, CT scan, or other monitoring methods known to the skilled artisan, including palpitation of the cancer or methods to monitor tumor biomarker levels. Progression of the cancer during treatment with gefitinib and/or erlotinib indicates gefitinib and/or erlotinib resistance. A rise in level of tumor biomarkers indicates tumor progression. Thus, a rise in tumor biomarker levels during treatment with gefitinib and/or erlotinib indicates gefitinib and/or erlotinib resistance. Detection of new tumors or detection of metastasis indicates tumor progression. Cessation of tumor shrinkage indicates tumor progression. Growth of the cancer is indicated by, for example, increase in tumor size, metastasis or detection of new cancer, and/or a rise in tumor biomarker levels.

The development of gefitinib and/or erlotinib resistance may be monitored by means of testing for presence of a gefitinib and/or erlotinib resistance associated mutation in circulating tumor cells obtained from the circulation, or other bodily fluid, of the subject. Presence of gefitinib and/or erlotinib resistance associated mutations in tumor cells from the subject is indicative of a gefitinib and/or erlotinib resistant tumor.

In one embodiment, the subject's tumor harbors mutations indicative of gefitinib and/or erlotinib sensitivity, yet it is resistant to gefitinib and/or erlotinib treatment. In one embodiment, the subject's tumor harbors mutations indicative gefitinib and/or erlotinib sensitivity and harbors mutations indicative of gefitinib and/or erlotinib resistance, e.g., the T790M mutation, that is, where a methione residue is substituted for the native threonine residue, in EGFR, e.g. increased EGFR internalization. In one embodiment, the subject's tumor does not harbor mutations indicative of gefitinib and/or erlotinib sensitivity and does harbor mutations indicative of gefitinib and/or erlotinib resistance, e.g., the T790M mutation in EGFR, e.g., increased EGFR internalization.

In connection with the administration of the drug, an "effective amount" indicates an amount that results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease load, reduction in tumor mass or cell numbers, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disease or condition.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration and the severity of the condition being treated. The skilled artisan is aware of the effective dose for each patient, which may vary with disease severity, individual genetic variation, or metabolic rate. However, in general, satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.5 to about 1000 mg/kg of body weight, optionally given in divided doses two to four times a day, or in sustained release form. The total daily dosage is projected to be from about 1 to 1000 mg, preferably from about 2 to 500 mg. Dosage forms suitable for internal use comprise from about 0.5 to 1000 mg of the active compound in

intimate admixture with a solid or liquid pharmaceutically acceptable carrier. This dosage regimen may be adjusted to provide the optimal therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The route of administration may be intravenous (I.V.), intramuscular (I.M.), subcutaneous (S.C.), intradermal (I.D.), intraperitoneal (I.P.), intrathecal (I.T.), intrapleural, intrauterine, rectal, vaginal, topical, intratumor and the like. The compounds of the invention can be administered parenterally by injection or by gradual infusion over time and can be delivered by peristaltic means.

Administration may be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays, for example, or using suppositories. For oral administration, the compounds of the invention are formulated into conventional oral administration forms such as capsules, tablets and tonics.

For topical administration, the pharmaceutical composition (inhibitor of kinase activity) is formulated into ointments, salves, gels, or creams, as is generally known in the art.

The therapeutic compositions of this invention, e.g. irreversible EGFR inhibitors, are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluents; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

The therapeutic composition useful for practicing the methods of the present invention, e.g. irreversible EGFR inhibitors, are described herein. Any formulation or drug delivery system containing the active ingredients, which is suitable for the intended use, as are generally known to those of skill in the art, can be used. Suitable pharmaceutically acceptable carriers for oral, rectal, topical or parenteral (including inhaled, subcutaneous, intraperitoneal, intramuscular and intravenous) administration are known to those of skill in the art. The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal without the production of undesirable physiological effects.

Formulations suitable for parenteral administration conveniently include sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient. Thus, such formulations may conveniently contain distilled water, 5% dextrose in distilled water or saline. Useful formulations also include concentrated solutions or solids containing the compound which upon dilution with an appropriate solvent give a solution suitable for parental administration above.

For enteral administration, a compound can be incorporated into an inert carrier in discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a suspension or solution in an aqueous liquid or non-aqueous liquid, e.g., a syrup, an elixir, an emulsion or a draught. Suitable carriers may be starches or sugars and include lubricants, flavorings, binders, and other materials of the same nature.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form, e.g., a powder or granules, optionally mixed with accessory ingredients, e.g., binders, lubricants, inert diluents, surface active or dispersing agents. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered active compound with any suitable carrier.

A syrup or suspension may be made by adding the active compound to a concentrated, aqueous solution of a sugar, e.g., sucrose, to which may also be added any accessory ingredients. Such accessory ingredients may include flavoring, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredient, e.g., as a polyhydric alcohol, for example, glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a conventional carrier, e.g., cocoa butter or Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany), for a suppository base.

Formulations for oral administration may be presented with an enhancer. Orally-acceptable absorption enhancers include surfactants such as sodium lauryl sulfate, palmitoyl carnitine, Laureth-9, phosphatidylcholine, cyclodextrin and derivatives thereof; bile salts such as sodium deoxycholate, sodium taurocholate, sodium glycochlate, and sodium fusidate; chelating agents including EDTA, citric acid and salicylates; and fatty acids (e.g., oleic acid, lauric acid, acylcarnitines, mono- and diglycerides). Other oral absorption enhancers include benzalkonium chloride, benzethonium chloride, CHAPS (3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate), Big-CHAPS (N, N-bis(3-D-gluconamidopropyl)-cholamide), chlorobutanol, octoxynol-9, benzyl alcohol, phenols, cresols, and alkyl alcohols. An especially preferred oral absorption enhancer for the present invention is sodium lauryl sulfate.

Alternatively, the compound may be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are well known to those of skill in the art. U.S. Pat. No. 4,789,734, the contents of which are hereby incorporated by reference, describes methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is provided by G. Gregoriadis, Chapter 14, "Liposomes," Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979).

Microspheres formed of polymers or proteins are well known to those skilled in the art, and can be tailored for

passage through the gastrointestinal tract directly into the blood stream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time ranging from days to months. See, for example, U.S. Pat. Nos. 4,906,474, 4,925,673 and 3,625,214, and Jein, TIPS 19:155-157 (1998), the contents of which are hereby incorporated by reference.

In one embodiment, the tyrosine kinase inhibitor of the present invention can be formulated into a liposome or microparticle which is suitably sized to lodge in capillary beds following intravenous administration. When the liposome or microparticle is lodged in the capillary beds surrounding ischemic tissue, the agents can be administered locally to the site at which they can be most effective. Suitable liposomes for targeting ischemic tissue are generally less than about 200 nanometers and are also typically unilamellar vesicles, as disclosed, for example, in U.S. Pat. No. 5,593,688 to Baldeschweiler, entitled "Liposomal targeting of ischemic tissue," the contents of which are hereby incorporated by reference.

Preferred microparticles are those prepared from biodegradable polymers, such as polyglycolide, polylactide and copolymers thereof. Those of skill in the art can readily determine an appropriate carrier system depending on various factors, including the desired rate of drug release and the desired dosage.

In one embodiment, the formulations are administered via catheter directly to the inside of blood vessels. The administration can occur, for example, through holes in the catheter. In those embodiments wherein the active compounds have a relatively long half life (on the order of 1 day to a week or more), the formulations can be included in biodegradable polymeric hydrogels, such as those disclosed in U.S. Pat. No. 5,410,016 to Hubbell et al. These polymeric hydrogels can be delivered to the inside of a tissue lumen and the active compounds released over time as the polymer degrades. If desirable, the polymeric hydrogels can have microparticles or liposomes which include the active compound dispersed therein, providing another mechanism for the controlled release of the active compounds.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or a finely divided solid carrier and then, if necessary, shaping the product into desired unit dosage form.

The formulations may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

Compounds of the present methods (i.e. irreversible EGFR inhibitors) may be presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less than 50 microns, preferably less than 10 microns, more preferably between 2 and 5 microns.

Generally for nasal administration a mildly acid pH will be preferred. Preferably the compositions of the invention have a pH of from about 3 to 5, more preferably from about 3.5 to about 3.9 and most preferably 3.7. Adjustment of the pH is achieved by addition of an appropriate acid, such as hydrochloric acid.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The irreversible kinase inhibitors of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

DEFINITIONS

The terms "ErbB1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to native sequence EGFR as disclosed, for example, in Carpenter et al. Ann. Rev. Biochem. 56:881-914 (1987), including variants thereof (e.g. a deletion mutant EGFR as in Humphrey et al. PNAS (USA) 87:4207-4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product. As used herein, the EGFR protein is disclosed as GenBank accession no. NP_005219 (SEQ ID NO: 1) which is encoded by the erbB1 gene, GenBank accession no. NM_005228 (SEQ ID NO: 2). Nucleotide and amino acid sequences of erbB1/EGFR may be found in FIG. 5.

The term "kinase activity increasing nucleic acid variance" as used herein refers to a variance (i.e. mutation) in the nucleotide sequence of a gene that results in an increased kinase activity. The increased kinase activity is a direct

result of the variance in the nucleic acid and is associated with the protein for which the gene encodes.

The term "drug" or "compound" as used herein refers to a chemical entity or biological product, or combination of chemical entities or biological products, administered to a person to treat or prevent or control a disease or condition. The chemical entity or biological product is preferably, but not necessarily a low molecular weight compound, but may also be a larger compound, for example, an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof.

As used herein, the terms "effective" and "effectiveness" includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the treatment to result in a desired biological effect in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (often referred to as side-effects) resulting from administration of the treatment. "Less effective" means that the treatment results in a therapeutically significant lower level of pharmacological effectiveness and/or a therapeutically greater level of adverse physiological effects.

Nucleic acid molecules can be isolated from a particular biological sample using any of a number of procedures, which are well-known in the art, the particular isolation procedure chosen being appropriate for the particular biological sample. For example, freeze-thaw and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from solid materials; heat and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from urine; and proteinase K extraction can be used to obtain nucleic acid from blood (Rolff, A et al. PCR: Clinical Diagnostics and Research, Springer (1994).

As used herein, a "cancer" in a subject or patient refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. In some circumstances, cancer cells will be in the form of a tumor, or such cells may exist locally within an animal, or circulate in the blood stream as independent cells.

EXAMPLES

Compounds

Compounds used herein, including EKB-569, HK1-357, and HK1-272 as described in U.S. Pat. No. 6,002,008; Greenberger et al., Proc. 11th NCI EORTC-AACR Symposium on New Drugs in Cancer Therapy, Clinical Cancer Res. Vol. 6 Supplement, November 2000, ISSN 1078-0432; in Rabindran et al., Cancer Res. 64: 3958-3965 (2004); Holbro and Hynes, Ann. Rev. Pharm. Tox. 44:195-217 (2004); and Tejpar et al., J. Clin. Oncol. ASCO Annual Meeting Proc. Vol. 22, No. 14S: 3579 (2004).

Analysis of Recurrent NSCLC and Generation of Gefitinib-Resistant NCI-H1650 Cells.

Clinical specimens of recurrent NSCLC were obtained at autopsy after appropriate consent. The entire kinase domain of EGFR was sequenced after analysis of uncloned PCR products. Multiple clones of exon 20 were sequenced to examine codon 790. Mutational analysis of EGFR (exons 1-28), ERBB2 (exons 1-24), PTEN (exons 1-9), Kras (codons 12, 13, and 61), and p53 (exons 5-8) in gefitinib-resistant clones as well as the parental NCI-H1650 cell line was performed by automated sequencing of individual exons and flanking intronic sequence (PCR conditions available on request) with bidirectional sequencing by using dye terminator chemistry (BIGDYE version 1.1, Applied Biosystems). Sequencing reactions were run on an ABI3100 sequencer (Applied Biosystems), and electropherograms were analyzed by using SEQUENCE NAVIGATOR and FACTURA software (Applied Biosystems).

To generate resistant subclones of NCI-H1650 cells, these were treated with ethyl methane sulfonate (EMS; 600 μg/ml), allowed to recover for 72 h, and then seeded at a density of 6×10^4 cells per 10-cm^2 dish in 20 μM gefitinib. Relative resistance of these cells to gefitinib, compared with the irreversible inhibitors, was achieved by seeding 5×10^4 cells in six-well plates in 5% FCS and 100 ng/ml EGF (Sigma), in the presence of varying concentrations of drugs, followed after 72 h by fixing cells with 4% formaldehyde, staining with 0.1% crystal violet, and quantifying cell mass by using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, Nebr.). For small interfering RNA (siRNA) knockdown experiments, cells were transfected with double-stranded RNA oligonucleotides targeting EGFR, ERBB2 (both SMARTpool from Dharmacon, Lafayette, Colo.), or nonspecific control (LRT1B), using X-treme GENE transfection reagent (Roche Applied Science). After 72 h, cells were stained with crystal violet and analyzed on the Odyssey Infrared scanner.

Immunoblotting and Signaling Studies.

Inhibition of EGFR signaling by increasing concentrations of gefitinib or the irreversible inhibitors was determined by seeding 9×10^4 cells in 24-well plates, adding the drugs to medium containing 5% FCS for 15 min, followed by a 2-h pulse with 100 ng/ml EGF, and harvesting of lysates. Lysates were prepared in 2× gel loading buffer, sonicated, boiled, and then separated by 10% SDS/PAGE, followed by electrotransfer to polyvinylidene fluoride (PVDF) membranes, and immunoblotting. Antibodies used were phospho-EGFR Y1068 and phospho-mitogen-activated protein kinase (MAPK) (Cell Signaling Technology, Beverly, Mass.), phospho-AKT (BioSource International, Camarillo, Calif.), and total EGFR, MAPK, AKT, and tubulin (Santa Cruz Biotechnology).

Analysis of EGFR Internalization.

To demonstrate internalization of EGFR by fluorescence microscopy, cells were grown on coverslips and incubated with 1 ng/ml recombinant human (rh) EGF (Molecular Probes, Eugene, Oreg.) for various intervals before fixing in 4% paraformaldehyde for 10 min. Coverslips were washed in PBS and mounted with ProLong Gold antifade reagent (Molecular Probes). To quantify EGFR internalization by cell surface biotinylation, cells were grown to confluency, pretreated with cyclohexamide, incubated on ice for 1 h with 1.5 mg/ml sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate (sulfo-NHS-SS-biotin; Pierce), and washed with blocking buffer (50 nM NH_4CL/1 mM MgCl/0.1 mM $CaCl_2$ in PBS) to quench free sulfo-NHS-SS-biotin, followed by several further washes with PBS. The cells were then incubated in culture medium at 37° C. for various intervals to allow internalization of the biotinylated molecules, washed twice for 20 min in a glutathione solution (50 mM glutathione/75 mM NaCl/75 mM NaOH/1% BSA) on ice to strip all of the biotinyl groups from the cell surface, and then scraped and lysed in 500 μM radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl, pH 7.4, with 150 mM NaCL/0.1% SDS/1% Triton X-100) supplemented with NaF, Na-orthovanadate, and protease inhibitors. Cell

extracts were centrifuged, and the supernatants were incubated with streptavidin beads (Sigma) to collect the biotinylated proteins, which were then analyzed by SDS/PAGE and immunoblotting with anti-EGFR antibody (SC-03, Santa Cruz Biotechnology) or antibody against transferrin receptor (Santa Cruz Biotechnology).

Results and Discussion

Analysis of Recurrent Lung Cancers with Acquired Resistance to Gefitinib.

Recurrent gefitinib-resistant NSCLC developed in two patients whose tumors had harbored an activating mutation of the EGFR kinase at the time of diagnosis and who had shown a dramatic initial clinical response to the drug (1). In both cases, progressive metastatic disease in the liver led to the patients' demises, 1-2 years after initiation of treatment. In case 1, analysis of the major liver metastasis obtained at the time of autopsy indicated persistence of the sensitizing EGFR mutation (L858R), as well as the presence of a newly acquired T790M mutation (FIG. 1A). Interestingly, analysis of uncloned PCR products showed the initial L858R mutation to be present at an abundance consistent with a heterozygous mutation that is present in all tumor cells, whereas the secondary T790M mutation was seen at approximately one-fifth the abundance of the corresponding wild-type allele. Thus, this resistance-associated mutation seems to be present in only a fraction of cells within the recurrent tumor.

Case 2 involved eight distinct recurrent metastases in the liver after the failure of gefitinib therapy. In all of these independent lesions, the sensitizing L861Q EGFR mutation was present at the expected ratio for a heterozygous mutation. No secondary EGFR mutation was detectable by analysis of uncloned PCR products from any of these metastases. However, after subcloning of the PCR products, the T790M mutation was found to be present at very low frequency in two of the four metastatic tumors analyzed (T790M, 2 of 50 clones sequenced from lesion 1 and 1 of 56 from lesion 2), but not from two other recurrent metastases (0 of 55 clones from lesion 3 and 0 of 59 from lesion 4), or the primary tumor (0 of 75 clones) (FIG. 1B and Table 1). Taken together, these results are consistent with previous reports that the T790M mutation is present in some, but not all, cases of acquired gefitinib resistance (three of seven tumors; see refs. 17, 18, and 21). Furthermore, as previously noted (18), even in some cases with this resistance-associated mutation, it seems to be present in only a small fraction of tumor cells within a recurrent lesion. These observations suggest that additional mechanisms of resistance are involved in cases without a secondary EGFR mutation and that such mechanisms coexist with the T790M mutation in other cases.

Generation of Gefitinib-Resistant Cell Lines with Susceptibility to Irreversible Inhibitors.

