UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN, INC.,
 	
    Plaintiff,
 	
    v.
 	
F. HOFFMANN-LA ROCHE, LTD.,
ROCHE DIAGNOSTICS GMBH, and
HOFFMANN-LA ROCHE, INC.
 	
    Defendants.
	Civil Action No. 05-CV-12237 WGY

DECLARATION OF ALEXANDER M. KLIBANOV, Ph.D. IN SUPPORT OF DEFENDANTS' OPPOSITION TO AMGEN'S MOTION FOR SUMMARY JUDGMENT OF INFRINGEMENT OF '422 CLAIM 1, '933 CLAIM 3, AND '698 CLAIM 6

1.   I, Alexander M. Klibanov, Ph.D., submit this Declaration in support of Roche's

Opposition to Amgen's Motion for Summary Judgment of Infringement of '422 Claim 1, '933

Claim 3, and '698 Claim 6. My opinions and analysis submitted in this Declaration were

previously disclosed in expert reports submitted in this litigation.

2.   I am a Professor of Chemistry and of Bioengineering at the Massachusetts

Institute of Technology ("M.I.T.").

3.   In 1971, I earned a Masters of Science degree in Chemistry from Moscow

University in Russia and continued there to earn a Ph.D. in Chemical Enzymology in 1974.

4.   Between 1974 and 1977, Following my immigration to the United States, I was

employed as a Research Chemist in the Chemistry Department of Moscow University. I was a

Postdoctoral Associate at the Chemistry Department of the University of California, San Diego

from 1977 to 1979. In 1979, I accepted a position as Assistant Professor of Applied

Biochemistry at M.I.T. I was promoted to Associate Professor in 1983, and then to full Professor

in the Department of Applied Biological Sciences in 1987. In 1988, I moved to the Department

of Chemistry at M.I.T., where I assumed a full professorship, a position I still hold today. Since

2000, I also hold a joint appointment as Professor of Bioengineering in the Department of

Biological Engineering at M.I.T.

5.   My research is multi-faceted. Over the course of my career, I have had extensive

experience studying, working with, and publishing about, enzymes, hormones, and other

proteins, including their chemistry, biochemistry, purification, characterization, chemical

modification (including chemical reactions with poly(ethylene glycol) ("PEG") reagents to make

new chemical substances, henceforth referred to as pegylation), biological effects, and synthesis.

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In particular, for the last 30 years or so, I have been active in developing and studying protein formulations, including their stability, administration, delivery, and biological evaluation.

6.   During over 30 years of scholarly work, I have earned numerous prestigious professional awards and honors. For example, I was elected to the United States National Academy of Sciences (considered among the highest professional honors that can be bestowed on an American scientist) and to the United States National Academy of Engineering (considered among the highest professional honors that can be bestowed on an American engineer or applied scientist). I am also a Founding Fellow of the American Institute for Medical and Biological Engineering and a Corresponding Fellow of the Royal Society of Edinburgh (Scotland's National Academy of Science and Letters). In addition, I have received the Arthur C. Cope Scholar Award, the Marvin J. Johnson Award, the Ipatieff Prize, and the Leo Friend Award, all from the American Chemical Society, as well as the International Enzyme Engineering Prize. I also have given 17 distinguished named lectureships all over the world.

7.   I currently serve on the Editorial Boards of eight scientific journals: Applied Biochemistry and Biotechnology, Biocatalysis and Biotransformation, Biotechnology Progress, Journal of Molecular Catalysis - Enzymatic, Microbial Biotechnology, Central European Journal of Chemistry, Biotechnology & Bioengineering, and Patents in Biotechnology. In the past, I was also an Editorial Board member for other scientific journals, e.g., Proceedings of the National Academy of Sciences of the U.S.A. (1999 - 2005), and Biochimica et Biophysica Acta - Protein Structure & Molecular Enzymology (1994 - 1996).

8.   I have published over 260 scientific papers, many dealing with chemical reactions of proteins, purification, stability, formulation, and delivery. I am also a named inventor of 16 issued United States patents, including several dealing with pharmaceutical formulations and the

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delivery of proteins. Finally, I have given over 350 invited lectures at professional conferences, universities, and corporations (including both Amgen and Roche) all over the world, many dealing with formulation, stability, delivery, and biological evaluation of proteins.

9.   Over my 27 years at M.I.T., I have taught numerous undergraduate and graduate courses in general (freshman) chemistry, organic chemistry, biological chemistry, enzyme and protein chemistry, biotechnology, and analytical biochemistry. I have also been a lecturer in summer courses at M.I.T. directed to industrial scientists, including "Controlled Release of Pharmaceuticals" and "Analytical Biochemistry in Process Monitoring and Validations." I have co-authored and co-edited several books including the "Handbook of Pharmaceutical Controlled Release Technology," published by Marcel Dekker in 2000.

10.   In addition to my research and teaching duties at M.I.T., I have consulted widely for pharmaceutical, chemical, and biotechnology companies. I have also founded four biotechnology companies and have been on the scientific advisory boards and/or boards of directors of those companies and many others.

11.   Many of these consulting, advisory, and directorship activities have dealt specifically with formulation, stability, delivery, and biological evaluation of therapeutic proteins.

12.   A copy of my Curriculum Vitae detailing my awards, appointments, publications, and patents is attached as Exhibit A.

I.   **FUNDAMENTALS OF ORGANIC CHEMISTRY AND BIOCHEMISTRY**

    A.   **Atoms, Molecules, and Chemical Bonds**

13.   Atoms are the basic building blocks of all molecules, which, in turn, comprise all chemical compounds. An atom is composed of a nucleus, which includes protons and neutrons, surrounded by electrons. The identity of an atom is defined by the number of protons in the

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nucleus (which necessarily equals the number of electrons). This is also called the atomic number. A chemical element is a substance made up of only one type of atoms, those having the same atomic number. For example, the simplest chemical element hydrogen is composed of atoms having one proton and one electron. Thus, hydrogen has an atomic number of 1. Carbon atoms have six protons, hence atomic number 6; nitrogen atoms have seven protons, hence atomic number 7; oxygen atoms have eight protons, hence atomic number 8; etc.

14.   Protons are positively charged particles, and electrons are equally but negatively charged. When the number of electrons equals the number of protons (as in an atom), there is no net charge. Conversely, when the number of electrons is less or greater than the number of protons, the substance, called an ion, has a net positive or negative charge, respectively.

15.   If the charge of an ion is positive it is called a cation; if the charge is negative it is called an anion. The net charge of an ion is an important physical attribute which influences its physical, chemical, and biological properties.

16.   The atomic mass of an atom is the sum of the number of protons plus the number of neutrons (yet another, electrically neutral subatomic particle; the mass of electrons can be neglected because an electron is roughly 2,000 times lighter than a proton or a neutron). For example, a carbon atom that has 6 protons and 6 neutrons possesses an atomic mass of 12.

17.   A different arrangement of atoms within a molecule corresponds to a different compound with its own unique set of chemical and physical properties. (Ex. 242, Morrison & Boyd (1983) at 79)[1]. Molecules are the smallest individual particles of a chemical compound. They typically are groups of atoms held together by chemical bonds. When one atom of element

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[1] All Exhibits cited herein are attached to the Declaration of Keith E. Toms in Support of Defendants' Opposition to Amgen's Motion for Summary Judgment of Infringement of '422 Claim 1, '933 Claim 3, and '698 Claim 6.

A bonds with one atom of element B, a molecule AB, or A-B results. It is important to note that molecule A-B is neither A nor B. In other words, A-B is substantially different from either A or B with its own physical and chemical properties. The molecule A-B does not "contain" or comprise either A or B, because both A and B significantly change upon their bonding, leading to the formation of A-B.

18.   Molecular weight is the total weights of all the individual atoms that make up the molecule. Molecular weight is a fundamental property of a chemical compound. Two substances having different molecular weights are by definition different chemical entities. Large differences in molecular weight typically are an indication that the substances are substantially different. This is because additional atoms that lead to higher molecular weights necessarily affect the properties of the substance.

19.   Depending on the types of atoms in a molecule, each molecule possesses a specific charge distribution within it. Charges, negative and positive, are typically localized in certain regions of a molecule.

20.   The field of organic chemistry involves the study of structure, properties, and synthesis of chemical compounds consisting of at least carbon and hydrogen atoms. "Carbon atoms can attach themselves to one another to an extent not possible for atoms of any other element. Carbon atoms can form chains thousands of atoms long, or rings of all sizes; the chains and rings can have branches and cross-links." (Ex. 242 at 2). Morrison and Boyd also state in their classic organic chemistry textbook:

> Each different arrangement of atoms corresponds to a different compound, and each compound has its own characteristic set of chemical and physical properties. It is not surprising that more than a million compounds of carbon are known today and that thousands of new ones are being made each year. It is not surprising that the study of their chemistry is a special field.

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> Organic chemistry is a field of immense importance to technology:
> it is the chemistry of dyes and drugs, paper and ink, paints and
> plastics, gasoline and rubber tires; it is the chemistry of the food
> we eat and the clothing we wear.
>
> Organic chemistry is fundamental to biology and medicine. Aside
> from water, living organisms are made up chiefly of organic
> compounds; the molecules of "molecular biology" are organic
> molecules. Biology, on the molecular level, is organic chemistry.
> (Ex. 242 at 2-3).

21.   Chemists use various pictures, letters, symbols, and words to represent molecules. These representations are typically shorthand notations used for convenience in a particular situation. Undergraduate students taking their first course in organic chemistry are taught that the pictures need to be interpreted to understand what is meant: "These crude pictures and models are useful to us only if we understand what they are intended to mean." (Ex. 242 at 3).

22.   Organic chemists have devised a standard system for representing the structure of molecules. In general, atoms are represented by their one- or two-letter symbols in the periodic table of the elements. For example, hydrogen is H, carbon is C, nitrogen is N, and oxygen is O.

23.   Organic molecules are formed by the joining of atoms through covalent bonds. Covalent bonding occurs when two atoms share electrons. The sharing of electrons creates a force that holds the atoms together. Covalent bonds are considered strong chemical bonds compared to other chemical bonds that can hold atoms or molecules together.

24.   Molecules made up exclusively of groups of carbon and hydrogen atoms are called hydrocarbons. These molecules can have different orientations in space. The carbon and hydrogen atoms in molecules can be connected in chains that can be linear, branched, or cyclic. Carbon in organic molecules always has four and only four covalent bonds. These bonds can be single bonds, double bonds, or triple bonds. A carbon atom covalently bonded to 4 hydrogen atoms constitutes one of the simplest organic substances called "methane". Methane (the main

component of natural gas) is a flammable gas. Its properties are dramatically different from elemental carbon (which occurs in nature as either diamonds or graphite) and elemental hydrogen. Methane can be represented by the following equivalent formulas:

$$
\begin{array}{c}
\text{H} \\
| \\
\text{H} - \text{C} - \text{H} \qquad\qquad CH_4 \\
| \\
\text{H}
\end{array}
$$
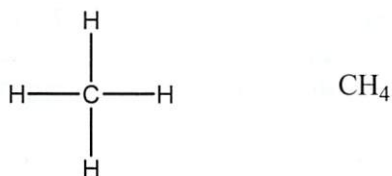
25.   Covalent bonds between atoms are represented by single straight lines (or dashes). Each line represents one bond or one pair of shared electrons. Thus, a single bond is represented by a single line, a double bond is represented by two parallel lines, and a triple bond by three parallel lines. Chemists have refined the system for visual simplicity so that carbons are represented by an intersection of lines or simply by the end of a line, and hydrogen atoms bonded to carbon atoms and the lines representing C-H bonds are often not drawn at all. Thus, for all carbon atoms, if only two bonds are actually depicted, it is understood that there are also two bonds to hydrogen atoms implied, but not drawn. Inother words, because chemists understand that in organic molecules, carbon always has four bonds, chemists do not always write out the carbon and hydrogen atoms on a molecule. Chemists understand a notation where every straight line represents a carbon-to-carbon bond and any carbon atom with fewer than four bonds depicted by implication includes enough single bonds to hydrogen atoms to make four bonds. This is illustrated below for the hydrocarbon compound called propane:

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C_3H_8
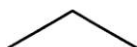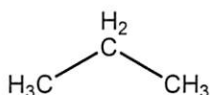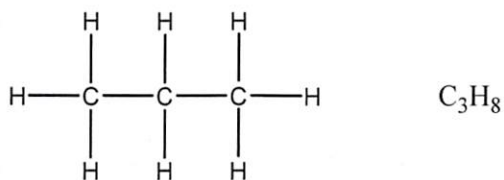
26.   Each of these equivalent representations of propane is understood to refer to a molecule having three carbon atoms and eight hydrogen atoms. As molecules become larger, these shorthand notations are used more frequently. However, the mere fact that a shorthand representation is used does not mean that any atom or bond is insignificant. To the contrary, to understand the nature of a chemical substance, every atom and every bond within its molecules should be considered important.

27.   There are three distinct two-carbon hydrocarbon molecules: ethane (C_2H_6), ethylene (C_2H_4), and acetylene (C_2H_2).


C_2H_6

C_2H_4

C_2H_2
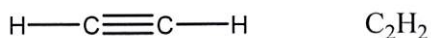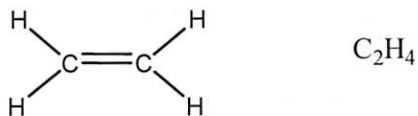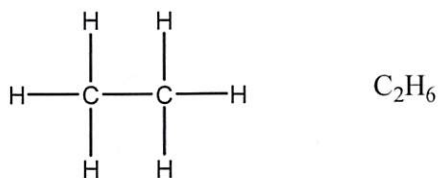
28.   In ethane, the two carbon atoms are attached to each other by a single bond. To fill out the required number of four bonds, each carbon atom is also bonded to three hydrogen

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atoms. In ethylene, the two carbon atoms are bonded to each other by a double bond. To fill out

the required number of four bonds, each carbon atom is also bonded to two hydrogen atoms. In

acetylene, the two carbon atoms are bonded to each other by a triple bond. To fill out the

required number of four bonds, each carbon atom is also bonded to only one hydrogen atom.

Although these three molecules structurally differ only by the type of covalent bond and the

number of hydrogens attached to the carbons, they have very different chemical and physical

properties.

29.   Organic molecules often have structures in the form of rings. In particular, six-

membered ring structures are common. Consider two simple 6-membered ring hydrocarbon

substances that differ in the type of covalent bonds and the number of hydrogen atoms attached

to the ring: cyclohexane (C_6H_{12}) and benzene (C_6H_6). Despite what might appear as only a small

difference in structure, benzene and cyclohexane have markedly different chemical and physical

properties. Thus it can be misleading to superficially look at such ring structures to

simplistically derive any inference about the properties of molecules.

cyclohexane C_6H_{12} benzene C_6H_6
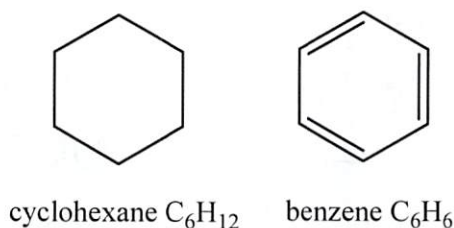
30.   The molecular structure of the common household drug aspirin is shown below.

One can discern in it the presence of a six-membered ring that resembles benzene. However, no

scientist would say that aspirin has benzene in it. The two compounds are strikingly distinct in

their properties: aspirin is a white, odorless, pain-relieving, crystalline solid, while benzene is a

sweet-smelling, volatile, cancer-causing liquid. It is my opinion that if a patent claim were

directed to benzene, aspirin would not infringe.

