Appeal Nos. 2018-1551, 2018-1552

United States Court of Appeals for the Federal Circuit

AMGEN INC., AMGEN MANUFACTURING, LIMITED,

Plaintiffs-Appellants,

– v. –

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, SANDOZ GMBH,

Defendants-Appellees.

Appeal from the United States District Court for the Northern District of California in No. 3:14-cv-04741-RS, Judge Richard Seeborg

AMGEN INC., AMGEN MANUFACTURING, LIMITED,

Plaintiffs-Appellants,

– v. –

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, SANDOZ GMBH, LEK PHARMACEUTICALS, D.D.,

Defendants-Appellees.

Appeal from the United States District Court for the Northern District of California in No. 3:16-cv-02581-RS, Judge Richard Seeborg

NON-CONFIDENTIAL BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED

(For Appearances See Inside Cover)

April 13, 2018

WENDY A. WHITEFORD
LOIS M. KWASIGROCH
KIMBERLIN L. MORLEY
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320
(805) 447-1000

NICHOLAS GROOMBRIDGE
ERIC ALAN STONE
JENNIFER H. WU
JENNIFER GORDON
PETER SANDEL
ARIELLE K. LINSEY
STEPHEN A. MANISCALCO
JACOB T. WHITT
GOLDA LAI
PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
1285 Avenue of the Americas
New York, New York 10019
(212) 373-3000

Attorneys for Plaintiffs-Appellants Amgen Inc. and Amgen Manufacturing, Limited

U.S. Patent No. 8,940,878, Claim 7

7. A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising:
(a) expressing a protein in a non-native limited solubility form in a non-mammalian cell;
(b) lysing a non-mammalian cell;
(c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following:
(i) a denaturant;
(ii) a reductant; and
(iii) a surfactant;
(d) forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of the following:
(i) a denaturant;
(ii) an aggregation suppressor;
(iii) a protein stabilizer; and
(iv) a redox component;
(e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix;
(f) washing the separation matrix; and
(g) eluting the protein from the separation matrix, wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin.

U.S. Patent No. 6,162,427, Claim 1

1. A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment, comprising administering to the patient a hematopoietic stem cell mobilizing-effective amount of G-CSF; and thereafter administering to the patient a disease treating-effective amount of at least one chemotherapeutic agent.

CERTIFICATE OF INTEREST

The full name of every party represented by me is:

AMGEN INC. and AMGEN MANUFACTURING, LIMITED

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

AMGEN INC. and AMGEN MANUFACTURING, LIMITED

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

AMGEN INC.

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

SIDLEY AUSTIN LLP: Vernon M. Winters, Sue Wang, and Alexander David Baxter who is no longer with the firm

PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP: Michael T. Wu and Ana J. Friedman who are each no longer with the firm

The title and number of any case known to counsel to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal. *See* Fed. Cir. R. 47.4(a)(5) and 47.5(b).

None

Date: April 13, 2018

/s/ Nicholas Groombridge
Nicholas Groombridge

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CONFIDENTIAL MATERIAL OMITTED

Pursuant to Federal Circuit Rule 28(d)(2)(B), Plaintiffs-Appellants prepared this public version of their brief which redacts certain information designated confidential pursuant to the district court's Protective Orders, entered on February 9, 2015 and January 17, 2017. Specifically, the material omitted on pages 11, 16-22, 25-26, 50, 53, and 55 contains references to Defendants-Appellees' accused processes, and was designated confidential by Defendants-Appellees during discovery under the terms of the Protective Orders. Prior to filing this public version of the brief, counsel for Plaintiffs-Appellants conferred with counsel for Defendants-Appellees, and Defendants-Appellees confirmed that they continue to consider the omitted material to be confidential.

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STATEMENT OF RELATED CASES

This Court (Judges Newman, Lourie, and Chen) previously considered Amgen's appeal from the United States District Court for the Northern District of California in Case No. 3:14-cv-04741-RS. *See Amgen Inc. v. Sandoz Inc.*, 794 F.3d 1347 (Fed. Cir. 2015) (No. 2015-1499). This Court issued an opinion on July 21, 2015, and the mandate issued on October 23, 2015. The Supreme Court granted each of the parties' petitions for writs of certiorari (U.S. No. 15-1039, 1195), and issued an opinion on June 12, 2017, remanding certain issues to this Court. *See Sandoz Inc. v. Amgen Inc.*, 137 S. Ct. 1664 (2017). This Court (again, Judges Newman, Lourie, and Chen) issued an opinion on the remand issues on December 14, 2017, *see Amgen Inc. v. Sandoz Inc.*, 877 F.3d 1315 (Fed. Cir. 2017) (No. 2015-1499), and the mandate issued on January 23, 2018.

No other related cases are known to counsel for Amgen to be pending in this or any other court that will directly affect or be affected by this Court's decision on appeal.

STATEMENT OF JURISDICTION

The district court has subject-matter jurisdiction over these cases under 28 U.S.C. §§ 1331 and 1338(a). This Court has jurisdiction over this consolidated appeal under 28 U.S.C. § 1295(a)(1). Amgen timely appealed under 28 U.S.C. § 2107 and Fed. R. App. P. 4(a) on February 5, 2018. (Appx86-93.) The appeals are from final judgments that dispose of all parties' claims. (Appx1-4.)

STATEMENT OF THE ISSUES

1. Whether the district court erred as a matter of law in construing the "washing" and "eluting" elements of claim 7 of U.S. Patent No. 8,940,878 where:
   a. the district court originally interpreted "eluting the protein from the separation matrix" to occur after "washing the separation matrix" and required "washing" and "eluting" to be accomplished by applying "a solution";
   b. but on summary judgment, the district court imposed additional limitations that washing and eluting must be accomplished by applying different solutions that are temporally and compositionally distinct.
2. Whether the district court erred as a matter of law in granting summary judgment of non-infringement of claim 7 of the '878 Patent with respect to Sandoz's current process for manufacturing its biosimilar products where Amgen presented evidence that Sandoz's process meets the claim limitations.
3. Whether the district court erred as a matter of law in granting summary judgment of non-infringement of claim 7 of the '878 Patent and denying Amgen's motion for additional discovery pursuant to Rule 56(d) with

respect to Sandoz's modified process for manufacturing its biosimilar products (which were still under development and had not yet been submitted to FDA), where Sandoz did not provide complete information about the process, denying Amgen discovery on the very way Sandoz will make its biosimilar products going forward.

4. Whether the district court erred in construing "chemotherapeutic agent" as used in claim 1 of U.S. Patent No. 6,162,427 to be for treatment of disease rather than working in combination with G-CSF to enhance stem cell mobilization.

STATEMENT OF THE CASE

This consolidated appeal arises from two patent lawsuits under the Biologics Price Competition and Innovation Act of 2009 ("BPCIA"). The accused products and processes are described in Sandoz's abbreviated biologics license applications ("aBLAs") to FDA referencing Amgen's Neupogen® (filgrastim) and Neulasta® (pegfilgrastim) products. (Appx6, Appx2025, Appx7117-7118.) *See Sandoz Inc. v. Amgen Inc.*, 137 S. Ct. 1664, 1673 (2017). This Court is familiar with Neupogen® from the earlier appeal. *Amgen Inc. v. Sandoz Inc.*, 794 F.3d 1347, 1352 (Fed. Cir. 2015); *see Sandoz Inc.*, 137 S. Ct. at 1672-73. In March 2015, FDA approved Sandoz's aBLA for its biosimilar filgrastim product, and it is sold as Zarxio®. *Amgen Inc.*, 794 F.3d at 1353; *see Sandoz Inc.*, 137 S. Ct. at 1672-73. FDA has not approved Sandoz's aBLA for its pegfilgrastim product. (Appx6.) Sandoz intends to make further submissions to FDA to change its process for manufacturing both of its products. (Appx14.)

Amgen asserts that Sandoz infringes two patents: U.S. Patent No. 6,162,427 ("'427 Patent") and U.S. Patent No. 8,940,878 ("'878 Patent"). Amgen initially sued Sandoz under 35 U.S.C. § 271(e)(2)(C)(ii) for infringement of its method of treatment patent, the '427 Patent (Appx80-85), after Sandoz's submission of an application for FDA approval of its filgrastim biosimilar product for Neupogen®'s stem cell mobilization indication. (Appx250-288 at Appx263, Appx269-270,

Appx284-285.) *See Amgen Inc.*, 794 F.3d at 1353. A year later, Amgen also brought infringement claims under 35 U.S.C. § 271(g) and (e)(2)(C)(ii) of the '878 Patent, which issued after Amgen filed its original Complaint. (Appx60-78, Appx2003-2047 at Appx2043-2044.) In addition, Amgen sued Sandoz for infringement of the '878 Patent under 35 U.S.C. § 271(e)(2)(C) and (g) after Sandoz notified Amgen of Sandoz's aBLA submission for its proposed biosimilar pegfilgrastim product. (Appx7114-7135 at Appx7130-7132.) Amgen also sued Sandoz for infringement of another patent (U.S. Patent No. 5,824,784) under § 271(e)(2)(C); those claims and the related counterclaims were later dismissed following the parties' joint stipulation of dismissal. (Appx55-58.)

The district court then construed the claims of the '427 and '878 Patents (Appx16-48), after which Amgen and Sandoz stipulated to non-infringement of the '427 Patent (Appx49-54) and the district court entered final judgment as to that patent. (Appx1-2.) Subsequently, with respect to the '878 Patent, the district court granted Sandoz's motion for summary judgment that Sandoz's current manufacturing process and a modified process in development do not infringe, either literally or under the doctrine of equivalents, the claims of the '878 Patent. (Appx9-15.) The district court reached its conclusion based on a further claim construction. (*See* Appx10-11.)

In addition, the district court determined that Sandoz's planned modification to its manufacturing process was not "material to the finding of noninfringement." (Appx14.) It thus denied Amgen's motion to defer any judgment until Sandoz submits its application for approval of the modified process to FDA and provides discovery to Amgen. (Appx14-15, Appx5134-5147.) The district court then entered final judgment of non-infringement as to the '878 Patent. (Appx1-4.)

Amgen timely appealed in both cases. (Appx86-93.) This Court consolidated the two appeals. (Dkt. No. 2.)

STATEMENT OF THE FACTS

A. Amgen's Neupogen® and Neulasta® Products, and Sandoz's aBLAs Referencing Those Products

Amgen Inc. discovers, develops, manufactures, and sells innovative therapeutic products based on advances in molecular biology, recombinant DNA technology, and chemistry. (Appx2008, Appx7115.) Amgen Manufacturing, Limited manufactures and sells biologic medicines for treating human diseases. (Appx2008, Appx7115.) Amgen's Neupogen® (filgrastim) and Neulasta® (pegfilgrastim) are recombinantly produced proteins that stimulate the production of neutrophils, a type of white blood cell. (Appx6.) *Sandoz Inc.*, 137 S. Ct. at 1672-73. Filgrastim is a pharmaceutical analog of a protein that is naturally produced in humans called granulocyte-colony stimulating factor ("G-CSF"). (Appx20, Appx2018.) Pegfilgrastim is a form of filgrastim that is joined with monomethoxypolyethylene glycol, or PEG, which allows the protein to remain in circulation for a longer period of time. (Appx6, Appx7128.) One use of Amgen's products is to counteract neutropenia, a neutrophil deficiency that makes a person highly susceptible to life-threatening infections and is a common side effect of certain chemotherapeutic drugs. (Appx6, Appx2018, Appx7128-7129.) Neupogen® is also administered to mobilize hematopoietic stem cells from a patient's bone marrow into the peripheral blood for peripheral stem cell transplantations. (Appx20.)

Amgen's Neupogen® and Neulasta® were each approved by FDA under the traditional biologics regulatory pathway, 42 U.S.C. § 262(a), which requires that the applicant demonstrate that the biologic is "safe, pure, and potent." 42 U.S.C. § 262(a)(2)(C)(i)(I); *Sandoz Inc.*, 137 S. Ct. at 1670. In contrast, Sandoz—which develops, manufactures, and sells pharmaceuticals, including generic medicines (Appx2008-2009, Appx7115-7116)—filed aBLAs under the BPCIA's abbreviated pathway, 42 U.S.C. § 262(k), seeking approval to market products based on biosimilarity using Amgen's Neupogen® and Neulasta® as the reference products. (Appx6, Appx2025, Appx7117-7118.) *See Sandoz Inc.*, 137 S. Ct. at 1666.

B. The '878 Patent

1. Protein and Protein Purification

In every human cell, thousands of different proteins work together to perform virtually every process within the cell, including functions such as metabolic reactions, signaling, sensing, and growth. (Appx4901.) G-CSF is an example of a protein. (*Id.*) Proteins are made naturally in the human body usually in small amounts. (*Id.*) Using recombinant DNA technology, however, useful proteins can be produced in mass quantities in genetically-engineered organisms. (Appx4902, Appx34.) Generally, scientists isolate a human gene that encodes a particular protein, then insert it into host cells (such as bacteria), and culture the cells to express the human protein. (Appx4901-4902.) This technology allows for

the production of large amounts of proteins, which can be used therapeutically in humans. (*Id.*)

Proteins typically have three-dimensional structures which are critical for their biological activity. (Appx4906-4908, Appx34.) Non-mammalian (*e.g.*, bacterial) expression systems, however, produce misfolded and/or aggregated recombinant proteins. (Appx4906-4908.) These misfolded and/or aggregated proteins often precipitate within the host cells in limited solubility, inactive forms referred to as "inclusion bodies." (*Id.*) Thus, the expressed protein must undergo further processing before it can be therapeutically useful. (*Id.*) This processing includes solubilization and refolding. (*Id.*, Appx34-35.) Solubilization untangles the aggregated and misfolded proteins. It is accomplished by mixing the inclusion bodies with various chemicals that unfold the protein. '878 Patent, 2:22-28. During refolding, the protein is reconfigured into its proper (biologically active) three-dimensional structure. This is accomplished by mixing the protein with other chemicals that facilitate refolding. *Id.* Then, the refolded protein must be purified to remove the chemicals introduced by the prior processing steps and other contaminants, including proteins expressed by the host organism, also present in inclusion bodies. *Id.* at 14:67-15:5.