Given the excellent correlation between the clinical responsiveness of EGFR-mutant NSCLC and the enhanced gefitinib-sensitivity of NSCLC cell lines with these mutations (2, 6, 22, 23), and the limited availability of clinical specimens from relapsing patients, we modeled gefitinib resistance in vitro. We cultured the bronchoalveolar cancer cell line NCI-H1650, which has an in-frame deletion of the EGFR kinase (del E746-A750), in 20 μM gefitinib, either with or without prior exposure to the mutagen ethyl methane sulfonate. This cell line exhibits 100-fold increased sensitivity to gefitinib, compared with some NSCLC lines expressing wild-type EGFR (6). Whereas the vast majority of these cells are efficiently killed by 20 μM gefitinib, drug-resistant colonies were readily observed at a frequency of $\approx 10^{-5}$, irrespective of mutagen treatment. Forty-nine independent drug-resistant clones were isolated, showing an average 50-fold decrease in gefitinib sensitivity (FIG. 2A). All of these showed persistence of the sensitizing mutation without altered expression of EGFR, and none had acquired a secondary EGFR mutation or new mutations in ERBB2, p5.3, Kras, or PTEN. Gefitinib-resistant clones demonstrated comparable resistance to related inhibitors of the anilinoquinazoline class. Remarkably, however, they displayed persistent sensitivity to three inhibitors of the ERBB family (FIG. 2A): HKI-272 (24) and HKI-357 (compound 7f in ref. 25), which are dual inhibitors of EGFR and ERBB2 (IC_{50} values of 92 and 34 nM, respectively, for EGFR and 59 and 33 nM, respectively, for ERBB2), and EKB-569 (26), a selective inhibitor of EGFR (IC_{50} values of 39 nM for EGFR and 1.3 μM for ERBB2) (Wyeth) (FIG. 2B). All three drugs are irreversible inhibitors, most likely via a covalent bond with the cys773 residue within the EGFR catalytic domain or the cys805 of ERBB2. Like gefitinib, these compounds demonstrate increased killing of NSCLC cells harboring an EGFR mutation, compared with cells expressing wild-type receptor (FIG. 2A). However, in contrast to gefitinib, against which resistant clones are readily generated, even at high drug concentrations, we were unable to establish clones of cells that were resistant to the irreversible inhibitors at concentrations above 10 μM, even after ethyl methane sulfonate mutagenesis (FIG. 2C).

Dependence of Gefitinib-Resistant Cells on EGFR and ERBB2 Expression.

To gain insight into the mechanisms underlying the acquisition of gefitinib resistance and the persistent sensitivity to the irreversible inhibitors, we first determined whether resistant cell lines remain dependent upon EGFR for their viability. We have previously shown that siRNA-mediated knockdown of EGFR triggers apoptosis in cells harboring mutant EGFRs, but not in those with wild-type alleles (6). Significantly, parental NCI-H1650 cells as well as their gefitinib-resistant derivatives showed comparable reduction in cell viability after transfection with siRNA targeting EGFR (FIG. 3A). Thus, acquisition of gefitinib-resistance does not involve EGFR-independent activation of downstream effectors. Because HKI-272 and HKI-357 target both EGFR and ERBB2, we also tested suppression of this related receptor. Knockdown of ERBB2 in NCI-H1650 and its gefitinib-resistant derivatives also caused loss of viability (FIG. 3A), suggesting a role for EGFR-ERBB2 heterodimers in transducing essential survival signals in tumor cells harboring EGFR mutations. Inhibition of EGFR alone by an irreversible inhibitor seems to be sufficient to induce apoptosis in gefitinib-resistant cells, as demonstrated by the effectiveness of EKB-569, which primarily targets EGFR (26). However, given the potentially complementary effects of targeting both EGFR and ERBB2 by using siRNA and the availability of irreversible inhibitors that target both of these family members, the potential benefit of dual inhibition warrants consideration.

We compared the ability of gefitinib and irreversible ERBB family inhibitors to suppress signaling via downstream effectors of EGFR that mediate its proliferative and survival pathways. HKI-357 was 10-fold more effective than gefitinib in suppressing EGFR autophosphorylation (measured at residue Y1068), and AKT and MAPK phosphorylation in parental NCI-H1650 cells harboring the del E746-A750 EGFR mutation (FIG. 3B). In a gefitinib-resistant derivative, NCI-H1650(G7), gefitinib exhibited considerably reduced efficacy in suppressing AKT phosphorylation,

a key EGFR signaling effector linked to gefitinib responsiveness (6), whereas HKI-357 demonstrated persistent activity (FIG. 3B).

Altered EGFR Internalization in Gefitinib-Resistant Clones.

Given the absence of secondary mutations in EGFR and the persistent susceptibility of gefitinib-resistant cells to siRNA-mediated suppression of EGFR, we tested whether the mechanism underlying the differential inhibition of EGFR signaling in gefitinib-resistant cells by reversible and irreversible inhibitors might be correlated with alterations in receptor trafficking, a well documented modulator of EGFR-dependent signaling (20). Indeed, analysis of EGFR trafficking in NCI-H1650-derived resistant cells demonstrated a consistent increase in EGFR internalization, compared with the parental drug-sensitive cells, as measured both by internalization of fluorescein-labeled EGF (FIG. 3C) and quantitation of cytoplasmic biotinylated EGFR (FIG. 3D). No such effect was observed with the transferrin receptor, suggesting that this did not result from a generalized alteration in all receptor processing. Although further work is required to define the precise mechanism for this alteration in EGFR trafficking, a complex process in which numerous regulatory proteins have been implicated, these results suggest that gefitinib's ability to inhibit EGFR activation is compromised in these cells, whereas the action of the irreversible inhibitors are not detectably affected.

Inhibition of T790M EGFR Signaling and Enhanced Cell Killing by Irreversible Inhibitors.

The enhanced suppression of EGFR signaling by irreversible ERBB inhibitors raised the possibility that these drugs may also exhibit persistent activity in the context of cells harboring the T790M secondary mutation in EGFR. We therefore tested the effect of these inhibitors on the NCI-H1975 bronchoalveolar cancer cell line, which harbors both L858R and T790M mutations in EGFR (18). Significantly, this cell line was derived from a patient that had not been treated with an EGFR inhibitor, indicating that this mutation is not uniquely associated with acquired drug resistance. Both HKI-357 and HKI-272 were considerably more effective than gefitinib in suppressing ligand-induced EGFR autophosphorylation and its downstream signaling, as determined by AKT and MAPK phosphorylation (FIG. 4A). Similarly, all three irreversible inhibitors suppressed proliferation in this cell line under conditions where it is resistant to gefitinib (FIG. 4B). Thus, irreversible ERBB inhibitors seem to be effective in cells harboring the T790M EGFR as well as in cells with altered trafficking of the wild-type receptor.

Our results confirm the report of T790M mutations in EGFR as secondary mutations that arise in previously sensitive NSCLCs harboring an activating mutation, associated with the emergence of acquired resistance (17, 18). However, this mutation is present only in a subset of cases, and even tumors that harbor the T790M mutation may contain only a small fraction of cells with this mutation. These observations imply that multiple resistance mechanisms can coexist in recurrent tumors after an initial response to gefitinib or similar reversible EGFR inhibitors. Moreover, these findings suggest that T790M-independent resistance mechanisms may be equally, if not more, effective than the T790M substitution itself in conferring drug resistance and may explain why recurrent tumors rarely exhibit clonality for T790M (17, 18). In vitro mechanisms of acquired gefitinib resistance do not involve secondary EGFR mutations at a significant frequency, but instead are correlated with altered receptor trafficking. However, it should be noted that we have not examined EGFR trafficking in all of the resistant clones that we established in vitro, and it remains possible that additional mechanisms may contribute to gefitinib resistance in some of the clones. Nonetheless, virtually all gefitinib-resistant clones exhibited comparable sensitivity to the irreversible ERBB inhibitors.

Our results indicate striking differences between competitive EGFR inhibitors such as gefitinib, whose effectiveness is limited by the rapid development of drug resistance in vitro, and irreversible inhibitors, to which acquired resistance appears to be rare (FIG. 2C). We speculate that increased internalization of ligand-bound EGFR in resistant cells may be linked to dissociation of the gefitinib-EGFR complex at the low pH of intracellular vesicles. In contrast, irreversible cross-linking of the receptor would be unaffected by such alterations in receptor trafficking. Acquired resistance to gefitinib is stably maintained after passage of cells for up to 20 generations in the absence of drug, suggesting that genetic or epigenetic alterations in genes that modulate EGFR turnover may underlie this phenomenon. Because receptor trafficking cannot be readily studied by using available clinical specimens, identification of such genomic alterations may be required before clinical correlations are possible. Nonetheless, such a mechanism may contribute to in vivo acquired gefitinib-resistance in patients with recurrent disease who do not have secondary mutations in EGFR.

Irreversible ERBB inhibitors also seem to be effective in overcoming gefitinib resistance mediated by the T790M mutation, an effect that presumably results from the preservation of inhibitor binding despite alteration of this critical residue. While this work was in progress, another irreversible inhibitor of EGFR [CL-387,785, Calbiochem (27)] was shown to inhibit the kinase activity of the T790M EGFR mutant (17). The effectiveness of CL-387,785 in the context of T790M was proposed to result from the absence of a chloride at position 3 of the aniline group, which is present in gefitinib and was postulated to interfere sterically with binding to the mutant methionine at codon 790. However, EKB-569, HKI-272, and HKI-357 all have chloride moieties at that position in the aniline ring, suggesting that their shared ability to bind irreversibly to EGFR is likely to explain their effectiveness, rather than the absence of a specific steric interaction with T790M (24-26). Thus, these irreversible inhibitors may prove to be broadly effective in circumventing a variety of resistance mechanisms, in addition to the T790M mutation.

TABLE 1

Presence of EGFR T790M mutation at very low frequency in recurrent tumors from case 2

	No. of clones	
Tumor	T790M mutant	Wild type
Primary	0	75
Recurrent 1	2	48
Recurrent 2	1	55
Recurrent 3	0	55
Recurrent 4	0	59

Sequencing of large numbers of cloned PCR products revealed that a minority of alleles within two of four liver lesions contain the T790M mutation.

The references cited throughout the application are incorporated herein by reference in their entirety.

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SEQUENCE LISTING

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Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
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Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                85                  90                  95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
            100                 105                 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
        115                 120                 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160

Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175

Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190

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

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
                245                 250                 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300
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Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
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Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
            340                 345                 350

Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
        355                 360                 365

Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
    370                 375                 380

Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400

Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
                405                 410                 415

Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430

His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
        435                 440                 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460

Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465                 470                 475                 480

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
                485                 490                 495

Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
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Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
        515                 520                 525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
    530                 535                 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545                 550                 555                 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
                565                 570                 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
            580                 585                 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
        595                 600                 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
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Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625                 630                 635                 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
                645                 650                 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
            660                 665                 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
        675                 680                 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
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Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
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Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
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Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
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Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
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Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785                 790                 795                 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960

Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
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Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
   1010                1015                1020

Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025                1030                1035                1040

Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
               1045                1050                1055

Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
           1060                1065                1070

Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
       1075                1080                1085

Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
   1090                1095                1100

Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105                1110                1115                1120

Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
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Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
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Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
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ggccacctcg tcggcgtccg cccgagtccc cgcctcgccg ccaacgccac aaccaccgcg    180

cacggccccc tgactccgtc cagtattgat cgggagagcc ggagcgagct cttcggggag    240

cagcg atg cga ccc tcc ggg acg gcc ggg gca gcg ctc ctg gcg ctg ctg    290
      Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu
        1               5                  10                  15

gct gcg ctc tgc ccg gcg agt cgg gct ctg gag gaa aag aaa gtt tgc      338
Ala Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys
                20                  25                  30

caa ggc acg agt aac aag ctc acg cag ttg ggc act ttt gaa gat cat      386
Gln Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His
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ttt ctc agc ctc cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg      434
Phe Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly
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aat ttg gaa att acc tat gtg cag agg aat tat gat ctt tcc ttc tta      482
Asn Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu
    65                  70                  75

aag acc atc cag gag gtg gct ggt tat gtc ctc att gcc ctc aac aca      530
Lys Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr
80                  85                  90                  95

gtg gag cga att cct ttg gaa aac ctg cag atc atc aga gga aat atg      578
Val Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met
                100                 105                 110

tac tac gaa aat tcc tat gcc tta gca gtc tta tct aac tat gat gca      626
Tyr Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala
            115                 120                 125

aat aaa acc gga ctg aag gag ctg ccc atg aga aat tta cag gaa atc      674
Asn Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile
        130                 135                 140

ctg cat ggc gcc gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg      722
Leu His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val
    145                 150                 155

gag agc atc cag tgg cgg gac ata gtc agc agt gac ttt ctc agc aac      770
Glu Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn
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atg tcg atg gac ttc cag aac cac ctg ggc agc tgc caa aag tgt gat      818
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Gln Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys
        210                 215                 220

cgt ggc aag tcc ccc agt gac tgc tgc cac aac cag tgt gct gca ggc      962
Arg Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly
    225                 230                 235

tgc aca ggc ccc cgg gag agc gac tgc ctg gtc tgc cgc aaa ttc cga     1010
Cys Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg
240                 245                 250                 255

gac gaa gcc acg tgc aag gac acc tgc ccc cca ctc atg ctc tac aac     1058
Asp Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn
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ggt gcc acc tgc gtg aag aag tgt ccc cgt aat tat gtg gtg aca gat     1154
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        290                 295                 300

cac ggc tcg tgc gtc cga gcc tgt ggg gcc gac agc tat gag atg gag     1202
His Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu
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gaa gac ggc gtc cgc aag tgt aag aag tgc gaa ggg cct tgc cgc aaa     1250
Glu Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys
320                 325                 330                 335

gtg tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata     1298
Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile
                340                 345                 350

aat gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc     1346
Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
            355                 360                 365

gat ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat     1394
Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His
        370                 375                 380

act cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag     1442
Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
    385                 390                 395

gaa atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg     1490
Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
400                 405                 410                 415

gac ctc cat gcc ttt gag aac cta gaa atc ata cgc ggc agg acc aag     1538
Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
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caa cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc     1586
Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
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ttg gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att     1634
Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
        450                 455                 460

tca gga aac aaa aat ttg tgc tat gca aat aca ata aac tgg aaa aaa     1682
Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
    465                 470                 475

ctg ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt     1730
Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
480                 485                 490                 495

gaa aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc     1778
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aat gtc agc cga ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag     1874
Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu
        530                 535                 540

ggt gag cca agg gag ttt gtg gag aac tct gag tgc ata cag tgc cac     1922
Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His
    545                 550                 555

cca gag tgc ctg cct cag gcc atg aac atc acc tgc aca gga cgg gga     1970
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560                 565                 570                 575

cca gac aac tgt atc cag tgt gcc cac tac att gac ggc ccc cac tgc     2018
Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys
                580                 585                 590

gtc aag acc tgc ccg gca gga gtc atg gga gaa aac aac acc ctg gtc     2066
Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val
            595                 600                 605

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Trp Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn
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tgc acc tac gga tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat     2162
Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn
    625                 630                 635

ggg cct aag atc ccg tcc atc gcc act ggg atg gtg ggg gcc ctc ctc     2210
Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu
640                 645                 650                 655

ttg ctg ctg gtg gtg gcc ctg ggg atc ggc ctc ttc atg cga agg cgc     2258
Leu Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg
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cac atc gtt cgg aag cgc acg ctg cgg agg ctg ctg cag gag agg gag     2306
His Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu
            675                 680                 685

ctt gtg gag cct ctt aca ccc agt gga gaa gct ccc aac caa gct ctc     2354
Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu
        690                 695                 700

ttg agg atc ttg aag gaa act gaa ttc aaa aag atc aaa gtg ctg ggc     2402
Leu Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly
    705                 710                 715

tcc ggt gcg ttc ggc acg gtg tat aag gga ctc tgg atc cca gaa ggt     2450
Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly
720                 725                 730                 735

gag aaa gtt aaa att ccc gtc gct atc aag gaa tta aga gaa gca aca     2498
Glu Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr
                740                 745                 750

tct ccg aaa gcc aac aag gaa atc ctc gat gaa gcc tac gtg atg gcc     2546
Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala
            755                 760                 765

agc gtg gac aac ccc cac gtg tgc cgc ctg ctg ggc atc tgc ctc acc     2594
Ser Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr
        770                 775                 780

tcc acc gtg cag ctc atc acg cag ctc atg ccc ttc ggc tgc ctc ctg     2642
Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu
    785                 790                 795

gac tat gtc cgg gaa cac aaa gac aat att ggc tcc cag tac ctg ctc     2690
Asp Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu
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cgc ttg gtg cac cgc gac ctg gca gcc agg aac gta ctg gtg aaa aca     2786
Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr
            835                 840                 845

ccg cag cat gtc aag atc aca gat ttt ggg ctg gcc aaa ctg ctg ggt     2834
Pro Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly
        850                 855                 860

gcg gaa gag aaa gaa tac cat gca gaa gga ggc aaa gtg cct atc aag     2882
Ala Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys
    865                 870                 875

tgg atg gca ttg gaa tca att tta cac aga atc tat acc cac cag agt     2930
Trp Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser
880                 885                 890                 895

gat gtc tgg agc tac ggg gtg acc gtt tgg gag ttg atg acc ttt gga     2978
Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly
                900                 905                 910

tcc aag cca tat gac gga atc cct gcc agc gag atc tcc tcc atc ctg     3026
Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu
            915                 920                 925

gag aaa gga gaa cgc ctc cct cag cca ccc ata tgt acc atc gat gtc     3074
Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val
        930                 935                 940

tac atg atc atg gtc aag tgc tgg atg ata gac gca gat agt cgc cca     3122
Tyr Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro
    945                 950                 955

aag ttc cgt gag ttg atc atc gaa ttc tcc aaa atg gcc cga gac ccc     3170
Lys Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro
960                 965                 970                 975

cag cgc tac ctt gtc att cag ggg gat gaa aga atg cat ttg cca agt     3218
Gln Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser
                980                 985                 990

cct aca gac tcc aac ttc tac cgt gcc ctg atg gat gaa gaa gac atg     3266
Pro Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met
            995                1000                1005

gac gac gtg gtg gat gcc gac gag tac ctc atc cca cag cag ggc ttc     3314
Asp Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe
       1010                1015                1020

ttc agc agc ccc tcc acg tca cgg act ccc ctc ctg agc tct ctg agt     3362
Phe Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser
   1025                1030                1035

gca acc agc aac aat tcc acc gtg gct tgc att gat aga aat ggg ctg     3410
Ala Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu
1040                1045                1050                1055

caa agc tgt ccc atc aag gaa gac agc ttc ttg cag cga tac agc tca     3458
Gln Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser
                1060                1065                1070

gac ccc aca ggc gcc ttg act gag gac agc ata gac gac acc ttc ctc     3506
Asp Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu
           1075                1080                1085

cca gtg cct gaa tac ata aac cag tcc gtt ccc aaa agg ccc gct ggc     3554
Pro Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly
       1090                1095                1100

tct gtg cag aat cct gtc tat cac aat cag cct ctg aac ccc gcg ccc     3602
Ser Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro
   1105                1110                1115

agc aga gac cca cac tac cag gac ccc cac agc act gca gtg ggc aac     3650
Ser Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn
1120                1125                1130                1135
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Pro Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe
              1140                1145                1150

gac agc cct gcc cac tgg gcc cag aaa ggc agc cac caa att agc ctg     3746
Asp Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu
          1155                1160                1165

gac aac cct gac tac cag cag gac ttc ttt ccc aag gaa gcc aag cca     3794
Asp Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro
       1170                1175                1180

aat ggc atc ttt aag ggc tcc aca gct gaa aat gca gaa tac cta agg     3842
Asn Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg
   1185                1190                1195

gtc gcg cca caa agc agt gaa ttt att gga gca tga                     3878
Val Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
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      oligonucleotide

<400> SEQUENCE: 5

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The invention claimed is:

1. A method for treating gefitinib and/or erlotinib resistant non-small cell lung cancer in a patient in need thereof, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer a pharmaceutical composition comprising a unit dosage of an irreversible epidermal growth factor receptor (EGFR) inhibitor that covalently binds to cysteine 773 residue in the ligand-binding pocket of EGFR or cysteine 805 residue in the ligand-binding pocket of erb-B2.