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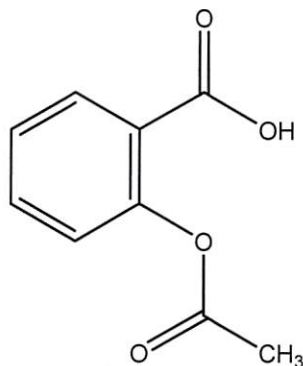
aspirin

31.   Organic molecules often have oxygen and/or nitrogen atoms in addition to carbons and hydrogens. A nitrogen typically forms three covalent bonds but can accommodate a fourth bond, resulting in a nitrogen that is positively charged. Oxygen usually forms only two covalent bonds.

32.   Certain groups of atoms, sometimes referred to as functional groups or residues, are designated by a specific name. For instance, a primary amine is a chemical group composed of a nitrogen atom attached to a carbon and two hydrogen atoms by three single covalent bonds. An alcohol group is –OH or a hydroxyl group typically attached to a carbon atom.


primary amine   primary alcohol
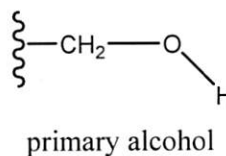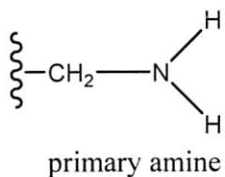
33.   An acid is a chemical compound that can donate a proton or positively charged hydrogen ion (H+) to another molecule. A carboxylic acid is an example of an organic acid having a group which has the formula -C(=O)OH, usually written -COOH or $-CO_2H$.

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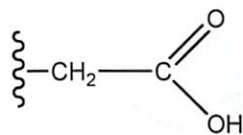
carboxylic acid

34.　　An amide forms when a carboxylic acid reacts with a primary amine. It has the following general structure:
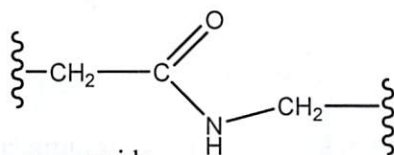
amide

35.　　Ethanol, also called ethyl alcohol, is the intoxicating substance known as alcohol in drinks like vodka, whiskey, beer, and wine. The molecular structure of ethanol (CH_3CH_2OH), shown below, may seem structurally similar to ethane (¶27 above). In fact, the molecular structures differ by a single atom: ethanol has an additional oxygen atom that is not present in ethane. The physical and chemical properties of the substances are nonetheless strikingly different. At room temperature and pressure, for example, ethanol is a colorless liquid, while ethane is a gas.
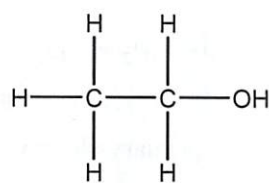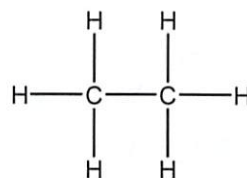
ethanol   ethane

36.　　This is just one example of the fundamental principle of organic chemistry that even seemingly small differences in molecular structure can and usually do have a profound impact on chemical, physical, and biological properties of a substance. The presence of the oxygen atom in ethanol, with all the other atoms being the same in kind and number, makes it a

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markedly distinct compound from ethane. The properties of a compound thus are a result of the
entire structure of the molecule. It is my opinion that if a patent claim were directed to ethane,
ethanol would not infringe. There is no ethane in ethanol.

37.   Organic synthesis involves the development of chemical reactions that transform
organic compounds into new ones through the formation of covalent bonds. It is the process of
making, breaking, and rearranging covalent bonds. The flow of electrons in the course of a
reaction can be depicted by curved arrows, a method of representation called "arrow pushing,"
where each arrow represents an electron pair. (*See, e.g.*, Ex. 242 at 157).

38.   The second of the two starting materials in the scheme below is called
formaldehyde (belonging to a large class of organic molecules called aldehydes). In this scheme,
it reacts with the simplest amine (ammonia, NH_3). This type of a reaction is relevant to some of
the discussions below.
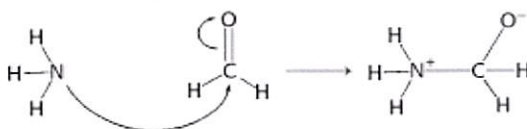
39.   The following pharmaceutical example might be instructive. In the early 1950s,
Dr. Jonas Salk of the University of Pittsburgh developed the first effective polio vaccine. This
medical breakthrough essentially eradicated polio in the United States and saved millions of
children from this debilitating disease. From the chemical standpoint, Dr. Salk's discovery
involved reacting a mixture of three active polio virus strains with formaldehyde.

40.   Formaldehyde predominantly forms covalent bonds with amino groups of the
protein subunits of polio virus, thereby rendering the latter non-virulent but still able to cause the
protective immune reaction in the human body. This type of a nucleophilic substitution reaction

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involving an amine, exemplified above, is conceptually similar to the chemical reaction of

epoetin beta's amino groups with m-PEG-SBA employed by Roche to produce CERA (see

below). In my opinion, the active ingredient in Dr. Salk's life-saving polio vaccine is materially

changed compared to the starting material, the disease-causing polio virus (otherwise, the FDA

presumably would have never approved its use to vaccinate children). If so, then CERA is

likewise materially changed compared to epoetin beta.

  41. Once a chemical reaction occurs, the original substances cease to exist and a new

and different substance (or substances) is (are) created. New covalent bonds are formed (and old

ones broken) producing new compounds with new properties that are distinct from those of the

starting materials or reagents. Pegylation is an example of an organic synthesis that transforms

starting materials into new chemical compounds through the formation of covalent bonds (see

explanation of pegylation technology below).

  42. For example, ethanol can be formed by the chemical addition of water across the

double bond of ethylene as described below:

ethylene   water (H_2O)   ethanol
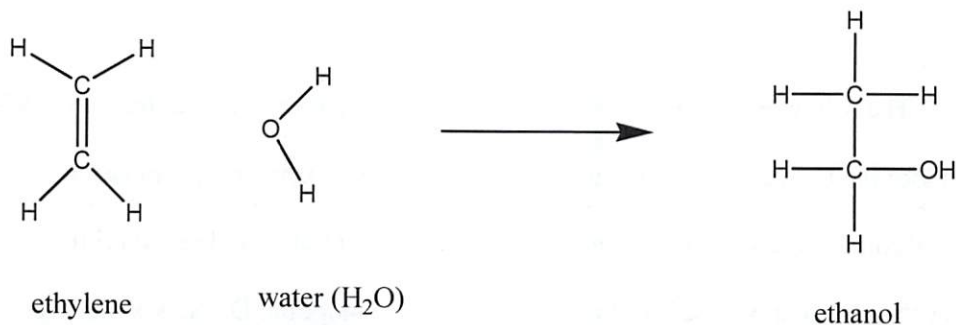
  43. This type of chemical reaction, called hydration, has been known for over a

century. However, the fact that this reaction seems relatively straightforward and is old does not

mean that changes that occur to the starting materials are not substantial. To the contrary,

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ethylene and water are both vastly different from ethanol in physical, chemical, and biological properties.

44.   One can inspect the structure of ethanol and mentally discern the elements of water (H_2O) in it (H and OH). This certainly does not mean that water is the same as ethanol and/or that the biological properties of ethanol are the same as, or even similar to, the biological properties of water. A healthy person can readily drink an eight ounce glass of pure water and feel perfectly fine. However, drinking an eight ounce glass of pure ethanol would cause significant biological effects (e.g., intoxication). Thus newly formed molecules cannot be viewed as physical assemblies of "parts" or "components", or of the starting materials. Nor are the properties of these newly formed molecules predictably derived from the properties of the starting materials.

ethanol
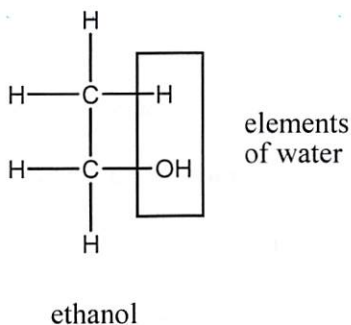
45.   There are many other examples in which seemingly small structural differences between two molecules result in large differences in the properties of the substances. For example, the difference between water, H_2O, and hydrogen sulfide, H_2S, is only one atom, but the two substances have drastically different properties. At room temperature and pressure, water is a liquid that supports life, while hydrogen sulfide is a poisonous gas. Similarly, hydrogen peroxide, H_2O_2, differs from water by only one extra oxygen atom. However, that one atom difference makes hydrogen peroxide highly reactive, toxic, and explosive.

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46.   With respect to the hydrogen peroxide H_2O_2, a superficial inspection may suggest that it consists of H_2 and O_2 molecules. Nothing can be further from the truth, however, as revealed even by the fact that H_2 and O_2 are both gases (the latter constitutes about 20% of the air we breathe) at room temperature and pressure, while H_2O_2 is a thick liquid.

47.   The chemical name for the active agent in household vinegar is acetic acid. This simple carboxylic acid has the formula:

acetic acid (vinegar)
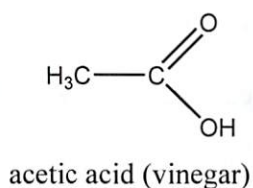
48.   The replacement of a single carbon-bonded hydrogen atom of acetic acid with a fluorine atom means the difference between a relatively harmless substance used in cooking and a severely toxic substance, mono-fluoroacetic acid. Although the chemical name mono-fluoroacetic acid includes the words acetic acid, this does not mean that mono-fluoroacetic acid contains or comprises acetic acid. It is my opinion that if a patent claim were directed to acetic acid, mono-fluoroacetic acid would not infringe.

49.   Besides covalent bonds, there are other types of chemical interactions between molecules. For example, a hydrogen bond is a ubiquitous chemical interaction in molecules between a hydrogen and another atom that is electronegative, particularly oxygen or nitrogen. An electronegative atom in a covalent bond draws electron density toward itself. In a hydrogen bond between hydrogen and oxygen, the slight negative charge on the electronegative oxygen is attracted to the slight positive charge on the relatively less electronegative hydrogen. A hydrogen bond is much weaker than a covalent bond. The existence of hydrogen bonds within a

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molecule or among molecules can have a significant influence on the physical, chemical and biological properties of the substance.

50.   Nucleophilic substitutions or additions are a type of organic chemical reaction in which a nucleophile (usually an electron-rich group that is therefore attracted to atoms having a localized positive charge) typically forms a new covalent bond to a carbon atom while displacing a leaving group from it.

B.   Amino Acids and Protein Chemistry

51.   Biochemistry is a subset of organic chemistry dealing with the chemistry of living organisms and the molecular basis of life. The fundamental principles of organic chemistry, such as the structure of molecules and chemical bonding, also apply to large biochemical molecules (also called biomacromolecules), such as proteins, nucleic acids (DNA and RNA), and carbohydrates. Furthermore, like in simpler organic molecules, the structure of biomacromolecules dictates their function: as is often stated, "structure begets function." A standard undergraduate textbook of Biochemistry states that "[t]he interplay between the three-dimensional structure of biomolecules and their biological function is the unifying motif of this book." (Ex. 285 at 4).

52.   The strongest bonds that are present in biochemicals, as in simpler organic chemicals, are covalent bonds. Indeed, covalent bonds can "withstand the thermal motions that tend to pull molecules apart." (Ex. 57, Alberts (1983) at 92). As stated above, covalent bonds are broken and formed when chemical reactions occur between atoms and/or molecules. In general, when covalent bonds are broken and new ones formed, large amounts of energy are involved. On the other hand, "noncovalent bonds are about 100 times weaker." (Ex. 57 at 92).

These noncovalent bonds are nevertheless vital for maintaining cellular functions that require molecules to associate and dissociate.

53.   Because biomacromolecules typically contain thousands of atoms, scientists will not usually draw detailed pictures of the structure. Rather, certain abbreviations or letter codes are often used to represent structure, as explained below.
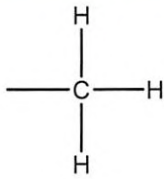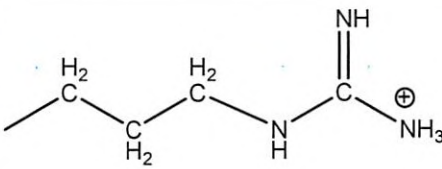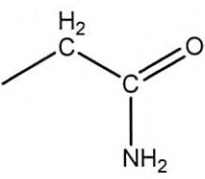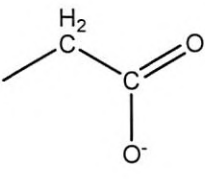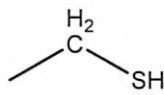
54.   Genes are nucleic acids that contain the information necessary for a cell to produce proteins. Genes and proteins are examples of chemical compounds that are biomacromolecules. It is noteworthy that my review of the Federal Circuit opinion in *Amgen, Inc. v. Chugai Pharmaceutical Co.*, a case involving an earlier patent granted to Amgen's Dr. Lin based on the same work and same patent application as the asserted Lin patents, indicates that the Court treats genes as chemical compounds: "A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it." 927 F.2d 1200, 1206 (Fed. Cir. 1991).

55.   Proteins are formed through the chemical reactions of certain natural amino acids in the cells through the biochemical process of translation. Amino acids are organic compounds containing an amino group and a carboxyl group. Although there is a potentially infinite number of various amino acids, there are only twenty (20) different, so-called standard amino acids that make up virtually all naturally occurring proteins.
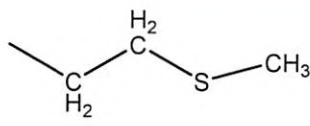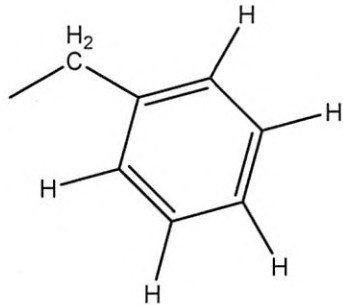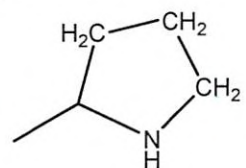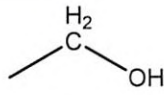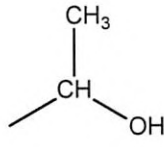
56.   Each such standard amino acid is composed of a central alpha carbon bonded to a hydrogen, to a carboxyl group, to an amino group, and to a unique side chain or R-group. In other words, the central alpha carbon bonded to a hydrogen, a carboxyl group and an amino group constitute a "core" structure that is common to each standard amino acid (except Pro). It
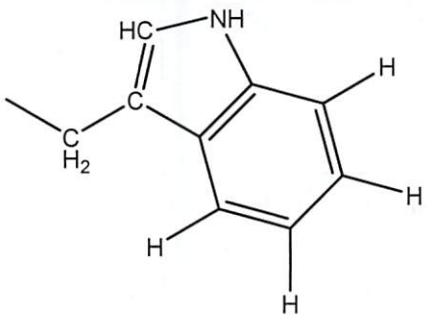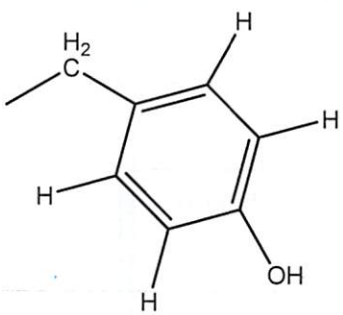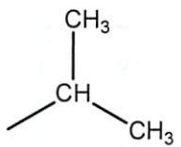
is the unique side chain that determines the individual chemical structure and properties of each standard amino acid. One standard amino acid is only distinguished from another by the unique side chain, which dictates the distinctive physicochemical properties of each amino acid.