Chromatography is one of a number of ways to purify proteins. *Id.* at 4:2-5; (Appx4908-4911.) Chromatography is a method of separating molecules in a

solution (the "mobile phase") on the basis of their chemical or physical interactions with a solid matrix (the "stationary phase" or "separation matrix"). (Appx4908-4911.) The separation matrix may be in the form of particles or beads, referred to as "resins," packed inside a column. (*Id.*) Chromatography resins may have certain functional groups attached to them that facilitate protein binding. (*Id.*) Sandoz currently uses and is in the process of developing a modified process that would use such chromatography resins, sold under the names [redacted] and [redacted] respectively. (*Id.*, Appx4949.) In large-scale purification, chromatography can be performed in large, steel columns (*see* '878 Patent, 12:16-26), as seen below.

(Appx3127.) A liquid phase containing the protein to be purified and other materials is introduced at the top of the column, flows downward, and exits at the bottom. (Appx4908-4910.)

There are two basic mechanisms of chromatography, and either can be used to purify proteins: adsorption and non-adsorptive. (Appx4908-4911.) In the case of adsorption, protein molecules are adsorbed, or bound, onto the separation matrix. (*Id.*) The types of adsorption-based chromatography differ in the nature of the binding interaction between the protein and the matrix. (*Id.*) Examples of adsorption-based chromatography include ion exchange chromatography ("IEX"). (*Id.*) IEX chromatography takes advantage of differences in the type and strength of ionic interactions of the different molecules in a sample with a charged resin. (*Id.*) Negatively charged molecules can bind to resins carrying positively charged groups (anion exchange chromatography, or "AEX"), or positively charged molecules can bind to resins carrying negatively charged groups (cation exchange chromatography, or "CEX"). (*Id.*)

Proteins contain both positively and negatively charged groups. (*Id.*) The isoelectric point ("pI") of a protein is the pH at which, theoretically, the protein carries no net charge. (*Id.*) Proteins in solution are commonly net positively charged or net negatively charged, depending on the pH of the solution. (*Id.*) At a pH below its pI, the protein carries a net positive charge; at a pH above its pI, the protein carries a net negative charge. (*Id.*) Proteins and other molecules with net negative charges can be adsorbed on anion exchange resins, while proteins and

other molecules with net positive charges can be adsorbed on cation exchange resins. (*Id.*)

Generally, proteins bind best to IEX resins at low salt concentrations and bind more weakly as the salt concentration increases. (*Id.*) High concentrations of salt ions interfere with the binding between the resin and the protein and can cause desorption. (*Id.*) In addition to separating proteins and other molecules according to whether their net charge is positive or negative, IEX can also separate proteins and other molecules according to the strength of their ionic interactions to the charged resin. (*Id.*) More negatively charged molecules, for example, can have stronger ionic interactions with a positively charged resin than do their less negatively charged counterparts. (*Id.*) Thus, molecules that do not bind to the separation matrix at all or ones that bind more weakly may be washed off the column first, while the binding of the more strongly interacting molecules is preserved. (*Id.*) Lastly, proteins in different folding states, or conformations, can differ in the strength of their ionic interactions to the charged resin. (*Id.*)

Desorption of a protein from a separation matrix can occur by changing the properties of the mobile phase to increase the concentration of ionic species, *e.g.*, salt ions, which competitively displace the bound protein, allowing it to "elute" from the matrix. (*Id.*) A change in pH can also result in elution, due to changes in the charge of the adsorbed protein and its binding capacity. (*Id.*) When the elution

of a protein of interest occurs later in time than when contaminants emerge from the column, a chromatographic separation has occurred and the protein of interest has become more purified than when it entered the column initially. '878 Patent, 6:64-7:3.

The output of a chromatography column is often routed through a meter measuring ultraviolet ("UV") absorbance, which is a proxy for protein concentration. (Appx4909.) The meter is thus used to detect when protein begins exiting the column. (*Id.*) When the UV meter detects protein in the liquid exiting the column, the UV trace begins to rise as protein concentration increases. The trace continues to rise until it peaks or plateaus and subsequently returns to baseline as the protein concentration in the liquid exiting the column decreases. (*Id.*) The liquid exiting the chromatography column may also be monitored for pH, conductivity (a measure of salt concentration) and the like, by various devices and in-line probes. (*Id.*) Traces of such measurements can also be plotted on a chromatogram as a function of time or volume of liquid exiting the column. (*Id.*)

2. The Invention

The '878 Patent is generally directed to improved methods for purifying proteins expressed in non-mammalian cells and, in certain embodiments, to the purification of proteins expressed as insoluble inclusion bodies in recombinant bacteria. *E.g.*, '878 Patent, 11:55-17:4. Prior to the invention of the '878 Patent, it

was believed in the art that certain of the specialized chemical compounds used to process inclusion bodies (so that the proteins in them can be solubilized and subsequently recovered in biologically active form) had to be diluted or reduced or removed prior to the application of a refold solution to a separation matrix to achieve purification. *Id.* at 12:16-20. The conventional wisdom before the '878 Patent was that these specialized chemical compounds in the refold solution could prevent or disrupt the interactions with a separation matrix necessary to achieve purification. *Id.* at 15:29-37. Thus, in the prior art, processing steps, such as a dilution, intervened between protein refolding and application to a first chromatographic separation matrix. *Id.* at 15:25-29.

The '878 Patent invention reflects the inventors' insight that protein purification can be achieved by ***directly*** applying a refold solution to a separation matrix, without intervening processing. *Id.* at 11:58-63, 15:25-42. Such additional processing can be costly and time-consuming, particularly at a large manufacturing scale. *Id.* at 11:58-63, 12:21-26, 15:30-42. The invention is applicable whether the first separation matrix to which the refold solution is directly applied captures an impurity protein or the protein of interest (*i.e.*, the desired protein at the end of the purification process). *Id.* at 14:65-15:5. Whatever the function of the first separation matrix in the overall purification scheme, the patent teaches eliminating

processing steps typically used in the prior art after protein refolding and prior to application to a first separation matrix. (Appx35.)

3. Sandoz's Manufacturing Process

As set forth in Sandoz's aBLAs for filgrastim and pegfilgrastim, and other Sandoz manufacturing documents, Sandoz expresses the filgrastim protein that is used in both Sandoz's Zarxio® and Sandoz's yet-to-be-approved pegfilgrastim product in limited solubility form in non-mammalian cells. (Appx4914-4919, Appx5522, Appx5598 (Sandoz's documents call its filgrastim product EP2006, and its pegfilgrastim product LA-EP2006).) Sandoz then solubilizes and refolds the filgrastim protein using various chemicals, resulting in a refold solution, which Sandoz's documents call "REF.E." (Appx4925, Appx5739.) In addition to filgrastim, Sandoz's refold solution contains various contaminants that need to be removed before the protein may be used therapeutically. These contaminants include process-related contaminants such as [redacted], [redacted] and [redacted] as well as product-related contaminants such as unwanted molecular variants and adducts of filgrastim. (Appx4916-4917, Appx5228-5229, Appx5395, Appx5536-5539.) Sandoz's refold solution also has a high concentration of salt. (Appx4893, Appx4929, Appx5251, Appx5310-5311, Appx5517-5518, Appx5620-5621, Appx5850, Appx5857.) Immediately after refolding, without performing any intervening processing steps, Sandoz loads the refold solution onto

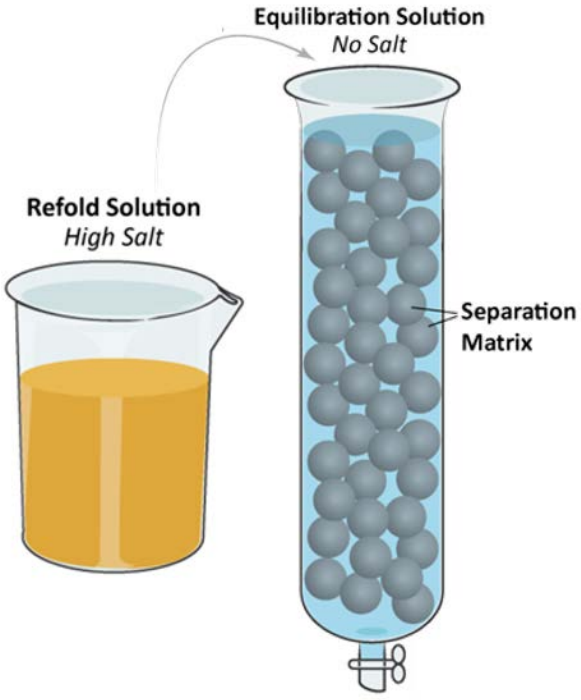
its first chromatography column, an anion exchange chromatography column (the "AEX Step"). (Appx4925-4926, Appx5247-5248, Appx5333-5336, Appx5340-5341, Appx5524, Appx5699, Appx5738, Appx5740, Appx5745-5750.)

a. Sandoz's AEX Step Causes Filgrastim To Bind ("Associate With") the Resin

In Sandoz's current process, Sandoz's AEX column contains [redacted] resin, the separation matrix.[1] (Appx4917, Appx4926-4927, Appx5247-5249, Appx5311, Appx5525-5526, Appx5542, Appx5552-5555, Appx5620-5621, Appx5851.) Before applying the refold solution, which contains the filgrastim and contaminants, a solution containing no salt, called the equilibration solution, is added to Sandoz's column. (Appx4893, Appx4929-4930, Appx5251-5252; *see* Appx5268, Appx5850, Appx5857.) The functional groups, ligands, on the outer surfaces of the resin beads are positively charged. (Appx4927-4928, Appx5248-5251, Appx5311, Appx5620, Appx5755-5756.) The pI of filgrastim is approximately 6, and the pH of Sandoz's refold solution ranges from [redacted]. (Appx4914, Appx4929, Appx5251, Appx5311, Appx5338-5339, Appx5522-5523, Appx5530, Appx5620-5621, Appx5695.) Thus, the filgrastim is negatively charged when it is directly applied to the column. (Appx4929, Appx4930.) Under the principles of anion exchange chromatography, *i.e.*, that negatively charged

molecules will bind positively charged ligands, the filgrastim in Sandoz's process can and does bind to the [redacted] beads. (Appx4893, Appx4930-4931.)

In addition, when the refold solution is loaded on the column, the column is already filled with Sandoz's equilibration solution which contains no salt, and thus the protein encounters low-salt conditions. (Appx4929-4930, Appx5251-5252.)



(Appx6991-7008 at Appx6994.) These low-salt conditions favor filgrastim binding. (Appx4929-4930, Appx5850, Appx5857.) Thus, the initial salt concentration on Sandoz's column provides conditions suitable for binding

[1] As discussed below, the [redacted] chromatographic resin in its AEX Step that Sandoz currently uses was discontinued and will be replaced with a [redacted] resin in late 2018 or 2019. (Appx14, Appx3750.)

filgrastim, and filgrastim binds to the beads. (Appx4929-4930, *see* Appx5345, Appx5919-5922, Appx5352.)

One of the purposes of the AEX Step is to remove Sandoz's detergent, [redacted], from its refold solution. (Appx4923, Appx4930, Appx5311, Appx5620.) The resin binds the detergent, and bound detergent is discarded with the resin at the end of the AEX Step. (Appx4923, Appx4930, Appx5311, Appx5357-5358, Appx5621, Appx5724.) Sandoz's process, however, creates a detergent-free zone in the column with binding sites that the detergent never occupies. (Appx4930; *see* Appx4876.) This zone, at the downstream end of the column, captures the filgrastim molecules by providing available sites to which filgrastim can (and does) bind. (Appx4930.) The detergent-free zone is created by using more resin in the column than needed to bind the total amount of detergent in the refold solution. (Appx4892, Appx4930, Appx5397, Appx5862.) In other words, Sandoz uses a quantity of resin with excess binding capacity to ensure that virtually all the detergent in the refold solution will be adsorbed on the matrix at the upstream end of the column.

Amgen's expert, Dr. Willson, analyzed the Sandoz process, including actual data from its batch records, to determine whether filgrastim binds to the resin in the column. Considering the loading time, the bed height and diameter of the column, the flow rate, and the volume of the piping between Sandoz's refold tank and the

column, Dr. Willson calculated how long it would take a molecule of similar size to filgrastim to move through the column if it did not bind to the resin. (Appx4895, Appx4898, Appx4931-4934, Appx5430.) He then compared that to the emergence time of filgrastim as shown in the batch records. (Appx4931-4934.) Dr. Willson provided evidence based on Sandoz's available batch records that filgrastim binds to Sandoz's column because it emerges significantly later in time than that of a hypothetical, non-binding molecule. (Appx4931-4934, Appx5430, Appx5571-5577, Appx5589-5593.) Specifically, Dr. Willson found from the batch records that filgrastim remained bound to the resin for an average of at least [redacted] minutes. (Appx4894, Appx4934, Appx5430.)

b. Sandoz's AEX Step Removes Contaminants From ("Washes") the Resin While Filgrastim Remains Bound

As the refold solution is loaded on the column and filgrastim binds to the resin for a period of time, other materials in the refold solution, *i.e.*, contaminants, continue to advance through and are washed off the column, separating the bound filgrastim from these contaminants. (Appx4936.) Sandoz does not begin collecting the liquid exiting the column when it begins loading the refold solution on the column or even at the time protein is first detected exiting the column. (Appx4895, Appx4938, Appx4947-4948, Appx5430.) Instead, Sandoz waits until the UV absorbance trace rises to a certain level before it begins collection.