2. The method of claim **1**, wherein the irreversible EGFR inhibitor is EKB-569 or HKI-357.

3. The method of claim **1**, wherein the irreversible EGFR inhibitor covalently binds to cysteine 773 residue of EGFR.

4. The method of claim **1**, wherein the irreversible EGFR inhibitor covalently binds to cysteine 805 residue of erb-B2.

5. The method of claim **1**, wherein the method further comprises administering at least one other tyrosine kinase inhibitor.

6. The method of claim **1**, wherein the method further comprises administering radiation.

7. The method of claim **1**, wherein the route of administering is intravenous, intramuscular, subcutaneous, intradermal, intraperitoneal, intrathecal, intrapleural, intrauterine, rectal, vaginal, topical, or intratumor.

8. The method of claim **1**, wherein the route of administering is transmucosal or transdermal.

9. The method of claim **1**, wherein the route of administering is oral.

* * * * *

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

(10) **Patent No.: US 10,596,162 B2**
(45) **Date of Patent: *Mar. 24, 2020**

(54) **METHOD FOR TREATING GEFITINIB RESISTANT CANCER**

(71) Applicants: **WYETH LLC**, New York, NY (US); **The General Hospital Corporation**, Boston, MA (US)

(72) Inventors: **Daniel A. Haber**, Chestnut Hill, MA (US); **Daphne Winifred Bell**, Chevy Chase, MD (US); **Jeffrey E. Settleman**, Newton, MA (US); **Raffaella Sordella**, Cold Spring Harbor, NY (US); **Nadia G. Godin-Heymann**, Middlesex (GB); **Eunice L. Kwak**, Marlborough, MA (US); **Sridhar Krishna Rabindran**, Eagleville, PA (US)

(73) Assignees: **Wyeth LLC**, New York, NY (US); **The General Hospital Corporation**, Boston, MA (US)

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This patent is subject to a terminal disclaimer.

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(52) **U.S. Cl.**

CPC ***A61K 31/4709*** (2013.01); ***A61K 31/4706*** (2013.01); ***A61K 38/17*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Peter J Reddig

(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

The present invention is directed to methods for the treatment of gefitinib and/or erlotinib resistant cancer. An individual with cancer is monitored for cancer progression following treatment with gefitinib and/or erlotinib. Progression of the cancer is indicative that the cancer is resistant to gefitinib and/or erlotinib. Once progression of cancer is noted, the subject is administered a pharmaceutical composition comprising an irreversible epidermal growth factor receptor (EGFR) inhibitor. In preferred embodiments, the irreversible EGFR inhibitor is EKB 569, HKI-272 and HKI-357.

4 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

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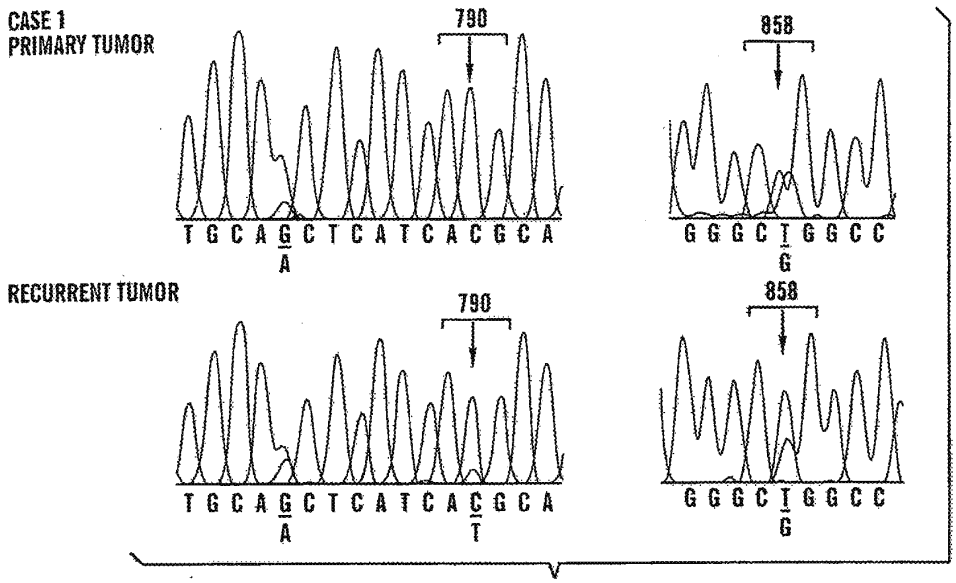

CASE 1
PRIMARY TUMOR
790
T G C A G C T C A T C A C G C A
A
858
G G G C T G G C C
G
RECURRENT TUMOR
790
T G C A G C T C A T C A C G C A
A
T
858
G G G C T G G C C
G

FIG. 1A

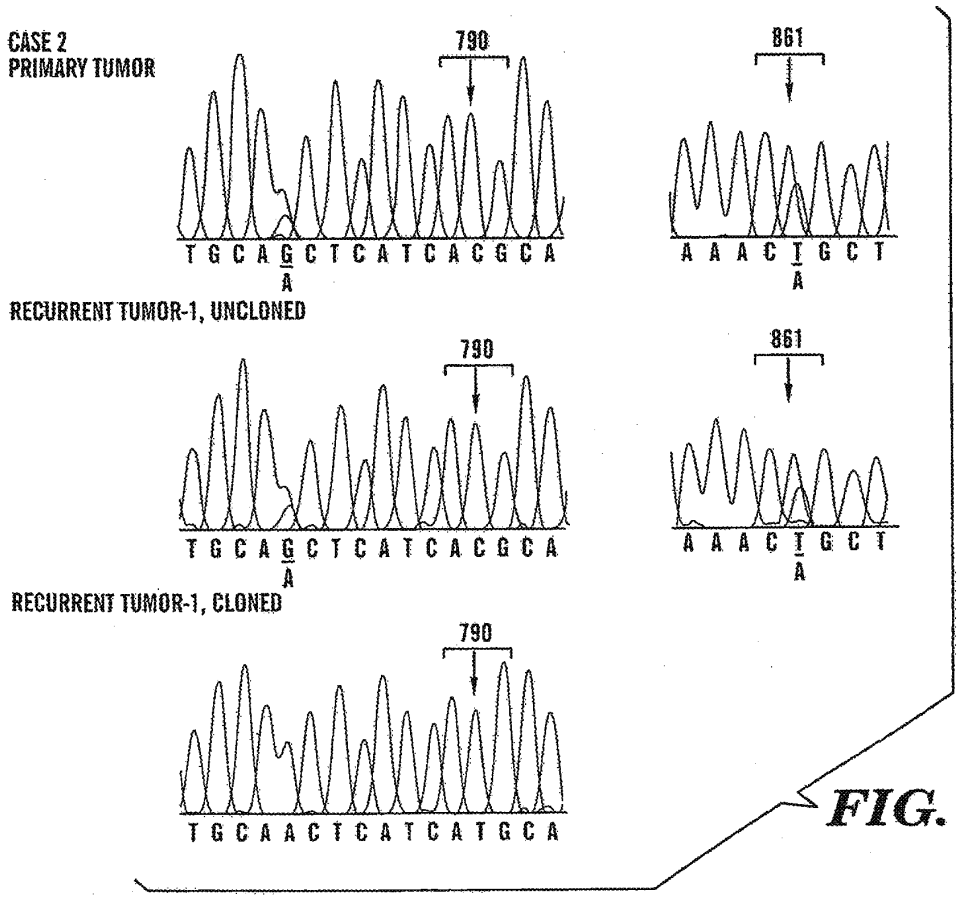
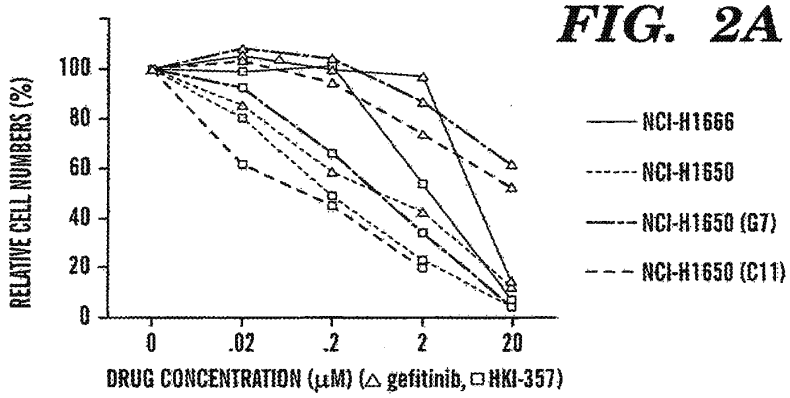
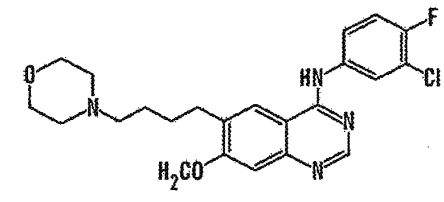
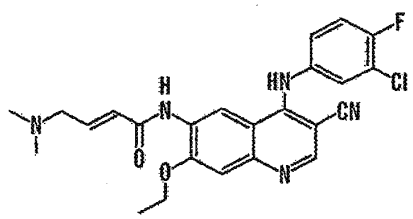

CASE 2
PRIMARY TUMOR
790
T G C A G C T C A T C A C G C A
A
861
A A A C T G C T
A
RECURRENT TUMOR-1, UNCLONED
790
T G C A G C T C A T C A C G C A
A
861
A A A C T G C T
A
RECURRENT TUMOR-1, CLONED
790
T G C A A C T C A T C A T G C A

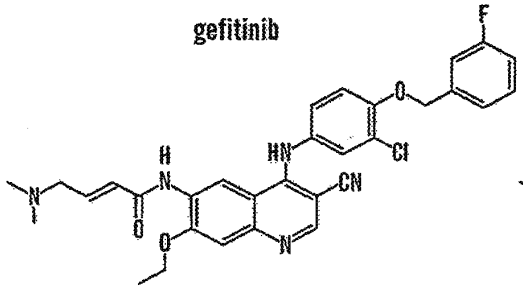
FIG. 1B

FIG. 2A









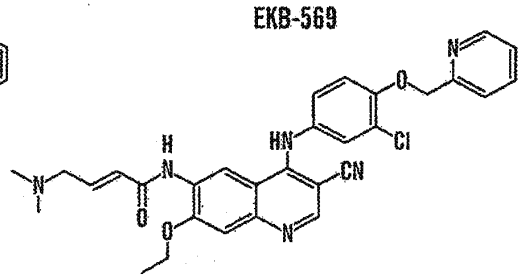


FIG. 2B

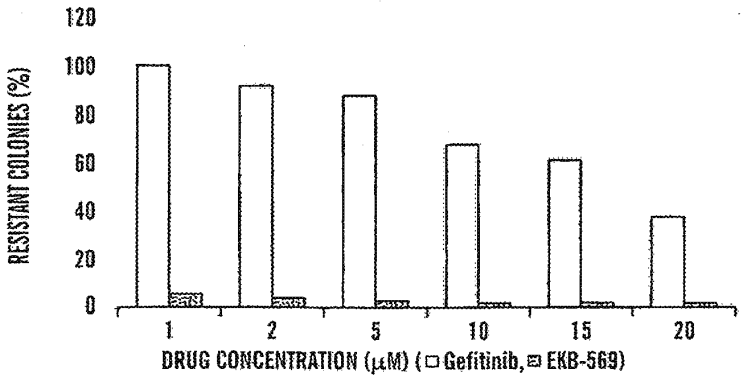


FIG. 2C

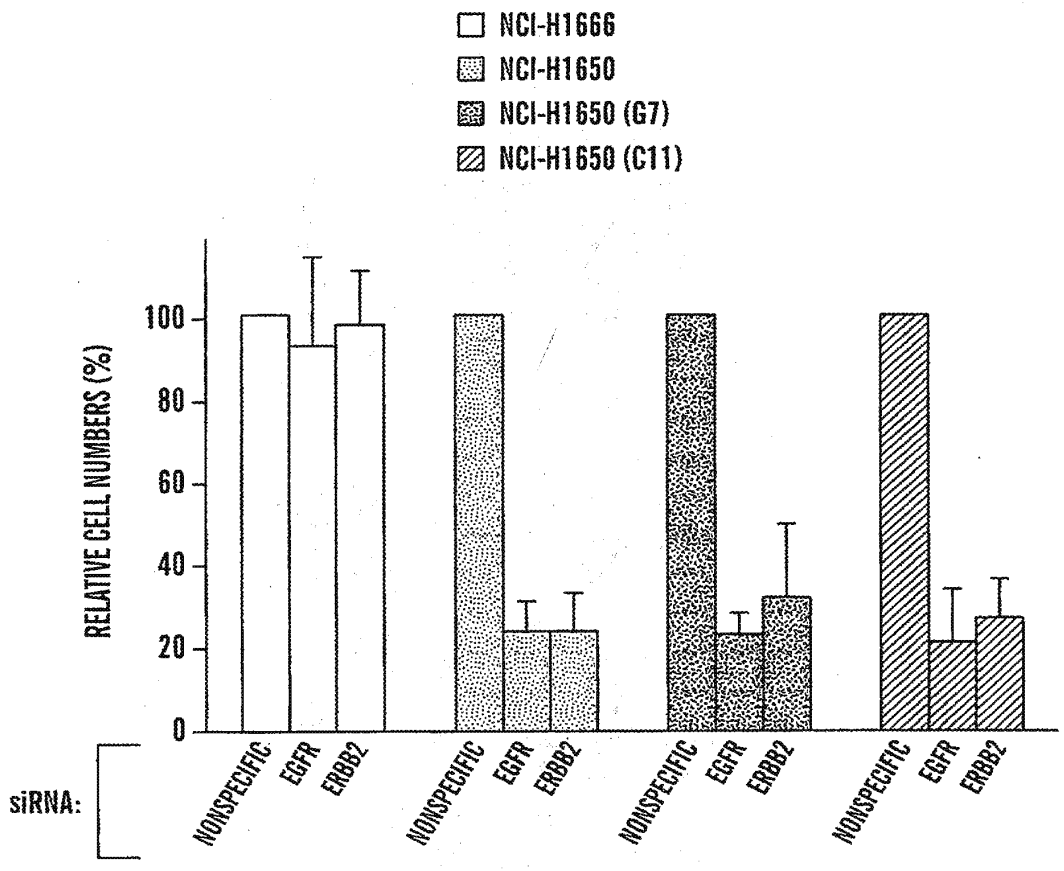

NCI-H1666
NCI-H1650
NCI-H1650 (G7)
NCI-H1650 (C11)
RELATIVE CELL NUMBERS (%)
0
20
40
60
80
100
siRNA:
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2
NONSPECIFIC
EGFR
ERBB2