57.   The side chains of the twenty standard amino acids (along with their 1-letter and 3-letter codes) in alphabetical order are:

	Amino Acid	Abbreviation	Side Chain's Structural Formula
1.	Alanine	A, Ala	
2.	Arginine	R, Arg	
3.	Asparagine	N, Asn	
4.	Aspartic acid	D, Asp	
5.	Cysteine	C, Cys	

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	Amino Acid	Abbreviation	Side Chain's Structural Formula
6.	Glutamine	Q, Gln	
7.	Glutamic acid	E, Glu	
8.	Glycine	G, Gly	—H
9.	Histidine	H, His	
10.	Isoleucine	I, Ile	
11.	Leucine	L, Leu	
12.	Lysine	K, Lys	

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	Amino Acid	Abbreviation	Side Chain's Structural Formula
13.	Methionine	M, Met	
14.	Phenylalanine	F, Phe	
15.	Proline	P, Pro	
16.	Serine	S, Ser	
17.	Threonine	T, Thr	

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	Amino Acid	Abbreviation	Side Chain's Structural Formula
18.	Tryptophan	W, Trp	
19.	Tyrosine	Y, Tyr	
20.	Valine	V, Val	

58.   Side chains of amino acids can be hydrophobic ("water-hating") or hydrophilic ("water-loving"). They also can be electrically neutral or charged and, if the latter, negatively or positively charged (i.e., anionic or cationic, respectively).
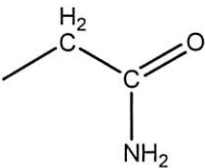
59.   Hydrophobic side chains tend to avoid water. Typical hydrophobic amino acids are alanine, valine, leucine, isoleucine, tryptophan, phenylalanine, and methionine. A hydrophilic side chain tends to interact with water, for example, via hydrogen bonding. The side chains of some of the hydrophilic standard amino acids, as depicted in the table above, are ionic

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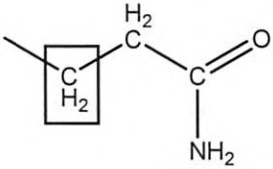
in aqueous solutions at physiological pH (see below). These include, for example, those of the negatively charged aspartic acid and glutamic acid, and of the positively charged lysine and arginine. (Ex. 215, Karp (2002) at 53).

60.   The pH of an aqueous solution is a measure of the solutions' acidity. The pH of the blood inside the human body is sometimes referred to as "physiological pH." It has the approximate value of 7.3.
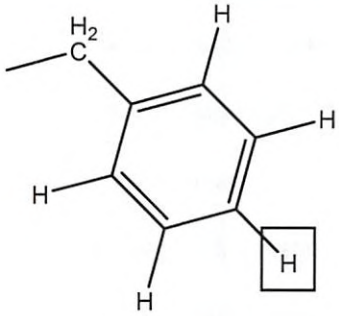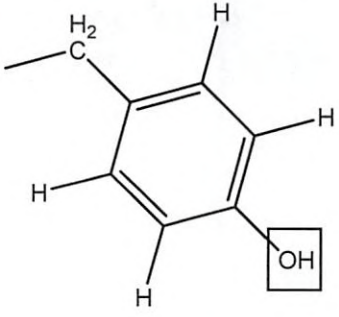
61.   Each amino acid's side chain imparts unique chemical properties on the amino acid and dictates how it will interact with other amino acid residues in a protein chain and with other molecules, such as receptors. Chemists, therefore, classify amino acids by the properties of their side chains. When the side chain of an amino acid reacts with another molecule and forms a covalent bond, the product of the reaction no longer has the structure and properties of the individual amino acid starting material.

62.   The amino acid glutamine only differs from the amino acid asparagine by one extra methylene group ($-CH_2-$), but scientists never say that glutamine has asparagine in it. Although glutamine and asparagine have a relatively small difference in molecular weight and all but one of their functional groups are the same, they are different chemical entities because they have different number and type of atoms in the molecular structures.

Amino Acid	Abbreviations	Side Chain's Structural Formula
Asparagine	N, Asn	

Amino Acid	Abbreviations	Side Chain's Structural Formula
Glutamine	Q, Gln	

63.   The amino acid tyrosine only differs from the amino acid phenylalanine by an extra O atom (it has an OH group instead of a hydrogen) on the side chain, but scientists never say that tyrosine has phenylalanine in it. Tyrosine and phenylalanine are different chemical entities because they have different number and type of atoms in the molecular structure.

Amino Acid	Abbreviations	Side Chain's Structural Formula
Phenylalanine	F, Phe	
Tyrosine	Y, Tyr	

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64.   It is well know in the field that the side chains of amino acids can be changed to create new amino acids that may not even occur in nature. Dr. Alton Meister, a renown authority in the field describes the synthesis of numerous amino acid analogs in his two-volume treatise on amino acid chemistry, generally regarded by protein chemists as one of the definitive references in the field. These synthetic amino acid analogs are referred to by Dr. Meister and those in the field by new chemical names (distinct from lysine). (Ex. 287, Alton Meister, BIOCHEMSTRY OF THE AMINO ACIDS, Preface and Chapter III (1965)).

65.   Amino acids can covalently bond with each other forming what is called a peptide bond (which is a type of an amide bond). Peptide bonds are formed between the carboxyl group (attached to the central alpha carbon) of one amino acid and the amino group (also attached to the central alpha carbon) of another amino acid, as a result of a so-called condensation reaction involving the release of a molecule of water. What remains of each amino acid is called an amino acid residue, either lacking a hydrogen atom of the amino group, or the hydroxyl (-OH) portion of the carboxyl group, or both. However, the amino acid residues retain the unique individual side chains that impart their properties and characteristics. (Ex. 215 at 52-53).

66.   A short chain of amino acids residues covalently bonded together via peptide bonds is called a peptide. Longer linear chains of standard amino acids residues made through biochemical processes in the cell are called proteins. The way amino acid residues arrange themselves in a protein leads to an amino group on one end, called the amino-terminus or N-terminus, and a carboxyl group on the other end, called the carboxyl terminus or C-terminus. Under physiological pH, the amino terminus of a protein is positively charged and the carboxyl terminus is negatively charged. An amino acid sequence in a protein contains specific amino acid residues possessing specific chemical structures (i.e., side chains) in a specific order.

67.   Note that an amino acid and its corresponding amino acid residue in a protein are distinct chemical entities. In fact, while the former is a real "free-standing" molecule, the latter is not.

68.   Consider the following protein:
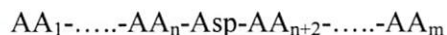
$$AA_1\text{-}.....\text{-}AA_n\text{-}Asp\text{-}AA_{n+2}\text{-}.....\text{-}AA_m$$

where AA stands for an amino acid residue, and n and m are integers. Now assume that the carboxyl group of the side chain of the aspartate (Asp) residue in the middle of this polypeptide chain is chemically amidated (i.e., covalently reacted with ammonia) to form the corresponding amide: $-CH_2\text{-}COO^- + NH_3 \rightarrow -CH_2\text{-}CONH_2$.

69.   As a result of this straightforward chemical reaction, the new protein will be:

$$AA_1\text{-}.....\text{-}AA_n\text{-}Asn\text{-}AA_{n+2}\text{-}.....\text{-}AA_m.$$
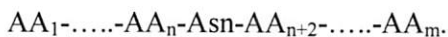
70.   No chemist or biochemist would say that the two proteins have the same amino acid sequences; they are obviously different because the second one has an asparagine (Asn) residue instead of an aspartate (Asp) residue.

71.   Likewise, consider the following protein:
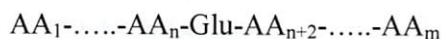
$$AA_1\text{-}.....\text{-}AA_n\text{-}Glu\text{-}AA_{n+2}\text{-}.....\text{-}AA_m$$

where AA stands for an amino acid residue, and n and m are integers. Now assume that the carboxyl group of the side chain of the glutamate (Glu) residue in the middle of this polypeptide chain is chemically amidated (i.e., covalently reacted with ammonia) to form the corresponding amide: $-CH_2\text{-}CH_2\text{-}COO^- + NH_3 \rightarrow -CH_2\text{-}CH_2\text{-}CONH_2$.

72.   As a result of this straightforward chemical reaction, the new protein will be:

$$AA_1\text{-}.....\text{-}AA_n\text{-}Gln\text{-}AA_{n+2}\text{-}.....\text{-}AA_m$$
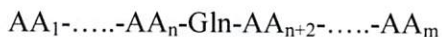
73.   No chemist or biochemist would say that the two proteins have the same amino acid sequences; they are obviously different because the second one has a glutamine (Gln) residue instead of a glutamate (Glu) residue.

74.   Replacing Asp with Asn or Glu with Gln in these examples results in a loss of a charge and in no change in mass. Upon pegylation of epoetin beta to form CERA, when the ε-amino group of one of the Lys residues is converted to an amide, there is also a loss of a charge plus a dramatic increase in mass (by some 30,000 daltons) brought about by thousands of new covalently bonded atoms. No chemist or biochemist can legitimately say, therefore, that in the Asp→Asn and Glu→Gln cases above the amino acid sequence has changed but in the case of pegylation it did not.

75.   Generally, peptide bonds are the sole covalent bonds between amino acid residues in proteins, except for disulfide bonds between the sulfur atoms of cysteine residues. These disulfide covalent bonds function as links between polypeptide chains or between "loops" in a single polypeptide chain in some proteins.

76.   The primary structure of a protein is the covalent structure, defined by the amino acid residue sequence (including subsequent post-translational modifications by the cell). The secondary structure of a protein stems from non-covalent interactions (primarily hydrogen bonding) among proximal amino acid residues and describes helices and sheets formed by the twists and turns of the peptide chains. The tertiary structure of a protein stems from non-covalent interactions among relatively distant amino acid residues in the peptide chain giving rise to the overall three-dimensional (3D), often globular, conformations. The secondary and tertiary structures are referred to as higher-order structures. The 3D arrangement in a protein, its

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shape or conformation, and its properties are determined by its primary structure and the aqueous medium in which the protein is dissolved. (Ex. 215 at 55-62).

77.   The chemical reactivity of a protein depends on the side chains of its amino acid residues, post-translational modifications by the cell (if any), and the higher-order structure, which dictate the accessibility and reactivity of the protein's functional groups towards other molecules. Thus, every protein interacts differently with other molecules, such as receptors.

78.   Protein conformations change when the delicate balance of non-covalent interactions maintaining the protein conformation, including ionic interactions, hydrogen bonds, and hydrophobic interactions, are changed. This is a fact well known to anyone who has taken undergraduate biochemistry. For example, the authoritative textbook BIOCHEMISTRY by J. David Rawn in a chapter dealing with protein structure states:

> All levels of protein structure from secondary to quaternary depend on a number of chemical interactions. The final conformation of a globular protein reflects the balance of these forces, which include the hydrophobic effect, hydrogen bonds, ionic interactions, van der Waals interactions, and covalent cross-links. (Ex. 261, Rawn (1989) at 106).

79.   Proteins are produced naturally by cells by a process called expression. Generally, expression is the result of transcription and translation. Protein synthesis in the cell starts with transcription, the copying of the DNA into "messenger" RNA ("mRNA"). Even before this copying process, a specific "transfer" RNA must be attached to one of the 20 amino acids and the ribosomal subunits must be preloaded with auxiliary molecules. These individual parts are brought together in the cell cytoplasm in a ribosome complex.

80.   An analog or a derivative is a chemical compound created as a result of the substitution of an atom or group of atoms in the parent compound with some other atoms or group of atoms. According to the Lin patents, analogs and derivatives of erythropoietin (EPO)

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are both compounds created through "modifications of cDNA and genomic EPO genes...accomplished by well-known site-directed mutagenesis techniques." (Ex. 47, '422 patent, col. 35, lns. 44-46). Chemically, an analog and a derivative are similar in this context. Analog (or homolog) usually refers to another member of a related series. Derivatives tend to be more structurally diverse than analogs.

81.   A mutant protein refers to a protein that differs from its parent by its amino acid composition as a result of changes in the encoding gene.

82.   It is understood among biochemists that one can change an amino acid sequence of a protein by substituting or chemically changing an existing residue, either way resulting in a new molecule. Modification of an amino acid residue here means the modification of the individual side chain, for example, through a chemical reaction involving the breaking and formation of covalent bonds (or, in other words, the covalent attachment of one atom or group of atoms in place of others).

83.   Changing even one bond in such a complex molecule as a protein, transforms it into a new molecular entity, with different (sometimes dramatically so) chemical structure and properties. For example, mutation of a single Glu amino acid residue into a Val in the amino acid sequence of human hemoglobin causes the potentially deadly human disease sickle cell anemia. Thus in this case, altering the side chain (without even changing either the number of carbon atoms or the total number of atoms in it) of less than one per hundred of amino acid residues drastically affects the functional properties of the protein and, as a result, the health of a person whose body uses this protein. (Ex. 215 at 56-57).

84.   If the methyl group of Ala were chemically modified to include an oxygen atom or to remove a methylene group, one would obtain a Ser or Gly amino acid residue, respectively.

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Regardless of what it is called, substitution of an amino acid residue or chemical modification of a side chain, the process involves the breaking of covalent bonds and the formation of new covalent bonds producing the same molecule.

85.   The hydrodynamic radius of a molecule is the radius of a spherical particle that has the same diffusion coefficient as that molecule in water. Hence the radius calculated from the diffusional properties of the particle is indicative of the apparent size of the hydrated molecule. The hydrodynamic radius of proteins includes effects arising from both shape and hydration. (Ex. 234, Mattison (2001) at 66-67).

C.   Carbohydrates

86.   Carbohydrates or sugar molecules are those with cyclic structures of carbon, oxygen, and hydrogen. Sucrose (table sugar) is a typical and familiar carbohydrate. Its molecular structures has two such cyclic structures (glucose and fructose residues) covalently bonded to each other. Like proteins, carbohydrates can form extended chains.

87.   Glycosylations are reactions whereby carbohydrates are covalently bonded to amino acid residues' side chains, thereby changing their chemical nature and structure. Glycosylations are called post-translational modifications because they are reactions of proteins and carbohydrates that occur naturally during and after translation in the eukaryotic cell. The result of such post-translational modifications of proteins is called glycoproteins.

88.   In N-linked glycosylation, carbohydrates covalently bond to an amide nitrogen in the side chain of Asn residues. O-linked glycosylation involves the covalent attachment of sugars to the hydroxyl's oxygen of Ser or Thr residues.

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89.   Sialic acids is a common name for certain sugars widely present in animal tissues and whose residues are found, in particular, in glycoproteins. An example of a sialic acid is N-acetylneuraminic acid (Neu5Ac), also known as NANA, whose structure is depicted below.
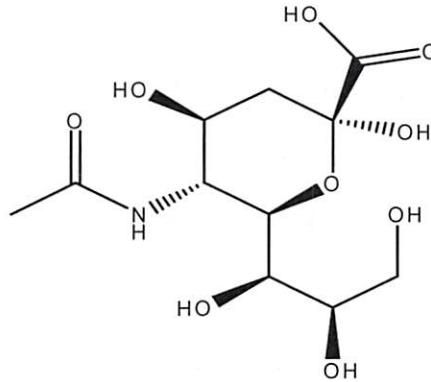
90.   Glycoproteins can be characterized by the number of branches in the carbohydrate chains. Biantennary denotes two "antennae" or branches in a glycoprotein molecule's carbohydrate. Triantennary denotes three, and tetraantennary denotes four.