(Appx4938, Appx4947-4948, *see* Appx5430, Appx5589-5593.) Specifically, collection can begin anywhere in the range [redacted] of the height on the ascending peak of the UV absorbance trace. (Appx4895, Appx5399, Appx5924-5925, Appx6117, *see* Appx6121, Appx6146.) Thus, when filgrastim binds to the anion exchange resin in the column, other materials of the refold solution exit the column first. (Appx4893-4896, Appx4936-4943.) They precede filgrastim off the column and so are removed from the refold solution. (Appx4893-4896, Appx4936-4943.) Because these materials are not collected, but rather are discarded, they never again comingle with the filgrastim, which consequently has been partially purified on the anion exchange column. (Appx4888, Appx4898.)

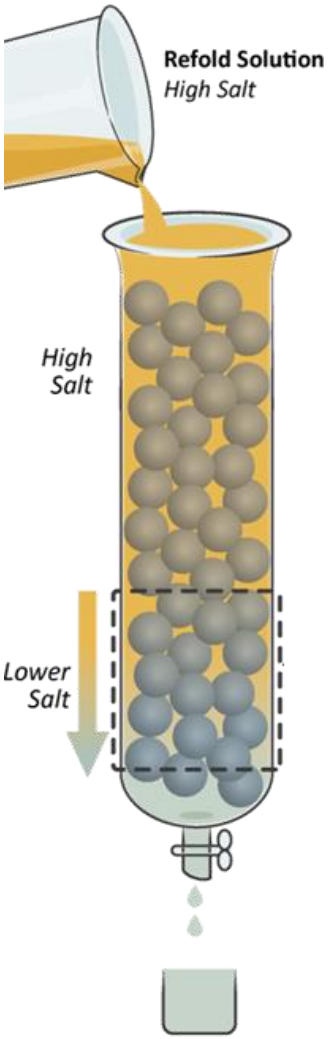
Amgen requested samples of the liquid exiting Sandoz's AEX column, particularly samples of the liquid exiting the column before Sandoz begins collection, *i.e.*, the discarded liquid. (Appx5411-5414, Appx5419.) Amgen wanted these samples to identify and quantify the materials removed during Sandoz's AEX Step. Sandoz refused, claiming that it is not feasible to take samples. (*See* Appx5346-5347.) Nevertheless, the evidence is that molecules that are positively charged (*e.g.*, [redacted] molecules that are uncharged (*e.g.*, oxidized [redacted] and molecules less negatively charged than filgrastim (*e.g.*, certain variants of filgrastim) travel through the column faster than the bound filgrastim, and are removed by Sandoz's AEX Step. (Appx4923-4924, Appx4937,

Appx5355-5366, Appx5720-5722, Appx5724, Appx5728.) For example, according to Sandoz's aBLA, removal of [redacted], which is identified as having a "very high criticality" for removal because of its toxicity to humans, "was demonstrated for early downstream process steps ([redacted], [redacted])," meaning that Sandoz's [redacted] step and a later chromatography step Sandoz calls "[redacted]," remove [redacted]. (Appx4916-4917, Appx4938-4939, Appx5311, Appx5356, Appx5621.) Sandoz's aBLA also states that [redacted] adducts, which are also highly critical contaminants, "are removed during the [redacted] [step]." (Appx4938, Appx5357, Appx5361-5362, Appx5728.) [redacted] and unwanted molecular variants of filgrastim are also depleted during the AEX Step. (Appx4924, Appx4940-4941, Appx5357, Appx5376-5378, Appx5395, Appx5660, Appx5722, Appx5725-5726, Appx5889-5890, Appx5895.)

c. Sandoz's AEX Step Causes Filgrastim To Come Off the Column After Contaminants Are Removed ("Eluting" After "Washing")

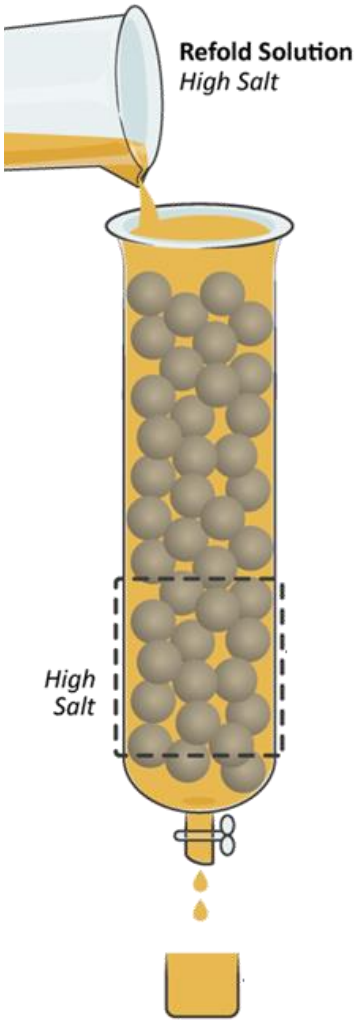
As described above, when the refold solution is first applied to Sandoz's column, it encounters low-salt conditions because the column is already filled with an equilibration solution that contains no salt. (Appx4929-4930.) These conditions favor binding. (*Id.*) Sandoz continues to apply its refold solution in an amount that is several times the volume of the column. (Appx4936-4937.) As a result, the conditions in the liquid phase in the column gradually change in a

number of ways, including changes to the salt concentration and pH. (Appx4945-4947.) The increasing salt concentration is depicted below:



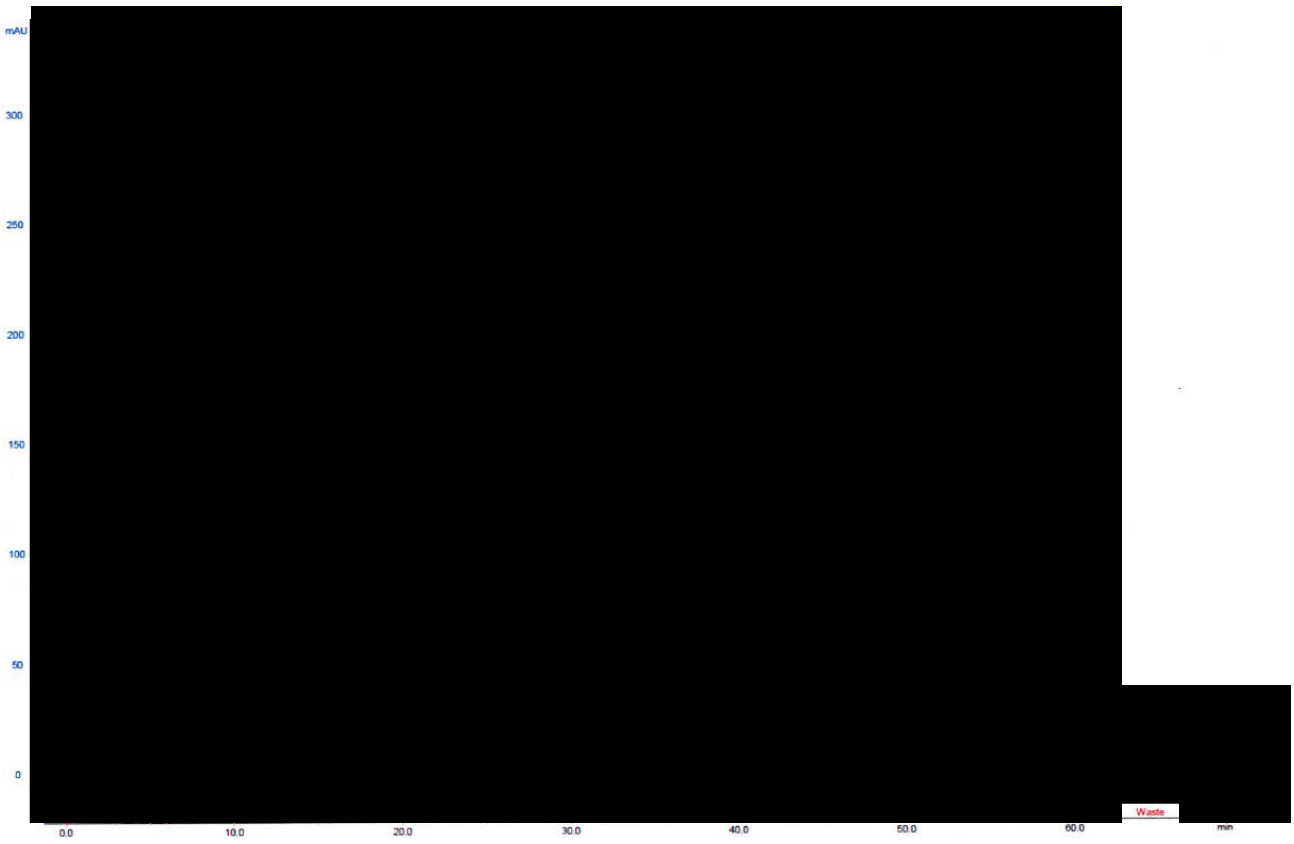
(Appx6996.)

This process continues until ultimately the conditions become such that the binding of filgrastim is reversed and the filgrastim elutes from the column. (Appx4945-4947.) One aspect of the changing conditions is that the refold solution eventually displaces all of the equilibration solution, resulting in high-salt concentration throughout the column as seen below.



(Appx7001.) These high-salt conditions cause the filgrastim to cease to be bound to the resin and thus to be carried out of the column, *i.e.*, eluted. (Appx4945-4947.)

That these changing conditions in Sandoz's process result in the elution of filgrastim is reflected in Sandoz's chromatograms from its AEX Step. (*See, e.g.*, Appx5391-5392.) The following is a chromatogram generated by Sandoz during a small-scale run of its AEX Step:



(Appx5392.) This illustrates the changing conditions over time on the [redacted] column. (Appx4946.) The blue line shows the UV absorbance, a measure of the protein concentration of the liquid exiting the column. (Appx5392.) The brown line shows the conductivity, reflecting salt concentration, of the liquid exiting the column, and the grey line reflects pH. (Appx4946, Appx5392.) When the high-salt refold solution is applied to the column (at the first vertical red line from the left), the conductivity trace is flat and then, over time, increases, plateaus, and finally decreases. (Appx4947, Appx5392.) As the salt concentration rises, there is a corresponding and dramatic rise in the UV trace, although the rise of the UV

trace is slightly delayed. (Appx4947, Appx5392.) This indicates that the high-salt concentration solution generated in the column is reversing the binding of filgrastim to [redacted] (Appx4947.) Additionally, the change in pH indicated by the grey line over time may also contribute to elution. (*Id.*)

As discussed above, Sandoz begins collection of the liquid exiting the column when the UV absorbance trace rises to a certain level, [redacted] of the height on the trace's ascending peak. (Appx4895, Appx5399, Appx5924-5925, Appx6117, *see* Appx6121, Appx6146.) In the chromatogram at Appx5392, Sandoz began to collect at the second vertical red line from the left. All of the material that exits the column before that collection, which, as explained above, is enriched in contaminants, is discarded. (Appx4893-4896, Appx4936-4943, Appx4947-4948.) When the UV trace returns to a level near baseline, indicating that the concentration of protein exiting the column has decreased, Sandoz stops collecting the column eluate, and discards the [redacted] (Appx5311, Appx5392, Appx5620-5621.)

4. The District Court's Rulings

The district court held in its claim-construction order that "washing" and "eluting" be accomplished by applying "a solution" and that eluting occurs after washing. (Appx42-46.) The parties submitted summary-judgment briefing based on this construction. (*See* Appx4850-4876, Appx4879-4955.) The district court

then granted summary judgment of non-infringement of Sandoz's current manufacturing process. (Appx5.) The district court departed from its original construction, and reached its conclusion of non-infringement based on a further claim construction that requires the washing and eluting elements to be "distinct," specifically requiring that different washing and eluting solutions be added to the column, and that washing be entirely concluded before any eluting begins, as discussed below. (Appx10-11.)

Further, the district court denied Amgen's request to defer ruling on Sandoz's motion for summary judgment until Sandoz submits an application for approval of its modified process (with the substitute anion exchange resin) to FDA and provides discovery to Amgen. (Appx14-15.)

C. The '427 Patent

1. The Invention

The '427 Patent is generally directed to an improved method of treating diseases requiring peripheral stem cell transplantation. (Appx80-85.) As part of the treatment regimen for, *e.g.*, certain blood cancers, it is necessary to completely ablate the contents of a patient's bone-marrow compartment with either high doses of radiation or high doses of cytotoxic chemicals. '427 Patent, 1:55-61, 2:11-14; (Appx2833-2834.) It is then necessary to repopulate the bone marrow compartment with transplanted stem cells so that the patient can resume

hematopoiesis, the making of blood cells to circulate in the peripheral blood system. '427 Patent, 1:28-31; (Appx2834-2835.) The transplant consists of the patient's own stem cells, collected by a process called leukapheresis, prior to the administration of the bone marrow-ablating doses of radiation or cytotoxic chemicals. '427 Patent, 1:18-27, 3:24-30; (Appx2834-2835.) After bone-marrow ablation, the stem cells are reinfused into the patient and "home" to the bone-marrow compartment where they can once again differentiate into blood cells which ultimately enter into circulation in the blood. '427 Patent, 1:18-31; (Appx2834-2835.) In this respect, the stem cell transplant is a necessary part of the treatment of the underlying diseases. '427 Patent, 1:18-31; (Appx2834-2835.)