FIG. 3A

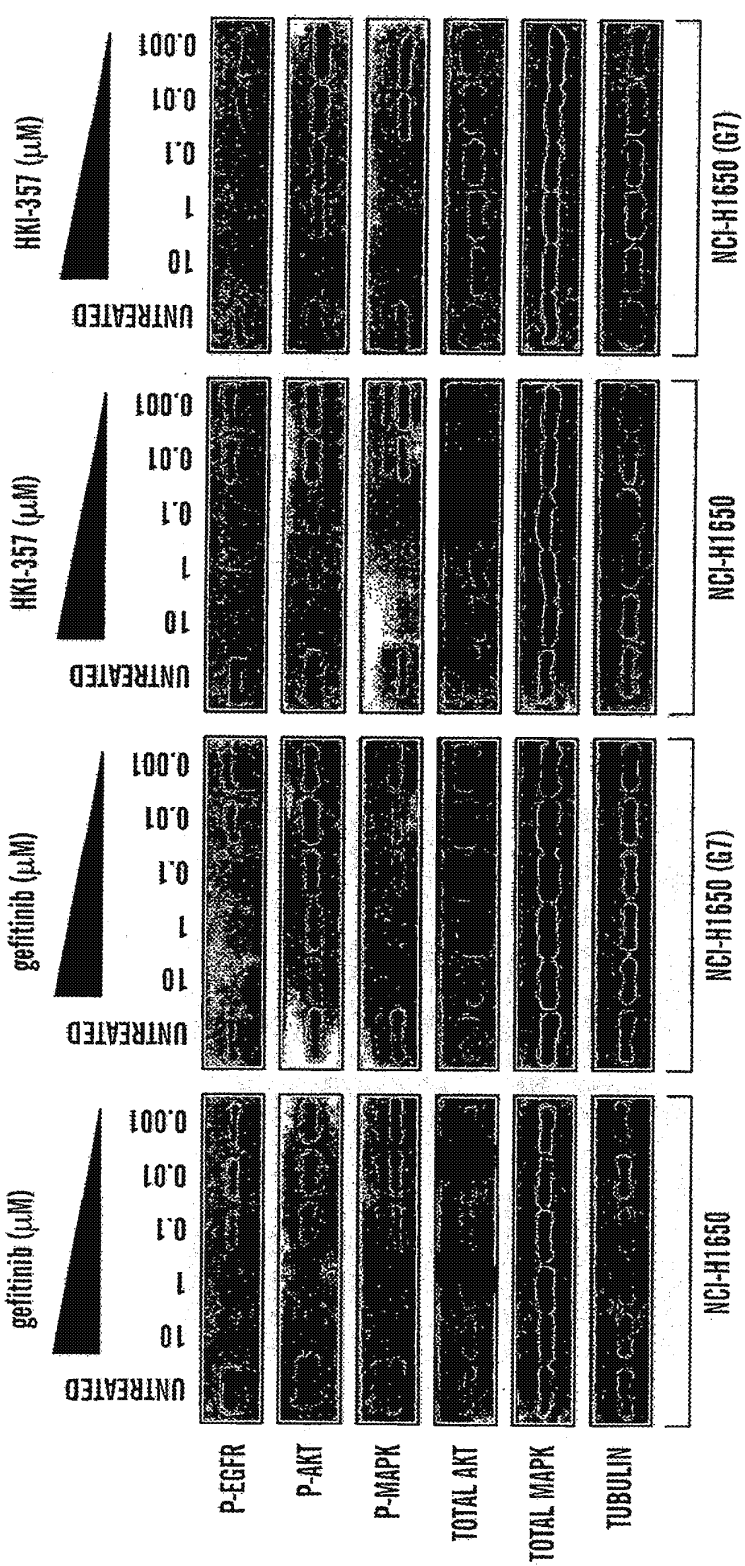

HKI-357 (μM)
UNTREATED
10
1
0.1
0.01
0.001
NCI-H1650 (G7)
NCI-H1650
gefitinib (μM)
P-EGFR
P-AKT
P-MAPK
TOTAL AKT
TOTAL MAPK
TUBULIN

FIG. 3B

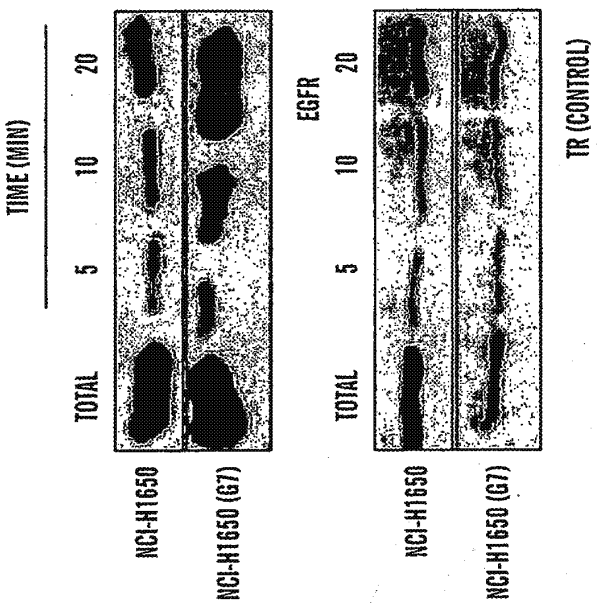

TIME (MIN)
TOTAL
5
10
20
EGFR
TOTAL
5
10
20
TR (CONTROL)
NCI-H1650
NCI-H1650 (G7)
NCI-H1650
NCI-H1650 (G7)

FIG. 3D

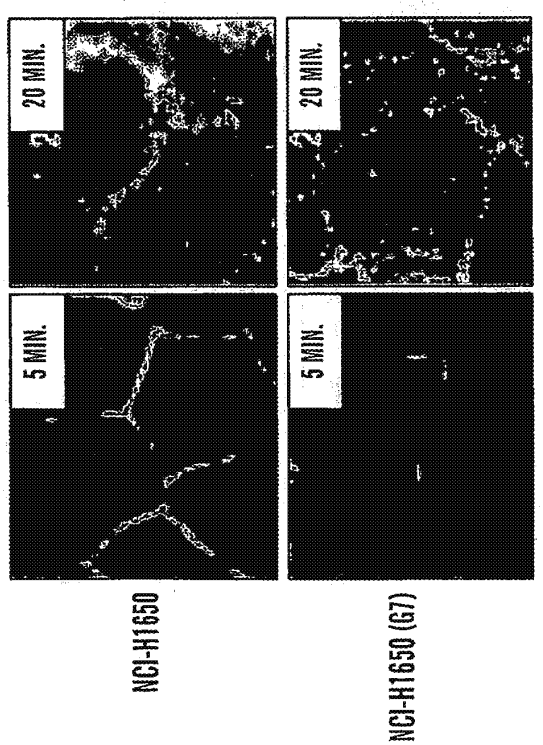

20 MIN.
20 MIN.
5 MIN.
5 MIN.
NCI-H1650
NCI-H1650 (G7)

FIG. 3C

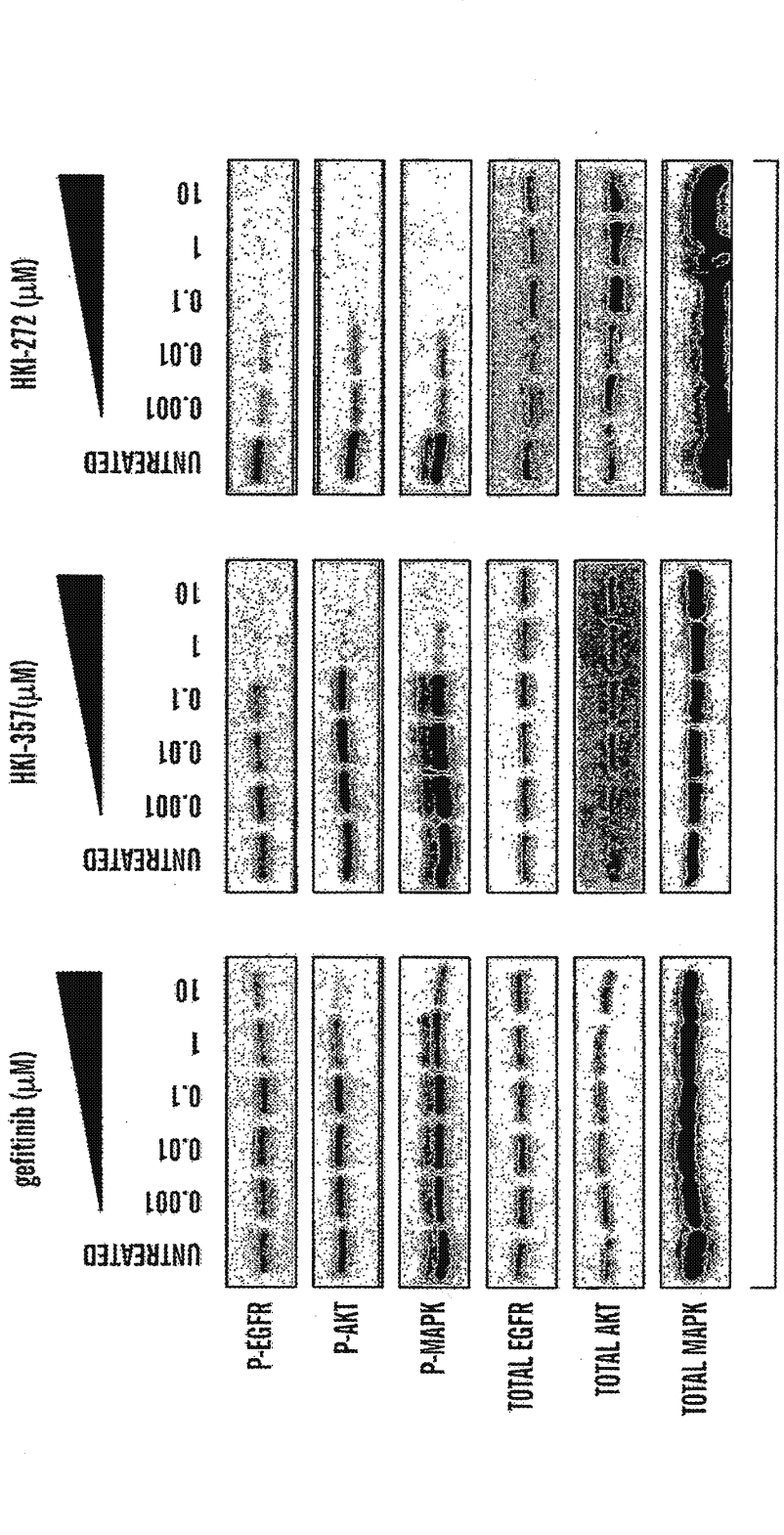

HKI-272 (μM)
UNTREATED
0.001
0.01
0.1
1
10
HKI-357(μM)
UNTREATED
0.001
0.01
0.1
1
10
gefitinib (μM)
UNTREATED
0.001
0.01
0.1
1
10
NCI-H1975
P-EGFR
P-AKT
P-MAPK
TOTAL EGFR
TOTAL AKT
TOTAL MAPK

FIG. 4A

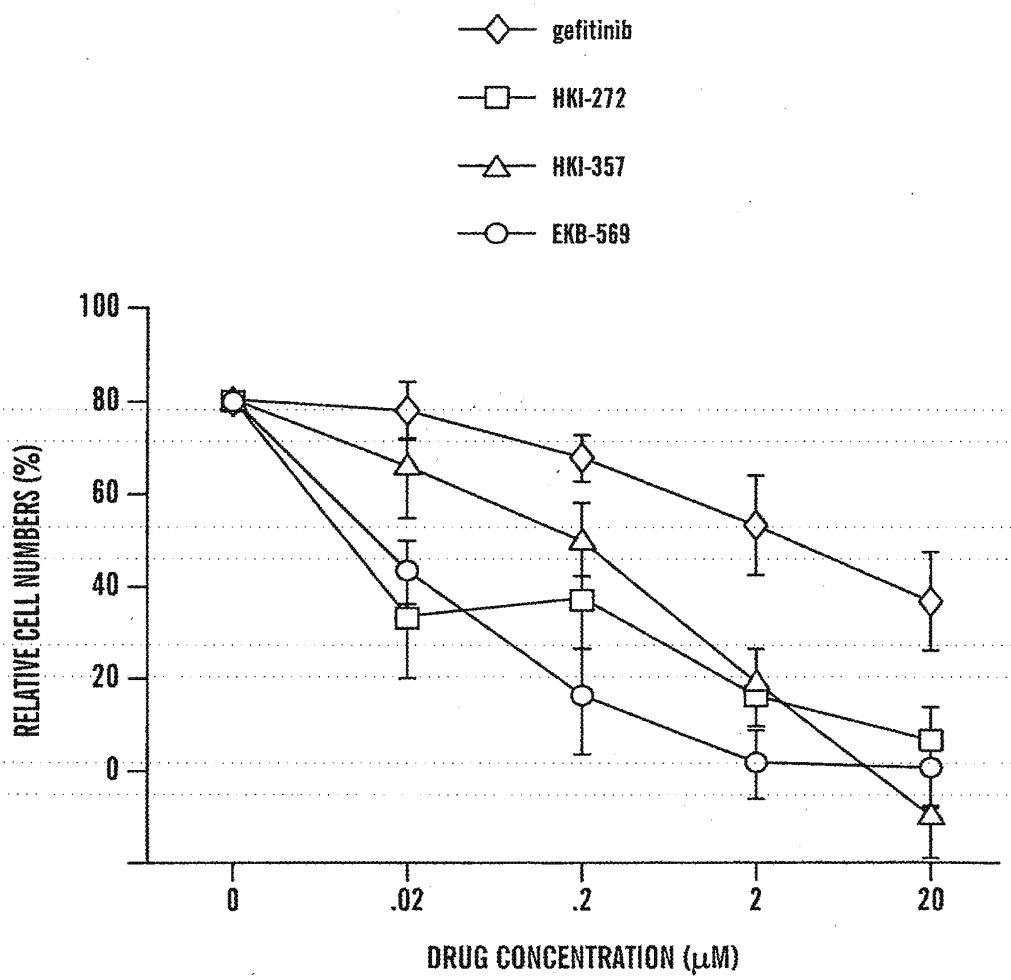

gefitinib
HKI-272
HKI-357
EKB-569
100
80
60
40
20
0
RELATIVE CELL NUMBERS (%)
0
.02
.2
2
20
DRUG CONCENTRATION (μM)

FIG. 4B

```
CCCGGCGCAGCGCGGCCGCAGCAGCCTCCGCCCCCCGCACGGTGTGAGCGCCCGACGCGG -185
.....

CCGAGGCGGCCGGAGTCCCGAGCTAGCCCCGGCGGCCGCCGCCGCCCAGACCGGACGACA -125
.....

GGCCACCTCGTCGGCGTCCGCCCGAGTCCCCGCCTCGCCGCCAACGCCACAACCACCGCG -65
.....

CACGGCCCCCTGACTCCGTCCAGTATTGATCGGGAGAGCCGGAGCGAGCTCTTCGGGGAG -5
.....

CAGCGATGCGACCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCT 55
.....-M--R--P--S--G--T--A--G--A--A--L--L--A--L--L--A--A--L-- 18

GCCCGGCGAGTCGGGCTCTGGAGGAAAAGAAAGTTTGCCAAGGCACGAGTAACAAGCTCA 115
C--P--A--S--R--A--L--E--E--K--K--V--C--Q--G--T--S--N--K--L-- 38

CGCAGTTGGGCACTTTTGAAGATCATTTTCTCAGCCTCCAGAGGATGTTCAATAACTGTG 175
T--Q--L--G--T--F--E--D--H--F--L--S--L--Q--R--M--F--N--N--C-- 58

AGGTGGTCCTTGGGAATTTGGAAATTACCTATGTGCAGAGGAATTATGATCTTTCCTTCT 235
E--V--V--L--G--N--L--E--I--T--Y--V--Q--R--N--Y--D--L--S--F-- 78

TAAAGACCATCCAGGAGGTGGCTGGTTATGTCCTCATTGCCCTCAACACAGTGGAGCGAA 295
L--K--T--I--Q--E--V--A--G--Y--V--L--I--A--L--N--T--V--E--R-- 98

TTCCTTTGGAAAACCTGCAGATCATCAGAGGAAATATGTACTACGAAAATTCCTATGCCT 355
I--P--L--E--N--L--Q--I--I--R--G--N--M--Y--Y--E--N--S--Y--A-- 118

TAGCAGTCTTATCTAACTATGATGCAAATAAAACCGGACTGAAGGAGCTGCCCATGAGAA 415
L--A--V--L--S--N--Y--D--A--N--K--T--G--L--K--E--L--P--M--R-- 138

ATTTACAGGAAATCCTGCATGGCGCCGTGCGGTTCAGCAACAACCCTGCCCTGTGCAACG 475
N--L--Q--E--I--L--H--G--A--V--R--F--S--N--N--P--A--L--C--N-- 158

TGGAGAGCATCCAGTGGCGGGACATAGTCAGCAGTGACTTTCTCAGCAACATGTCGATGG 535
V--E--S--I--Q--W--R--D--I--V--S--S--D--F--L--S--N--M--S--M-- 178

ACTTCCAGAACCACCTGGGCAGCTGCCAAAAGTGTGATCCAAGCTGTCCCAATGGGAGCT 595
D--F--Q--N--H--L--G--S--C--Q--K--C--D--P--S--C--P--N--G--S-- 198

GCTGGGGTGCAGGAGAGGAGAACTGCCAGAAACTGACCAAAATCATCTGTGCCCAGCAGT 655
C--W--G--A--G--E--E--N--C--Q--K--L--T--K--I--I--C--A--Q--Q-- 218

GCTCCGGGCGCTGCCGTGGCAAGTCCCCCAGTGACTGCTGCCACAACCAGTGTGCTGCAG 715
C--S--G--R--C--R--G--K--S--P--S--D--C--C--H--N--Q--C--A--A-- 238

GCTGCACAGGCCCCCGGGAGAGCGACTGCCTGGTCTGCCGCAAATTCCGAGACGAAGCCA 775
G--C--T--G--P--R--E--S--D--C--L--V--C--R--K--F--R--D--E--A-- 258

CGTGCAAGGACACCTGCCCCCCACTCATGCTCTACAACCCCACCACGTACCAGATGGATG 835
T--C--K--D--T--C--P--P--L--M--L--Y--N--P--T--T--Y--Q--M--D-- 278

TGAACCCCGAGGGCAAATACAGCTTTGGTGCCACCTGCGTGAAGAAGTGTCCCCGTAATT 895
V--N--P--E--G--K--Y--S--F--G--A--T--C--V--K--K--C--P--R--N-- 298
```

