EXHIBIT 2

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff, v. F. HOFFMANN-LA ROCHE LTD, a Swiss Company, ROCHE DIAGNOSTICS GMBH, a German Company, and HOFFMANN LA ROCHE INC., a New Jersey Corporation, Defendants.	Civil Action No.: 1:05-cv-12237 WGY

FIRST EXPERT REPORT OF

VLADIMIR P. TORCHILIN, Ph.D., D.Sc.

CONFIDENTIAL PURSUANT TO PROTECTIVE ORDER

CONTAINS CONFIDENTIAL DISCOVERY MATERIAL

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27. Nektar offers off-the-shelf PEG reagents with a variety of linking chemistries for the optimization of pegylated derivatization of any protein. These off-the-shelf PEG reagents include, mPEG-SPA (succinimidyl propionic acid), mPEG-SBA (succinimidyl butyric acid), $mPEG_2$-NHS (branched), mPEG-ALD (aldehyde), and mPEG-MAL (maleimide). (Exs. 18-19, Nektar Molecule Engineering Catalog 2003 ("Nektar 2003"); Nektar Advanced PEGylation Catalog 2004 ("Nektar 2004").) Nektar also advertises that it can make PEG with custom linking chemistries upon request. (Exs. 18-19, Nektar 2003; Nektar 2004.)

28. Currently, there exist many chemical approaches to synthesize derivatives of PEG that may be coupled to proteins. (Exs. 18-19, Nektar 2003; Nektar 2004.) A common site of attachment on proteins for PEG was and is the amino groups (NH_2) of a protein's lysine residues and its N-terminus. Such PEG derivatives, which attach to proteins via amide linkages at the protein's lysines or N-terminus amino groups, have been known and in use since the '70s. Today, Nektar sells a variety of mPEGs combined with different linkers. Some of these linkers have a terminal ester group that can in turn be activated by various chemistries. An appropriately activated mPEG-linker molecule can then react through its activated ester with a

primary amine of the protein (at the lysine or N-terminus in amino acid residues) to form an amide linked PEG conjugate.

29. Initially, PEGs were used that had a relatively low molecular weight range, typically around 1900 to 5000 Da and up to a maximum of about 12,000 Da. (Exs. 2 and 20, Abuchowski 1977; Harris et al., 2 NATURE 214 - 221, 215 (2003) ("Harris 2003").) Some early linking chemistries also tended to be less specific in the site of attachment to the protein, *i.e.*, some chemistries reacted with more than one type of functional group on the protein and so produced conjugates that were heterogeneous in site of attachment and "bond" of attachment. (Exs. 2 and 20, Abuchowski 1977; Harris 2003) Some linkers also contained bonds that were susceptible to hydrolysis after conjugation to a protein.

30. Later PEG and PEG linking technology used PEGs of larger molecular weight, typically 20,000 to 40,000 Da, and linking chemistries that were more specific in their site of attachment on the protein, and in some cases, gave exquisite specificity in the site of attachment. (Ex. 16, Kozlowski 2001.) These linkers used were also resistant to hydrolysis in aqueous solution. (Ex. 16, Kozlowski 2001.)

31. Over the last three decades, proteins modified with PEG have shown increased *in vivo* longevity and activity but frequently decreased *in vitro* specific activity when compared to the unmodified protein. (Ex. 6, Delgado 1992.) Modifying a protein with PEG increases the molecular weight of the PEG-protein, can increase the solubility of the protein in water, and can increase the solubility of the protein in certain organic solvents.

32. At least 40 proteins had been conjugated with PEG by the early 1990s. (Exs. 6 and 8, Delgado 1992; Fuertges 1990.) These pegylated proteins included the enzymes asparaginase, superoxide dismutase, uricase, adenosine deaminase, catalase, tPA, arginase, elastase, galactosidase, β-glucuronidase, lipase, phenylalanine ammonia lyase, streptokinase, trypsin, and urokinase. By 1990, five pegylated proteins had been used in clinical trials: peg-Asparaginase, peg-Superoxide Dismutase, peg-Uricase, peg-Adenosine Deaminase, and peg-Interleukin-2. As a rule, these pegylated proteins were conjugates between multiple PEG

molecules and a single protein, *i.e.*, these proteins were poly-pegylated with lower molecular weight PEG. These pegylated proteins showed increased *in vivo* longevity and activity but frequently decreased *in vitro* specific activity, consistent with earlier experience.