In the prior art, it was known that the number of collectable stem cells in the peripheral blood could be increased prior to leukapheresis by the administration of certain agents. '427 Patent, 1:32-54. Specifically, it was known that administration of G-CSF alone, certain chemotherapeutic agents alone, and the combination of certain chemotherapeutic agents followed by G-CSF caused an increase in the number of stem cells in peripheral blood. *Id.* The inventors of the '427 Patent surprisingly discovered that administration of G-CSF (*i.e.*, filgrastim) ***first, followed by*** at least one chemotherapeutic agent enhanced the number of collectable stem cells in peripheral blood more so than the prior-art approaches. *Id.* at 1:55-2:11, 3:13-46. In other words, the order of administration of the

chemotherapeutic agent ***after*** the G-CSF had an unexpected beneficial effect that would reduce the number of leukaphereses a patient would have to endure prior to bone-marrow ablation. *Id.* This improved method is claimed in the '427 Patent.

2. The District Court Rulings

The district court acknowledged that stem cell transplantation is "a component" of the disease treatment required by the '427 Patent claims. But the district court construed the relevant limitation—"method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment"—to exclude the possibility that both agents recited in the body of the claim are administered in combination to mobilize peripheral stem cells for the transplant that is a component of the treatment. (Appx24-26.) The district court also construed "disease treating-effective amount of at least one chemotherapeutic agent" to mean an "amount sufficient to treat a disease for which at least one chemotherapeutic agent is prescribed." (Appx28.) The district court thus imposes a requirement that the chemotherapeutic agent be prescribed as a disease treatment that is unrelated to stem cell mobilization and transplantation.

Following the district court's constructions of the '427 Patent (Appx16-34), Amgen and Sandoz stipulated to non-infringement (Appx49-54) and the district court entered final judgment as to that patent. (Appx1-2.)

SUMMARY OF THE ARGUMENT

The district court erred in construing the claims of both the '878 Patent and the '427 Patent. These errors then led to the final judgments of non-infringement for each of the patents. Correctly construed, there is at least a genuine issue of disputed material fact as to whether Sandoz infringes those patents. Accordingly, Amgen respectfully requests that the district court's judgments be reversed, vacated, and/or remanded.

The '878 Patent. The district court erred by imposing additional limitations on the "washing" and "eluting" terms of the '878 Patent and granting summary judgment of non-infringement. The district court's original claim-construction order interpreted "eluting the protein from the separation matrix" to occur after "washing the separation matrix," and required "washing" and "eluting" to be accomplished by applying "a solution." (Appx44-46.) Amgen submitted ample evidence that these limitations are literally met in Sandoz's process under this original claim construction. (*See infra*, II.A.) Specifically, during the accused process, Sandoz's refold solution is directly applied to its chromatography column pre-loaded with an equilibration solution. As the refold solutions enters and mixes into the equilibration solution, conditions are created that cause the filgrastim protein to bind to the column, satisfying the "associate" limitation. While the filgrastim binds to the column and more refold solution is applied, other materials

of the refold solution pass through and exit the column, which achieves purification of filgrastim and satisfies the "washing" limitation. These contaminating materials are discarded. Over time, the high-salt refold solution continues to be added and mixes with the no-salt equilibration solution in the column; when the salt concentration in the column becomes high enough to reverse the binding of the filgrastim, the filgrastim is eluted and collected. Because the column conditions do not change instantaneously across the entire length of the column, filgrastim molecules elute from an upstream location while washing is occurring at a different location downstream, but at each location on Sandoz's column, washing precedes eluting. This satisfies the claim elements under the district court's original construction of the "washing" and "eluting" terms of the '878 Patent.

The district court, however, granted summary judgment of non-infringement by requiring the "washing" and "eluting" elements to be compositionally and temporally distinct, *i.e.*, requiring the addition of different washing and eluting solutions to the column, and washing be concluded at all points throughout the column before eluting occurs. (Appx10-12.) The grant of summary judgment turned on these additional claim constructions having been newly imposed. This is error. The intrinsic evidence does not support the narrower reading of the claims imposed by the district court. Indeed, the district court's summary-judgment

construction is contrary to the specification's description of the washing and eluting solutions to be "any composition" that is "compatible with both the protein and the matrix" and any "appropriate solution," respectively. '878 Patent, 15:47-64. Limiting the washing and eluting terms to be compositionally and temporally distinct reads out teachings where eluting begins with the same solution used to wash the column and the salt concentration is gradually increased to cause the protein to unbind (*i.e.*, a gradient elution). *Id.* at 20:34-39.

Accordingly, the district court's construction in its summary-judgment order should be reversed. In any event, even if the district court's summary-judgment construction were correct, there is evidence that under the doctrine of equivalents Sandoz's process infringes the '878 Patent claims. The district court thus erred in holding that there is no infringement under the doctrine of equivalents. Rather than addressing Amgen's doctrine-of-equivalents evidence on a limitation-by-limitation basis as to the "washing" and "eluting" terms, the district court analyzed only whether the entire claimed method is equivalent to the accused Sandoz process as a whole. (Appx12-13.) The law is otherwise. *Warner-Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 29 (1997).

Further, the district court erred in granting summary judgment of non-infringement of Sandoz's modified process for manufacturing its biosimilar products, and denying Amgen's motion for additional discovery under Federal

Rule of Civil Procedure 56(d). As far as Amgen is aware, that process has not yet been submitted to FDA, and Sandoz never provided Amgen or the district court complete information about it, not even the type of information Dr. Willson considered in his infringement analysis of Sandoz's current process. The district court nevertheless granted summary judgment that the modified process does not infringe. (Appx14-15.) This is contrary to the required analysis for a technical act of infringement under 35 U.S.C. § 271(e)(2)(C). By ruling now that the modified process does not infringe, prior to Sandoz's submission of the modified process to FDA and prior to Sandoz providing Amgen complete information about its final process for which it will seek regulatory approval, the district court denied Amgen the opportunity to assess infringement based on "what [the Applicant] has asked the FDA to approve as a regulatory matter." *Sunovion Pharm., Inc. v. Teva Pharm. USA, Inc.*, 731 F.3d 1271, 1278 (Fed. Cir. 2013); *see Amgen Inc. v. Apotex Inc.*, 712 F. App'x 985, 992 (Fed. Cir. 2017) (importing the Court's decision in a Hatch-Waxman Act case "into the BPCIA context") (Appx7908-7914). The district court's decision should be reversed, and any judgment with respect to Sandoz's modified process should be deferred until Sandoz finalizes its process and submits its proposed modified process to FDA and that information is provided to Amgen.

The ’427 Patent. The district court erred in construing claim 1, which led to the parties’ stipulation of non-infringement. The district court incorrectly interpreted the claimed method so that the function of the chemotherapeutic agent is to treat the underlying disease rather than to enhance stem cell mobilization. (Appx24-28.) The district court came to this erroneous conclusion by holding that a “disease treating-effective amount” of a chemotherapeutic agent requires that the chemotherapeutic agent be prescribed as a disease treatment for, *e.g.*, cancer (and not as the amount to be used in conjunction with G-CSF for stem cell mobilization and subsequent peripheral stem cell transplantation). (*Id.*) That term simply defines the particular amount of a chemotherapeutic agent to be administered. The district court thus erred by imposing a limitation that the agent be used for treating an underlying disease. The district court acknowledged that the specification contains evidence “that the purpose of administering a chemotherapeutic agent is the same as that for G-CSF administration.” (Appx27.) That purpose is enhancing the number of collectable stem cells in peripheral blood to be used for the peripheral stem cell transplantation that is an aspect of the overall disease treatment. However, the district court then concluded that this teaching of the specification “cannot supplant the language of the claim itself,” and based the construction on its misreading of the term “disease treating-effective amount.”

ARGUMENT

This Court reviews summary-judgment decisions by applying the law of the regional circuit. *Unwired Planet, LLC v. Apple Inc.*, 829 F.3d 1353, 1356 (Fed. Cir. 2016). Under Ninth Circuit law, the district court's grant of summary judgment is reviewed *de novo*, reapplying the same standard applied by the district court. *Goodman v. Staples The Office Superstore, LLC*, 644 F.3d 817, 822 (9th Cir. 2011); *see Advanced Fiber Techs. (AFT) Tr. v. J & L Fiber Servs., Inc.*, 674 F.3d 1365, 1372 (Fed. Cir. 2012).

An assessment of infringement requires: "First, the claims of the patent must be construed to determine their scope" and "Second, a determination must be made as to whether the properly construed claims read on the accused device." *Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1304 (Fed. Cir. 1999). The proper construction of a claim is ultimately a legal question, and a purely legal question reviewed *de novo* where the district court reviews only intrinsic evidence to reach its construction. *Teva Pharm. USA, Inc. v. Sandoz, Inc.*, 135 S. Ct. 831, 841-42 (2015). If a district court makes "subsidiary factual findings," those factual findings are reviewed for clear error on appeal. *Id.* at 841.

"Summary judgment of noninfringement may only be granted if, after viewing the alleged facts in the light most favorable to the nonmovant and drawing all justifiable inferences in the nonmovant's favor, there is no genuine issue

whether [what has been accused] is encompassed by the patent claims." *Novartis Corp. v. Ben Venue Labs., Inc.*, 271 F.3d 1043, 1046 (Fed. Cir. 2001); *see Goodman*, 644 F.3d at 822. "Because infringement is itself a fact issue, however, a motion for summary judgment of infringement or noninfringement should be approached with a care proportioned to the likelihood of its being inappropriate." *D.M.I., Inc. v. Deere & Co.*, 755 F.2d 1570, 1573 (Fed. Cir. 1985).

In addition, this Court applies the law of the regional circuit "when reviewing the court's decision under Rule 56(d)." *Baron Services, Inc. v. Media Weather Innovations LLC,* 717 F.3d 907, 912 n.6 (Fed. Cir. 2013). The Ninth Circuit reviews a district court's denial of a request for time to conduct additional discovery under Federal Rule of Civil Procedure 56(d) for abuse of discretion. *See Jones v. Blanas*, 393 F.3d 918, 926, 930 (9th Cir. 2004) (reversing grant of summary judgment because district court abused discretion in not permitting further discovery before deciding summary-judgment motion); *Burlington N. Santa Fe R. Co. v. Assiniboine & Sioux Tribes of Fort Peck Reservation*, 323 F.3d 767, 774-75 (9th Cir. 2003) (same).

I. The District Court Erred in Construing Claim 7 of the '878 Patent to Require Temporally and Compositionally Distinct Washing and Eluting

The district court's original claim construction required, in relevant part, simply that "eluting" occur after "washing." (Appx44-46.) The district court, however, modified its original construction in its summary-judgment order to

require a process in which washing be completed throughout the chromatography column before any eluting begins and also compositionally distinct washing and eluting solutions are applied to the column. (Appx9-11.) Because the district court's modified construction is contrary to the intrinsic evidence, Amgen respectfully requests that the construction be reversed and the case remanded for a determination of infringement.

A. The District Court's Original Claim Construction of "Washing" and "Eluting" Was Simply that Eluting Occurs After Washing Using "a" Solution

The teaching of the '878 Patent is the surprising observation that protein is able to associate with the separation matrix even where there is direct application of the refold solution to the separation matrix. As the specification explains:

> Initially, it was expected that the highly ionic and/or chaotropic compounds and various other components of the refold solution would inhibit the association of the protein with the separation matrix. However, in contrast to reports in the literature . . . , it was surprising to observe that the protein was in fact able to associate with the separation matrix in the presence of the components of the refold solution.

'878 Patent, 15:30-37. Claim 7 of the '878 Patent recites a "method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system" which includes:

> (e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to ***associate*** with the matrix;
>
> (f) ***washing*** the separation matrix; and

> (g) ***eluting*** the protein from the separation matrix, wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin.

(emphases added).

The district court construed "associate" to mean "bind." (Appx44.) The district court construed "washing the separation matrix" to mean "adding ***a solution*** to the separation matrix to remove materials in the refold solution while preserving binding of the protein to be purified." (Appx44-45 (emphasis added).) In addition, the district court construed "eluting the protein from the separation matrix" to mean "applying ***a solution*** that reverses the binding of the purified protein to the separation matrix," and found that this "must occur after the step of 'washing the separation matrix.'" (Appx46 (emphasis added).) Regarding the latter construction, the district court explained that eluting comes after washing because if otherwise, "the protein captured by the separation matrix could once again comingle with the contaminants and components to be washed away." (*Id.*)

The original construction thus requires that eluting occur after washing. But nothing in the original construction requires that washing and eluting be "distinct" such that washing is completed throughout the column before any eluting begins or that the solution added to the column for washing be different from the solution added to the column for eluting. Indeed, with respect to the solutions used for washing and eluting, they are broadly defined in the patent as any compatible

composition (’878 Patent, 15:47-49) or any solution that interferes with binding (*id.* at 15:65-16:2, 16:28-30), respectively. Nothing precludes the patent claims from covering a purification process that adds a single solution to the column as long as it can achieve both functions.