FIG. 5

```
ATGTGGTGACAGATCACGGCTCGTGCGTCCGAGCCTGTGGGGCCGACAGCTATGAGATGG 955
Y--V--V--T--D--H--G--S--C--V--R--A--C--G--A--D--S--Y--E--M-- 318

AGGAAGACGGCGTCCGCAAGTGTAAGAAGTGCGAAGGGCCTTGCCGCAAAGTGTGTAACG 1015
E--E--D--G--V--R--K--C--K--K--C--E--G--P--C--R--K--V--C--N-- 338

GAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAAACACT 1075
G--I--G--I--G--E--F--K--D--S--L--S--I--N--A--T--N--I--K--H-- 358

TCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGGCATTTAGGGGTG 1135
F--K--N--C--T--S--I--S--G--D--L--H--I--L--P--V--A--F--R--G-- 378

ACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCTGAAAACCGTAA 1195
D--S--F--T--H--T--P--P--L--D--P--Q--E--L--D--I--L--K--T--V-- 398

AGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATG 1255
K--E--I--T--G--F--L--L--I--Q--A--W--P--E--N--R--T--D--L--H-- 418

CCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTTG 1315
A--F--E--N--L--E--I--I--R--G--R--T--K--Q--H--G--Q--F--S--L-- 438

CAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATG 1375
A--V--V--S--L--N--I--T--S--L--G--L--R--S--L--K--E--I--S--D-- 458

GAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAA 1435
G--D--V--I--I--S--G--N--K--N--L--C--Y--A--N--T--I--N--W--K-- 478

AACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGCT 1495
K--L--F--G--T--S--G--Q--K--T--K--I--I--S--N--R--G--E--N--S-- 498

GCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGG 1555
C--K--A--T--G--Q--V--C--H--A--L--C--S--P--E--G--C--W--G--P-- 518

AGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGT 1615
E--P--R--D--C--V--S--C--R--N--V--S--R--G--R--E--C--V--D--K-- 538

GCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCC 1675
C--N--L--L--E--G--E--P--R--E--F--V--E--N--S--E--C--I--Q--C-- 558

ACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACT 1735
H--P--E--C--L--P--Q--A--M--N--I--T--C--T--G--R--G--P--D--N-- 578

GTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAG 1795
C--I--Q--C--A--H--Y--I--D--G--P--H--C--V--K--T--C--P--A--G-- 598

TCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACC 1855
V--M--G--E--N--N--T--L--V--W--K--Y--A--D--A--G--H--V--C--H-- 618

TGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGA 1915
L--C--H--P--N--C--T--Y--G--C--T--G--P--G--L--E--G--C--P--T-- 638

ATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGG 1975
N--G--P--K--I--P--S--I--A--T--G--M--V--G--A--L--L--L--L--L-- 658

TGGTGGCCCTGGGGATCGGCCTCTTCATGCGAAGGCGCCACATCGTTCGGAAGCGCACGC 2035
V--V--A--L--G--I--G--L--F--M--R--R--R--H--I--V--R--K--R--T-- 678
```

FIG. 5 (con'd.)

```
TGCGGAGGCTGCTGCAGGAGAGGGAGCTTGTGGAGCCTCTTACACCCAGTGGAGAAGCTC 2095
L--R--R--L--L--Q--E--R--E--L--V--E--P--L--T--P--S--G--E--A-- 698

CCAACCAAGCTCTCTTGAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGG 2155
P--N--Q--A--L--L--R--I--L--K--E--T--E--F--K--K--I--K--V--L-- 718

GCTCCGGTGCGTTCGGCACGGTGTATAAGGGACTCTGGATCCCAGAAGGTGAGAAAGTTA 2215
G--S--G--A--F--G--T--V--Y--K--G--L--W--I--P--E--G--E--K--V-- 738

AAATTCCCGTCGCTATCAAGGAATTAAGAGAAGCAACATCTCCGAAAGCCAACAAGGAAA 2275
K--I--P--V--A--I--K--E--L--R--E--A--T--S--P--K--A--N--K--E-- 758

TCCTCGATGAAGCCTACGTGATGGCCAGCGTGGACAACCCCCACGTGTGCCGCCTGCTGG 2335
I--L--D--E--A--Y--V--M--A--S--V--D--N--P--H--V--C--R--L--L-- 778

GCATCTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTCC 2395
G--I--C--L--T--S--T--V--Q--L--I--T--Q--L--M--P--F--G--C--L-- 798

TGGACTATGTCCGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAACTGGTGTG 2455
L--D--Y--V--R--E--H--K--D--N--I--G--S--Q--Y--L--L--N--W--C-- 818

TGCAGATCGCAAAGGGCATGAACTACTTGGAGGACCGTCGCTTGGTGCACCGCGACCTGG 2515
V--Q--I--A--K--G--M--N--Y--L--E--D--R--R--L--V--H--R--D--L-- 838

CAGCCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGG 2575
A--A--R--N--V--L--V--K--T--P--Q--H--V--K--I--T--D--F--G--L-- 858

CCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTGCCTATCA 2635
A--K--L--L--G--A--E--E--K--E--Y--H--A--E--G--G--K--V--P--I-- 878

AGTGGATGGCATTGGAATCAATTTTACACAGAATCTATACCCACCAGAGTGATGTCTGGA 2695
K--W--M--A--L--E--S--I--L--H--R--I--Y--T--H--Q--S--D--V--W-- 898

GCTACGGGGTGACTGTTTGGGAGTTGATGACCTTTGGATCCAAGCCATATGACGGAATCC 2755
S--Y--G--V--T--V--W--E--L--M--T--F--G--S--K--P--Y--D--G--I-- 918

CTGCCAGCGAGATCTCCTCCATCCTGGAGAAAGGAGAACGCCTCCCTCAGCCACCCATAT 2815
P--A--S--E--I--S--S--I--L--E--K--G--E--R--L--P--Q--P--P--I-- 938

GTACCATCGATGTCTACATGATCATGGTCAAGTGCTGGATGATAGACGCAGATAGTCGCC 2875
C--T--I--D--V--Y--M--I--M--V--K--C--W--M--I--D--A--D--S--R-- 958

CAAAGTTCCGTGAGTTGATCATCGAATTCTCCAAAATGGCCCGAGACCCCCAGCGCTACC 2935
P--K--F--R--E--L--I--I--E--F--S--K--M--A--R--D--P--Q--R--Y-- 978

TTGTCATTCAGGGGGATGAAAGAATGCATTTGCCAAGTCCTACAGACTCCAACTTCTACC 2995
L--V--I--Q--G--D--E--R--M--H--L--P--S--P--T--D--S--N--F--Y-- 998

GTGCCCTGATGGATGAAGAAGACATGGACGACGTGGTGGATGCCGACGAGTACCTCATCC 3055
R--A--L--M--D--E--E--D--M--D--D--V--V--D--A--D--E--Y--L--I-- 1018

CACAGCAGGGCTTCTTCAGCAGCCCCTCCACGTCACGGACTCCCCTCCTGAGCTCTCTGA 3115
P--Q--Q--G--F--F--S--S--P--S--T--S--R--T--P--L--L--S--S--L-- 1038

GTGCAACCAGCAACAATTCCACCGTGGCTTGCATTGATAGAAATGGGCTGCAAAGCTGTC 3175
S--A--T--S--N--N--S--T--V--A--C--I--D--R--N--G--L--Q--S--C-- 1058
```

FIG. 5 (con'd.)

```
CCATCAAGGAAGACAGCTTCTTGCAGCGATACAGCTCAGACCCCACAGGCGCCTTGACTG 3235
P--I--K--E--D--S--F--L--Q--R--Y--S--S--D--P--T--G--A--L--T-- 1078

AGGACAGCATAGACGACACCTTCCTCCCAGTGCCTGAATACATAAACCAGTCCGTTCCCA 3295
E--D--S--I--D--D--T--F--L--P--V--P--E--Y--I--N--Q--S--V--P-- 1098

AAAGGCCCGCTGGCTCTGTGCAGAATCCTGTCTATCACAATCAGCCTCTGAACCCCGCGC 3355
K--R--P--A--G--S--V--Q--N--P--V--Y--H--N--Q--P--L--N--P--A-- 1118

CCAGCAGAGACCCACACTACCAGGACCCCCACAGCACTGCAGTGGGCAACCCCGAGTATC 3415
P--S--R--D--P--H--Y--Q--D--P--H--S--T--A--V--G--N--P--E--Y-- 1138

TCAACACTGTCCAGCCCACCTGTGTCAACAGCACATTCGACAGCCCTGCCCACTGGGCCC 3475
L--N--T--V--Q--P--T--C--V--N--S--T--F--D--S--P--A--H--W--A-- 1158

AGAAAGGCAGCCACCAAATTAGCCTGGACAACCCTGACTACCAGCAGGACTTCTTTCCCA 3535
Q--K--G--S--H--Q--I--S--L--D--N--P--D--Y--Q--Q--D--F--F--P-- 1178

AGGAAGCCAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAAATGCAGAATACCTAA 3595
K--E--A--K--P--N--G--I--F--K--G--S--T--A--E--N--A--E--Y--L-- 1198

GGGTCGCGCCACAAAGCAGTGAATTTATTGGAGCATGA 3633 (SEQ ID NO 2)
R--V--A--P--Q--S--S--E--F--I--G--A--*- 1210 (SEQ ID NO 1)
```

FIG. 5 (con'd.)

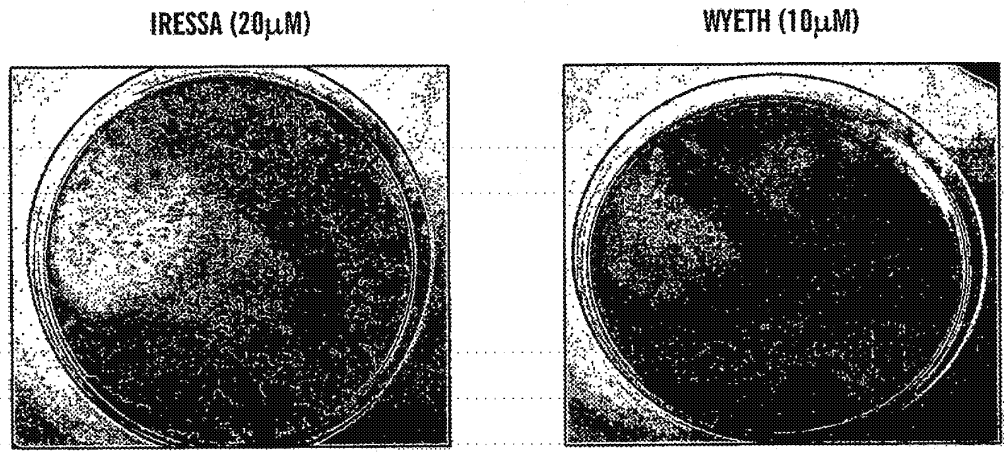


FIG. 6

METHOD FOR TREATING GEFITINIB RESISTANT CANCER

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. patent application Ser. No. 11/883,474, filed on Jul. 31, 2007 and having a 35 U.S.C. § 371(c) date of Aug. 5, 2008, which is the National Phase application of International Application No. PCT/US2006/003717, filed Feb. 2, 2006, which designates the United States and was published in English, and claims the priority benefit under 35 U.S.C. § 119(e) of U.S. provisional Patent Application No. 60/649,483, filed Feb. 3, 2005, and U.S. provisional Patent Application No. 60/671,989, filed Apr. 15, 2005. These applications, in their entirety, are incorporated herein by reference.

SEQUENCE LISTING

This application incorporates by reference in its entirety the Computer Readable Form (CRF) of a Sequence Listing in ASCII text format submitted via EFS-Web. The Sequence Listing text file submitted via EFS-Web, entitled 13120-023-999 _SUB_SEQ_LISTING.txt, was created on Oct. 9, 2019, and is 27,630 bytes in size.

BACKGROUND

Epithelial cell cancers, for example, prostate cancer, breast cancer, colon cancer, lung cancer, pancreatic cancer, ovarian cancer, cancer of the spleen, testicular cancer, cancer of the thymus, etc., are diseases characterized by abnormal, accelerated growth of epithelial cells. This accelerated growth initially causes a tumor to form. Eventually, metastasis to different organ sites can also occur. Although progress has been made in the diagnosis and treatment of various cancers, these diseases still result in significant mortality.

Lung cancer remains the leading cause of cancer death in industrialized countries. Cancers that begin in the lungs are divided into two major types, non-small cell lung cancer and small cell lung cancer, depending on how the cells appear under a microscope. Non-small cell lung cancer (squamous cell carcinoma, adenocarcinoma, and large cell carcinoma) generally spreads to other organs more slowly than does small cell lung cancer. About 75 percent of lung cancer cases are categorized as non-small cell lung cancer (e.g., adenocarcinomas), and the other 25 percent are small cell lung cancer. Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths in the United States, Japan and Western Europe. For patients with advanced disease, chemotherapy provides a modest benefit in survival, but at the cost of significant toxicity, underscoring the need for therapeutic agents that are specifically targeted to the critical genetic lesions that direct tumor growth (Schiller J H et al., N Engl J Med, 346: 92-98, 2002).

Epidermal growth factor receptor (EGFR) is a 170 kilodalton (kDa) membrane-bound protein expressed on the surface of epithelial cells. EGFR is a member of the growth factor receptor family of protein tyrosine kinases, a class of cell cycle regulatory molecules. (W. J. Gullick et al., 1986, Cancer Res., 46:285-292). EGFR is activated when its ligand (either EGF or TGF-α) binds to the extracellular domain, resulting in autophosphorylation of the receptor's intracellular tyrosine kinase domain (S. Cohen et al., 1980, J. Biol. Chem., 255:4834-4842; A. B. Schreiber at al., 1983, J. Biol. Chem., 258:846-853).

EGFR is the protein product of a growth promoting oncogene, erbB or ErbB1, that is but one member of a family, i.e., the ERBB family of protooncogenes, believed to play pivotal roles in the development and progression of many human cancers. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. The ERBB family of oncogenes encodes four, structurally-related transmembrane receptors, namely, EGFR, HER-2/neu (erbB2), HER-3 (erbB3) and HER-4 (erbB4). Clinically, ERBB oncogene amplification and/or receptor overexpression in tumors have been reported to correlate with disease recurrence and poor patient prognosis, as well as with responsiveness in therapy. (L. Harris et al., 1999, Int. J. Biol. Markers, 14:8-15; and J. Mendelsohn and J. Baselga, 2000, Oncogene, 19:6550-6565).

EGFR is composed of three principal domains, namely, the extracellular domain (ECD), which is glycosylated and contains the ligand-binding pocket with two cysteine-rich regions; a short transmembrane domain, and an intracellular domain that has intrinsic tyrosine kinase activity. The transmembrane region joins the ligand-binding domain to the intracellular domain. Amino acid and DNA sequence analysis, as well as studies of nonglycosylated forms of EGFR, indicate that the protein backbone of EGFR has a mass of 132 kDa, with 1186 amino acid residues (A. L. Ullrich et al., 1984, Nature, 309:418-425; J. Downward et al., 1984, Nature, 307:521-527; C. R. Carlin et al., 1986, Mol. Cell. Biol., 6:257-264; and F. L. V. Mayes and M. D. Waterfield, 1984, The EMBO J., 3:531-537).

The binding of EGF or TGF-α to EGFR activates a signal transduction pathway and results in cell proliferation. The dimerization, conformational changes and internalization of EGFR molecules function to transmit intracellular signals leading to cell growth regulation (G. Carpenter and S. Cohen, 1979, Ann. Rev. Biochem., 48:193-216). Genetic alterations that affect the regulation of growth factor receptor function, or lead to overexpression of receptor and/or ligand, result in cell proliferation. In addition, EGFR has been determined to play a role in cell differentiation, enhancement of cell motility, protein secretion, neovascularization, invasion, metastasis and resistance of cancer cells to chemotherapeutic agents and radiation. (M.-J. Oh et al., 2000, Clin. Cancer Res., 6:4760-4763).

A variety of inhibitors of EGFR have been identified, including a number already undergoing clinical trials for treatment of various cancers. For a recent summary, see de Bono, J. S. and Rowinsky, E. K. (2002), "The ErbB Receptor Family: A Therapeutic Target For Cancer", *Trends in Molecular Medicine,* 8, 519-26.

A promising set of targets for therapeutic intervention in the treatment of cancer includes the members of the HER-kinase axis. They are frequently upregulated in solid epithelial tumors of, by way of example, the prostate, lung and breast, and are also upregulated in glioblastoma tumors. Epidermal growth factor receptor (EGFR) is a member of the HER-kinase axis, and has been the target of choice for the development of several different cancer therapies. EGFR tyrosine kinase inhibitors (EGFR-TKIs) are among these therapies, since the reversible phosphorylation of tyrosine residues is required for activation of the EGFR pathway. In other words, EGFR-TKIs block a cell surface receptor responsible for triggering and/or maintaining the cell signaling pathway that induces tumor cell growth and division. Specifically, it is believed that these inhibitors interfere with the EGFR kinase domain, referred to as HER-1. Among the

more promising EGFR-TKIs are three series of compounds: quinazolines, pyridopyrimidines and pyrrolopyrimidines.

Two of the more advanced compounds in clinical development include Gefitinib (compound ZD1839 developed by AstraZeneca UK Ltd.; available under the tradename IRESSA; hereinafter "IRESSA") and Erlotinib (compound OSI-774 developed by Genentech, Inc. and OSI Pharmaceuticals, Inc.; available under the tradename TARCEVA; hereinafter "TARCEVA"); both have generated encouraging clinical results. Conventional cancer treatment with both IRESSA and TARCEVA involves the daily, oral administration of no more than 500 mg of the respective compounds. In May, 2003, IRESSA became the first of these products to reach the United States market, when it was approved for the treatment of advanced non-small cell lung cancer patients.

IRESSA is an orally active quinazoline that functions by directly inhibiting tyrosine kinase phosphorylation on the EGFR molecule. It competes for the adenosine triphosphate (ATP) binding site, leading to suppression of the HER-kinase axis. The exact mechanism of the IRESSA response is not completely understood, however, studies suggest that the presence of EGFR is a necessary prerequisite for its action.

A significant limitation in using these compounds is that recipients thereof may develop a resistance to their therapeutic effects after they initially respond to therapy, or they may not respond to EGFR-TKIs to any measurable degree at all. The response rate to EGFR-TKIs varies between different ethnic groups. At the low end of EGFR-TKI responders, in some populations, only 10-15 percent of advanced non-small cell lung cancer patients respond to EGFR kinase inhibitors. Thus, a better understanding of the molecular mechanisms underlying sensitivity to IRESSA and TARCEVA would be extremely beneficial in targeting therapy to those individuals whom are most likely to benefit from such therapy.

There is a significant need in the art for a satisfactory treatment of cancer, and specifically epithelial cell cancers such as lung, ovarian, breast, brain, colon and prostate cancers, which incorporates the benefits of TKI therapy and overcoming the non-responsiveness exhibited by patients. Such a treatment could have a dramatic impact on the health of individuals, and especially older individuals, among whom cancer is especially common.