33. Today, several pegylated proteins have been approved by the FDA for human therapy including: peg-Interferon (Pegasys®), peg-Mutant hGH (Somavert®), peg-Adenosine Deaminase (Adagen®), peg-G-CSF (Neulasta®), and peg-Asparaginase (Oncaspar®). Other recombinant proteins that have been PEGylated include, interleukin-2, interleukin-6, growth hormone releasing factor, bone derived neutrophic factor, and human growth hormone.

C. PEG PROVIDES A "SHIELD" THAT CAN PROVIDE PROTECTION TO THE PROTEIN.

34. My laboratory investigated the mechanism of PEG protective action with liposomes. (Ex. 21, Torchilin et al., 1195 BIOCHIM. BIOPHYS. ACTA 11-20 (1994) ("Torchilin 1994").) On the biological level, PEG sterically hinders interactions of blood components, such as opsonins, with the surface of the target, *e.g.*, a liposome or a protein. (Exs. 21-23, Torchilin 1994; Allen et al., 13 ADV. DRUG DELIV. REV. 285-309 (1994) ("Allen 1994"); Woodle, 64 CHEM. AND PHYSICS OF LIPIDS 249-262 (1993) ("Woodle 1993").) In this context, steric hindrance refers to the physical exclusion by PEG of opsonins or other blood components from the target surface. This prevents target interaction with opsonins and blocks fast capture of liposomes by the RES. (Ex. 21, Torchilin 1994.) The reduced binding of blood components to PEG-liposomes and PEG-proteins was demonstrated in several studies. (Exs. 6, 22, 24-26, Delgado 1992; Allen 1994; Senior et al., 1062 BIOCHIM. BIOPHYS. ACTA. 77-82 (1991); Chonn et al., 1070 BIOCHIM. BIOPHYS. ACTA. 215-222 (1991); Chonn et al., 267 J. BIOL. CHEM. 18759-18765 (1992).)

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65. Similarly, Roche told the U.K. Clinical Trials Unit that:

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Like peg Interleukin-2 and Interleukin-2, peg-EPO and EPO are similar in their toxicity pattern, *in vivo* activity, and *in vitro* activity. Thus, peg-EPO and EPO have the same biological function.

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(*Id.* at ITC-R-IND-00067153 (emphasis added).)

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c. The Nomenclature Roche Used Also Shows That the EPO Was Not Materially Changed

73. The nomenclature used to describe pegylated proteins also reflects the conventional understanding that the critical structure and function of the therapeutic protein is maintained after pegylation. Once pegylated, the therapeutic protein's name is preceded by the prefix "peg," to indicate that the protein has been pegylated. The pegylated proteins discussed in the Hershfield et al., Jurgens et al., Chua et al., Meyers et al., and Monkarsh et al. articles follow this convention and were named peg-Adenosine

Deaminase, peg-Asparaginase, peg-Uricase, peg-Interleukin-2, and peg-Interferon. (Exs. 53 and 73-76, Meyers et al., 49 CLIN. PHARMACOL. THER. 307-313 (1991); Hershfield et al., 316 N. ENGL. J. MED. 589-596 (1987); Jurgens et al., 200 KLIN. PEDIATR. 184-189 (1988); Chua et al., 109 ANN. INT. MED. 114-117 (1988); and Monkarsh et al., 247 ANAL. BIOCHEM. 434-40 (1997).) This is the general rule for the nomenclature of pegylated proteins. This nomenclature system reflects, in part, the fact that the biological function of the pegylated protein has not changed, *e.g.*, peg-Adenosine Deaminase is still an Adenosine Deaminase, peg-Asparaginase is still an Asparaginase, peg-Uricase is still a Uricase, peg-Interleukin-2 is still an Interleukin-2, and peg-Interferon is still an Interferon. That is, the PEG conjugates of these proteins still have the biological activity of the unmodified parent protein.

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78. As discussed above in paragraphs 9-33, scientists have conjugated therapeutic proteins with water-soluble polymers to improve their *in vivo* longevity and *in vivo* biological activity for over thirty years. Today, conjugation with the water-soluble polymer PEG is a conventional modification for improving the pharmacokinetics of a therapeutic protein. PEG was selected as the preferred water-soluble polymer for these conjugates, in part, because it did not change the biological function of the conjugated protein.

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number of proteins used for patient therapy have now been modified with PEG and evaluated clinically. These include adenosine deaminase, asparaginase, superoxide dismutase, uricase, and interleukin-2 (23, 24, 25, 26, 27). ***All of these pegylated proteins have increased half-lives relative to the unmodified proteins***.

A pegylated version of epoetin beta (PEG-EPO, Ro 50-3821) has been synthesized by chemically conjugating one linear methoxy-PEG molecule to epoetin beta (EPO, Ro 205-3859). Data from animal studies indicate that PEG-EPO has enhanced pharmacokinetic properties compared with EPO that may allow it to be given once weekly or once very two weeks.