II.   HUMAN ERYTHROPOIETIN

91.   Biochemically, epoetin beta is a glycoprotein consisting of 165 amino acid residues; it has two disulfide bridges, one between Cys amino acid residues at positions 7 and 161 and another between Cys amino acid residues at positions 29 and 33. Carbohydrates are bonded to three Asn residues at positions 24, 38, and 83 and to Ser at position 126. All other residues have standard amino acids' side chains. The molecular mass of epoetin beta is approximately 30 kDa. (Ex. 171, ITC-R-BLA-00004659).

92.   To my knowledge, it has not been possible to elucidate the three-dimensional structure of erythropoietin by either NMR spectroscopy or X-ray crystallography. The closest to that goal is the paper published by Syed et al. which was an attempt to elucidate the structure of erythropoietin using X-ray crystallography. In that study, a mutant called "r-met lys hu

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erythropoietin" expressed in *E. coli* was used instead of actual erythropoietin. (Ex. 270, Syed (1998) at 515). This multiply mutated protein has different amino acid sequence than human erythropoietin because the amino acid residues 24, 38, 83, 121, and 122 were substituted to improve the resolution of the crystal structure or to increase polarity in compensation for the absence of glycosylation. (*Id.*). Additionally, the protein used in the Syed et al. experiments consisted of 166 (not 165) amino acid residues.

93.   In another study, Cheetham et al. stated that "(a)ttempts to perform structural studies on unglycosylated, bacterially expressed native rHuEPO using either X-ray or NMR methods, have been unsuccessful due to the limited solubility and stability of the protein in the absence of its native carbohydrate chains." (Ex. 97, Cheetham et al. (1998) at 861). Therefore, Cheetham et al. made a model compound that also was significantly different in amino acid sequence from human erythropoietin. Data obtained by them from NMR spectroscopy on the model compound were extrapolated in an attempt to understand the 3D structure of erythropoietin. Thus, the NMR structure determined by them was not that of human erythropoietin. It is speculative to assume that the structure of this model is identical to the structure of human erythropoietin.

III.   **MIRCERA IS NOT THE PRODUCT OF THE LIN-PATENTED PROCESS AND IT HAS BEEN MATERIALLY CHANGED BY SUBSEQUENT PROCESSES NOT RECITED IN THE LIN CLAIMS**

94.   CERA, the active ingredient in MIRCERA is not the product of growing or culturing cells, and CERA is not isolated from cells or cell culture. CERA is the product of a chemical synthesis performed free of cells, and it is a new molecule different in structure and properties from any compositions isolated from cells.

95.   The product Roche isolates from cells or cell media is not a polypeptide "*expressed by said cells*" but rather is the product of further processing to a fragment of an

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expressed peptide (Ex. 179, ITC-R-BLA-00004722; Ex. 183, ITC-R-BLA-00005616-32; *see*

Ex. 262, Recny et al. (1987) at 17156-63; *see also* Ex. 87, AM-ITC-00929860-862).   I refer to

the product of Roche's cells as harvested cell-free supernatant ("crude harvest" or "crude

isolate").

<p style="text-align:center">A.   <u>Roche Practices Several Processes Subsequent to the Harvesting of a Product
from Cells that Result in a Material Change to the Product Harvested from
Cells</u></p>

96.   The Roche crude harvest undergoes a process whereby impurities and byproducts

are extracted and certain isoforms are selected using five specific chromatography steps. (Ex.

177, ITC-R-BLA-00004682-83). This process yields a selection of predominantly 6 isoforms

("epoetin beta") (Ex. 160, ITC-R-BLA-00004059). The term isoforms, in this context, refers to

substances with different amount of sialic acid residues.

97.   The epoetin beta produced in the process is then used as a starting material in a

chemical reaction with an activated PEG reagent to produce a crude reaction product.

98.   This crude reaction product is further processed by two additional steps to

produce CERA. (Ex. 159, ITC-R-BLA-00004046-51). Finally, CERA is formulated into

MIRCERA. (Ex. 147, ITC-R-BLA-00003366; Ex. 151, ITC-R-BLA-00003429).

<p style="text-align:center">a.   <u>Purification of Crude Harvest</u></p>

99.   After crude material is harvested from the cells, it is subsequently processed to

give epoetin beta. (Ex. 174, ITC-R-BLA-00004663). Figure 2 entitled "Flow Chart of the EPO

Purification Process" in Roche's BLA (Ex. 174, ITC-R-BLA-00004664) outlines the several

steps employed to purify epoetin beta. The BLA also describes a summary of the purification

process in narrative form (See Ex. 177, ITC-R-BLA-00004682 - 4704). The five purification

steps that Roche employs on the cell-free culture supernatant are subsequent processes to the

Lin-patented process.

<p style="text-align:center">32</p>

100.   As I understand it, the five-step process takes crude harvested material, removes unwanted impurities and selects for particular isoforms. In 2002, Roche scientists were awarded two U.S. patents for their procedure of purification. (Ex. 49, U.S. Patent Nos. 6,399,333 and Ex. 50, 6,471,500). This is an indication that Roche's purification process is not trivial. The PTO recognized that Roche's purification of crude compositions to pure substances has valuable utility that was not taught by others, such as Amgen.

101.   The purification process Roche employs converts a therapeutically useless composition into a useful therapeutic product. The crude product from the harvest would not be a useful starting material for the manufacture of CERA because the activated PEG reagent would, in all likelihood, fail to react properly, if at all, when contacted with unpurified compositions.

102.   In addition, the purification of crude harvest material produces a material change because, as stated above, it selects out predominately 6 isoforms. (Ex. 203, ITC-R-BLA-0005775-81). In contrast, one expects erythropoietin to have 14 or more different isoforms. (Ex. 46, U.S. Patent No. 5,856,298 col. 5, lns. 33-50).

103.   Therefore, even just the purification of epoetin beta, so that it does not include all possible isoforms, is a material change from crude composition isolated from cells.

b.   Chemical Reaction to Produce CERA Creates a New Molecule

104.   The chemical reaction of epoetin beta with an active PEG reagent produces CERA, the active ingredient in MIRCERA. The laboratory code for this active ingredient is RO0503821. (Ex. 285, ITC-R-BLA-00004026). I may refer to RO0503821 as CERA, which is an acronym for Continuous Erythropoietin Receptor Activator.

105.   CERA is the product of a chemical synthesis involving two reagents or starting materials: epoetin beta and a N-hydroxysuccinimidyl ester of the methoxy-poly(ethylene glycol)-

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butanoic acid (Roche's activated PEG reagent, m-PEG SBA) having an average molecular weight of 30 kDa. (Ex. 152, ITC-R-BLA-00004027). CERA is created via a synthetic chemical process and not biologically using live cells. CERA is a unique substance materially different in structure and physicochemical and biological properties from products that can be produced by cells.

106.   Chemically reacting a PEG reagent with another substance (e.g., a protein) is called pegylation. Pegylation requires an activated PEG reagent. Activation means that a derivative of the PEG is prepared with a group that can covalently react with another reagent.

107.   Epoetin beta has multiple potential reactive sites. However, there are just nine (9) reactive sites (primary amino groups) for the synthesis that leads to CERA using Roche's m-PEG SBA as a starting material. The reaction between a free amino group in epoetin beta and m-PEG-SBA can occur at the epsilon amino groups of Lys residues 20, 45, 52, 97, 116, 140, 152, 154, and at the alpha amino group of the N-terminal Ala residue. These amino groups are predominantly protonated at physiological pH and therefore carry a positive charge ($-NH_3^+$).

108.   m-PEG SBA reacts with the unprotonated form (which is in a dynamic equilibrium with the protonated form) of one of the amino groups of epoetin beta mentioned above. The unprotonated form of amino groups in epoetin beta carries out a nucleophilic attack on the carbonyl group in the m-PEG SBA releasing N-hydroxysuccinimide (NHS), the leaving group, and forming a covalent amide bond with m-PEG-butanoyl group, thus producing the new chemical entity CERA and other substances. (Ex. 153, ITC-R-BLA-00004028). This reaction is irreversible and forms a strong covalent bond. Once the reaction takes place and CERA is produced, one cannot get back the starting materials from it.

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109.   The nitrogen atoms on the amino groups of lysine residues and N-terminus in epoetin beta, as stated above, bear predominantly 3 hydrogens (protons) each at physiological pH of the solution and thus carry a positive charge. Hydrogens of epoetin beta are hence lost in the pegylation reaction.

110.   Therefore CERA lacks at least one primary amino group present in epoetin beta. At least one nitrogen in CERA that was in a free amino group in epoetin beta becomes covalently bonded to a carbon atom of a carbonyl group (C=O), i.e., becomes an amide. Since the amide nitrogen is not protonated under physiological conditions, it carries no positive charge. (Ex. 152). Thus CERA has a distinct charge from that of epoetin beta plus many other differences (e.g., twice the size). Consequently, pegylation alone materially changes CERA, not only from epoetin beta, but also from the crude harvested material from cells.

111.   It follows, then, that eliminating a positive charge in one of the amino acid residues slone should change this "balance of forces", at least with respect to ionic interactions (both attractive and repulsive) because a positively charged side chain can participate in them, while a neutral side chain cannot. Moreover, ethylene oxide residues in CERA can additionally participate in hydrophobic interactions and in hydrogen bonding, thus disturbing this "delicate balance" of forces even further. The inescapable conclusion from this analysis is that the structure of CERA must be different from that of epoetin beta.

        c.   **Purification of CERA Results in a Material Change**

112.   Subsequent purification of CERA further materially changes the molecule from crude composition harvested cells. This process purifies the molecules using two chromatographic steps. (Ex. 129, ITC-R-BLA-00004035; Ex. 159). In the first step, such impurities as oligoforms and other undesirable chemicals are removed producing CERA with less than 20% of impurities. Oligoforms are the unwanted products formed when epoetin beta

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reacts with too many (above 1:1 on the molecular basis) activated PEG reagents. It can be difficult to separate these from CERA. (Ex. 161, ITC-R-BLA-00004126, Ex. 158, ITC-R-BLA-00004043). The second step further removes the remaining impurities producing CERA with less than 0.5% of impurities. (Ex. 129, Ex. 158). There is no evidence that the unreacted epoetin beta starting material still remains.

113.   The crude product of the chemical reaction of epoetin beta and Roche's activated PEG reagent would not be therapeutically useful without first being purified because it would not be formulated into a pharmaceutical.

d.   The Chemical Reaction that Produces CERA Materially Changes Epoetin Beta Starting Material

i)   CERA is a New Chemical Entity of Novel Structure

114.   Once the chemical synthesis of CERA takes place, epoetin beta starting material ceases to exist. CERA's structure is drastically different from that of the starting reagent epoetin beta. CERA does not have the same amino acid residues as epoetin beta because at least one of the amino acid residues present in epoetin beta is not found in CERA. In epoetin beta, the N-terminal Ala residue and Lys residues at positions 20, 45, 52, 97, 116, 140, 152 and 154, are present. In contrast, in CERA either the N-terminal Ala or one of the Lys residues at positions 20, 45, 52, 97, 116, 140, 152 and 154 is no longer present.

115.   Where there was a free amino group containing a protonated nitrogen and thus having a positive charge, there is now a chemically distinct amide group that lacks the positive charge. (Ex. 152). Moreover, where only two or three hydrogen atoms were bonded to that nitrogen atom, there are now thousands of covalently bonded atoms. This change far exceeds any conventional mutation that those Lys residues or N-terminal Ala can possibly undergo.

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amine group protonated

amide group
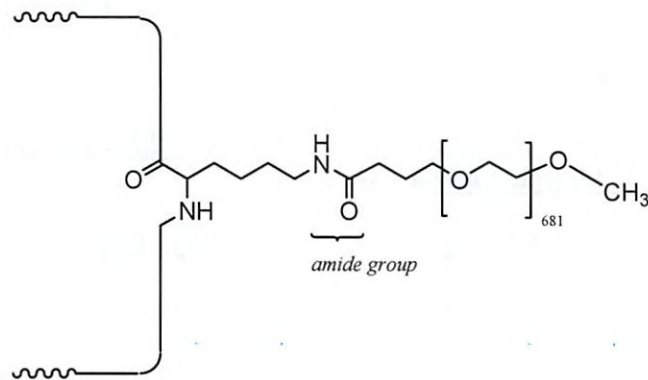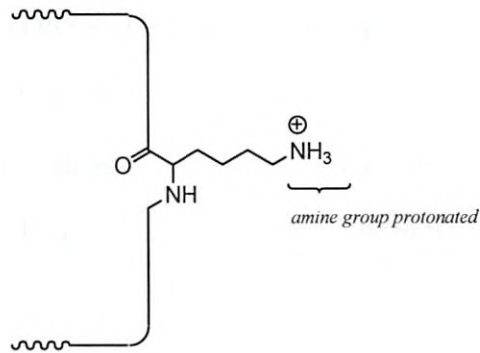
116.   The reaction between m-PEG SBA and epoetin beta drastically and irreversibly

changes the individual amino acids residues where the reaction occurs. A Lys residue in a

protein sequence has this structure:

Lysine Side Chain at physiological pH

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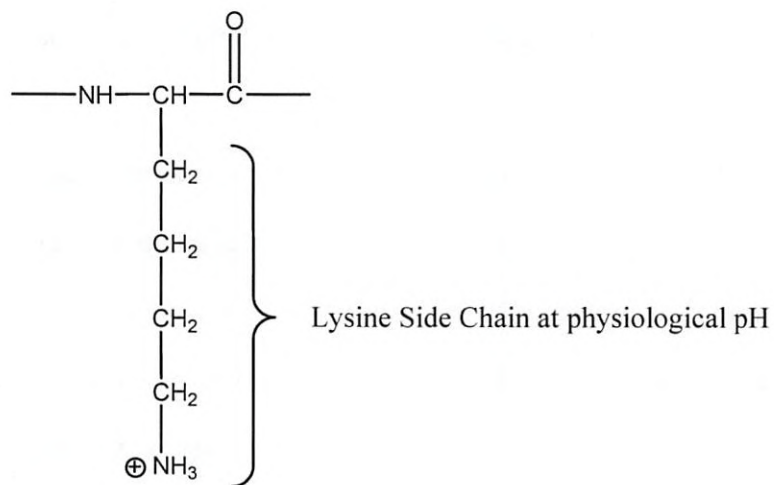
117.   In CERA, the structure represented above does not exist. Hydrogens are lost and an amide bond has been formed, thus making it structurally different. This difference in structure is much greater than a change from asparagine residue to a glutamine residue that I discussed in the tutorial.