SUMMARY

The inventors of the present invention have surprisingly discovered that irreversible EGFR inhibitors are effective in the treatment of cancer in subjects who are no longer responding to gefitinib and/or erlotinib therapies. Thus, in one embodiment, the present invention provides a method for the treatment of gefitinib and/or erlotinib resistant cancer. In this embodiment, progression of cancer in a subject is monitored at a time point after the subject has initiated gefitinib and/or erlotinib treatment. Progression of the cancer is indicative of cancer that is resistant to gefitinib and/or erlotinib treatment and the subject is administered a pharmaceutical composition comprising an irreversible epidermal growth factor receptor (EGFR) inhibitor.

In preferred embodiments, the irreversible EGFR inhibitor EKB-569, HKI-272 or HKI-357. Alternatively, the irreversible EGFR inhibitor may be any compound which binds to cysteine 773 of EGFR (SEQ ID NO: 1).

The progression of cancer may be monitored by methods well known to those of skill in the art. For example, the progression may be monitored by way of visual inspection of the cancer, such as, by means of X-ray, CT scan or MRI. Alternatively, the progression may be monitored by way of tumor biomarker detection.

In one embodiment, the patient is monitored at various time points throughout the treatment of the cancer. For example, the progression of a cancer may be monitored by analyzing the progression of cancer at a second time point and comparing this analysis to an analysis at a first time point. The first time point may be before or after initiation of gefitinib and/or erlotinib treatment and the second time point is after the first. An increased growth of the cancer indicates progression of the cancer.

In one embodiment, the progression of cancer is monitored by analyzing the size of the cancer. In one embodiment, the size of the cancer is analyzed via visual inspection of the cancer by means of X-ray, CT scan or MRI. In one embodiment, the size of the cancer is monitored by way of tumor biomarker detection.

In one embodiment, the cancer is epithelial cell cancer. In one embodiment, the cancer is gastrointestinal cancer, prostate cancer, ovarian cancer, breast cancer, head and neck cancer, esophageal cancer, lung cancer, non-small cell lung cancer, cancer of the nervous system, kidney cancer, retina cancer, skin cancer, liver cancer, pancreatic cancer, genital-urinary cancer and bladder cancer.

In one embodiment, the size of the cancer is monitored at additional time points, and the additional time points are after the second time point.

In one embodiment, the later time point is at least 2 months after the preceding time point. In one embodiment, the later time point is at least 6 months after preceding time point. In one embodiment, the later time point is at least 10 months after preceding time point. In one embodiment, the later time point is at least one year after preceding time point.

In another embodiment, the present invention provides a method of treating cancer, comprising administering to a subject having a mutation in EGFR, namely, a substitution of a methionine for a threonine at position 790 (T790M) of SEQ ID. No. 1, a pharmaceutical composition comprising an irreversible EGFR inhibitor. The T790M mutation confers resistance to gefitinib and/or erlotinib treatment.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A-**1**B show EGFR sequence analysis in recurrent metastatic lesions from two NSCLC patients with acquired gefitinib resistance. FIG. **1**A shows sequence analysis for Case 1. The T790M mutation in EGFR is present in a recurrent liver lesion after the development of clinical gefitinib resistance. (Left) The mutation was not detected in the primary lung lesion at the time of diagnosis. (Right) Both the primary lung tumor and the recurrent liver lesion harbor the L858R gefitinib-sensitizing mutation. Of note, the L858R mutation is present in the expected ratio for a heterozygous mutation in both primary and recurrent lesions, whereas T790M is detectable at low levels compared with the wild-type allele. A polymorphism (G/A) is shown in the same tracing to demonstrate equivalent representation of the two alleles in the uncloned PCR product (SEQ ID NOS 3 & 4 disclosed respectively, in order of appearance). FIG. **1**B shows sequence analysis for Case 2. The T790M mutation is present within a small minority of gefitinib-resistant cells. (Left) The T790M mutation was undetectable either in the lung primary tumor or in eight recurrent liver lesions from this case by sequencing uncloned PCR products. Heterozygosity at an adjacent poly-

morphism (G/A) confirms amplification of both EGFR alleles from these specimens. The heterozygous gefitinib-sensitizing mutation, L861Q, was detected at the expected ratio within the primary lung tumor as well as each of the eight recurrent liver lesions (SEQ ID NOS 3 & 5 disclosed respectively, in order of appearance).

FIGS. **2**A-2C show acquired resistance to gefitinib in bronchoalveolar cancer cell lines and persistent sensitivity to irreversible ERBB family inhibitors. FIG. **2**A shows inhibition by tyrosine kinase inhibitors of proliferation of bronchoalveolar cancer cell lines with wild-type EGFR (NCI-H1666), the activating delE746-A750 mutation in EGFR (NCI-H1650), or two representative gefitinib-resistant subclones of NCI-H1650 (G7 and C11). The effect of the reversible inhibitor gefitinib is compared with that of the irreversible inhibitor HKI-357. Comparable results were observed with the other irreversible inhibitors. Cell numbers were measured by crystal violet staining, after culture in 5% FCS, with 100 ng/ml EGFR, at 72 h after exposure to indicated drug concentrations. Each data point represents the mean of four samples. FIG. **2**B shows the chemical structure of gefitinib, a reversible inhibitor of EGFR; EKB-569, an irreversible inhibitor of EGFR; and HKI-272 and HKI-357, two irreversible dual inhibitors of EGFR and ERBB2. FIG. **2**C shows generation of drug-resistant NCI-H1650 cells after treatment with varying concentrations of gefitinib or the irreversible ERBB inhibitor EKB-569. Colonies were stained after 12 days in culture in the presence of inhibitors.

FIGS. **3**A-3D show persistent dependence on EGFR and ERBB2 signaling in gefitinib-resistant cells, and altered receptor trafficking. FIG. **3**A shows cell viability after siRNA-mediated knockdown of EGFR and ERBB2 in bronchoalveolar cell lines with wild-type EGFR (NCI-H1666), compared with cells with the activating delE746-A750 mutation in EGFR (NCI-H1650) and two gefitinib-resistant derivatives (G7 and C11). Viable cells were counted 72 h after treatment with double-stranded RNA and are shown as a fraction relative to cells treated with nonspecific siRNA, with standard deviations based on triplicate samples. FIG. **3**B shows inhibition of EGFR autophosphorylation (Y1068) and phosphorylation of downstream effectors AKT and MAPK (ERK) in cells treated with increasing concentrations of gefitinib or the irreversible inhibitor HKI-357, followed by a 2-h pulse with EGF. The parental cell line NCI-H1650 is compared with a representative gefitinib-resistant line, G7. Total AKT and MAPK are shown as controls; tubulin is used as loading control for total EGFR levels, which are at the lower limit of detection in these cells. FIG. **3**C shows altered EGFR internalization in gefitinib-resistant NCI-H1650 (G7) cells, compared with the sensitive NCI-H1650 parental cell line. Rhodamine-tagged EGF is used to label EGFR at 5 and 20 rain, after addition of ligand. The increased internalization of EGFR in NCI-H1650 (G7) cells is most evident at 20 min. (Zeiss microscope, ×63 magnification). FIG. **3**D shows immunoblotting of internalized EGFR from NCI-H1650 parental cells and the resistant derivative G7 after pulse labeling of cell surface proteins by biotinylation and chase over 20 min. The increased intracellular EGFR in NCI-H1650 (07) cells is compared with the unaltered transferrin receptor (TR) internalization.

FIGS. **4**A-4B show Effectiveness of irreversible ERBB inhibitors in suppressing the T790M EGFR mutant. FIG. **4**A shows comparison of gefitinib and two irreversible inhibitors, HK-357 and HKI-272, in their ability to suppress EGFR autophosphorylation (Y1068) and phosphorylation of downstream effectors AKT and MAPK (ERK) in the NCI-H1975 bronchoalveolar cell line, harboring both a sensitizing mutation (L858R) and the resistance-associated mutation (T790M). Total EGFR, AKT, and MAPK are shown as loading controls. FIG. **4**B shows suppression of proliferation in NCI-H1975 cells harboring the L858R and T790M mutations by the three irreversible ERBB family inhibitors, compared with gefitinib.

FIG. **5** shows the nucleotide sequence (SEQ ID NO: 2) and the amino acid sequence (SEQ ID NO: 1) of EGFR.

FIG. **6** shows that like gefitinib, HKI 357 and EKB 569 (labeled "Wyeth") demonstrated increased cell killing of NSCLC cells harboring an EGFR mutation, but unlike gefitinib, clones resistant to these drugs were not readily generated in vitro and they retained their effectiveness against gefitinib-resistant clones.

DETAILED DESCRIPTION

Gefitinib and Erlotinib Resistant Cancers

Gefitinib (compound ZD1839 developed by AstraZencca UK Ltd.; available under the tradename IRESSA) and erlotinib (compound OSI-774 developed by Genentech, Inc. and OSI Pharmaceuticals, Inc.; available under the trade name TARCEVA) induce dramatic clinical responses in cases of non-small cell lung cancers (NSCLCs) harboring activating mutations in the EGF receptor (EGFR) (1-3), which is targeted by these competitive inhibitors of ATP binding (4, 5). The effectiveness of these tyrosine kinase inhibitors may result both from alterations in the ATP cleft associated with these mutations, which lead to enhanced inhibition of the mutant kinase by these drugs, and from biological dependence of these cancer cells on the increased survival signals transduced by the mutant receptors, a phenomenon described as "oncogene addiction" (6, 7).

Although therapeutic responses to both gefitinib and erlotinib can persist for as long as 2-3 years, the mean duration of response in most cases of NSCLC is only 6-S months (8-10). The mechanisms underlying acquired drug resistance are not well understood. By analogy with imatinib (GLBEVEC), which inhibits the BCR-ABL kinase involved in chronic myeloid leukemias (CMLs), the C-KIT kinase implicated in gastrointestinal stromal tumors (GISTs), and the FIP1L1-PDOFR-α kinase in idiopathic hypereosinophilic syndrome (HES), secondary kinase domain mutations can potentially suppress drug binding (11-16). However, recurrent NSCLC is not readily biopsied; hence, only limited clinical specimens are available for analysis. Recently, a single secondary mutation, T790M, within the EGFR kinase domain has been reported in three of six cases with recurrent disease after gefitinib or erlotinib therapy (17, 18). Codon 315 of BCR-ABL, which is analogous to EGFR codon 790, is frequently mutated in imatinib-resistant CML (11, 12), and mutation of the corresponding residue in C-KIT (codon 670) and FIP1L1-PDGFR-α (codon 674) is associated with imatinib-resistant GIST and HES, respectively (15, 16). Early in vitro modeling of resistance to EGFR inhibitors indicated that mutation of codon 790 within the wild-type receptor would similarly suppress inhibition by an EGFR tyrosine kinase inhibitor (19). Recently, transfected EGFR proteins containing activating mutations together with the T790M substitution were shown to exhibit reduced inhibition by gefitinib and erlotinib (17, 18). Although the T790M mutation seems to contribute to acquired resistance in some cases of NSCLC, the mechanisms underlying treatment failure in cases lacking secondary EGFR mutations remain unexplained.

In contrast to the cytoplasmic kinase BCR-ABL, signaling by the membrane-bound EGFR involves a complex

pathway of ligand binding, receptor homodimerization, and heterodimerization with ERBB2 and other family members, followed by internalization and recycling of the ligand-bound receptor or ubiquitin-mediated receptor degradation (20). Significant EGF-dependent signaling is thought to occur during the process of internalization, which is also associated with the dissociation of EGFR complexes at the low pH of intracellular vesicles. As such, multiple factors modulate the strength and quality of the signal transduced by the receptor, and alterations in EGFR trafficking have been closely linked with the regulation of EGF-dependent cellular responses (20).

The present invention is based on the discovery that gefitinib resistant cancers can include those wherein the T790M EGFR mutation is only present in a subset of resistant tumor cells and those wherein the T790M mutation is not observed, but increased EGFR internalization is observed. The invention is further based on the discovery that irreversible EGFR inhibitors, which covalently cross-link the receptor, are effective in inhibiting cancers with the T790M mutation and in cancers with altered EGFR trafficking that can make such cancers resistance to treatment with gefitinib and/or erlotinib. Accordingly, the present invention provides a method of treating gefitinib and/or erlotinib resistant cancers comprising administering irreversible EGFR inhibitors.

Method of Treating a Patient

In one embodiment, the invention provides a method for treating gefitinib/erlotinib resistant cancer. The method comprises administering to a patient in need of such treatment an effective amount of certain irreversible EGFR inhibitors, including EKB-569 (4-anilinoquinoline-3-carbonitrile; Greenberger et al., 11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy, Amsterdam, Nov. 7-10, 2000, abstract 388; Wyeth), HKI-357 (a derivative of 4-anilinoquinoline-3-carbonitrile; Tsou et al. J. Med. Chem. 2005, 48: 1107-1131; Wyeth) and/or HKI-272 (a derivative of 4-anilinoquinoline-3-carbonitrile; Rabindran et al., Cancer Res. 2004, 64, 3958-3965; Wyeth). In one preferred embodiment, the invention provides a method comprising administering to a patient in need of such treatment an effective amount of EKB-569. In one preferred embodiment, the invention provides a method comprising administering to a patient in need of such treatment an effective amount of HKI-357.

The treatment may also involve a combination of treatments, including, but not limited to a tyrosine kinase inhibitor in combination with other tyrosine kinase inhibitors, chemotherapy, radiation, etc.

Cancers may initially be diagnosed as gefitinib/erlotinib sensitive or predicted to be gefitinib/erlotinib sensitive by means of the methods described in Lynch et al., 2004; 350:2129-2139. Gefitinib/erlotinib sensitivity may be predicted by the presence in the tumor of EGFR mutations including, for example, deletion of residues 747 (lysine) to 749 (glutamic acid) combined with a mutation in 750 (alanine), deletion of residues 747 (lysine) to 750 (alanine), substitution of arginine for leucine at residue 858, of substitution of glutamine for leucine at residue 861.

Cancers may be diagnosed as gefitinib and/or erlotinib resistant after treatment with gefitinib and/or erlotinib has commenced. Alternatively, cancers may be diagnosed as gefitinib and/or erlotinib resistant prior to initiation of treatment with such compounds. Gefitinib and/or erlotinib resistance in the tumor may occur after, e.g., 6 months or longer of gefitinib and/or erlotinib treatment. Alternatively, gefitinib and/or erlotinib resistance of the tumor may be diagnosed less than 6 months after gefitinib and/or erlotinib treatment has commenced. Diagnosis of gefitinib and/or erlotinib resistance may be accomplished by way of monitoring tumor progression during gefitinib and/or erlotinib treatment. Tumor progression may be determined by comparison of tumor status between time points after treatment has commenced or by comparison of tumor status between a time point after treatment has commenced to a time point prior to initiation of gefitinib and/or erlotinib treatment. Tumor progression may be monitored during gefitinib and/or erlotinib treatment visually, for example, by means of radiography, for example, X-ray, CT scan, or other monitoring methods known to the skilled artisan, including palpitation of the cancer or methods to monitor tumor biomarker levels. Progression of the cancer during treatment with gefitinib and/or erlotinib indicates gefitinib and/or erlotinib resistance. A rise in level of tumor biomarkers indicates tumor progression. Thus, a rise in tumor biomarker levels during treatment with gefitinib and/or erlotinib indicates gefitinib and/or erlotinib resistance. Detection of new tumors or detection of metastasis indicates tumor progression. Cessation of tumor shrinkage indicates tumor progression. Growth of the cancer is indicated by, for example, increase in tumor size, metastasis or detection of new cancer, and/or a rise in tumor biomarker levels.

The development of gefitinib and/or erlotinib resistance may be monitored by means of testing for presence of a gefitinib and/or erlotinib resistance associated mutation in circulating tumor cells obtained from the circulation, or other bodily fluid, of the subject. Presence of gefitinib and/or erlotinib resistance associated mutations in tumor cells from the subject is indicative of a gefitinib and/or erlotinib resistant tumor.

In one embodiment, the subject's tumor harbors mutations indicative of gefitinib and/or erlotinib sensitivity, yet it is resistant to gefitinib and/or erlotinib treatment. In one embodiment, the subject's tumor harbors mutations indicative gefitinib and/or erlotinib sensitivity and harbors mutations indicative of gefitinib and/or erlotinib resistance, e.g., the T790M mutation, that is, where a methione residue is substituted for the native threonine residue, in EGFR, e.g. increased EGFR internalization. In one embodiment, the subject's tumor does not harbor mutations indicative of gefitinib and/or erlotinib sensitivity and does harbor mutations indicative of gefitinib and/or erlotinib resistance, e.g., the T790M mutation in EGFR, e.g., increased EGFR internalization.

In connection with the administration of the drug, an "effective amount" indicates an amount that results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease load, reduction in tumor mass or cell numbers, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disease or condition.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration and the severity of the condition being treated. The skilled artisan is aware of the effective dose for each patient, which may vary with disease severity, individual genetic variation, or metabolic rate. However, in general, satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.5 to about 1000 mg/kg of body weight, optionally given in divided doses two to four times a day, or in sustained release form. The total daily dosage is projected

to be from about 1 to 1000 mg, preferably from about 2 to 500 mg. Dosage forms suitable for internal use comprise from about 0.5 to 1000 mg of the active compound in intimate admixture with a solid or liquid pharmaceutically acceptable carrier. This dosage regimen may be adjusted to provide the optimal therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The route of administration may be intravenous (I.V.), intramuscular (I.M.), subcutaneous (S.C.), intradermal (I.D.), intraperitoneal (I.P.), intrathecal (I.T.), intrapleural, intrauterine, rectal, vaginal, topical, intratumor and the like. The compounds of the invention can be administered parenterally by injection or by gradual infusion over time and can be delivered by peristaltic means.

Administration may be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays, for example, or using suppositories. For oral administration, the compounds of the invention are formulated into conventional oral administration forms such as capsules, tablets and tonics.

For topical administration, the pharmaceutical composition (inhibitor of kinase activity) is formulated into ointments, salves, gels, or creams, as is generally known in the art.