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These five articles are:

- Hershfield et al., 316 N. ENGL. J. MED. 589-596 (1987) entitled "Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase." (Ex. 73.)
- Jurgens et al., 200 KLIN. PEDIATR. 184-189 (1988) entitled "Clinical experience with polyethylene glycol coupled *E. coli* asparaginase in patients with recurrent ALL." (Ex. 74.)
- Chua et al., 109 ANN. INT. MED. 114-117 (1988) entitled "Use of polyethylene glycol-modified uricase (PEG-uricase) to treat hyperuricemia in a patient with non-Hodgkin's lymphoma." (Ex. 75.)
- Meyers et al., 49 CLIN. PHARMACOL. THER. 307-313 (1991) entitled "A phase I study including pharmacokinetics of polyethylene glycol conjugated interleukin-2." (Ex. 53.)
- Monkarsh et al., 247 ANAL. BIOCHEM. 434-40 (1997) entitled "Positional isomers of monopegylated interferon alpha-2a: isolation, characterization, and biological activity." (Ex. 76.)

83. I have reviewed these articles and they confirm that pegylation is a conventional approach to improve the *in vivo* longevity and *in vivo* biological activity of a protein therapeutic. These articles present information on two peg-protein therapeutics, peg-Adenosine Deaminase and peg-Asparaginase, that have been approved by the FDA for the treatment of adenosine deaminase deficiency and acute lymphoblastic leukemia, respectively. The Monkarsh et al. article also presents information on a monopegylated interferon that was made by Roche. This article from 1997 states that it was known that one way to "improve serum half-life, decrease frequency of dosing, and reduce immunogenicity of protein therapeutics is to modify lysine residues on the surface of the protein with polyethylene glycol (PEG) . . ." (Ex. 76, Monkarsh et al., 247 ANAL. BIOCHEM. 434-40 (1997).)

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[7] These three articles are:

- Meyers et al., 49 CLIN. PHARMACOL. THER. 307-313 (1991) entitled "A phase I study including pharmacokinetics of polyethylene glycol conjugated interleukin-2." (Ex. 53.)
- Harris et al., 40 CLIN. PHARAMACOKINET. 539-551 (2001) ("Harris 2001") entitled "PEGylation a novel process for modifying pharmacokinetics." (Ex. 111.)
- Bailon et al., 1 PHARMACEUT. SCI. TECHNOL. TODAY 352-356 (1998) ("Bailon 1998") entitled "Polyethylene glycol-conjugated pharmaceutical proteins." (Ex. 112.)

85. The Meyers article was also cited in Redacted The Harris and Bailon publications that are newly cited in also identify the well known effects of pegylation as increased *in vivo* longevity, increased *in vivo* activity, and reduced *in vitro* activity. The Harris and Bailon publications also note that pegylation is a common approach for producing these well known effects on the pharmacokinetics of a protein

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therapeutic. These three articles state that one mechanism for increasing *in vivo* longevity by pegylation is reduced renal clearance of the conjugate due to its increased mass.

86. Pegylation of the therapeutic proteins in these articles increased the half-life of the peg-protein conjugate by 10 fold (peg-Interleukin-2) to 50 fold (peg-Adenosine Deaminase).

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Other cytokines show similar decreases in their *in vitro* activity and receptor binding after pegylation. For example, peg-Interferon-α2a has reduced *in vitro* activity (about 14 fold less) and reduced binding to its receptor (about 90 fold less), peg-hGH has reduced *in vitro* activity (about 50 – 100 fold less) and reduced binding to its receptor (about 60 – 150 fold less), and peg-GHA (growth hormone antagonist) has reduced *in vitro* activity (about 65 fold less).[8] (Exs. 113-116, Bailon et al., 12 BIOCONJUGATE CHEM. 195 – 202 (2001) ("Bailon 2001"); Foser et al., 3 PHARMACOGEN. J. 312 – 319 (2003) ("Foser 2003"); Clark et al., 271 J. BIOL. CHEM. 21969 – 77 (1996) ("Clark 1996"); Olson et al., POLY(ETHYLENE GYCOL) CHEMISTRY AND BIOLOGICAL APPLICATIONS (Eds. Harris et al.) Am. Chem. Soc. (1997), pp. 170-181.)

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(Ex. 35, Micera BLA, Drug Substance – RO0503821, 3.2.S.3.1, at p. 246; ITC-R-BLA-00004232 – 4330 at 4269.)