CERA Side Chain
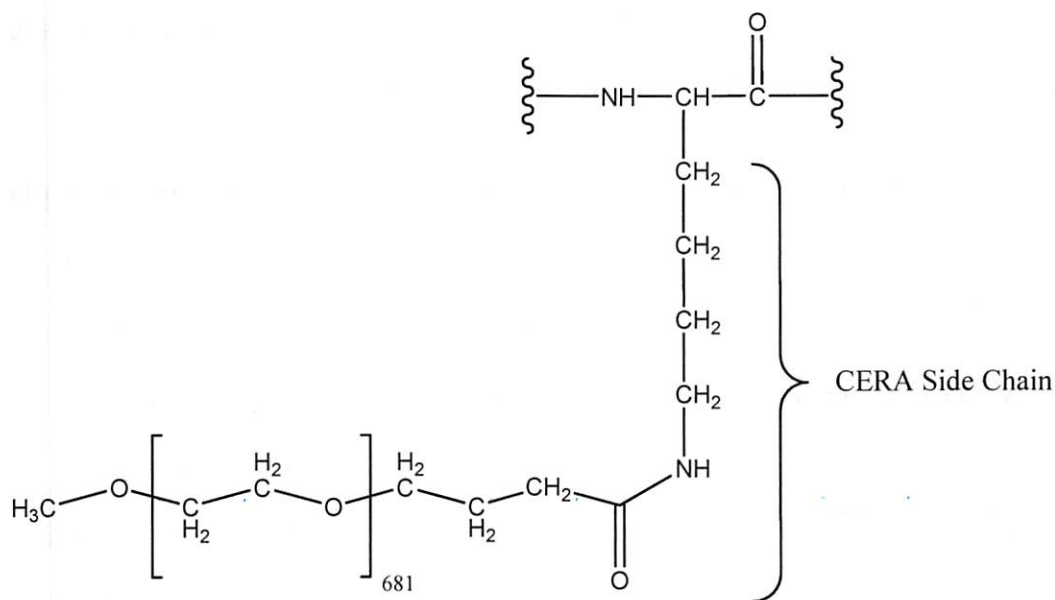
118.   Since the amino acid sequence is the linear order in which particular amino acid residues are bonded to each other, it follows that changing the nature of any one of these amino acid residues necessarily changes the primary structure. Thus, as all amino acid residues present in epoetin beta are not present in CERA (and vice versa), it cannot be legitimately said that epoetin beta and CERA have the same amino acid residues.

119.   Striking structural differences between CERA and epoetin beta can be clearly illustrated by comparing the different number of atoms and covalent bonds in both compounds. Compared to the starting material epoetin beta, CERA has thousands of additional atoms that can interact with other atoms in CERA and with other molecules. Namely, CERA has 1367 additional carbon atoms (1 + (2 x 681) + 4), 2733 additional hydrogen atoms (3 + (4 x 681) + 6),

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and 683 additional oxygen atoms (1 + (1 x 681) + 1), totaling 4783 more atoms than epoetin beta starting material. Moreover, CERA has thousands of additional covalent bonds that epoetin beta does not have. These are huge differences in structure that make CERA a structurally different and substantially changed compound from epoetin beta.

ii)   Properties of CERA are Substantially Changed from Epoetin Beta Staring Material

120.   In addition to having a different structure, CERA possesses different physicochemical, pharmacodynamic, and pharmacokinetic properties compared to epoetin beta. For example, CERA has a different charge, a different molecular weight, and behaves very differently when subjected to various characterization and separation techniques. CERA has different hydrodynamic properties as measured by dynamic light scattering and analytical centrifugation. (Ex. 260, R11-000636121-129). In addition, MIRCERA has an extended pharmacokinetic half-life that allows for much less frequent administration than epoetin beta in humans. It has different interactions at the EPO receptor level revealed by a vastly different (at least some 30-fold) binding affinity, as well as different association and dissociation rate constants with the receptor. It is also more stable in aqueous solution and less prone to aggregation.

iii)   Molecular Weight is Different

121.   CERA has a very different molecular mass (weight) from epoetin beta. As explained in my tutorial, molecular weight is an important and fundamental physicochemical characteristic of a molecule and is typically one of the first properties chemists determine when producing a new and unknown molecule.

122.   Roche determined the mean molecular mass of CERA to be 61.3 kDa. (Ex. 167, ITC-R-BLA-00004271-4275). In contrast, the average molecular mass of epoetin beta is only

some 30 kDa. (Ex. 171) A head-to-head comparison using analytical centrifugation separately

conducted by Roche scientists also proves that the molecular mass of CERA is far different from

that of epoetin beta: 63.4 kDa as compared to 31.4 kDa, respectively. (Ex. 254, R005312436;

Ex. 260, R11-000636122-23, 29).

123.   Thus CERA has about double the molecular weight of epoetin beta. This

necessarily has profound effects on molecular properties and the way CERA and epoetin interact

with other molecules, such as receptors.

iv)   CERA and Epoetin beta Migrate Differently in SDS-PAGE, (RP)-HPLC and (SE)-HPLC

124.   Roche ran numerous experiments to characterize the physicochemical properties

of epoetin beta and CERA using various techniques, such as SDS-PAGE, (RP)-HPLC, and (SE)-

HPLC. These are different techniques chemists use to probe differences in properties of

compounds. The results of these experiments, as discussed below, are additional evidence that

CERA and epoetin beta are distinct chemical entities with different structures and

physicochemical characteristics. (Ex. 168, ITC-R-BLA-00004296-4316).

125.   Experiments using SDS-PAGE confirm that the CERA and epoetin beta are

different. (Ex. 168, ITC-R-BLA-00004297-4300.) The apparent molecular weight of CERA

was found to be much higher than that of epoetin beta: CERA migrates around 97.4 kDa, as

compared to 34 to 41 kDa for epoetin beta. (Ex. 172, ITC-R-BLA-00004660; Ex. 132, ITC-R-

BLA-00005580; Ex. 185, ITC-R-BLA-00005770-73; Ex. 169, ITC-R-BLA-00004299-300).

v)   CERA and Epoetin Have Different Carbohydrate Compositions

126.   Review of Roche's experimental data also indicates that the glycosylation of

CERA is different from that of the epoetin beta starting material. The carbohydrate structures of

the two were assessed with respect to the *N*-linked glycosylation, *O*-linked glycosylation, and the

total sialic acid content. (Ex. 163, ITC-R-BLA-00004233; Ex. 164, ITC-R-BLA-00004241-4268).

127.   *N*-glycosylation of CERA is different from that of epoetin beta. (Ex. 163). The relative amounts of tetraantennary and lactosamine structures in CERA was found to be decreased compared to those in epoetin beta starting material, while the amount of biantennary and triantennary structures was increased. (Ex. 165, ITC-R-BLA-00004247-4256). Similarly, the *O*-linked glycosylation was compared between CERA and epoetin beta and found to be different. (Ex. 166, ITC-R-BLA-00004257-4268).

128.   In addition, the sialic acids enzymatically released from CERA were analyzed. The data show that there is a difference in the total sialic acid content between CERA and epoetin beta. (Ex. 164, ITC-R-BLA-00004241-4246).

vi) CERA and Epoetin beta Have Different Hydrodynamic Properties

129.   Hydrodynamic properties of CERA and epoetin beta were also compared by Roche scientists. The essential hydrodynamic properties are diffusion coefficient, hydrodynamic radius, sedimentation coefficient, fractional ratio, and second virial coefficient. (Ex. 260).

130.   The profound differences in the hydrodynamic properties of CERA compared to the epoetin beta are well illustrated by the difference in their hydrodynamic radii, among others. Strikingly the hydrodynamic radius of CERA is twice as large as that of epoetin beta (6.37 versus 3.19 nm, respectively). (Ex. 260, R11-000636129)

131.   Aggregation is a non-specific and undesirable association of molecules with each other. In measuring the hydrodynamic properties of CERA and epoetin beta, Roche's researchers also found that CERA is less prone to aggregation than epoetin beta, which is beneficial from the pharmaceutical standpoint because it maximizes the biological activity and

minimizes potential complications. (Ex. 254, Ex. 260, R11-000636128-29).   Also, aggregates

may lead to immunogenic reactions and cause problems in the administration and delivery of the

drug into the body.

<div align="center">

**vii)   CERA and Epoetin beta Have Different Interaction with the
EPO-Receptor**

</div>

132.   Roche used two methods to examine the binding affinity of CERA and of epoetin

beta to the EPO-receptor: competitive binding analysis using UT-7 cells (Ex. 191, ITC-R-BLA-

00007256-70) and direct measurement of binding kinetics using surface plasmon resonance (Ex.

189, ITC-R-BLA-00007243-55). The results of these two experiments show that CERA has a

30- to 100-fold lower receptor binding affinity than epoetin beta. Even a 30-fold (let alone a

100-fold) difference in binding affinity between compounds is very substantial in terms of the

biological responsiveness of a system.

133.   CERA also has a dramatically different half-life (the time required for half of a

substance to be eliminated from the organism) than epoetin beta. In clinical trials, MIRCERA

has an *in vivo* serum half-life of 134 ± 19 hours (IV) and 139 ± 20 hours (SC). (Ex. 199, ITC-R-

BLA-00019402-84 at 19445). In contrast, the *in vivo* serum half-life of formulated epoetin beta

is over ten times shorter - merely between 4 and 12 hours. (Ex. 258, R-10-002641295; *c. f.* Ex.

128, ITC-R-BLA 00000091).

134.   Generally, half-life can be thought of as a measure of the length of time a

substance is present in the body after administration. Other things being equal between two

drugs the one with a longer in vivo half-life will usually translate into less frequent dosing

because a single dose will last longer. (Ex. 239, Molineux Tr. (3/28/07) 43:19-50:6; Ex. 236,

Molineux (2002) at 15). As a result of a difference in half-life, patients will require less frequent

dosing of MIRCERA compared to existing formulation of erythropoietin, resulting in a better

<div align="center">42</div>

quality of life and a better compliance. This is a material change that goes to the basic utility of the product. (Ex. 230, MacDougall (2005) at 439; Ex. 128).

135.   In the pharmaceutical industry, drugs are often characterized as being "me too" products if they provide only minimal differences from other already marketed products or, alternatively, "next generation" products if they provide significant extra benefits. With this in mind, it is worth nothing that CERA has been recognized by some as a "third generation" erythropoietic agent, having significant benefits over both EPO and Aranesp. (Ex. 230 at 436). As discussed below, this supports my opinions on lack of enablement and written description in the Lin patents

viii)   Changes to Structure of Erythropoietin Are Known to Affect Biological Activity

136.   Other structural changes to erythropoietin, even though far less profound than pegylation, are known to markedly affect its activity. For instance, Amgen reported that substituting the lysine residue at position 45 with another amino acid residue drastically lowers the biological activity, essentially rendering the new structure inactive. (Ex. 105, Elliott (2003) at 247). Since Lys45 is one of the main sites where epoetin beta and PEG reagents can react, in light of this Amgen publication, the pegylation reaction used by Roche was one that skilled chemists would have been discouraged to attempt. Another scientific article from 2003 indicates that reacting the lysine amino groups of erythropoietin with an activated PEG reagent gave isomers that "can contribute to antigenicity of the drug and poor clinical outcomes." (Ex. 121, Harris & Chess (2003) at 215).

137.   Iodination is a process whereby an iodine (denoted as I; a bulky atom that belongs to a family of chemical elements called halogens) is substituted for an atom (typically hydrogen) in a protein. For instance, Amgen's expert, Dr. Goldwasser, substituted a hydrogen in the

aromatic ring of tyrosine residues in erythropoietin with iodine through the formation of a new (I-C) covalent bond. This reaction rendered erythropoietin inactive. (Ex. 117, Goldwasser Tr. 2/14/07, pp. 177:18-178:11).

ix)   The Chemical Reaction used to Produce CERA is not Conventional and Not Predictable

138.   Pegylation is not a conventional, routine, or predictable process. In fact, the reactions of protein molecules with activated PEG reagents is a complex chemical reaction, requiring the evaluation of numerous variables and yielding new molecules with unpredictable physiochemical and biological properties. It is by no means predictable or trivial even today to create a useful therapeutic through chemical reactions with PEG reagents.

139.   PEG molecules vastly vary in size and shape. As Amgen's Dr. Graham Molineux explains, PEG molecules can be either linear branched[4], forked, or branched-forked. (Ex. 241, Molineux, Pharmacotherapy (2003) at 5S). Commercially available PEG molecules can have molecular weights ranging from a few hundred to tens of thousand Daltons. For example, PEG molecules with 50,000 Da have been reported.

140.   It is important to stress that the starting materials involved in a pegylation reaction cease to exist after the reaction. Proteins have many different reaction sites, including cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, lysine, threonine, tyrosine, N-terminal amino group, C-terminal carboxylic acid, and carbohydrates. There are numerous types of reactive groups that can be introduced into PEG molecules. The reactive groups in the PEG reagent and the protein reagent may not be chemically compatible or suitable, thus adding another degree of uncertainty and variability in pegylation reactions. If the reactive groups are not suitable, the reaction will not succeed. Different pegylation chemistries and reaction

conditions lead to different compounds with different therapeutic properties, if any. (Ex. 122, Harris (2001), 542-549).

141.   From my own professional experience, successful pegylation greatly depends on numerous factors, including the protein's concentration and primary structure, as well as the activated PEG reagent's concentration, molecular weight, distribution, activation site, and structure; and also reaction time, temperature, pH, and ionic strength. Amgen's publications corroborate this view. In a 2002 article, Dr. Molineux indicates that pegylation methods must be determined for each protein. "Optimal pegylation is product-specific, and can vary depending on the site of attachment, the chemistry used to create the conjugate, and the characteristics of the PEG used." (Ex. 236, Molineux (2002) at 15). The size of the PEG molecule and the reaction site critically influence the properties of the final product. In fact, that was the main basis for the transition from the first-generation pegylation technology to the second generation (see below).

142.   Chemical reactions between proteins and activated PEG reagents invariably produces a mixture of distinct molecules. Precisely characterizing products of a pegylation reaction is arduous and sometimes impossible because of the presence of multiple different products, or species.

143.   Amgen scientists agree that the product of pegylation is a new molecule distinct in structure and properties from the starting materials. (See Ex. 236 at 13-14; Ex. 240, Molineux Anti-Cancer Drugs (2003) at 260, citing to Ex. 286, Bailon and Berthold (1998)). For instance, according to Dr. Molineux, the products of the pegylation reaction have different physiochemical properties from the starting reagents including "changes in conformation, electrostatic binding properties and hydrophobicity." (Ex. 240 at 260.) Moreover, the products of a pegylation reaction have not only different physicochemical and formulation properties, but also different

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pharmacokinetic and pharmacodynamic properties. (Ex. 240 at 259; See also Ex. 239 at 72:1-72:20).

144.   Pegylation may have major and opposing effects on the product's biological activity, such as receptor binding affinity (the strength of the attraction between a protein and the receptor). The relative balance and the overall impact of these effects are unpredictable. (Ex. 217, Kinstler et al. (2002) at 478). Researchers cannot anticipate in advance the outcome of a pegylation reaction and what chemical and biological properties the resulting product will have.

145.   The development of new therapeutically useful biopolymers using PEG reagents in the mid-1980's was a trial and error undertaking without a reasonable expectation of success using any of the technologies of that time (or even today). Amgen scientist Dr. Molineux has published on the problems encountered by researchers using first-generation PEG reagents (Ex. 236 at 14).

146.   The chemical reagents used to carry out pegylation reactions available in the mid-1980's were few and inadequate. The pegylation reactions using the chemical reagents of that era were generally unpredictable with respect to the structure and activity of the products that would be produced. All these reagents had drawbacks and limitations that would require extensive experimentation to overcome if one were to use them to produce a therapeutically useful biopolymer having erythropoietic activity.

I.   First-Generation Pegylation Technology

147.   Although the potential benefits of chemical reactions with activated PEG reagents were desired in the art in 1984, the technology for obtaining such therapeutics was not generally obtainable without difficult experimental efforts.

148.   As of 1992, the experience with pegylation technology was limited and rather unsatisfactory. In particular, pegylation procedures employed during the late 1970s and 1980s

were plagued by difficulties, including restriction to PEGs with low molecular weights, relatively

unstable activated PEGs, and lack of selectivity in protein modification. This gave rise to impure

and heterogeneous substances difficult to purify. (Ex. 263, Roberts et al. (2002) at 462). As a

matter of fact, researchers at the time considered pegylation to be "a failing technology".