The therapeutic compositions of this invention, e.g. irreversible EGFR inhibitors, are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluents; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

The therapeutic composition useful for practicing the methods of the present invention, e.g. irreversible EGFR inhibitors, are described herein. Any formulation or drug delivery system containing the active ingredients, which is suitable for the intended use, as are generally known to those of skill in the art, can be used. Suitable pharmaceutically acceptable carriers for oral, rectal, topical or parenteral (including inhaled, subcutaneous, intraperitoneal, intramuscular and intravenous) administration are known to those of skill in the art. The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal without the production of undesirable physiological effects.

Formulations suitable for parenteral administration conveniently include sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient Thus, such formulations may conveniently contain distilled water, 5% dextrose in distilled water or saline. Useful formulations also include concentrated solutions or solids containing the compound which upon dilution with an appropriate solvent give a solution suitable for parental administration above.

For enteral administration, a compound can be incorporated into an inert carrier in discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a suspension or solution in an aqueous liquid or non-aqueous liquid, e.g., a syrup, an elixir, an emulsion or a draught. Suitable carriers may be starches or sugars and include lubricants, flavorings, binders, and other materials of the same nature.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form, e.g., a powder or granules, optionally mixed with accessory ingredients, e.g., binders, lubricants, inert diluents, surface active or dispersing agents. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered active compound with any suitable carrier.

A syrup or suspension may be made by adding the active compound to a concentrated, aqueous solution of a sugar, e.g., sucrose, to which may also be added any accessory ingredients. Such accessory ingredients may include flavoring, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredient, e.g., as a polyhydric alcohol, for example, glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a conventional carrier, e.g., cocoa butter or Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany), for a suppository base.

Formulations for oral administration may be presented with an enhancer. Orally-acceptable absorption enhancers include surfactants such as sodium lauryl sulfate, palmitoyl carnitine, Laureth-9, phosphatidylcholine, cyclodextrin and derivatives thereof; bile salts such as sodium deoxycholate, sodium taurocholate, sodium glycochlate, and sodium fusidate; chelating agents including EDTA, citric acid and salicylates; and fatty acids (e.g., oleic acid, lauric acid, acylcarnitines, mono- and diglycerides). Other oral absorption enhancers include benzalkonium chloride, benzethonium chloride, CHAPS (3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate), Big-CHAPS (N, N-bis(3-D-gluconamidopropyl)-cholamide), chlorobutanol, octoxynol-9, benzyl alcohol, phenols, cresols, and alkyl alcohols. An especially preferred oral absorption enhancer for the present invention is sodium lauryl sulfate.

Alternatively, the compound may be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are well known to those of skill in the art. U.S. Pat. No. 4,789,734, the contents of which are hereby incorporated by reference, describes methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is provided by G. Gregoriadis,

Chapter 14, "Liposomes," Drug Carriers in Biology and Medicine, pp. 287-341 (Academic Press, 1979).

Microspheres formed of polymers or proteins are well known to those skilled in the art, and can be tailored for passage through the gastrointestinal tract directly into the blood stream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time ranging from days to months. See, for example, U.S. Pat. Nos. 4,906,474, 4,925,673 and 3,625,214, and Jein, TIPS 19:155-157 (1998), the contents of which are hereby incorporated by reference.

In one embodiment, the tyrosine kinase inhibitor of the present invention can be formulated into a liposome or microparticle which is suitably sized to lodge in capillary beds following intravenous administration. When the liposome or mnicroparticle is lodged in the capillary beds surrounding ischemic tissue, the agents can be administered locally to the site at which they can be most effective. Suitable liposomes for targeting ischemic tissue are generally less than about 200 nanometers and are also typically unilamellar vesicles, as disclosed, for example, in U.S. Pat. No. 5,593,688 to Baldeschweiler, entitled "Liposomal targeting of ischemic tissue," the contents of which are hereby incorporated by reference.

Preferred microparticles are those prepared from biodegradable polymers, such as polyglycolide, polylactide and copolymers thereof. Those of skill in the art can readily determine an appropriate carrier system depending on various factors, including the desired rate of drug release and the desired dosage.

In one embodiment, the formulations are administered via catheter directly to the inside of blood vessels. The administration can occur, for example, through holes in the catheter. In those embodiments wherein the active compounds have a relatively long half life (on the order of 1 day to a week or more), the formulations can be included in biodegradable polymeric hydrogels, such as those disclosed in U.S. Pat. No. 5,410,016 to Hubbell et al. These polymeric hydrogels can be delivered to the inside of a tissue lumen and the active compounds released over time as the polymer degrades. If desirable, the polymeric hydrogels can have microparticles or liposomes which include the active compound dispersed therein, providing another mechanism for the controlled release of the active compounds.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or a finely divided solid carrier and then, if necessary, shaping the product into desired unit dosage form.

The formulations may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

Compounds of the present methods (i.e. irreversible EGFR inhibitors) may be presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less than 50 microns, preferably less than 10 microns, more preferably between 2 and 5 microns.

Generally for nasal administration a mildly acid pH will be preferred. Preferably the compositions of the invention have a pH of from about 3 to 5, more preferably from about 3.5 to about 3.9 and most preferably 3.7. Adjustment of the pH is achieved by addition of an appropriate acid, such as hydrochloric acid.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The irreversible kinase inhibitors of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers am sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

Definitions

The terms "ErbB1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to native sequence EGFR as disclosed, for example, in Carpenter et al. Ann. Rev. Biochem. 56:881-914 (1987), including variants thereof (e.g. a deletion mutant EGFR as in Humphrey et al. PNAS (USA) 87:4207-4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product. As used herein, the EGFR protein is disclosed as GenBank accession no. NP_005219 (SEQ ID NO: 1) which is encoded by the erbB1 gene, GenBank accession no. NM_005228 (SEQ ID NO: 2). Nucleotide and amino acid sequences of erbB1/EGFR may be found in FIG. 5.

The term "kinase activity increasing nucleic acid variance" as used herein refers to a variance (i.e. mutation) in the nucleotide sequence of a gene that results in an increased

kinase activity. The increased kinase activity is a direct result of the variance in the nucleic acid and is associated with the protein for which the gene encodes.

The term "drug" or "compound" as used herein refers to a chemical entity or biological product, or combination of chemical entities or biological products, administered to a person to treat or prevent or control a disease or condition. The chemical entity or biological product is preferably, but not necessarily a low molecular weight compound, but may also be a larger compound, for example, an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof.

As used herein, the terms "effective" and "effectiveness" includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the treatment to result in a desired biological effect in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (often referred to as side-effects) resulting from administration of the treatment. "Less effective" means that the treatment results in a therapeutically significant lower level of pharmacological effectiveness and/or a therapeutically greater level of adverse physiological effects.

Nucleic acid molecules can be isolated from a particular biological sample using any of a number of procedures, which are well-known in the art, the particular isolation procedure chosen being appropriate for the particular biological sample. For example, freeze-thaw and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from solid materials; heat and alkaline lysis procedures can be useful for obtaining nucleic acid molecules from urine; and proteinase K extraction can be used to obtain nucleic acid from blood (Rolff, A et al. PCR: Clinical Diagnostics and Research, Springer (1994).

As used herein, a "cancer" in a subject or patient refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. In some circumstances, cancer cells will be in the form of a tumor, or such cells may exist locally within an animal, or circulate in the blood stream as independent cells.

EXAMPLES

Compounds. Compounds used herein, including EKB-569, HK1-357, and HK1-272 as described in U.S. Pat. No. 6,002,008; Greenberger et al., Proc. 11th NCI EORTC-AACR Symposium on New Drugs in Cancer Therapy, Clinical Cancer Res. Vol. 6 Supplement, November 2000, ISSN 1078-0432; in Rabindran et al., Cancer Res. 64: 3958-3965 (2004); Holbro and Hynes, Ann. Rev. Pharm. Tox. 44:195-217 (2004); and Tejpar et al., J. Clin. Oncol. ASCO Annual Meeting Proc. Vol. 22, No. 14S: 3579 (2004).

Analysis of Recurrent NSCLC and Generation of Gefitinib-Resistant NCI-H1650 Cells. Clinical specimens of recurrent NSCLC were obtained at autopsy after appropriate consent. The entire kinase domain of EGFR was sequenced after analysis of uncloned PCR products. Multiple clones of exon 20 were sequenced to examine codon 790. Mutational analysis of EGFR (exons 1-28), ERBB2 (exons 1-24), PTEN (exons 1-9), Kras (codons 12, 13, and 61), and p53 (exons 5-8) in gefitinib-resistant clones as well as the parental NCI-H1650 cell line was performed by automated sequencing of individual exons and flanking intronic sequence (PCR conditions available on request) with bidirectional sequencing by using dye terminator chemistry (BIGDYE version 1.1, Applied Biosystems). Sequencing reactions were run on an ABI3100 sequencer (Applied Biosystems), and electropherograms were analyzed by using SEQUENCE NAVIGATOR and FACTURA software (Applied Biosystems).

To generate resistant subclones of NCI-H1650 cells, these were treated with ethyl methane sulfonate (EMS; 600 μg/ml), allowed to recover for 72 h, and then seeded at a density of 6×10^4 cells per 10-cm^2 dish in 20 μM gefitinib. Relative resistance of these cells to gefitinib, compared with the irreversible inhibitors, was achieved by seeding 5×10^4 cells in six-well plates in 5% FCS and 100 ng/ml EGF (Sigma), in the presence of varying concentrations of drugs, followed after 72 h by fixing cells with 4% formaldehyde, staining with 0.1% crystal violet, and quantifying cell mass by using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, Nebr.). For small interfering RNA (siRNA) knockdown experiments, cells were transfected with double-stranded RNA oligonucleotides targeting EGFR, ERBB2 (both SMARTpool from Dharmacon, Lafayette, Colo.), or nonspecific control (LRT1B), using X-treme GENE transfection reagent (Roche Applied Science). After 72 h, cells were stained with crystal violet and analyzed on the Odyssey Infrared scanner.

Immunoblotting and Signaling Studies. Inhibition of EGFR signaling by increasing concentrations of gefitinib or the irreversible inhibitors was determined by seeding 9×10^4 cells in 24-well plates, adding the drugs to medium containing 5% FCS for 15 min, followed by a 2-h pulse with 100 ng/ml EGF, and harvesting of lysates. Lysates were prepared in 2× gel loading buffer, sonicated, boiled, and then separated by 10% SDS/PAGE, followed by electrotransfer to polyvinylidene fluoride (PVDF) membranes, and immunoblotting. Antibodies used were phospho-EGFR Y1068 and phospho-mitogen-activated protein kinase (MAPK) (Cell Signaling Technology, Beverly, Mass.), phospho-AKT (BioSource International, Camarillo, Calif.), and total EGFR, MAPK, AKT, and tubulin (Santa Cruz Biotechnology).

Analysis of EGFR Internalization. To demonstrate internalization of EGFR by fluorescence microscopy, cells were grown on coverslips and incubated with 1 ng/ml recombinant human (rh) EGF (Molecular Probes, Eugene, Oreg.) for various intervals before fixing in 4% paraformaldehyde for 10 min. Coverslips were washed in PBS and mounted with ProLong Gold antifade reagent (Molecular Probes). To quantify EGFR internalization by cell surface biotinylation, cells were grown to confluency, pretreated with cyclohexamide, incubated on ice for 1 h with 1.5 mg/ml sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate (sulfo-NHS-SS-biotin; Pierce), and washed with blocking buffer (50 nM NH_4CL/1 mM $MgCl_2$/0.1 mM $CaCl_2$ in PBS) to quench free sulfo-NHS-SS-biotin, followed by several further washes with PBS. The cells were then incubated in culture medium at 37° C. for various intervals to allow internalization of the biotinylated molecules, washed twice for 20 min in a glutathione solution (50 mM glutathione/75 mM NaCl/75 mM NaOH/1% BSA) on ice to strip all of the biotinyl groups from the cell surface, and then scraped and lysed in 500 μM radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl, pH 7.4, with 150 mM NaCL/0.1% SDS/1% Triton X-100) supplemented with NaF, Na-orthovanadate, and protease inhibitors. Cell extracts were centrifuged, and the supernatants were incubated with streptavidin beads (Sigma) to collect the biotinylated proteins, which were then analyzed by SDS/PAGE and immunoblotting with

anti-EGFR antibody (SC-03, Santa Cruz Biotechnology) or antibody against transferrin receptor (Santa Cruz Biotechnology).

Results and Discussion

Analysis of Recurrent Lung Cancers with Acquired Resistance to Gefitinib. Recurrent gefitinib-resistant NSCLC developed in two patients whose tumors had harbored an activating mutation of the EGFR kinase at the time of diagnosis and who had shown a dramatic initial clinical response to the drug (1). In both cases, progressive metastatic disease in the liver led to the patients' demises, 1-2 years after initiation of treatment. In case 1, analysis of the major liver metastasis obtained at the time of autopsy indicated persistence of the sensitizing EGFR mutation (L858R), as well as the presence of a newly acquired T790M mutation (FIG. 1A). Interestingly, analysis of uncloned PCR products showed the initial L858R mutation to be present at an abundance consistent with a heterozygous mutation that is present in all tumor cells, whereas the secondary T790M mutation was seen at approximately one-fifth the abundance of the corresponding wild-type allele. Thus, this resistance-associated mutation seems to be present in only a fraction of cells within the recurrent tumor.

Case 2 involved eight distinct recurrent metastases in the liver after the failure of gefitinib therapy. In all of these independent lesions, the sensitizing L861Q EGFR mutation was present at the expected ratio for a heterozygous mutation. No secondary EGFR mutation was detectable by analysis of uncloned PCR products from any of these metastases. However, after subcloning of the PCR products, the T790M mutation was found to be present at very low frequency in two of the four metastatic tumors analyzed (T790M, 2 of 50 clones sequenced from lesion 1 and 1 of 56 from lesion 2), but not from two other recurrent metastases (0 of 55 clones from lesion 3 and 0 of 59 from lesion 4), or the primary tumor (0 of 75 clones) (FIG. 1B and Table 1). Taken together, these results are consistent with previous reports that the T790M mutation is present in some, but not all, cases of acquired gefitinib resistance (three of seven tumors; see refs. 17, 18, and 21). Furthermore, as previously noted (18), even in some cases with this resistance-associated mutation, it seems to be present in only a small fraction of tumor cells within a recurrent lesion. These observations suggest that additional mechanisms of resistance are involved in cases without a secondary EGFR mutation and that such mechanisms coexist with the T790M mutation in other cases.

Generation of Gefitinib-Resistant Cell Lines with Susceptibility to Irreversible Inhibitors. Given the excellent correlation between the clinical responsiveness of EGFR-mutant NSCLC and the enhanced gefitinib-sensitivity of NSCLC cell lines with these mutations (2, 6, 22, 23), and the limited availability of clinical specimens from relapsing patients, we modeled gefitinib resistance in vitro. We cultured the bronchoalveolar cancer cell line NCI-H1650, which has an in-frame deletion of the EGFR kinase (delE746-A750), in 20 μM gefitinib, either with or without prior exposure to the mutagen ethyl methane sulfonate. This cell line exhibits 100-fold increased sensitivity to gefitinib, compared with some NSCLC lines expressing wild-type EGFR (6). Whereas the vast majority of these cells are efficiently killed by 20 μM gefitinib, drug-resistant colonies were readily observed at a frequency of $\approx 10^{-5}$, irrespective of mutagen treatment. Forty-nine independent drug-resistant clones were isolated, showing an average 50-fold decrease in gefitinib sensitivity (FIG. 2A). All of these showed persistence of the sensitizing mutation without altered expression of EGFR, and none had acquired a secondary EGFR mutation or new mutations in ERBB2, p53, Kas, or PTEN. Gefitinib-resistant clones demonstrated comparable resistance to related inhibitors of the anilinoquinazoline class. Remarkably, however, they displayed persistent sensitivity to three inhibitors of the ERBB family (FIG. 2A): HKI-272 (24) and HKI-357 (compound 7f in ref. 25), which are dual inhibitors of EGFR and ERBB2 (IC_{50} values of 92 and 34 nM, respectively, for EGFR and 59 and 33 nM, respectively, for ERBB2), and EKB-569 (26), a selective inhibitor of EGFR (IC_{50} values of 39 nM for EGFR and 1.3 μM for ERBB2) (Wyeth) (FIG. 2B). All three drugs are irreversible inhibitors, most likely via a covalent bond with the cys773 residue within the EGFR catalytic domain or the cys805 of ERBB2. Like gefitinib, these compounds demonstrate increased killing of NSCLC cells harboring an EGFR mutation, compared with cells expressing wild-type receptor (FIG. 2A). However, in contrast to gefitinib, against which resistant clones are readily generated, even at high drug concentrations, we were unable to establish clones of cells that were resistant to the irreversible inhibitors at concentrations above 10 μM, even after ethyl methane sulfonate mutagenesis (FIG. 2C).

Dependence of Gefitinib-Resistant Cells on EGFR and ERBB2 Expression. To gain insight into the mechanisms underlying the acquisition of gefitinib resistance and the persistent sensitivity to the irreversible inhibitors, we first determined whether resistant cell lines remain dependent upon EGFR for their viability. We have previously shown that siRNA-mediated knockdown of EGFR triggers apoptosis in cells harboring mutant EGFRs, but not in those with wild-type alleles (6). Significantly, parental NCI-H1650 cells as well as their gefitinib-resistant derivatives showed comparable reduction in cell viability after transfection with siRNA targeting EGFR (FIG. 3A). Thus, acquisition of gefitinib-resistance does not involve EGFR-independent activation of downstream effectors. Because HKI-272 and HKI-357 target both EGFR and ERBB2, we also tested suppression of this related receptor. Knockdown of ERBB2 in NCI-H1650 and its gefitinib-resistant derivatives also caused loss of viability (FIG. 3A), suggesting a role for EGFR-ERBB2 heterodimers in transducing essential survival signals in tumor cells harboring EGFR mutations. Inhibition of EGFR alone by an irreversible inhibitor seems to be sufficient to induce apoptosis in gefitinib-resistant cells, as demonstrated by the effectiveness of EKB-569, which primarily targets EGFR (26). However, given the potentially complementary effects of targeting both EGFR and ERBB2 by using siRNA and the availability of irreversible inhibitors that target both of these family members, the potential benefit of dual inhibition warrants consideration.