B. The District Court Construed “Washing” and “Eluting” in its Summary-Judgment Order to Require the Application of Different Solutions That Are Temporally and Compositionally Distinct

Departing from its claim-construction order, the district court granted Sandoz’s motion for summary judgment of non-infringement. (Appx5.) Even though the district court said it was not modifying its earlier claim construction of washing and eluting (Appx10), the district court’s summary-judgment order further construed the meaning of these terms (Appx10-12). Specifically, the district court held in its summary-judgment order that having “sequential washing and eluting” as required by claim 7 means that they be both temporally and compositionally distinct. (*Id.*)

First, the district court stated that “continuously pumping a refold solution” into a column containing a separation matrix does not meet this limitation because “there is no pause in the pumping of the refold solution.” (Appx10.) The district court rejected as a matter of law an interpretation of the claims—as was permitted by its original claim-construction order—that eluting occur after washing via a continuous pumping process where conditions in the column are ***changing*** such

that washing and eluting ***are*** occurring sequentially at particular points in the column. (*See* Appx10-11.) The district court thus made clear that it further construed the claims to exclude processes in which washing and eluting occur in a continuous, integrated process; in other words, washing must be completed throughout the entire length of the column before any eluting from the column begins.

Second, the district court held that the washing and eluting solutions are compositionally "distinct" and cannot be satisfied by a single solution because the patent specification describes a "wash buffer" that is "optimized to preserve protein binding" and an eluting solution that "interferes with the binding." (Appx11.) According to the district court, "The opposite purposes of these two solutions suggests that ***they must indeed be distinct***, and cannot be, as Amgen contends, a single solution achieving different ends, due to different conditions, at different points in time." (*Id.* (emphasis added).) The district court further explained: "Nor is there any point at which Sandoz adds a second solution to the column that is compositionally different than the refold." (Appx10-11.)

C. The District Court's Construction in the Summary-Judgment Order is Erroneous

The district court's summary-judgment construction of "washing" and "eluting" to be temporally and compositionally "distinct" imposes limitations on

the claims that are not supported by the intrinsic evidence and were not a requirement of the original claim-construction order.

The Claims. Nothing in the claims requires that washing and eluting be temporally distinct such that washing be entirely completed, for example, even at the downstream end of the column, before any eluting can begin, for example, at the upstream start of the column, or that there be a pause between washing and eluting. Nor do the claims require that two distinct solutions be added to a column. Elements (f) and (g) of the claim simply start with "washing" and "eluting," respectively, and certainly do not say "add solution #1 to wash" and then, "add solution #2 to elute." '878 Patent, 22:24-25. Claim 7 does not specify what solutions are used for washing and eluting, let alone require that two different solutions be added. *Id.* Indeed, claim elements (f) and (g) do not even use the word "solution" but merely refer to the actions of "washing and "eluting." Where the patentee intended a particular solution to be used, the patent claims specify that solution, such as a regeneration reagent solution for washing in dependent claim 18 (and the claims that depend from it). *Id.* at 22:62-63.

The Specification. The district court's summary-judgment construction is contrary to the specification. As an initial matter, the invention of the '878 Patent describes novel processes for purifying proteins, of which washing and eluting are only two of the required elements (as claim 7 recites). The purpose of washing and

eluting is simply to wash away materials that did not bind and elute the protein that did, thereby achieving purification. This is consistent with the disclosure in the specification that the wash solution "can be of any composition" that is "compatible with both the protein and the matrix" and that the elution solution can be any "appropriate solution." '878 Patent, 15:47-64. As the specification explains: "The protein of interest associates with the matrix in the presence of the components of refold buffer, ***impurities are washed away and the protein is eluted.***" *Id.* at 4:52-55 (emphasis added).

The district court's summary-judgment construction incorrectly limits the claims to washing and eluting by the addition of two different solutions to the column and ignores teachings that describe adding a solution whose salt concentration gradually changes over time to achieve washing and eluting. *See, e.g.*, *id.* at 20:34-41. The specification is clear that elution can occur using ion exchange chromatography by increasing the salt concentration.

> In the cases that utilize ***ion exchange***, mixed-mode, or hydrophobic interaction chromatography, the ***concentration of salt can be increased*** or decreased to disrupt ionic interaction between bound protein and a separation matrix.

Id. at 16:18-22 (emphases added). Example 3 then discloses such gradient elution, making clear that eluting begins with the ***same*** solution used to wash the column—

30 mM MES[2] solution at pH 6.0—and then the salt concentration is gradually increased to cause the protein to become unbound. *Id.* at 20:34-41. Further, there is no temporal pause or other break between washing and eluting in that example. *Id.* Rather, the conditions in the column of this example gradually change from those favoring binding of the protein to those favoring elution of the protein.

The district court ignored this in narrowing its construction to require that the added washing and eluting solutions be different. Instead, the district court relied on column 15, lines 55-62 in holding that the "opposite purposes of these two [washing and eluting] solutions suggests that they must indeed be distinct." (Appx11.) This is error. As the specification describes elsewhere, the purpose of washing and eluting is simply to wash away unbound protein and contaminants and to elute the bound protein. '878 Patent, 4:52-55. Nothing in the specification requires a buffer "optimized to preserve protein binding," which is added for purposes of washing, be different from the solution that "interferes with binding," used to elute. (Appx11.)

The specification does not say that the wash buffer is "optimized to preserve protein binding," even though the district court puts that phrase in quotation marks. Instead, the specification makes clear that the wash buffer encompasses "any composition" which "will vary with the protein being purified." '878 Patent,

[2] "MES" refers to 2-(*N*-morpholino)ethanesulfonic acid, a buffering agent.

15:47-59. It is the pH range of the wash buffer that is "chosen to optimize the chromatography conditions, preserve protein binding, and to retain the desired characteristics of the protein of interest." *Id.* The specification similarly defines the elution solution broadly to encompass "an appropriate solution (*e.g.*, a low pH buffered solution or a salt solution) to form an elution pool comprising the protein of interest." *Id.* at 15:60-64, 16:28-30. Thus, the specification does not exclude a solution added to the column for washing from being the solution added to the column for eluting; if anything, the specification makes clear that the solutions are defined broadly to include all appropriate compositions, even ones that, as applied to the column, are the same.

Accordingly, the district court erred in determining that compositionally distinct solutions must be added to a column to accomplish the "opposite purposes" of washing and eluting. They can be generated *in situ*. (*See* Appx4893-4894, Appx6784-6785.) As discussed above, the '878 Patent contemplates the use of a gradient for washing and eluting where, like in Example 3, eluting is accomplished with a solution whose conditions are changing over time and eluting begins with the same solution used to wash the column. '878 Patent, 20:34-41.

Thus, the district court's further claim construction in its summary-judgment order should be reversed, and the original construction adopted.

II. The District Court Erred as a Matter of Law in Granting Summary Judgment that Sandoz Does Not Infringe Claim 7 of the '878 Patent

Based on its incorrect summary-judgment claim construction, the district court granted summary judgment of non-infringement as to both Sandoz's current manufacturing process and its planned modified manufacturing process. This was error. Under the original claim construction, there is ample evidence of literal infringement and at the very least, there are genuine issues of disputed material fact that preclude grant of summary judgment. Indeed, Amgen's expert, Dr. Willson, provided a 74-page declaration that explained in detail how Sandoz's AEX Step literally practices elements (e), (f), and (g) of claim 7, including how eluting occurs after washing. (Appx4879-4955.) The district court was required to credit Dr. Willson's testimony in evaluating Sandoz's motion for summary judgment, and erred in not doing so. That the district court disregarded Amgen's evidence of infringement is confirmed by the district court's statement that "[t]he materials Sandoz seeks to strike are not relied on in this order." (Appx5.) The materials in question include large portions of the declaration of Dr. Willson explaining the sequence of events at each location on Sandoz's accused column. (Appx5, Appx4883-4885, Appx4887-4899, Appx4933-4934, Appx4942-4943, Appx4947-4955, Appx6255.) The district court denied the motion to strike, making clear these materials are properly part of the record, but then failed to consider them. This was legal error. *U.S. Water Servs., Inc. v. Novozymes A/S*,

843 F.3d 1345, 1351-52 (Fed. Cir. 2016) (holding that the district court improperly granted summary judgment because it disregarded the non-movant's evidence, including its experts' testimony, "improperly ma[king] credibility determinations and weigh[ing] conflicting evidence.").

A. Under the Original Claim Construction, There is Evidence of Infringement that Raises Genuine Issues of Disputed Material Fact Which Defeat Summary Judgment

Amgen submitted ample factual evidence that Sandoz's AEX Step includes washing and eluting—occurring in that order—literally satisfying the requirements of claim 7 as construed by the district court in its August 4, 2016 claim-construction order. Amgen's expert Dr. Willson provided extensive testimony, based on Sandoz's aBLAs and other manufacturing documents, on the changing conditions in Sandoz's column as the refold solution is added over time which satisfy the claim elements. For example, he opined:

> [T]aking a single location within Sandoz's column where a protein molecule binds, the order of events is binding, followed by washing, followed by elution – the sequence recited in the claim. Taking such locations as the frame of reference, as I do in my analysis, eluting occurs after washing, as required by the Court's claim construction.

(Appx4891.)

As explained above, the conditions in the Sandoz column at first favor filgrastim binding. (Appx4926-4936.) While the binding of filgrastim is preserved, contaminants in the refold solution become removed from the protein to

be purified, *i.e.*, filgrastim. (Appx4936-4944.) These contaminants are discarded, never again to comingle with the purified filgrastim. (*Id.*, Appx4888, Appx4898.) As the refold solution continues to be added, the chemical composition of the mobile phase in the column gradually continues to change, allowing more components of the refold solution to be removed until such time as the conditions change again to favor reversal of binding of filgrastim. (Appx4944-4949.) Specifically, there is an increase in the salt concentration (*see* Appx5392), which, as disclosed by the '878 Patent, can be used to "disrupt ionic interaction between bound protein and a separation matrix." *See* '878 Patent, 16:18-22. At that time, the filgrastim elutes from the column in a more highly purified state than when it was added to the column. (Appx4944-4949.)

The district court was also wrong to read claim 7 as requiring that filgrastim elute in a completely purified state from the column to which the refold solution is applied. (Appx13.) The specification says that the eluted protein may only be partially purified: "As used herein, the terms 'isolate' and 'purify' are used interchangeably and mean to reduce by 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95%, or more, the amount of heterogeneous elements, for example biological macromolecules such as proteins or DNA, that may be present in a sample comprising a protein of interest." '878 Patent, 6:64-7:3.

In other words, partial purification can occur on the separation matrix to which the refold solution is directly applied. That is exactly what happens on Sandoz's AEX column. Because contaminants enter and are washed through and out of the column before collection begins of the UV-absorbing materials (which include filgrastim), partial purification of filgrastim is achieved on Sandoz's AEX column. (Appx4888-4889, Appx4893-4895, Appx4947-4948.)

Dr. Willson explained that the conditions in Sandoz's column do not change instantaneously throughout the length of the column—*i.e.*, conditions change over time across the length of the column. (Appx4891-4894.) As Sandoz applies its refold solution to its column, the salt concentration, for example, will increase first at the top end of the column, and over time, through to the bottom end of the column. (Appx4875, Appx4893-4894.) This means that conditions will favor elution at the top of the column before they favor elution at the bottom of the column, such that filgrastim elutes first at the top of the column while filgrastim remains bound to the resin at the bottom end of the column where contaminants may still be washing away. The sequence of events at any specific location on Sandoz's column is ***always*** washing then eluting—the order of claim 7 as construed by the district court. (Appx4891-4892.)

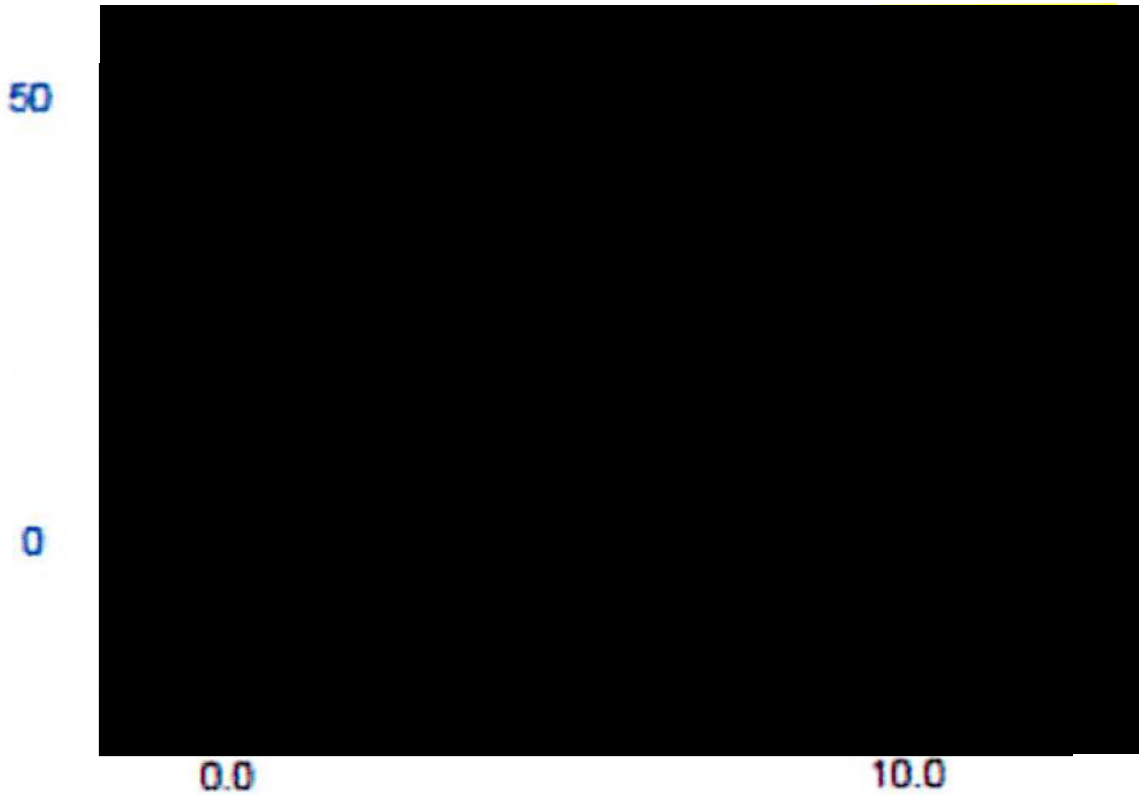
The changing conditions in Sandoz's column are shown in Sandoz's chromatograms from its AEX Step, which satisfy the claimed "washing" and

"eluting" that is described by the '878 Patent. They are akin to changing conditions taught in the patent. Example 3 says:

> After loading, the column was ***washed*** with 30 mM MES; pH 4.5, for 3 CV at 60 cm/hr, then ***washed*** with an additional 3 CV of ***30 mM MES; pH 6.0***. The protein of interest was recovered from the resin by ***gradient elution*** over 25 CV between ***30 mM MES; pH 6.0*** and 30 mM MES, 500 mM NaCl; pH 6.0 at 60 cm/hr.