(Ex. 263 at 474). For instance, Veronese et al. reported the difficulties encountered when

attempting to create biopolymers using enzymic proteins and PEG reagents. (Ex. 284, Veronese

et al. (1983) at 757).

149.   Delgado summarized the drawbacks and limitations associated with first-

generation pegylation technology:

> Despite the well-established advantages of PEG-proteins over their
> unmodified counterparts, this technology has not yet been very
> widely exploited commercially because until recently most
> methods were suboptimal; they use conditions which are likely to
> reduce biological activity, they are not readily controlled with
> respect to the number of PEG molecules attached, and they often
> require purification steps which themselves endanger labile
> proteins. They also leave part of the coupling moiety attached to
> the protein, where it may serve as an antigen… (Ex. 101, Delgado
> (1992) at 274).

150.   As Amgen's Dr. Molineux explains, "(e)arly attempts at pegylation were

hampered by technical problems..." (Ex. 236, Molineux (2002) at 14). Also, "low molecular

weights were used because of the presence of large amounts of diol. The activation of diol leads

to a difunctional contaminant that can produce crosslinking and aggregation." (Ex. 236 at 15).

A person of ordinary skill in the art would have understood that "(l)ack of optimal pegylation"

resulted in molecules with less activity than the starting reagents. (Ex. 236 at 15). Other

scientific publications corroborate those statements. (Ex. 121 at 215). Thus, there is a consensus

that first-generation technology generally did not result in useful therapeutic compounds.

(Ex. 263 at 462, 474).

151.   Several references, reported that products made using first generation succinimidyl reagents were readily susceptible to hydrolysis. A 1985 reference explains: "succinimidylsuccinates of PEGs have been prepared; however, this procedure is limited by easy hydrolysis of the ester between PEG and succinic acid." (Ex. 283, Veronese (1985) at 142). Similarly, Dr. Delgado states in 1992 that succinimidyl active esters are "limited by the easy hydrolysis of the ester between PEG and the acid." (Ex. 101 at 276).

152.   In addition to the general problems with first-generation pegylation reagents, reacting them with the ε-amino groups of Lys residues was specifically discouraged and considered a potential problem in the art during the early 1980s. Amgen's expert Dr. Katre reported that lysine residues that are part of the active site of a protein reagent are unsuitable for pegylation reactions. (Ex. 216, Katre (1993) at 95).

153.   Likewise, in their 2003 review article, Harris and Chess stress the difficulties encountered by researchers using first-generation pegylation technology, particularly difficulties with reactions at the ε-amino groups of Lys residues:

> The first-generation pegylation methods were fraught with difficulties. With first-generation pegylation, the PEG polymer was generally attached to the ε amino groups of lysine. This resulted in the modification of multiple lysines, and gave mixtures of PEG isomers with different molecular masses. The existence of these isomers makes it difficult to reproduce drug batches, and can contribute to the antigenicity of the drug and poor clinical outcomes.(Ex. 121 at 215).

154.   Harris and Chess further stressed in that 2003 review article that, "PEGylation has taken 20 years to emerge as a viable pharmaceutical tool. During this time there have been important advances in the chemistry of PEGylation, in the generation of biomolecule therapeutics and in understanding PEG-biomolecule conjugates." (Ex. 121 at 220).

155.   Regulatory bodies consider products of stable pegylation to be new chemical entities. "The organic and polymer chemistry of PEG activation has now matured, and protein pegylation is becoming viable commercially. However, the technique needs significant know-how, and the modified protein is considered to be a new chemical entity from a regulatory point of view." (Ex. 90, Banga (2006) at 205).

II.   Second-Generation Pegylation Technology

156.   Since it took close to 20 years for pegylation to emerge as a viable pharmaceutical tool. (Ex. 121 at 220), researchers were beginning to give up on the idea of chemically reacting proteins with PEG reagents as a general pharmaceutically useful technology until higher molecular weight PEGs became available and until chemistries were developed to optimize reaction and workup conditions to avoid unstable activated PEGs, side reactions, and diol contamination. (Ex. 220, Kozlowski and Harris (2001) at 219-20). One of the first examples of second-generation pegylation chemistry was described only in 1993 with the preparation of methoxyPEG-propionaldehyde. (Ex. 220 at 220).

III.   Amgen's Scientists' Statements Support My View That Pegylation Was in the Mid-1980's, and Is Still Today, an Unpredictable Art.

157.   I reviewed portions of the Lin deposition transcript and noted that Dr. Lin was aware of pegylation in November 1984 but did not disclose it in his patents. (Ex. 225, Lin Tr. (3/28/07) at 91:19-25; see also 91:4-122:25). Moreover, Dr. Lin agreed that he, who is at least one of ordinary skill in the art, would not be able to predict whether reactions with PEG reagents would result in biologically active new biopolymers. (Ex. 225 at 100:9-22).

158.   Mr. Boone, Amgen's corporate representative, stated that Amgen had not fully tested compounds using PEG and EPO as reagents and that scientists at Amgen could not have predicted the activity and half-life of those compounds. (Ex. 94, Boone Tr. (3/30/07) at 76:6 -

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77:16, 92:24 - 93:3, and, generally, 94:14 - 96:24). Indeed, according to Mr. Boone, Amgen experimented with pegylating EPO in the late 1980's and early 1990's, but never advanced a new biopolymer of this nature even to pre-clinical, let alone clinical, trials. (Ex. 94 at 24:22-26:1).

159.   Statements in Amgen's patents and prosecution histories indicate that Amgen believed that prior references disclosing reactions between PEG reagents and protein reagents did not teach or suggest to a person of ordinary skill in the art how to obtain therapeutically useful biopolymers by reacting EPO with PEG reagents.

160.   For Example, in Amgen's prosecution for the U.S. Patent No. 6,586,398 ("the '398 patent"), which is directed to PEG-NESP, Amgen stated, "not all proteins respond equally to PEGylation and there is no guarantee of improved performance." (Ex. 52, '398 patent at col. 2, lns. 19-21). Mr. Boone, one of the inventors of this PEG-NESP patent, agreed that this statement is true. (Ex. 94 at 159:16-24). Indeed, Amgen considered pegylation technology unpredictable, especially with respect to molecules like EPO, as evidenced by the '398 patent specification which states, "[i]t was thus evident that the PEGylation of non-glycosylated proteins was not necessarily predictive of the PEGylation of glycosylated proteins." (Ex. 52 at col. 6, lns. 1-3).

161.   The specification of the '398 patent calls the results of the pegylation reactions "surprising". According to Amgen's own language, its patent is "based upon the surprising finding that a highly glycosylated protein ... can be PEGylated to provide a pharmaceutical composition with an even more dramatic sustained duration profile than NESP, allowing for a once every 4-6 week dosing for raising hematocrit and treating anemia, and thus providing tremendous therapeutic advantage." (Ex. 52 at col. 2, lns. 22-29).

162.   In Dr. Katre's patents directed to pegylated compounds and their file histories, I find that she made numerous statements to the PTO regarding the unpredictability of pegylation reactions. Discussing prior art to her patent application, Dr. Katre stated:

> None of these references, however, discloses details of how to modify CSF-1 with a polymer such as PEG or polyvinyl alcohol so as to retain its biological activity while also increasing its circulating half-life or efficacy. **Furthermore, it is not generally possible to predict the extent of protein modification or the nature of the reaction conditions that are desirable, because some proteins are much more susceptible to inactivation through conjugation than others.** (Ex. 42, U.S. Patent No. 5,153,265 at col. 4, lns. 4-12; emphasis added).

163.   Likewise, during the prosecution of U.S.S.N. 06/866,459, a patent application directed to chemical reactions of proteins, Dr. Katre argued that "one cannot predict [without experimentation with the particular protein] whether selective conjugation of a given protein with a given chemical reagent such as PEG or POG will be successful to retain biological activity and confer water solubility." (Ex. 280, U.S.S.N. 06/866,459, Paper No. 5, dated 8/3/87 at pp. 4-5). Stressing the unpredictability of reactions involving proteins, Dr. Katre argued: "[i]t is impossible to predict with any degree of certainty the outcome of a chemical reaction involving different proteins and a given reagent." (Ex. 280 at 6).

164.   Amgen's expert witness Dr. Katre also stated that the "specific physicochemical and biological properties of a protein molecule including its immunogenic and solubility and stability properties, are determined by the overall structural architecture of the molecules." (Ex. 280 at 5) and "[i]t is not a priori possible to predict which selected proteins would be favorably responsive to treatment with polymers due to the vast difference in the pharmacokinetics and physical properties among different proteins." (Ex. 280 at p. 7.)

165.   Dr. Katre further concluded that "until actual empirical data are obtained, it is impossible to predict that any given protein will remain active after conjugation." (Ex. 280 at p.

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9). Dr. Katre explained, "it is impossible to predict the outcome of a chemical conjugation of a different protein, as regards to water solubility, in vivo half-life, biological activity, stability, and immunogenicity. . . . No generalization can be made between such proteins and that of applicants, as proteins are very complex molecules." (Ex. 280 at p. 10).

IV. Experience at AMGEN Confirms Pegylation is Neither Routine Nor Conventional

166.   Dr. Lin, the named inventor of the Lin patents, acknowledges that pegylation of erythropoietin is unpredictable. According to him, there is no way to know the outcome of a pegylation reaction and whether the product would be biologically active until one performs the experiments:

> Q: So you understood that modifications, like pegylation, could be done to EPO?
>
> A: Yes. Yes.
>
> Q: But the book didn't tell you whether or not, when you made those modifications, the protein would be active; correct?
>
> . . . .
>
> THE WITNESS: You had to do it yourself. For any particular procedure, you had to do it yourself to see if the end product that you modified—the way you did it—would be active or not. You had to check it out, experimentary [sic]. Yes. (Ex. 225 at 100:9-22; *see also* 94:2-95:12).

167.   Dr. Lin further testified during his deposition that he was aware of the pegylation technology, a chemical process, at the time the patents were filed, but failed to disclose it in the specifications of the patents at issue. Dr. Lin explained that at the time, the literature showed chemical reactions of PEG reagents with some compounds with clinical uses, but he did not disclose them in the patents. (Ex. 225 at 304:12-305:5). Also, Mr. Boone, Amgen's spokesman in this litigation on Amgen's efforts to create a product from the reaction of erythropoietin and

PEG reagents, and one of the named inventors of U.S. Patent No. 6,586,398 to pegylated

Aranesp®, has stated the following about pegylation:

> Q: Do you have any reason to believe that scientists at
> Amgen were able to predict the in vivo biological activity
> of a PEG-EPO compound prior to testing?
>
> . . . .
>
> THE WITNESS: I don't know.
>
> Q: You don't know if you have any reasons or not?
>
> A: I have no reason to believe that Amgen scientists would
> be able to predict in vivo biological activity of a PEG-EPO
> compound. (Boone Tr., 3/30/07, pp. 46:10-22).
>
> * * *
>
> THE WITNESS: Based on your definition of in vivo
> activity, I would say that the assays that Amgen run—ran at
> that time were not very predictable.
>
> Q: Was Amgen able to predict from the structure of its
> PEG-EPO whether or not the compound would be in vivo
> active?
>
> A: No, we were not able to predict from the structure.
> (93:1-10)
>
> Q: So, with the information Amgen had available to it in
> 1991, they were not able to predict whether an EPO
> produced in CHO cells which was modified at the amines
> to contain PEG would be activity in vivo as I defined it?
>
> A: I believe—I believe the assay that we were using for in
> vivo activity would not, with a hundred percent guaranty,
> predict the activity as you defined it.
>
> (94:21-95:4)
>
> Q: So every time you attach a PEG to EPO, you don't
> necessarily end up with a biologically active compound;
> right?
>
> . . . .

THE WITNESS: All I can say is there's no guaranty that if
you attach a PEG to EPO you will have a biologically
active material as defined in your assay. (Ex. 94 at 96:12-
21).

168.   Dr. Elliott, in charge of developing improved erythropoietic compounds at

Amgen, also testified that pegylation is unpredictable and there is no way to know what

properties the product of the pegylation reaction will have. For example, he asserted that

whether the product of a pegylation reaction will be immunogenic can only be determined

experimentally:

Q: So this would be another instance where you just have
to do the test, the trial and see what happens?

A: With a given molecule, looking at the variable nature of
the human population and what we know, if we're going to
speak about erythropoietin specifically . . . .

Q: Yes.

A: We know that there's a potential for immunogenicity
which is caused by whole bunch of variables, some of
which have to do with manufacturing processes.

And so one could imagine that if you were to do pegylation
of EPO, there might be some conditions under which you
pegylate that would result in immunogenicity and other
conditions where it might be less likely to get
immunogenicity.

It's a combination not only of whether peg is there or not,
but also how you make the protein.

**So because of all of these variables, one would need to
do an experiment to find out.**

And, then, even when you do the experiment, it's not
necessarily conclusive because you might only have a
limited number of samples or time that is involved in the
experiment.

(Ex. 104, Elliot Tr. (3/29/07) at pp. 198:12-199:11,
emphasis added).

V.   Amgen Tried but Failed to Develop a Product Through the Chemical Reaction of Erythropoietin and Activated PEG Reagents

169.   As part of its erythropoietin program, Amgen tried to develop a product through the chemical reactions of erythropoietin and activated PEG reagents. Mr. Boone, an Amgen scientist and named inventor of the PEG-NESP patent, testified at his deposition that Amgen did not attempt to react erythropoietin with PEG reagents until after 1985. Amgen experimented with different reagents, including branched and linear activated PEG reagents of different molecular weights, such as 30 kDa PEG-NHS, 10 kDa branched PEG-NHS, and 30 kDa PEG-Ald. (Ex. 61, AM44 0003879).

170.   The difficulty, unpredictability, and uniqueness of the synthesis used by Roche to create CERA is, therefore, highlighted by the fact that Amgen, despite numerous attempts, was unable to synthesize a new therapeutic product by reacting EPO and a PEG reagent.

171.   In Mr. Boone's testimony taken in the present litigation, he makes a number of statements that support my opinion that pegylation is a complex process, requiring the evaluation of numerous variables, and yielding new molecules with unpredictable biological activities. For example, Mr. Boone testified that Amgen attempted between 1985 and approximately 2000 to react erythropoietin and PEG reagents. Amgen, however, did not advance any product into development. According to him, "(t)here were several things that needed to be developed. The chemistry to do the attachment needed to be developed. There were different types of polyethylene glycol, different sizes. And there were different residues to the protein that could be attached to." (Ex. 94 at 31:6-11). Further, he stated, "I have no reason to believe that Amgen scientists would be able to predict *in vivo* biological activity of a PEG-EPO compound" (Ex. 94 at 46:20-22) and that "...(T)here's no guaranty that if you attach a PEG to EPO you will have a biologically active material as defined in your assay." (Ex. 94 at 96:18-21).