We compared the ability of gefitinib and irreversible ERBB family inhibitors to suppress signaling via downstream effectors of EGFR that mediate its proliferative and survival pathways. HKI-357 was 10-fold more effective than gefitinib in suppressing EGFR autophosphorylation (measured at residue Y1068), and AKT and MAPK phosphorylation in parental NCI-H1650 cells harboring the delE746-A750 EGFR mutation (FIG. 3B). In a gefitinib-resistant derivative, NCI-H1650(G7), gefitinib exhibited considerably reduced efficacy in suppressing AKT phosphorylation, a key EGFR signaling effector linked to gefitinib responsiveness (6), whereas HKI-357 demonstrated persistent activity (FIG. 3B).

Altered EGFR Internalization in Gefitinib-Resistant Clones. Given the absence of secondary mutations in EGFR

and the persistent susceptibility of gefitinib-resistant cells to siRNA-mediated suppression of EGFR, we tested whether the mechanism underlying the differential inhibition of EGFR signaling in gefitinib-resistant cells by reversible and irreversible inhibitors might be correlated with alterations in receptor trafficking, a well documented modulator of EGFR-dependent signaling (20). Indeed, analysis of EGFR trafficking in NCI-H1650-derived resistant cells demonstrated a consistent increase in EGFR internalization, compared with the parental drug-sensitive cells, as measured both by internalization of fluorescein-labeled EGF (FIG. 3C) and quantitation of cytoplasmic biotinylated EGFR (FIG. 3D). No such effect was observed with the transferrin receptor, suggesting that this did not result from a generalized alteration in all receptor processing. Although further work is required to define the precise mechanism for this alteration in EGFR trafficking, a complex process in which numerous regulatory proteins have been implicated, these results suggest that gefitinib's ability to inhibit EGFR activation is compromised in these cells, whereas the action of the irreversible inhibitors are not detectably affected.

Inhibition of T790M EGFR Signaling and Enhanced Cell Killing by Irreversible Inhibitors. The enhanced suppression of EGFR signaling by irreversible ERBB inhibitors raised the possibility that these drugs may also exhibit persistent activity in the context of cells harboring the T790M secondary mutation in EGFR. We therefore tested the effect of these inhibitors on the NCI-H1975 bronchoalveolar cancer cell line, which harbors both L858R and T790M mutations in EGFR (18). Significantly, this cell line was derived from a patient that had not been treated with an EGFR inhibitor, indicating that this mutation is not uniquely associated with acquired drug resistance. Both HKI-357 and HKI-272 were considerably more effective than gefitinib in suppressing ligand-induced EGFR autophosphorylation and its downstream signaling, as determined by AKT and MAPK phosphorylation (FIG. 4A). Similarly, all three irreversible inhibitors suppressed proliferation, in this cell line under conditions where it is resistant to gefitinib (FIG. 4B). Thus, irreversible ERBB inhibitors seem to be effective in cells harboring the T790M EGFR as well as in cells with altered trafficking of the wild-type receptor.

Our results confirm the report of T790M mutations in EGFR as secondary mutations that arise in previously sensitive NSCLCs harboring an activating mutation, associated with the emergence of acquired resistance (17, 18). However, this mutation is present only in a subset of cases, and even tumors that harbor the T790M mutation may contain only a small fraction of cells with this mutation. These observations imply that multiple resistance mechanisms can coexist in recurrent tumors after an initial response to gefitinib or similar reversible EGFR inhibitors. Moreover, these findings suggest that T790M-independent resistance mechanisms may be equally, if not more, effective than the T790M substitution itself in conferring drug resistance and may explain why recurrent tumors rarely exhibit clonality for T790M (17, 18). In vitro mechanisms of acquired gefitinib resistance do not involve secondary EGFR mutations at a significant frequency, but instead are correlated with altered receptor trafficking. However, it should be noted that we have not examined EGFR trafficking in all of the resistant clones that we established in vitro, and it remains possible that additional mechanisms may contribute to gefitinib resistance in some of the clones. Nonetheless, virtually all gefitinib-resistant clones exhibited comparable sensitivity to the irreversible ERBB inhibitors.

Our results indicate striking differences between competitive EGFR inhibitors such as gefitinib, whose effectiveness is limited by the rapid development of drug resistance in vitro, and irreversible inhibitors, to which acquired resistance appears to be rare (FIG. 2C). We speculate that increased internalization of ligand-bound EGFR in resistant cells may be linked to dissociation of the gefitinib-EGFR complex at the low pH of intracellular vesicles. In contrast, irreversible cross-linking of the receptor would be unaffected by such alterations in receptor trafficking. Acquired resistance to gefitinib is stably maintained after passage of cells for up to 20 generations in the absence of drug, suggesting that genetic or epigenetic alterations in genes that modulate EGFR turnover may underlie this phenomenon. Because receptor trafficking cannot be readily studied by using available clinical specimens, identification of such genomic alterations may be required before clinical correlations are possible. Nonetheless, such a mechanism may contribute to in vivo acquired gefitinib-resistance in patients with recurrent disease who do not have secondary mutations in EGFR.

Irreversible ERBB inhibitors also seem to be effective in overcoming gefitinib resistance mediated by the T790M mutation, an effect that presumably results from the preservation of inhibitor binding despite alteration of this critical residue. While this work was in progress, another irreversible inhibitor of EGFR [CL-387,785, Calbiochem (27)] was shown to inhibit the kinase activity of the T790M EGFR mutant (17). The effectiveness of CL-387,785 in the context of T790M was proposed to result from the absence of a chloride at position 3 of the aniline group, which is present in gefitinib and was postulated to interfere sterically with binding to the mutant methionine at codon 790. However, EKB-569, HKI-272, and HKI-357 all have chloride moieties at that position in the aniline ring, suggesting that their shared ability to bind irreversibly to EGFR is likely to explain their effectiveness, rather than the absence of a specific steric interaction with T790M (24-26). Thus, these irreversible inhibitors may prove to be broadly effective in circumventing a variety of resistance mechanisms, in addition to the T790M mutation.

TABLE 1

Presence of EGFR T790M mutation at very tow frequency in recurrent tumors from case 2

	No. of clones	
Tumor	T790M mutant	Wild type
Primary	0	75
Recurrent 1	2	48
Recurrent 2	1	55
Recurrent 3	0	55
Recurrent 4	0	59

Sequencing of large numbers of cloned PCR products revealed that a minority of alleles within two of four liver lesions contain the T790M mutation.

The references cited throughout the application are incorporated herein by reference in their entirety.

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Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
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Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
   1010                1015                1020

Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025                1030                1035                1040

Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
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Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
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Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
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Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
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Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105                1110                1115                1120

Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
                1125                1130                1135

Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
            1140                1145                1150

Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
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Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
   1170                1175                1180
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ggccacctcg tcggcgtccg cccgagtccc cgcctcgccg ccaacgccac aaccaccgcg     180

cacggccccc tgactccgtc cagtattgat cgggagagcc ggagcgagct cttcggggag     240

cagcg atg cga ccc tcc ggg acg gcc ggg gca gcg ctc ctg gcg ctg ctg      290
      Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu
        1               5                  10                  15

gct gcg ctc tgc ccg gcg agt cgg gct ctg gag gaa aag aaa gtt tgc      338
Ala Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys
                20                  25                  30

caa ggc acg agt aac aag ctc acg cag ttg ggc act ttt gaa gat cat      386
Gln Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His
            35                  40                  45

ttt ctc agc ctc cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg      434
Phe Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly
        50                  55                  60

aat ttg gaa att acc tat gtg cag agg aat tat gat ctt tcc ttc tta      482
Asn Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu
    65                  70                  75

aag acc atc cag gag gtg gct ggt tat gtc ctc att gcc ctc aac aca      530
Lys Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr
80                  85                  90                  95

gtg gag cga att cct ttg gaa aac ctg cag atc atc aga gga aat atg      578
Val Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met
                100                 105                 110

tac tac gaa aat tcc tat gcc tta gca gtc tta tct aac tat gat gca      626
Tyr Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala
            115                 120                 125

aat aaa acc gga ctg aag gag ctg ccc atg aga aat tta cag gaa atc      674
Asn Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile
        130                 135                 140

ctg cat ggc gcc gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg      722
Leu His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val
    145                 150                 155

gag agc atc cag tgg cgg gac ata gtc agc agt gac ttt ctc agc aac      770
Glu Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn
160                 165                 170                 175

atg tcg atg gac ttc cag aac cac ctg ggc agc tgc caa aag tgt gat      818
Met Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp
                180                 185                 190

cca agc tgt ccc aat ggg agc tgc tgg ggt gca gga gag gag aac tgc      866
Pro Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys
            195                 200                 205

cag aaa ctg acc aaa atc atc tgt gcc cag cag tgc tcc ggg cgc tgc      914
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Arg Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly
    225                 230                 235

tgc aca ggc ccc cgg gag agc gac tgc ctg gtc tgc cgc aaa ttc cga     1010
Cys Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg
240                 245                 250                 255

gac gaa gcc acg tgc aag gac acc tgc ccc cca ctc atg ctc tac aac     1058
Asp Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn
                260                 265                 270

ccc acc acg tac cag atg gat gtg aac ccc gag ggc aaa tac agc ttt     1106
Pro Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe
            275                 280                 285

ggt gcc acc tgc gtg aag aag tgt ccc cgt aat tat gtg gtg aca gat     1154
Gly Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp
        290                 295                 300

cac ggc tcg tgc gtc cga gcc tgt ggg gcc gac agc tat gag atg gag     1202
His Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu
    305                 310                 315

gaa gac ggc gtc cgc aag tgt aag aag tgc gaa ggg cct tgc cgc aaa     1250
Glu Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys
320                 325                 330                 335

gtg tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata     1298
Val Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile
                340                 345                 350

aat gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc     1346
Asn Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly
            355                 360                 365

gat ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat     1394
Asp Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His
        370                 375                 380

act cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag     1442
Thr Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys
    385                 390                 395

gaa atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg     1490
Glu Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr
400                 405                 410                 415

gac ctc cat gcc ttt gag aac cta gaa atc ata cgc ggc agg acc aag     1538
Asp Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys
                420                 425                 430

caa cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc     1586
Gln His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser
            435                 440                 445

ttg gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att     1634
Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile
        450                 455                 460

tca gga aac aaa aat ttg tgc tat gca aat aca ata aac tgg aaa aaa     1682
Ser Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys
    465                 470                 475

ctg ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt     1730
Leu Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly
480                 485                 490                 495

gaa aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc     1778
Glu Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser
                500                 505                 510

ccc gag ggc tgc tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg     1826
Pro Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg
            515                 520                 525
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aat gtc agc cga ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag     1874
Asn Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu
        530                 535                 540

ggt gag cca agg gag ttt gtg gag aac tct gag tgc ata cag tgc cac     1922
Gly Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His
    545                 550                 555

cca gag tgc ctg cct cag gcc atg aac atc acc tgc aca gga cgg gga     1970
Pro Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly
560                 565                 570                 575

cca gac aac tgt atc cag tgt gcc cac tac att gac ggc ccc cac tgc     2018
Pro Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys
                580                 585                 590

gtc aag acc tgc ccg gca gga gtc atg gga gaa aac aac acc ctg gtc     2066
Val Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val
            595                 600                 605

tgg aag tac gca gac gcc ggc cat gtg tgc cac ctg tgc cat cca aac     2114
Trp Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn
        610                 615                 620

tgc acc tac gga tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat     2162
Cys Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn
    625                 630                 635

ggg cct aag atc ccg tcc atc gcc act ggg atg gtg ggg gcc ctc ctc     2210
Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu
640                 645                 650                 655

ttg ctg ctg gtg gtg gcc ctg ggg atc ggc ctc ttc atg cga agg cgc     2258
Leu Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg
                660                 665                 670

cac atc gtt cgg aag cgc acg ctg cgg agg ctg ctg cag gag agg gag     2306
His Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu
            675                 680                 685

ctt gtg gag cct ctt aca ccc agt gga gaa gct ccc aac caa gct ctc     2354
Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu
        690                 695                 700

ttg agg atc ttg aag gaa act gaa ttc aaa aag atc aaa gtg ctg ggc     2402
Leu Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly
    705                 710                 715

tcc ggt gcg ttc ggc acg gtg tat aag gga ctc tgg atc cca gaa ggt     2450
Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly
720                 725                 730                 735

gag aaa gtt aaa att ccc gtc gct atc aag gaa tta aga gaa gca aca     2498
Glu Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr
                740                 745                 750

tct ccg aaa gcc aac aag gaa atc ctc gat gaa gcc tac gtg atg gcc     2546
Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala
            755                 760                 765

agc gtg gac aac ccc cac gtg tgc cgc ctg ctg ggc atc tgc ctc acc     2594
Ser Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr
        770                 775                 780

tcc acc gtg cag ctc atc acg cag ctc atg ccc ttc ggc tgc ctc ctg     2642
Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu
    785                 790                 795

gac tat gtc cgg gaa cac aaa gac aat att ggc tcc cag tac ctg ctc     2690
Asp Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu
800                 805                 810                 815

aac tgg tgt gtg cag atc gca aag ggc atg aac tac ttg gag gac cgt     2738
Asn Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg
                820                 825                 830

cgc ttg gtg cac cgc gac ctg gca gcc agg aac gta ctg gtg aaa aca     2786
Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr
            835                 840                 845
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ccg cag cat gtc aag atc aca gat ttt ggg ctg gcc aaa ctg ctg ggt     2834
Pro Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly
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gcg gaa gag aaa gaa tac cat gca gaa gga ggc aaa gtg cct atc aag     2882
Ala Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys
    865                 870                 875

tgg atg gca ttg gaa tca att tta cac aga atc tat acc cac cag agt     2930
Trp Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser
880                 885                 890                 895

gat gtc tgg agc tac ggg gtg acc gtt tgg gag ttg atg acc ttt gga     2978
Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly
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tcc aag cca tat gac gga atc cct gcc agc gag atc tcc tcc atc ctg     3026
Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu
            915                 920                 925

gag aaa gga gaa cgc ctc cct cag cca ccc ata tgt acc atc gat gtc     3074
Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val
        930                 935                 940

tac atg atc atg gtc aag tgc tgg atg ata gac gca gat agt cgc cca     3122
Tyr Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro
    945                 950                 955

aag ttc cgt gag ttg atc atc gaa ttc tcc aaa atg gcc cga gac ccc     3170
Lys Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro
960                 965                 970                 975

cag cgc tac ctt gtc att cag ggg gat gaa aga atg cat ttg cca agt     3218
Gln Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser
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cct aca gac tcc aac ttc tac cgt gcc ctg atg gat gaa gaa gac atg     3266
Pro Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met
            995                1000                1005

gac gac gtg gtg gat gcc gac gag tac ctc atc cca cag cag ggc ttc     3314
Asp Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe
       1010                1015                1020

ttc agc agc ccc tcc acg tca cgg act ccc ctc ctg agc tct ctg agt     3362
Phe Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser
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gca acc agc aac aat tcc acc gtg gct tgc att gat aga aat ggg ctg     3410
Ala Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu
1040                1045                1050                1055

caa agc tgt ccc atc aag gaa gac agc ttc ttg cag cga tac agc tca     3458
Gln Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser
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gac ccc aca ggc gcc ttg act gag gac agc ata gac gac acc ttc ctc     3506
Asp Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu
           1075                1080                1085

cca gtg cct gaa tac ata aac cag tcc gtt ccc aaa agg ccc gct ggc     3554
Pro Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly
       1090                1095                1100

tct gtg cag aat cct gtc tat cac aat cag cct ctg aac ccc gcg ccc     3602
Ser Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro
   1105                1110                1115

agc aga gac cca cac tac cag gac ccc cac agc act gca gtg ggc aac     3650
Ser Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn
1120                1125                1130                1135

ccc gag tat ctc aac act gtc cag ccc acc tgt gtc aac agc aca ttc     3698
Pro Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe
               1140                1145                1150

gac agc cct gcc cac tgg gcc cag aaa ggc agc cac caa att agc ctg     3746
Asp Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu
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gac aac cct gac tac cag cag gac ttc ttt ccc aag gaa gcc aag cca     3794
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       1170                1175                1180

aat ggc atc ttt aag ggc tcc aca gct gaa aat gca gaa tac cta agg     3842
Asn Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg
   1185                1190                1195

gtc gcg cca caa agc agt gaa ttt att gga gca tga                    3878
Val Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
1200                1205                1210


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The invention claimed is:

1. A method of treating gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 in a patient, comprising administering daily to the patient having gefitinib and/or erlotinib resistant non-small cell lung cancer having a T790M mutation in SEQ ID NO: 1 a pharmaceutical composition comprising a unit dosage of 2-500 mg of an irreversible EGFR inhibitor that covalently binds to cysteine 773 of the catalytic domain within the SEQ ID NO: 1 having a T790M mutation;

wherein the irreversible EGFR inhibitor is not CL-387, 785.

2. The method of claim **1**, wherein the irreversible EGFR inhibitor is selected from the group consisting of EKB-569, HKI-272 and HKI-357.

3. The method of claim **1**, wherein the irreversible EGFR inhibitor is HKI-272.

4. The method of claim **1**, wherein the irreversible EGFR inhibitor covalently binds to cysteine 805 in the ligand-binding pocket of ERBB2.

* * * * *

CERTIFICATE OF COMPLIANCE

1. This brief complies with the type-volume limitations of Federal Rule of Appellate Procedure 32(a)(7)(B) and Federal Circuit Rule 32(b)(1) because it contains 13,941 words, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(f) and Federal Circuit Rule 32(b)(2).

2. This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6) because it has been prepared in a proportionally spaced typeface using Microsoft Word for Microsoft 365 in Times New Roman 14-point font.

Dated: December 18, 2024

/s/ *Jennifer L. Swize*
Jennifer L. Swize
JONES DAY
51 Louisiana Avenue, N.W.
Washington, DC 20001
(202) 879-3939