'878 Patent, 20:34-39 (emphases added). Thus, the gradient elution begins with the same solution as the wash (30 mM MES; pH 6.0) and then gradually increases the salt concentration until it reaches 500 mM, while keeping the other components the same.

Like the salt-gradient used for elution in Example 3 of the patent, Sandoz's process begins elution with the same solution as used for the wash and, as shown in the chromatogram, includes an increasing salt concentration over time (*i.e.*, gradient elution). Specifically, as the brown line increases (measuring conductivity, a reflection of salt concentration) in Sandoz's AEX Step, so does the blue line (which measures UV absorbance, a proxy for protein concentration).



(Appx5389-5392.)

As Dr. Willson explains, it is the increase in the salt concentration that causes the protein to elute and exit the Sandoz AEX column. (Appx4946-4947.) Further, the wash element is satisfied because Sandoz discards the liquid exiting the column between the time the refold solution was first loaded on the column (first red dashed vertical line, "Load") to the time at which liquid begins to be collected. The discarded liquid (corresponding to the "F3" fraction in the figure) contains contaminants (Appx4947), and their removal results in purification of Sandoz's filgrastim. *See* '878 Patent, 6:64-7:3; (Appx4888, Appx4894-4895, Appx4936, Appx4948.) That Sandoz's AEX Step "entails continuously pumping a

refold solution" onto the column and that "there is no pause in the pumping of the refold solution" (Appx11-12), do not negate Amgen's factual evidence that during Sandoz's AEX Step, a solution is added to the column that removes contaminants while binding of filgrastim is preserved and thereafter a solution is applied which reverses the binding of the filgrastim from the resin.

Because the district court should have applied its original claim construction and credited Amgen's evidence, the judgment of non-infringement should be reversed, vacated, and/or remanded.

B. Even if the District Court's Summary-Judgment Construction Requiring Washing and Eluting To Be "Distinct" Were Adopted, There Is Evidence Raising a Genuine Issue of Material Fact that Sandoz Infringes Under the Doctrine of Equivalents

As an initial matter, Amgen did not have the opportunity to present its infringement case under the narrowed construction of the summary-judgment order. Accordingly, even if the modified construction is affirmed, Amgen should be given the opportunity to present its infringement case under the narrowed construction. Nevertheless, the materials that Amgen submitted under the original construction is evidence of infringement under the doctrine of equivalents applying the narrowed construction.

Even if the district court were correct that claim 7 requires temporally distinct washing and eluting and also compositionally distinct solutions for washing and eluting, Sandoz's process infringes under the doctrine of equivalents.

Amgen's evidence is that the continuous addition of the refold solution during Sandoz's AEX Step accomplishes washing and eluting (in that order) and generates compositionally distinct solutions for washing and eluting within Sandoz's column. (Appx4893-4894, *see* Appx6784-6785.) *In situ* generation of compositionally distinct washing and eluting solutions over time is insubstantially different than seriatim addition of separate washing and eluting solutions. *In situ* generation achieves the same functions (washing and eluting), in substantially the same way (binding protein preferentially compared to contaminants, and then raising salt concentration to reverse protein binding) to achieve the same result (protein purification). (*See* Appx4890-4894, Appx4937-4949, Appx5265-5266, Appx5271-5272.) As this Court has previously recognized, where a claim calls for a particular composition, the fact that the composition is formed *in situ* does not defeat infringement. *See In re Omeprazole Patent Litigation*, 536 F.3d 1361, 1376 (Fed. Cir. 2008) (a subcoating that forms *in situ* can be "disposed on said core region"). Thus, there are insubstantial differences between Sandoz's process and the claimed process.

In addition, in performing its doctrine-of-equivalents analysis, the district court erroneously analyzed "the differences between the ***method claimed by the '878 Patent*** and the ***accused AEX step***," and whether the ***claimed method*** and the ***AEX step*** perform the same function, in the same way, to achieve the same result.

(Appx12-13 (emphases added).) This is legal error because the district court did not evaluate infringement on a limitation-by-limitation basis. An analysis under the doctrine of equivalents "must be applied to individual elements of the claim, not to the invention as a whole." *Warner-Jenkinson*, 520 U.S. at 29. To determine equivalence, one examines whether an asserted equivalent "plays a role substantially different from the claimed element" or "whether a substitute element matches the function, way, and result of the claimed element." *Id.* at 40.

Moreover, the district court erroneously viewed the fact that Sandoz's AEX Step removes its detergent, [redacted], as antithetical to the invention of the '878 Patent. (Appx12-13.) But, as recognized by the district court, the invention of the '878 Patent is "that [a] refold solution could be applied directly to a separation matrix without removing components of or diluting the solution." (Appx12.) The district court ignored that Sandoz's AEX Step is a first chromatography step to which a refold solution is directly applied and serves a purification function beyond the removal of the detergent. As explained above, because of the column's excess capacity (*i.e.*, that there is more [redacted] in Sandoz's column than is needed for detergent removal), the column binds filgrastim, contaminants (other than the detergent) are washed away, and filgrastim is eluted in a more purified state.

The district court wrongly held that in the claimed method "[t]here are no steps beyond the eluting step" and that because the material resulting from the AEX Step in the Sandoz process "requires further purification," the results of the two methods are substantially different. (Appx13.) This is a misreading of the '878 Patent: as noted above, the patent expressly defines "purify" to include the removal of as little as 1% of the contaminants ('878 Patent, 6:64-7:3), and states that "[i]n some cases, the method can also reduce or eliminate the need for subsequent purification steps" (*id.* at 4:60-62), making clear that the use of such subsequent steps is permitted.

Accordingly, if this Court agrees with the district court's new construction, the district court's judgment of non-infringement should nevertheless be vacated.

III. The District Court Erred in Granting Summary Judgment of Non-Infringement as to Sandoz's Yet-To-Be Submitted Modified Process for Making Its Biosimilar Products

The district court erred by granting summary judgment of non-infringement as to a modified process that Sandoz, at least as of the time of the summary-judgment proceedings, was still developing, had not submitted to FDA, and for which Amgen never had complete information. Relatedly, the district court abused its discretion in denying Amgen's motion pursuant to Federal Rule of Civil Procedure 56(d). *See Baron Services*, 717 F.3d at 912-14. *Baron* reversed the district court's decision that "prematurely granted summary judgment of

noninfringement" as "it was improper for the district court to have refused Baron's request to delay ruling on MWI's summary judgment motion until Baron had the opportunity" to take discovery. *Id.* The same result is compelled here where Amgen was denied the opportunity to take discovery of information essential to its opposition to Sandoz's motion for summary judgment.

The [redacted] resin used by Sandoz has been discontinued, and Sandoz will deplete its supply of this resin in late 2018 or 2019. (Appx14, Appx3750.) Sandoz plans to replace that resin with a [redacted] resin. (Appx3750.) As far as Amgen is aware, Sandoz has yet to submit amended aBLAs to FDA for either its filgrastim or pegfilgrastim products (Appx14), which submissions will reflect the final process parameters of the new AEX Step for which Sandoz seeks approval. Prior to the grant of summary judgment, Sandoz said that it expected to make these submissions in the first half of 2018 (Appx7054), and promised to produce the documentation to Amgen at that time. (Appx5155-5160, Appx5123-5124, Appx5132-5133.)

Sandoz concedes that details and process parameters of the modified process will differ from the current method that Sandoz uses: "there will be a handful of immaterial differences in process" involving technical details that are listed at Appx3751. (Appx3750-3751, Appx3804-3805.) But those technical details are hardly immaterial and, in fact, are among the details that Amgen's expert relied on

in his infringement analysis of Sandoz's current process. Specifically, to calculate the emergence time of Sandoz's filgrastim, Dr. Willson considered each of the allegedly "immaterial" details that Sandoz either admits will be different or has not provided to Amgen; Amgen thus needs further information to have Dr. Willson perform the same calculation for the modified step. (Appx4931-4934, Appx5160.) Moreover, the process parameters and analytical data in Sandoz's forthcoming FDA submissions and the underlying source documents provide the most probative evidence of Sandoz's infringement. But neither Amgen nor the Court has the factual details regarding the modified process because Sandoz only agreed to provide the factual details when the modified process is submitted to FDA. (Appx5123.) And, at least as of the summary-judgment proceedings in the district court, Sandoz had not made such FDA submissions.

Nevertheless, the district court addressed the merits of Sandoz's motion for summary judgment as to the modified process, determining that no issues of material fact existed to preclude summary judgment. (Appx14.) But the question is not whether there are issues of material fact now, before an FDA application for the modified process has been submitted to FDA and discovery has been provided. Rather, the question is whether there are disputed issues of material fact after the modified process has been finalized and Sandoz has disclosed the details of it to Amgen. The district court's decision is also wrong because it appears to

conclusively find non-infringement even if Sandoz makes further, entirely new changes to the modified process before submitting it for FDA approval. And it is not clear how Amgen or the district court would even know of such new changes. The district court's decision effectively deprives Amgen the ability to allege infringement in the future and permits Sandoz to make any changes it wishes to the modified process because it has been declared non-infringing in advance.

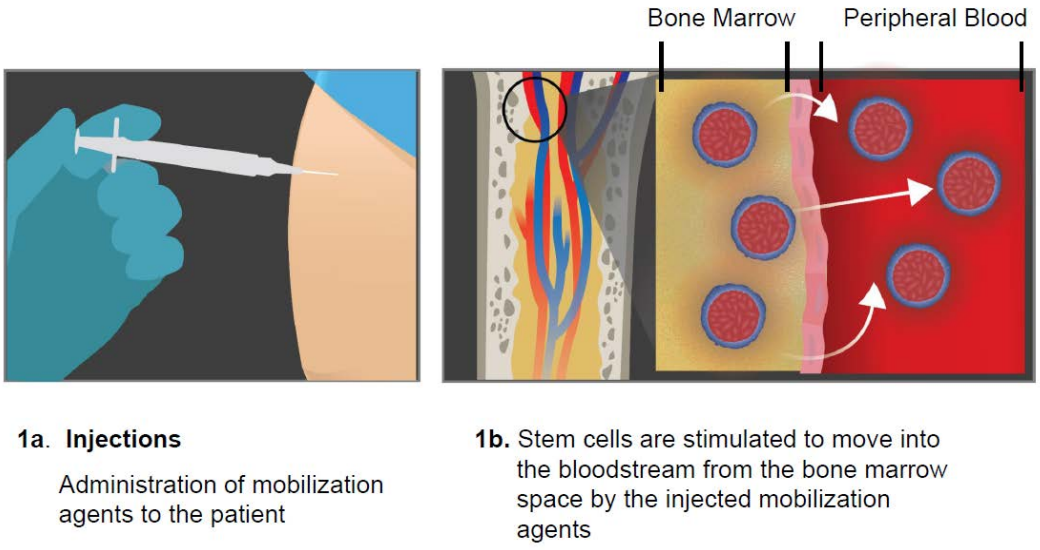
Further, the district court's decision is contrary to the required analysis for the technical act of infringement. As the Court is well-aware, Sandoz's submissions of its aBLAs for its biosimilar filgrastim product and its proposed biosimilar pegfilgrastim product are technical acts of infringement of the '878 Patent under 35 U.S.C. § 271(e)(2)(C). (Appx2043-2044, Appx7116-7119.) *See Sandoz Inc.*, 137 S. Ct. at 1670. The relevant inquiry for infringement in this context is a comparison of the patent claims to the process that Sandoz will likely use to make its products. *See Glaxo, Inc. v. Novopharm, Ltd.*, 110 F.3d 1562, 1570 (Fed. Cir. 1997) ("What is likely to be sold, or, preferably, what will be sold, will ultimately determine whether infringement exists."). Infringement is assessed against "[w]hat [the Applicant] has asked the FDA to approve as a regulatory matter." *Sunovion Pharm., Inc.*, 731 F.3d at 1278; *see Apotex Inc.*, 712 F. App'x at 992 (importing the Court's decision in a Hatch-Waxman Act case "into the BPCIA context") (Appx7908-7914).