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172.   Dr. Elliott also stated that pegylation "...causes a number of effects *depending on how it is done*." (Ex. 106, Elliott Tr. 5/20/98 at 142:19-20; emphasis added). He further testified that during the course of Amgen's EPO analog program, Amgen scientists often had no indication of how to alter proteins for improved activity:

> And so it was kind of like, you know, a rat in the maze
> where we're going down a path. We bump into a wall. We
> back up and have to backtrack and try another path.
>
> We would be trying to find the end of this path, but we
> didn't know which direction it was. It's only when we
> emerged at the end that we can actually figure out, you
> know, which way to go...(A)t the beginning, we didn't even
> know if this program would be successful, if there was
> something that you could find that would work...*in vitro*
> mutagenesis and changing proteins by its very nature is an
> unpredictable process. (Ex. 108, (10/15/98) at 3354:22-
> 3355:17)

VI. Boehringer Mannheim's and Roche's Pegylation Efforts

173.   Roche's MIRCERA is the product of nearly a decade of research and experimentation toward the development of a new erythropoietic agent. It was by no means a trivial undertaking. This work was first conducted at Boehringer Mannheim (BM), beginning in 1993, and later at Roche in Nutley, New Jersey.

174.   Roche's efforts clearly demonstrate the unpredictability and difficulty of pegylation technology in that it was impossible to know in advance which pegylation method and conditions might work, if any, for obtaining the compound that eventually became CERA. It was only through extensive experimentation and trial-and-error that scientists at Roche, were able to find the suitable synthesis that led to CERA.

175.   BM and Roche were forced to analyze a number of complicated synthesis factors. For example, they needed to determine the optimal PEG reagent to be used by evaluating activated reagents, chain length, molecular weight, structure, the optimal temperature and pH of

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the reaction, appropriate purification procedures, and whether a mono-pegylated, di-pegylated, or tri-pegylated molecule would result in a compound with the right therapeutic properties.

176.   With experimental results showing no increased half-life in pharmacokinetic studies in rats, BM decided that reacting epoetin beta with a PEG reagent was not useful. Consequently, in early 1997 BM ceased efforts to develop a new erythropoietic compound. (Ex. 251, R004961456). Afterit acquired BM, Roche decided to restart the program to develop a new erythropoietic compound. Several factors contributed to this decision. When Roche restarted efforts to react PEG reagents with epoetin beta, suitable PEG reagents with much longer chain lengths became available. Roche started to experiment with them and with different reactions conditions and played an important role in the evolution of the second-generation pegylation technology. Even with these advantageous changes in reaction conditions, however, numerous variables were still involved in the development of a new compound and the outcome was still unpredictable.

177.   Results suggested to Roche scientists that the most active substance was made from a 30-kDa mPEG-SBA reagent. (Ex. 249, R001572230). This new chemical entity was selected for further testing and evaluation. This new compound had enhanced, as well as prolonged, pharmacological effect as shown by its longer half-life. *Id.* The product of nearly 10 years of extensive research and experimentation, what ultimately became CERA serves as the active ingredient in Roche's MIRCERA product. Roche obtained patent protection for CERA in 2003 (Ex. 51, U.S. Patent No. 6,583,272).

e.   Formulation of MIRCERA

178.   A final additional material change occurs when CERA is formulated into MIRCERA. The formulation process for MIRCERA involves the following: first, the chemical compound poloxamer 188 is dissolved in water for injection (WFI). Part of the required amount

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of WFI is placed in the compounding vessel, and a nitrogen gas atmosphere is maintained within it. L-Methionine, sodium sulfate anhydrous, sodium dihydrogen phosphate monohydrate, and mannitol are separately dissolved and mixed, and the pH is adjusted to 6.2. The poloxamer 188 solution is added to the solution containing these four different substances. Then sufficient WFI is added to bring to final volume, followed by mixing. (Ex. 151, ITC-R-BLA-00003428-29).

179.   Roche creates this formulation with the drug substance CERA to be used as an injectable. CERA could not be used as an injectable without an acceptable formulation. Formulation of MIRCERA also contributes to the stability of the product, which is important for handling and distribution. Unstable formulations at best add costs and inconvenience to pharmaceuticals and at worst are unusable and dangerous.

IV.   CERA is Not Human Erythropoietin or Its Equivalent

180.   CERA is not human erythropoietin. Nor is CERA equivalent to human erythropoietin since it is structurally distinct and has very different properties from human erythropoietin, as described above. Moreover, CERA is the product of a chemical synthesis.

181.   CERA is not "human erythropoietin" because it is a new and distinct molecule with different structure and properties from human erythropoietin. CERA is not human erythropoietin because it does not have the amino acid sequence of human erythropoietin. CERA does not share the 3D structure of human erythropoietin and does not have the same physical, chemical and biological properties as human erythropoietin.

182.   Chemists do not look to merely a part of the molecular structure to discern the identity of the entire molecule. As I pointed out above, each different arrangement of atoms corresponds to a different compound with its own characteristic set of chemical and physical properties. Just like it would be improper to conclude that aspirin meets the definition of benzene because it has a ring "composed of 6 carbons with alternating single and double bonds",

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it is improper to say that CERA contains human erythropoietin or that it contains the expression product in a mammalian host cell of DNA encoding human erythropoietin.

183.   CERA, like all organic molecules is defined by the specific connectivity of all it atoms through covalent bonds. If one looks at only some of the atoms and bonds, (s)he is conducting an improper analysis that is doomed to lead to an incorrect conclusion. Epoetin beta is no more a "part" or "component" of CERA, as Amgen's experts contend, than, for example, acetic acid – or vinegar – is a "part" or "component" of the anti-inflammatory drug aspirin (see paragraphs 30 and 47 above), which it certainly is not.

184.   One cannot read the depiction of CERA $[CH_3(CH_2CH_2O)_nCH_2CH_2CH_2CO-NH]_m$—EPO in a "mechanical" fashion, or one would arrive at the wrong view that the "NH" does not originate in "EPO" because it is separated from it in the structure above. In other words, the "—EPO" in the structure above is demonstrably distinct from "EPO" (just like a lysine residue in "–Lys-" is different from the amino acid lysine, "Lys"), because "—EPO" could not be a stand alone molecule. It defies the fundamentals of chemical bonding.

185.   Even if one were to ignore the fact that Amgen takes Roche's BLA statements out of the context, Amgen's basic tenet that "EPO" and "—EPO" are the same thing is factually untenable from the chemical point of view. While the difference between "EPO" and "—EPO" may seem minor if analyzed superficially, when one takes into account the universally accepted chemical nomenclature and the meaning that it assigns to the dash symbol ("—"), the difference between the two species covered by these names is large. For example, using Amgen's misleading reasoning, one would conclude that "OH" and "—OH" are also essentially the same thing. But a chemist would vehemently disagree knowing that, among other things, "OH" is a deadly and highly reactive free radical causing mutagenesis and cancer in people, whereas

"—OH" is a harmless residue in water that we drink and bathe in. It is as undeniable that "—EPO"

is materially changed compared to "EPO'" as that "—OH" is materially different from "OH".

186.   Chemists, including protein chemists do not look to merely a part of the molecular

structure to discern the identity of the entire molecule.   My opinions in this matter therefore rest

on examining the whole molecules. Even the so-called "non-functional" rest of the molecule is

important for such aspects as folding, stability, conformational mobility, etc. In other words, the

whole protein molecule plays a role, not just "chunks" of it.

187.   If the biological activities of two molecules are the same, the underlying

structures need not be the same. For example, the enzymatic proteins bovine pancreatic α-

chymotrypsin and trypsin have the same activity in the hydrolysis of para-nitrophenyl acetate.

(Ex. 265, Stewart (1959); Ex. 119, Gutfreund (1955)) Yet, the structures of these two proteins

are undeniably different. Likewise, there are many commercial drugs (e.g., pain relievers) that

have the same activity but indisputably different structures.

188.   CERA is distinct from human erythropoietin based on its molecular structure and

physical, chemical, and biological properties. Rather, CERA is an erythropoietic compound that

is synthesized by chemically reacting a 30-kDa average molecular weight m-PEG-SBA with one

of 9 primary amino groups of epoetin beta starting material. (Ex. 152, ITC-R-BLA-00004027).

189.   Human erythropoietin is a glycoprotein hormone weighing approximately 34 kDa.

(Ex. 47 at col. 5, line 50-51.) The Lin patents identify the amino acid sequence of mature human

erythropoietin as having 166 amino acids, with specific sites for glycosylation at positions 24,

38, and 83. The rest of the residues have the standard amino acid side chains. (Ex. 47 at col. 20,

lns. 66 - col. 21, lns. 15). The glycosylation of erythropoietin is essential for *in vivo* activity.

(Ex. 105, Elliott (2003) at 245; Ex. 116, Goldwasser et al. (1974) at 4202-4206; Ex. 103,

Delorme et al. (1992) at 9876).

190.   According to the teachings of the Lin patents, human erythropoietin has 166

amino acid residues specified in Figure 6 of the patents:

> **FIG. 6 thus serves to identify the primary structural
> conformation (amino acid sequence) of mature human EPO as
> including 166 specified amino acid residues (estimated
> M.W.=18,399).** Also revealed in the Figure is the DNA sequence
> coding for a 27 residue leader sequence along with 5' and 3' DNA
> sequences which may be significant to promoter/operator functions
> of the human gene operon. **Sites for potential glycosylation of
> the mature human EPO polypeptide are designated in the
> Figure by asterisks.** It is worthy of note that the specific amino
> acid sequence of FIG. 6 likely constitutes that of a naturally
> occurring allelic form of human erythropoietin. Support for this
> position is found in the results of continued efforts at sequencing
> of urinary isolates of human erythropoietin which provided the
> finding that a significant number of erythropoietin molecules
> therein **have a methionine at residue 126 as opposed to a serine
> as shown in the Figure.**(Ex. 47 at col. 20, ln. 66 to col. 21, ln. 15;
> emphasis added).

191.   Further in the file history of the '422 patent Amgen defined "urinary EPO" by the

sequence fragments disclosed in Table 1 of Example 1 of the Lin patents. (Ex. 134, AM-ITC

00899473). Fragment T28 of Table 1 indicates that somewhere in the sequence of "urinary

EPO" there is a sequence of amino acid residues corresponding to "E-A-I-S-P-P-D-A-A-M-A-A-

P-L-R". (Ex. 47 at col. 15, lns. 35-54).

192.   Since CERA does not have the 166 amino acid residues of Figure 6, it is not

human erythropoietin as defined in the Lin patents. CERA has only 165 residues. (Ex. 152,

ITC-R-BLA-00004027). Further, CERA has neither a methionine residue at position 126 nor the

sequence "E-A-I-S-P-P-D-A-A-M-A-A-P-L-R", which the Lin patents disclose as a primary

structure characteristic of human erythropoietin isolated from urine. The facts that CERA does

not have 166 amino acid residues and does not have M (Met) at position 126 are evident from

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Figure 1 of Roche's BLA which describes the manufacture of CERA. (Ex. 154, ITC-R-BLA-00004029). Based on this evidence alone, CERA is not human erythropoietin.

193.   Additionally, the amino acid sequence of human erythropoietin has eight lysine (Lys or K) residues. As pointed out above, a Lys residue is fully and unequivocally defined by a side chain that has four CH_2- groups covalentkly bonded to each other linearly, the last being also covalently bonded to an NH_2 (amino) group. In the Lin patents, the Lys residues of human erythropoietin are indicated by the standard three-letter abbreviation "Lys" in Figure 6 and by the standard one letter abbreviation "K" in Figure 9 at positions 20, 45, 52, 97, 116, 140, 152, and 154. Although the patent does expressly indicate that certain residues of Figure 6 can be glycosylated (see the quote above), there is nothing in the Lin patents that teaches or even suggests that any of the 8 Lys residues of human erythropoietin (or any other erythropoiesis stimulating agent) can be chemically reacted in any way to produce a biologically active substance.

194.   Also, the amino acid sequence of human erythropoietin has a free amino group on the N-terminus. The amino acid residue identified as residue "1" in Figure 6 in the Lin patents is Ala. Under standard convention in the field, proteins are numbered from the amino terminus. Therefore, it is understood that Ala is the N-terminal residue of human erythropoietin. Being the N-terminal amino acid residue means that there are no other amino acid residues attached to the alpha-amino group of this residue. Therefore, it is understood that in human erythropoietin the amino group of Ala at position 1 is "free" to be protonated and thus positively charged under physiological conditions.

195.   The eight Lys residues and a free amino group of Ala of position 1 are structural attributes of human erythropoietin. CERA does not include all of these structural attributes. One

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of the nine primary amino groups of human erythropoietin (eight lysines and one amino terminal alanine) is absent in every molecule of CERA. In its place, CERA has an amide group. By virtue of being in an amide instead of an amino group, the corresponding nitrogen atom of CERA is not positively charged under physiological conditions. CERA also has thousands of new atoms and covalent bonds that are not found in the molecular structure of human erythropoietin.

196.   At the time of the filing of the Lin patents, the amino acid sequence of erythropoietin isolated from human urine was defined only by what the patents disclosed. By all indications in the Lin patents, erythropoietin isolated from human urine has the sequences of the fragments given in Table 1 and by the representation that at position 126 there is a methionine. ('422 patent, col. 15, lns 35-54). Epoetin beta does not have the sequence of Fragment T28 of Table 1 (which Amgen used to define human urinary erythropoietin in the file history of the '422 patent) and epoetin beta does not have a methionine at position 126, as can be seen from Roche's regulatory documents. Thus epoetin beta is not human erythropoietin as defined by the Lin patents. (Ex. 154; see also Ex. 183, ITC-R-BLA-00005616 - 5632 at 5619).

197.   CERA does not have the 3D structure of human erythropoietin. Human erythropoietin is a glycoprotein which means that, aside from an amino acid sequence and carbohydrate structure, it also possesses higher-order structure, that is, a specific three-dimensional (3D) structure. The Lin patents teach that the invention, should share the 3D structure of human erythropoietin:

> These sequences, by virtue of sharing primary, secondary or tertiary structural and conformational characteristics with naturally-occurring erythropoietin may possess biological activity and/or immunological properties in common with the naturally-occurring product such that they may be employed as biologically active or immunological substitutes for erythropoietin in

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therapeutic and immunological processes. (Ex. 47 at col. 10, lns. 56-63).

198.   CERA has a different 3D structure than human erythropoietin due to the presence of thousands of additional atoms and numerous non-covalent interactions not present in human erythropoietin.

199.   The physicochemical and biological properties of a compound, along with its complete structure, define the chemical identity of the compound. As explained above, CERA's molecular weight, charge, hydrodynamic radius, and binding affinity for the erythropoeitin receptor are greatly different from those of epoetin beta. The differences in these properties between CERA and epoetin beta are further evidence that CERA is not "*human erythropoietin.*"

V.   CERA Is Not *"purified from mammalian cells grown in culture"*

200.   The Court has construed the term "*purified from mammalian cells grown in culture*" to mean "obtained in substantially homogeneous form from the mammalian cells." I explained previously the steps in the manufacture of CERA and that CERA is not purified from cell culture. Rather CERA is a synthetic substance, and thus it cannot meet the limitation "purified from mammalian cells grown in culture". No known cellular process can produce CERA. CERA can only be produced through the use of synthetic organic chemistry which does not involve the use of cells or cell culture.

201.   If "purified from mammalian cells grown in culture" imports a limitation on the structure or source of substance that fall within the scope of the claim, the limitation is not satisfied by CERA. CERA is substantially different in terms of structure from a compound that is obtained from cell culture; if CERA is deemed an analog of erythropoietin, it is not one that is taught by the Lin patents.