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peg-Interferon-α2a has a molecular weight of about 60 kDa, which is about

[8] The publications for peg GHA did not present comparative receptor binding data.

three times the molecular weight of recombinant Interferon-α2a, peg-hGH has a molecular weight of about 47-52 kDa, which is about twice the molecular weight of recombinant hGH, and peg-Interleukin-2 had a molecular weight of about 29-36 kDa, which is about twice the molecular weight of recombinant Interleukin-2.

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(Exs. 113, 115 and 117, Bailon 2001; Clark 1996; Zimmerman et al., 8 J. BIOL. RESP. MOD. 329-30 (1989).)

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These other pegylated proteins, peg-Interferon-α2a, peg-hGH, peg-Interleukin-2, peg-GHA, and peg-Adenosine Deaminase were not described as new proteins that were no longer interferon, Interleukin-2, GHA, or Adenosine Deaminase, respectively.

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91. Before 1999, other companies had also demonstrated that pegylation could improve *in vivo* longevity and *in vivo* activity of other cytokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), CD4-IgG, and interferon- γ (INF-γ). (Ex. 112, Bailon 1998.).

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95. Publications from Nektar/Shearwater also point out the conventional nature of pegylation to improve the *in vivo* longevity and activity of therapeutic proteins.

> ***A well known method for increasing the blood circulation lifetime*** of a protein pharmaceutical is to covalently attach chains of the water-soluble polymer, poly(ethyleneglycol) or PEG, to the protein [3].

(Ex. 16, Kozlowski 2001 (emphasis added).)

> PEGylation now represents a promising ***sustained-action delivery method*** for injectable medications. * * * The potential advantages of pegylation include ***increased circulating exposure*** to the therapeutic protein, decreased acute adverse effects, more convenient dosage regimens, and increased health-related quality of life.

(Ex. 111, Harris 2001 (emphasis added).)

> . . . pegylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, such as ***sustained blood levels that enhance effectiveness***, fewer adverse reactions, longer shelf life and improve patient convenience[21]. However, pegylation can produce ***a decrease in the in vitro activity*** of proteins, but generally this negative effect is offset in biological systems by an increased half-life.

(Ex. 20, Harris 2003, emphasis added.)

96. It is common to make new dosage forms of a drug that have a sustained duration of action. As described in detail above, pegylation is a well-known method for increasing the duration of action of drugs. Thus, in my opinion pegylation of EPO is a conventional technique for making a sustained duration form of EPO.

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109. Many of the concepts concerning changes caused by an increase in molecular weight (Paragraph 111, 1st category) are well known and understood. For example, it has long been known that peg-proteins move much slower in PAGE than the unpegylated parent protein because the peg-conjugate has a larger size. (Exs. 6 and 112, Delgado 1992; Bailon 1998.) It was already known that increasing the molecular weight increases the hydrodynamic radius. (Exs. 6, 16 and 20, Delgado 1992; Kozlowski 2001; Harris 2003.) Also, it is well known and understood that diffusion, sedimentation, and the virial coefficient are effected by the size and density of the molecule. Proteins like EPO are tightly packed molecules compared to water-soluble polymers like PEG and so conjugating proteins with PEG will change the overall density of the conjugate from the protein.

110. The changes caused by modifying an amine to make an amide by the conjugation of PEG (Paragraph 111, 2nd category) was also well-known, and many groups had used such linking chemistry to attach PEG to many proteins. The change of amine to an amide reduces the positive charge on the protein at the site of pegylation. This reduction of one positive charge for the protein will alter the pI and the pKa of the protein which both correlate with the charges on the protein. (Exs. 6, 20, 112, Delgado 1992 ; Harris 2003 ; Bailon 1998.)

111. The general properties of PEG have been known for decades and are discussed at length above. For example, pegylation extends the circulating serum half-life of the protein by enlarging its effective size. In fact, there is a direct correlation between the molecular size of the attached PEG group and the *in vivo* half-life of a pegylated protein. It has also been suggested that the hydrophilic nature of the ethylene oxide unit and the mobility of the PEG in aqueous

solution result in a molecule that may appear to be 5-10 times larger than a protein of comparable weight. (Ex. 13, Wang 2002.) It has also been known that pegylating decreases the rate of proteolytic degradation. (Exs. 6, 15 and 20, Delgado 1992; Nucci 1991; Harris 2003.)

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I declare under penalty of perjury in accordance with the laws of the United States that the foregoing is true and correct and that this declaration and expert report was executed in Boston, Massachusetts, on April 06, 2007.

Vladimir P. Torchilin, Ph.D., D.Sc.