Here, Amgen cannot compare the patent claims to the process Sandoz will likely use because Amgen does not have the details of the modified process which, as far as Amgen is aware, is still under development and will be finalized in the near future. Yet rather than deny summary judgment with respect to this modified process or wait to decide the motion until the process was finally fixed, submitted to FDA, and discovery provided, the district court ruled as a matter of law that the modified process does not infringe. This was error. The district court's decision effectively prevents Amgen from learning the details of Sandoz's modified process in this litigation, and relieves Sandoz of its obligations to disclose information to Amgen about its filgrastim biosimilar product and proposed pegfilgrastim biosimilar product and the process used for their manufacture that would allow Amgen to evaluate whether the finalized modified process will infringe. While Sandoz is free to change and seek FDA approval for a modified manufacturing process, it cannot be the law that biosimilar applicants can obtain final judgments of non-infringement as to modified processes without providing details about that process to the patentee or the court.

IV. The District Court Erred in its Construction of Claim 1 of the '427 Patent By Ascribing Meanings to Claim Terms that Are Contradicted by the Intrinsic Record

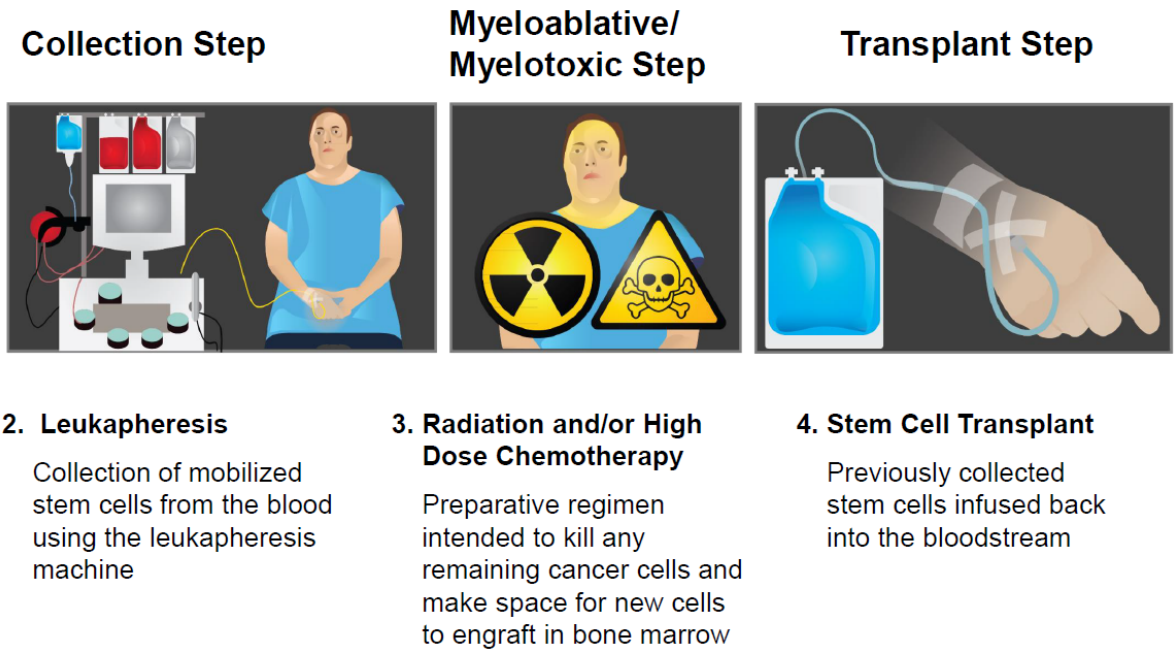
A. Background of the Invention: Stem Cell Mobilization, Collection, and Transplantation in the Treatment of Diseases

Hematopoietic stem cells are the cells in our bodies capable of proliferating and differentiating into cells that make up our blood and our immune systems. (Appx19.) After birth, hematopoietic stem cells self-renew, proliferate, differentiate, and reside primarily in the bone marrow. (*Id.*) Certain cancers of the blood or bone marrow and certain genetic diseases require a peripheral stem cell transplant as part of the treatment of the underlying disease. *See* '427 Patent, 1:5-11, 1:55-61, 2:11-14. Stem cell transplantation is accomplished by first "mobilizing" hematopoietic stem cells from the bone marrow into the peripheral blood (*i.e.*, the blood circulating through our veins and arteries). *Id.* at 3:13-30; (Appx20.) This mobilization step is depicted below.



(Appx3141, Appx2834, Appx2987.) The stem cells are subsequently collected from the peripheral blood by a process known as leukapheresis. '427 Patent, 1:55-61; (Appx20, Appx2834.) The collected cells may be stored while the patient undergoes a preparative regimen that employs high doses of radiation or cytotoxic chemicals. *See* '427 Patent, 1:18-31, 1:55-61; (Appx20, Appx2834.) This regimen reduces or obliterates ("ablates") the cells in the patient's bone marrow in order to clear the bone marrow out so that it may receive a stem cell transplant. *See* '427 Patent, 1:5-11, 3:24-30; (Appx2834-2835.) Following the preparative regimen, the stored stem cells are reinfused or "transplanted" back into the patient, restoring the bone marrow and its ability to produce blood cells and reconstitute the hematopoietic system. *See* '427 Patent, 1:18-31; (Appx2834-2835.) The

transplant is part of the treatment of the underlying disease. These steps are depicted below.



(Appx3142, Appx2834, Appx2988.)

Before the invention of the '427 Patent, it was known that administering G-CSF alone, certain chemotherapeutic agents alone, or certain chemotherapeutic agents followed by G-CSF could mobilize stem cells for collection by leukapheresis. '427 Patent, 1:32-54; (Appx20.) The prior-art approaches typically required several leukapheresis sessions to collect a sufficient number of stem cells for a successful transplantation. '427 Patent, 1:24-27, 1:55-61. Additionally, certain patients fail to mobilize sufficient numbers of collectable stem cells necessary for successful transplant using the prior art mobilization approaches. *Id.*

The inventors of the ’427 Patent discovered that when G-CSF was administered ***first*** followed by administration of a chemotherapeutic agent for the purpose of stem cell mobilization, the yield of hematopoietic stem cells in peripheral blood could be enhanced compared to the prior art approaches. *See* ’427 Patent, 1:62-65, 3:13-17; (Appx20-21.) This, in turn, leads to more efficient collection of stem cells, fewer leukaphareses, more effective transplants, and, consequently, better patient outcomes. *See* ’427 Patent, 1:55-61, 3:24-30.

B. The Specification and the Prosecution History of the ’427 Patent Make Clear that the Chemotherapeutic Agent Recited in Claim 1 Is Used in Conjunction with G-CSF for the Purpose of Enhancing Stem Cell Mobilization

The district court relied on claim language used as adjectives to modify the amounts of G-CSF and the chemotherapeutic agent administered, and thus lost sight of the sole purpose for which ***both*** agents are administered: to enhance stem cell mobilization to make a more effective transplant for treatment of a disease requiring such a transplant. Instead, the district court reached the erroneous conclusion that only the G-CSF mobilizes stem cells while the role of the chemotherapeutic agent is something different: “to treat a disease for which at least one chemotherapeutic agent is prescribed.” (Appx28.)

Although the district court acknowledged that stem cell transplantation is “a component of disease treatment,” it construed a “method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such

treatment" to exclude the possibility that both agents recited in the body of the claim are administered in combination to mobilize peripheral stem cells for the transplant that is a component of the treatment. (Appx24-26.) Next, the district court construed "disease treating-effective amount of at least one chemotherapeutic agent" to mean an "amount sufficient to treat a disease for which at least one chemotherapeutic agent is prescribed." (Appx28.) Taken together, these constructions impose a requirement that the chemotherapeutic agent be prescribed as a disease treatment that is unrelated to stem cell mobilization and transplantation, and improperly exclude situations where the chemotherapeutic agent is prescribed only for stem cell mobilization rather than treatment of an underlying disease.

In reaching this conclusion, the district court relied upon the words "disease treating" in the claims as a description of the chemotherapeutic agent. (Appx25-28.) But those words appear only as part of the phrase "disease treating-effective amount" making plain that they refer simply to the amount of chemotherapeutic agent used to enhance mobilization and not a purpose related to treatment of the underlying disease. The district court's constructions are contrary to the intrinsic evidence.

It is axiomatic that the specification of a patent is the single best tool for interpreting claims. *See Phillips v. AWH Corp.*, 415 F.3d 1303, 1315 (Fed. Cir.

2005) (en banc) (explaining that the specification is usually "dispositive," as "it is the single best guide to the meaning of a disputed term."). The role of the chemotherapeutic agent in claim 1 is clear, starting with the Abstract which states: "The invention relates to the ***use of G-CSF in combination with a chemotherapeutic agent*** . . . to produce a pharmaceutical preparation for ***boosting the mobilization of hematopoietic stem cells*** from bone marrow." '427 Patent, Abstract (emphases added). Similarly, the specification describes:

- "The present invention relates to ***the novel use of G-CSF and a chemotherapeutic agent*** or a combination of chemotherapeutic agents to produce a pharmaceutical preparation ***for enhanced mobilization of hematopoietic stem cells*** in the treatment of diseases requiring peripheral stem cell transplantation." *Id.* at 1:5-9.

- "In addition, the invention is directed to a pharmaceutical packaging unit containing G-CSF, chemotherapeutic agent(s) and informational instructions regarding ***the application of the G-CSF and the chemotherapeutic agent or the combination of chemotherapeutic agents for enhanced mobilization of hematopoietic stem cells prior to the onset of a corresponding therapy***." *Id.* at 1:11-18.

- "Surprisingly, it has now been found that an ***unexpectedly high stem cell concentration in blood can be achieved when administering G-CSF in combination with a chemotherapeutic agent*** (chemotherapeutic agents)." *Id.* at 1:62-65.

- "Therefore, the invention is directed to ***the use of G-CSF and a chemotherapeutic agent or a combination of chemotherapeutic agents to produce a pharmaceutical preparation for enhanced mobilization of hematopoietic stem***

> ***cells in the treatment of diseases requiring peripheral stem cell transplantation*** . . ." *Id*. at 1:66-2:4.

- "The administration of chemotherapeutic agent(s) is initiated either immediately after the second or third G-CSF injection or on the fourth day. . . . ***Surprisingly, it was determined that administration of G-CSF prior to opening of the endothelial barrier induced by chemotherapeutic agents significantly increases the stem cell mobilization and thus, can improve leukapheresis efficiency***." *Id*. at 3:7-17.

(emphases added throughout).

Nothing in the specification suggests that the claimed method—of administering G-CSF first and then a chemotherapeutic agent—is used as a disease treatment that is unrelated to stem cell transplantation such that one could mistake the role of the chemotherapeutic agent as the means to treat the underlying disease, rather than as an enhancer of stem cell mobilization in conjunction with G-CSF. Instead, the specification distinguishes between the claimed method and the actual therapy for the underlying disease, which, as the specification makes clear, could be accomplished not only with chemical agents but also with radiation:

> In addition, ***administration of G-CSF and a chemotherapeutic agent in the run-up to a, e.g., antitumor therapy*** offers the opportunity of recovering the stem cells mobilized in large amounts from the blood with higher efficiency (e.g., using leukapheresis), ***then performing the antitumor therapy using a cytostatic agent or irradiation*** and subsequently, conducting the peripheral stem cell transplantation.

'427 Patent, 3:24-30 (emphases added). Yet, in spite of these repeated disclosures in the specification that ***both*** G-CSF and the chemotherapeutic agent are used in the claimed method to enhance stem cell mobilization to make a transplant that

would be effective in the treatment of diseases requiring stem cell transplants, the district court thought otherwise. It erroneously concluded that "[t]he specification bears out [an] interpretation" that "one substance mobilizes stem cells [G-CSF], while the other [the chemotherapeutic agent] treats a disease." (Appx25.)

Not only is this conclusion untenable in view of the specification, it also ignores the assessment made by the PCT Examiner in the International Preliminary Examination Report, which forms part of the prosecution history of the '427 Patent. (Appx2568-2572.) The PCT Examiner had no difficulty understanding that the chemotherapeutic agent, in combination with G-CSF, was being used in the claimed method to enhance stem cell mobilization. (Appx2570-2571.) Specifically, the PCT Examiner stated that the claims cover "the use of ***a combination of G-CSF and chemotherapy*** (cyclophosphamide) ***to mobilize stem cells*** in the treatment of malignant diseases requiring peripheral stem cell transplantation." (Appx2570-2571 (emphases added).)

The district court justified its "one substance mobilizes stem cells, while the other treats a disease" interpretation based on its view that other claim language, specifically, "stem cell mobilizing-effective amount of G-CSF" and "disease treating-effective amount of at least one chemotherapeutic agent" negated the teaching of the specification, such that the inventors chose to claim one of the prior-art methods it had distinguished (use of G-CSF alone to mobilize stem cells)

as their invention. As the specification explains, there may be ***some*** chemotherapeutic agents that mobilize stem cells on their own but not all: "Some chemotherapeutic agents are also known to possess the ability of mobilizing bone marrow stem cells." '427 Patent, 1:35-37. The claims thus use the term "disease treating-effective amount" for the chemotherapeutic agent, because that agent does not necessarily itself mobilize stem cells. As the specification explains, it is the ***combination*** of G-CSF and the chemotherapeutic agent which mobilizes stems cells; the amount of chemotherapeutic agent that is required to be administered in that combination is simply the amount effective to treat diseases requiring stem cell transplants, regardless of whether the chemotherapeutic agent is itself a stem cell mobilizer or, by some other mechanism, enhances the stem cell mobilizing effect of G-CSF.