202.   The Lin patents provide no support for chemically synthesized substances. The

PTO and the Courts have previously determined that the Lin patents do not support claims to all

"EPO-like" substances, such as analogs, or derivatives of human erythropoietin. For example,

when Amgen tried to claim synthetic substances during the prosecution of the Lin patents, the

patent examiner rejected the claims. The PTO examiner stated:

> Claims to "synthetic polypeptides" are not enabled by this
> disclosure. "Synthetic," as opposed to "recombinant," is an art
> recognized term which indicates a chemically derived rather than
> genetically engineered protein. No support for chemical synthesis
> of EPO or EPO fragments is shown by this disclosure. (Ex. 83,
> AM-ITC 00941090-1099, at 1094).

VI.   MIRCERA Is Not a "Pharmaceutical Composition" According to Claim 1 of the '422 Patent or Equivalent

203.   MIRCERA does not have just one *"diluent, adjuvant, or carrier"* or an equivalent

as required by claim 1 of the '422 patent.

204.   I understand the Court has ruled that this phrase means "a composition suitable

for administration to humans containing a diluent, adjuvant, or carrier." I understand that one

interpretation of this claim requires there to be just one diluent, adjuvant, or carrier in the

pharmaceutical composition. MIRCERA, in contrast, has multiple ingredients considered a

carrier or a diluent.

205.   The MIRCERA formulation contains several different substances, CERA (the

active ingredient); L-methionine; sodium sulfate; sodium phosphate monobasic; mannitol;

poloxamer 188; hydrochloric acid and sodium hydroxide for pH adjustment; and water.

(Ex. 148, ITC-R-BLA-00003366). The inactive ingredients (as opposed to the active ingredient

CERA) in MIRCERA should not together be considered a single "diluent or carrier" not only

because there are several of them but also because they serve separate and distinct functions in

the formulation. For example, poloxamer 188 is a surfactant; it does not serve a "diluent or carrier".

206.   In addition, pharmaceutical compositions can include other inactive ingredients that are not diluents, adjuvants or carriers. For example, preservatives, colorants, and flavorants are often added to pharmaceutical compositions.

VII.   CERA Is Not the Product of the Expression in a Mammalian Host Cell Of An Exogenous DNA Sequence Encoding Human Erythropoietin As Required by Claims of the '933 Patent or Its Equivalent

207.   Claim 3 of the '933 patent recites *"a glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence."* The phrase *"glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence"* refers to the product of transcription and translation in a mammalian cell from a DNA sequence that does not originate in the genome of the host.

208.   In the prosecution history of the '933 patent Amgen stated "(a)ll product claims in the subject application are now product-by-process claims." Independent Claim 67, and thus all of the pending claims, specifically define the erythropoietin of the subject invention as a "glycoprotein product of the expression of an exogenous DNA sequence," and also that "(t)hese product-by-process claims are presented . . . to further define the product of the subject invention since the recombinant erythropoietin claimed cannot be precisely defined except by the process by which it is produced." (Ex. 281, U.S.S.N. 07/133,178, Paper No.11, dated 6/29/89 at 3-4). Dr. Lin confirmed this in his prior testimony. (Ex. 226, Lin Tr. 6/7/00 at 965:8-14).

209.   Thus, Amgen defined claimed products in the '933 patent by the process of producing them, i.e., the invention as being the product of expression. CERA is not the product of expression. Even if epoetin beta were the product of the expression of an exogenous DNA encoding human erythropoietin (which in my opinion, it is not as explained below), that does not

mean that CERA is. In fact, the opposite must be the case because since CERA is the result of a

chemical reaction involving epoetin beta it cannot also be the product of expression. After the

pegylation reaction, in fact, the epotein beta starting material no longer exists as such.

210.   Epoetin beta is not the expression product of a DNA encoding human

erythropoietin in a mammalian host cell. Roche's BLA states that

> The Epoetin beta (EPO) coding information within the cDNA
> probe used is encoded in 579 nucleotides, coding for a 27
> amino acid leader peptide and a 166 amino acid protein. This
> is further processed into a 165 amino acid protein by removal
> of one C-terminal amino acid." (Ex. 179, ITC-R-BLA-
> 00004722)

211.   This indicates that the expression product has 166 amino acid residues and a

subsequent cellular process cleaves the 166th amino acid residue to produce a 165 amino acid

residue sequence. Moreover, the Lin patents do not disclose the further processing of the

expression product into a fragment of 165 amino acid residues. As I understand it, Amgen's

competitors at Genetics Institute were the first to report the truncation of an amino acid residue

off of the C-terminus of the expression product. (Ex. 262, Recny et al. (1987)). Therefore, even

epoetin beta is not the expression product in a mammalian host cell of exogenous DNA encoding

human erythropoietin, but rather the product further processing after expression.

A.   CERA Is Not The Equivalent of Erythropoietin Or Human Erythropoietin

212.   CERA is not the equivalent of "erythropoietin", "human erythropoietin", "a

glycoprotein product in a mammal host cell of an exogenous DNA encoding human

erythropoietin", or a "glycosylated erythropoietin polypeptide."

213.   CERA performs the functions of increasing hemoglobin in mammals and others

described above in a different way than epoetin beta. This difference is not insubstantial; it is

substantial. CERA works in a substantially different way than epoetin beta. It works by not

binding nearly as tightly as epoetin beta and by having a longer *in vivo* half-life. CERA has a

different affinity to the EPO receptor which stems from its different structure compared to

epoetin beta. These differences are not insubstantial, they are substantial.

214.   The different ways in which CERA and the epoetin beta increase hemoglobin and

impart other therapeutic responses produce a different end result, which is significant. While the

FDA has not approved MIRCERA yet based on the BLA, a dose of CERA causes a sustained

increase in hemoglobin levels which translates into in an improved quality of life for patients

who take it. In turn, it results in a product which does not need to be given as often to benefit

patients. In other words CERA's different way of functioning produces a different result that is

not insubstantial; rather, it is substantial. I also understand that some patients who take Amgen's

Epogen have developed potentially harmful antibodies to erythropoietin. In clinical trials for

MIRCERA, on the other hand, there has been no indication of any patient raising antibodies

against CERA or erythropoietin.

B.   **Aranesp, Not MIRCERA, Is Structurally and Functionally EPO-Like**

215.   The fact that CERA can stimulate erythropoiesis through the erythropoeitin

receptor is not a basis to conclude that CERA is covered by the claims because, as Amgen

scientists have stated, a "number of different molecules . . . can effect the dimerization and

signal transduction of EPOR" (erythropoeitin receptor). (Ex. 246, Ossulund (2003) at 32-33).

Not all of these molecules are human erythropoietins. In fact, Dr. Elliott, Amgen's research

scientist in charge of Amgen's erythropoietin analog program, has written that molecules other

than erythropoietin can homodimerize and activate the EPOR (*see* Ex. 105) Dr. Elliott also

presents evidence that "EPO was not essential for receptor activation (Fig. 2)." (Ex. 105 at 249).

The caption of Figure 2 states that: "The EPO mimetic compounds; agonist antibody [53], EPO

dimer [46, 47], EPO mimetic peptide [54], compound 5 [55], and small-molecule mimetics [55,

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56] can all homodimerize and activate EPOR in a manner similar to that of rHuEPO." *(Ex. 105 at 249).*

216.   In 1985, Amgen started a research program directed at constructing analogs with different biological activity and elucidating the biologically active sites of human erythropoietin that led to the development of Aranesp®. (Ex. 79, AM-ITC 00052992-95). As part of this program, Amgen experimented with various modifications to erythropoietin, including altering the carbohydrate structure, and substituting various amino acids residues. (Ex. 79).

217.   Dr. Elliott found this after being involved in Amgen's program attempting to develop other erythropoietic stimulating agents. In 1992, after making hundreds of analogs Amgen created "Analogue 321", which became what is now called NESP or New Erythropoietin Stimulating Protein. (Ex. 108 at 3421:8-3424:20).

218.   NESP is formulated and marketed as Aranesp®. Amgen maintains that Aranesp® will activate the erythropoietin receptor and stimulate erythropoiesis. (Ex. 60, AM44 0445261; Ex. 62, AM44 0004809). Yet Amgen has maintained that Aranesp® (also called darbepoetin and DA) is not covered by any of the asserted claims of the Lin patents (Ex. 75, Amgen Response to Interrogatory No. 8., February 10, 2007).

219.   Aranesp is claimed to stimulate erythropoiesis by the same mechanism as human erythropoietin. (Aranesp Label). However, the half-life of Aranesp is almost 3 times longer (17.8 hours vs. 6.3 hours) and clearance is over 4 times slower (2 mL/h/kg vs. 8.58 mL/h/kg) than those of human erythropoietin (Ex. 59, AM 44 0197792). Recall the half-life of CERA is exceeds 100 hours.

220.   Dr. Elliott has testified that Aranesp® is similar and acts through the same mechanism as human EPO. (Ex. 108 at 23:16-19; 24:13-15; 24:23-25:2).

221.   Along the same lines, in his recent presentations, Dr. Elliott depicted Aranesp®
and pegylated proteins as distinct from rHuEPO, with Aranesp® being closer to rHuEPO than
either "Peg-DA" or "Peg-rHuEPO", which are grouped together. (Ex. 60, AM 44 0445251-5274
at 61; Ex. 58, AM 44 0004799-834 at 809; Ex. 108 at 216:16-220:12, 229:4-232:21, 238:13-
241:24).

222.   As stated above, CERA is not, and cannot be, the product of expression of a DNA
sequence encoding human erythropoietin. Amgen's own documents that compare CERA with
Aranesp® emphasize the fact that CERA is made by a chemical process and Aranesp® is
manufactured by recombinant DNA technology. (Ex. 68, AM44 0897376-82).

223.   U.S. Patent No. Ex. 53, 7,217,689 ("the '689 patent") issued on May 15, 2007,
from an application filed on June 6, 1995. The patent identifies Steven G. Elliott and Thomas E.
Byrne as the inventors and Amgen as the assignee of the patent. The claims of the '689 patent
are directed to "analog[s] of human erythropoietin having the in vivo biological activity of
increasing hematocrit." (Ex. 53 at col. 45, ll. 21-22).

224.   The specification of the '689 patent indicates that "[i]ncreasing the number of
carbohydrate chains of erythropoietin, and therefore the number of sialic acids per erythropoietin
molecule, may confer advantageous properties such as increased solubility, greater resistance to
proteolysis, reduced immunogenicity, increased serum half-life, and increased biological
activity." (Ex. 53 at col. 9, ll. 17-21).

225.   It is my understanding that the named inventor Steven G. Elliott is the same
person who was deposed in this case on March 29, 2007, and whose deposition transcript I have
reviewed and cited above. I further understand that the party controlling the prosecution of the
'689 patent is the same Amgen company that initiated the present litigation against Roche. It is

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my additional understanding that the '689 patent is directed to NESP, the active ingredient in

Amgen's Aranesp product and that the file history of the '689 patent was not available to the

public before the '689 patent issued on May 15, 2007. Amgen, of course, had access to this file

throughout the prosecution of the patent. (Ex. 104 at 13:5-16; 201:25-202:22).

226.   Upon reviewing the prosecution file history of the '689 patent, I found that a

number of the positions taken by Amgen and Amgen's experts support my opinions about the

state of the art, the level of skill in the art, and the unpredictability in the art as it relates to

proteins having in vivo erythropoietic activities that are therapeutically useful.

227.   For example, in February 2004, prior to the commencement of the present lawsuit

against Roche, Amgen attorneys submitted to the PTO Board of Patent Appeals and

Interferences (BPAI) an appeal brief (Ex. 279, U.S.S.N. 08/479,892, "Brief for Party Elliott"

dated February 11, 2004).

228.   In characterizing the art prior to the '689 patent, Amgen stated that "Human Epo

was known to be a glycoprotein with three N-linked carbohydrate chains and one O-linked

carbohydrate chain." (Ex. 279 at 9). In making its argument to the PTO, Amgen went on to

state that "even if one could predict that a new N-linked chain would be added to an amino acid

residue in Epo, one skilled in the art could not be assured that the resulting Epo molecule would

retain biological activity (Cummings declaration, paragraphs 7 and 8)." (Ex. 279 at 15).

229.   Amgen stated that there was "no knowledge of the amino acid residues in Epo

that were important for receptor binding (Cummings declaration at paragraph 8)", and that

"[O]ne skilled in the art is left with no guidance as to what regions of the Epo polypeptide may

be modified with new carbohydrate chains and still retain biological activity." (Ex. 279 at 16).

230.   In addition, Amgen characterized the unpredictability of the art by stating:

[I]t is clear that no methods existed as of 1989, that would reliably direct one skilled in the art to any region of a protein such as Epo that would add new carbohydrate. In view of this, addition of new carbohydrate to Epo in 1989 was no better than a trial and error approach with no assurance that any given amino acid residue could be successfully modified such that a new carbohydrate chain was added. (Ex. 279 at 16)

As of the priority date of the application, no information was available in the prior art on the location of the active site of Epo or an identification of the regions of Epo that were important for maintaining proper three-dimensional structure and in vivo biological activity (Cummings declaration at paragraphs 7 and 8). Therefore one of ordinary skill in the art had no prior knowledge of what amino acid residues in Epo could be modified to add carbohydrate chains without altering in vivo biological activity. Addition of new carbohydrate chains to Epo could have disrupted Epo synthesis, folding and/or biological activity. There was no reasonable expectation of success in adding a chain to Epo such that the resulting glycosylation analog had the in vivo biological activity of raising hematocrit. (Ex. 279 at 26).

VIII. ROCHE DOES NOT INFRINGE UNDER THE REVERSE DOCTRINE OF EQUIVALENTS

231.   From my review of the Lin patents and the prosecution file history, the spirit and intent of Dr. Lin's invention was to produce products that can be made from host cells by means of DNA technology. For example, for the claim term "erythropoietin" and the similar terms "erythropoietin glycoprotein product," "erythropoietin polypeptide," "glycosylated erythropoietin polypeptide", and "human erythropoietin," are all characterized in the specification by Amgen as "uniquely characterized by being the product of prokaryotic or eucaryotic host expression (e.g., by bacterial, yeast and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis." (Ex. 44 at col. 10, lns. 15-20). The idea of Dr. Lin was to use "the existing machinery for gene expression in the transformed or transfected microbial host cells... to construct the desired product" (Ex. 44 at col. 2, lns. 28-31) It is also clear that the desired product was to have the

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same in vivo biological activity as naturally derived human erythropoietin. (Ex. 44 at col. 10 lns. 9-16).

232.   The prosecution history also supports the notion that Dr Lin's invention was to make and use products that are produced by cells. As I stated above, in characterizing the invention to the PTO Amgen asserted that the only way to describe the invention was: "recombinant erythropoietin claimed cannot be precisely defined except by the process by which it is produced." Additionally, the only way Amgen was able to overcome PTO's novelty rejections was by distinguishing its host cell produced erythropoietin from erythropoietin from other sources, including naturally derived urinary erythropoietin. (Ex. 281 at 4).

233.   As explained above, CERA is a novel patented molecule made by a chemical reaction of epoetin beta and a highly specific activated PEG reagent. Amgen admits CERA was not, and cannot be, produced by cells. (Ex. 94 at 122:13-15). Notably, Amgen's second generation product Aranesp is produced in cells because, as Dr. Elliott testified, it is the only way he knew how to go about it. (Ex. 108 at 3636:1-10).

234.   I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

DATE: June 28, 2007

/s/ Alexander M. Klibanov
Alexander M. Klibanov, PH.D.

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CERTIFICATE OF SERVICE

I hereby certify that the redacted version of this document was filed through the ECF system and was sent electronically to the registered participants as identified on the Notice of Electronic Filing (NEF) and paper copies were sent to those indicated as non registered participants on the above date.

/s/ Kregg T. Brooks
Kregg T. Brooks

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