In short, "stem cell mobilizing-effective" and "disease treating-effective" are adjectival phrases specifying the ***amounts*** of G-CSF and the chemotherapeutic agent, respectively, to be administered for the ***same*** and ***sole*** purpose of mobilizing stem cells to make the transplant with which to treat the disease requiring the transplant. G-CSF has several uses, only one of which is stem cell mobilization. '427 Patent, 1:32-34; (Appx2830-2831.) "Stem cell mobilizing-effective amount" modifying G-CSF simply means that the amount of G-CSF administered to the patient is that normally used for G-CSF's stem cell mobilization use (and not, *e.g.*,

the amount given for treatment of neutropenia). "Disease treating-effective amount" modifying the chemotherapeutic agent is standard pharmaceutical claim parlance that indicates the amount administered is the amount effective to achieve the goal of the claimed method. *See Abbott Labs. v. Baxter Pharm. Prod., Inc.*, 334 F.3d 1274, 1277-80 (Fed. Cir. 2003) (noting that the term "effective amount" has a customary usage and using the specification to determine the desired effect); *see also Geneva Pharm., Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373, 1383-84 (Fed. Cir. 2003) (explaining that "'effective amount' is a common and generally acceptable term for pharmaceutical claims"). Here, the claimed method treats a disease requiring peripheral stem cell transplantation, and it could not be clearer from the intrinsic record that the chemotherapeutic agent is part of the pair of stem cell mobilizing agents, along with G-CSF. That stem cell mobilization is part of a larger procedure used in the treatment of an underlying disease does not mean that the chemotherapeutic agent must itself be prescribed for treatment of that disease.

The district court's reliance on dependent claim 4 is equally unavailing because it ignores the specification's repeated teachings regarding chemotherapeutic agents' ability to enhance the mobilization of stem cells. The district court construed claim 4's limitation "opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells" to mean "disrupts the bone marrow endothelial barrier to facilitate permeability of the

endothelial barrier for stem cells" (Appx34), *i.e.*, one mechanism of action by which a chemotherapeutic agent might enhance stem cell mobilization. However, the district court went on to say that claim 4 "demonstrates the patentee's ability to differentiate between two of chemotherapeutic agents' known ***functions***: opening the endothelial barrier and treating disease (typically cancer)." (Appx28 (emphasis added).) This is incorrect. Claim 4 does not specify a different role or function for the chemotherapeutic agent in claim 4 (stem cell mobilization) than in claim 1 (cancer treatment). Rather, claim 4 specifies a mechanism of action by which the chemotherapeutic agent is participating in the enhancement of stem cell mobilization. Dependent claim 4 thus supports Amgen's interpretation of the role of the chemotherapeutic agent in claim 1 (stem cell mobilization).

Finally, Amgen's construction is consistent with the interpretation given to the term "disease treating-effective amount" by another district court for the same patent: an "amount sufficient to enhance the mobilization of stem cells for recovery from the blood for subsequent peripheral transplantation." *Amgen, Inc. v. Apotex Inc.*, No. 15-61631-CIV, 2016 WL 1375566, at *6 (S.D. Fla. Apr. 7, 2016) (Appx2780-2791 at Appx2791).

CONCLUSION

For the foregoing reasons, Amgen respectfully requests that this Court reverse, vacate and/or remand the district court judgments.

Dated: April 13, 2018

Respectfully submitted,

/s/ Nicholas Groombridge
Nicholas Groombridge
Eric Alan Stone
Jennifer H. Wu
Jennifer Gordon
Peter Sandel
Arielle K. Linsey
Stephen A. Maniscalco
Jacob T. Whitt
Golda Lai
PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
1285 Avenue of the Americas
New York, NY 10019
(212) 373-3000

Wendy A. Whiteford
Lois M. Kwasigroch
Kimberlin L. Morley
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, CA 91320
(805) 447-1000

Attorneys for Plaintiffs-Appellants

ADDENDUM

INDEX TO ADDENDUM

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

AMGEN INC. and AMGEN MANUFACTURING, LIMITED, Plaintiffs, v. SANDOZ INC., SANDOZ INTERNATIONAL GMBH, and SANDOZ GMBH, Defendants.	Case No. 3:14-cv-04741-RS **~~[PROPOSED]~~ FINAL JUDGMENT** The Honorable Richard Seeborg

[~~PROPOSED~~] FINAL JUDGMENT

It is hereby ORDERED and ADJUDGED:

Pursuant to the Court's Order Granting Summary Judgment of Noninfringement and Denying Rule 56(d) Motion (Dkt. No. 346) and Stipulation and Order for Entry of Judgment Regarding U.S. Patent 6,162,427 (Dkt. No. 272), the Court hereby ENTERS FINAL JUDGMENT in this matter:

(i) against Amgen Inc. and Amgen Manufacturing, Limited (collectively, "Amgen") and in favor of Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH (collectively, "Sandoz") on Amgen's third and fourth causes of action for infringement of U.S. Patent No. 6,162,427 ("the '427 patent") and U.S. Patent No. 8,940,878 ("the '878 patent");

(ii) in favor of Sandoz Inc. and against Amgen on Sandoz Inc.'s sixth and eighth counterclaims for noninfringement of the '427 and '878 patents; and

(iii) dismissing without prejudice Sandoz Inc.'s seventh and ninth counterclaims for invalidity of the '427 and '878 patents.

Dated: 1/8/18

Honorable Richard Seeborg
United States District Court Judge

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

AMGEN INC. and AMGEN MANUFACTURING, LIMITED,

Plaintiffs,

v.

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, SANDOZ GMBH, and LEK PHARMACEUTICALS, D.D.,

Defendants.

Case No. 3:16-cv-02581-RS

[~~PROPOSED~~] FINAL JUDGMENT

The Honorable Richard Seeborg

~~[PROPOSED]~~ FINAL JUDGMENT

It is hereby ORDERED and ADJUDGED:

Pursuant to the Court's Order Granting Summary Judgment of Noninfringement and Denying Rule 56(d) Motion (Dkt. No. 183) and Joint Stipulation of Dismissal of All Claims and Counterclaims Related to U.S. Patent No. 5,824,784, and Order (Dkt. No. 61), the Court hereby ENTERS FINAL JUDGMENT in this matter:

(i) against Amgen Inc. and Amgen Manufacturing, Limited (collectively, "Amgen") and in favor of Sandoz Inc., Sandoz International GmbH, Sandoz GmbH, and Lek Pharmaceuticals d.d. (collectively, "Sandoz") on Amgen's first and second causes of action for infringement of U.S. Patent No. 8,940,878 ("the '878 patent");

(ii) in favor of Sandoz Inc. and against Amgen on Sandoz Inc.'s first counterclaim for noninfringement of the '878 patent;

(iii) in favor of Lek Pharmaceuticals d.d. ("Lek") and against Amgen on Lek's first counterclaim for noninfringement of the '878 patent;

(iv) dismissing without prejudice Amgen's third cause of action for infringement of U.S. Patent No. 5,824,784 ("the '784 patent");

(v) dismissing without prejudice Sandoz Inc.'s second, third, and fourth counterclaims for invalidity of the '878 patent, noninfringement of the '784 patent, and invalidity of the '784 patent; and

(vi) dismissing without prejudice Lek's second counterclaim for invalidity of the '878 patent.

Dated: 1/8/18

Honorable Richard Seeborg
United States District Court Judge

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

AMGEN INC., et al.,

Plaintiffs,

v.

SANDOZ INC., et al.,

Defendants.

Case No. 14-cv-04741-RS
Case No. 16-cv-02581-RS

ORDER GRANTING SUMMARY JUDGMENT OF NONINFRINGEMENT AND DENYING RULE 56(D) MOTION

I. INTRODUCTION

Defendants Sandoz Inc., Sandoz International GmbH, Sandoz GmbH, and Lek Pharmaceuticals d.d. (collectively, "Sandoz") move for summary judgment as to both noninfringement and damages. Plaintiffs Amgen Inc. and Amgen Manufacturing, Limited (collectively, "Amgen") oppose summary judgment and move, in the alternative, pursuant to Rule 56(d), to defer a ruling on noninfringement until additional information is produced regarding a pending modification to Sandoz's allegedly infringing process. For the reasons explained below, Sandoz's motion for summary judgment of noninfringement is granted. The motion for summary judgment regarding damages is denied as moot. Amgen's Rule 56(d) motion is denied.[1]

[1] Sandoz and Amgen have filed multiple sealing motions regarding materials submitted as part of their summary judgment filings. Those motions (14-cv-04741, Dkt. No.'s 278, 289, 295, 298, 312, 324, 328, 332, 337; 16-cv-02581, Dkt. No.'s 116, 133, 134, 137, 151, 162, 166, 169, 174) are granted. Amgen additionally moved for leave to file an opposition to a request to strike made by Sandoz in one of its replies. The materials Sandoz seeks to strike are not relied on in this order. Accordingly, Sandoz's request to strike and Amgen's motion (14-cv-04741, Dkt. No. 333, 16-cv-02581, Dkt. No. 170) are denied.

II. BACKGROUND

Amgen and Sandoz compete to develop, manufacture, promote, and sell biopharmaceutical products. The products at issue here are filgrastim and pegfilgrastim. Filgrastim is the pharmaceutical analog of a protein that naturally occurs in the human body. It stimulates the production of a type of white blood cells ("neutrophils") vital to the human immune system and, accordingly, is useful for treating patients undergoing certain forms of cancer therapy (e.g., chemotherapy) that can cause neutrophil deficiency ("neutropenia"). Pegfilgrastim is a modified version of filgrastim that remains in the circulatory system for a substantially longer period of time and thus is "long acting." Amgen began selling filgrastim in 1991 under the brand name Neupogen® and launched a pegfilgrastim product, Neulasta®, in 2002. Sandoz brought to market an FDA-approved biosimilar filgrastim product, Zarxio®, in 2015. Sandoz also has submitted an application to offer a biosimilar pegfilgrastim product that is pending before the FDA.

As explained in the claim construction order, recombinant proteins like filgrastim are manufactured in a multi-step process. The process begins when scientists introduce human DNA into a host cell of a different species, such as *E. Coli* bacteria, causing the bacteria to produce human proteins. Before these proteins can be therapeutically useful, however, they must attain a three-dimensional shape. Trouble arises when the host cells produce proteins that lack this proper shape. These "unfolded" proteins accumulate in the host cell and form insoluble aggregates called "inclusion bodies." To remedy the problem, scientists break open (lyse) the host cell to release the inclusion bodies. They solubilize the inclusion bodies, mixing the proteins with various chemicals to create a solution. They then combine that solution with a "refold buffer" to cause the protein to take a workable, three-dimensional shape.

Once the protein has refolded, it must be separated from the chemicals used for solubilization and refolding. This step is called purification and typically involves applying the solution containing the refolded protein to a "separation matrix." Generally, the separation matrix can function in one of two ways. In "flow-through" purification the separation matrix attracts one or more of the unwanted chemicals used to solubilize and refold the protein. The protein itself,

however, does not attach to the matrix and thus "flows through" and is collected. By contrast, in "capture purification" the separation matrix attracts and binds *the protein* so that the unwanted contaminants and chemicals flow through the matrix and are discarded. The purified protein is then eluted (i.e., released) from the separation matrix and collected.

The present dispute between Amgen and Sandoz began in 2014. Over the past three years, the litigation between the parties has involved multiple issues and multiple patents. The only patent that remains at issue, however, is U.S. Patent No. 8,940,878 ("the '878 patent"), entitled "Capture Purification Processes for Proteins Expressed in a Non-Mammalian System." As the name suggests, the '878 patent generally relates to processes for purifying proteins. Claim 7 of the patent claims one such method. Amgen asserts that one of the steps in Sandoz's process for making and purifying filgrastim and pegfilgrastim ("the AEX step") infringes claim 7. Sandoz contends the AEX step does not infringe because it does not satisfy elements (e), (f) and (g) of claim 7:

> (e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix;
> (f) washing the separation matrix; and
> (g) eluting the protein from the separation matrix, wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin.

While Amgen Inc. retains ownership of the '878 patent, Amgen Manufacturing Limited ("AML") is responsible for manufacturing Neupogen and Neulasta. AML does not practice the '878 patent method in manufacturing either product.[2]

III. LEGAL STANDARD

Summary judgment is proper "if the pleadings and admissions on file, together with the affidavits, if any, show that there is no genuine issue as to any material fact and that the moving party is entitled to judgment as a matter of law." Fed. R. Civ. P. 56(c). The purpose of summary

[2] Additional background information regarding how recombinant proteins are genetically engineered and purified can be found in the claim construction order (14-cv-04741, Dkt. No. 205).

judgment "is to isolate and dispose of factually unsupported claims or defenses." *Celotex v. Catrett*, 477 U.S. 317, 323-24 (1986). The moving party "always bears the initial responsibility of informing the district court of the basis for its motion, and identifying those portions of the pleadings and admissions on file, together with the affidavits, if any, which it believes demonstrate the absence of a genuine issue of material fact." *Id*. at 323 (citations and internal quotation marks omitted). If it meets this burden, the moving party is then entitled to judgment as a matter of law when the non-moving party fails to make a sufficient showing on an essential element of the case with respect to which he bears the burden of proof at trial. *Id*. at 322-23.

The non-moving party "must set forth specific facts showing that there is a genuine issue for trial." Fed. R. Civ. P. 56(e). The non-moving party cannot defeat the moving party's properly supported motion for summary judgment simply by alleging some factual dispute between the parties. To preclude the entry of summary judgment, the non-moving party must bring forth material facts, i.e., "facts that might affect the outcome of the suit under the governing law . . . . Factual disputes that are irrelevant or unnecessary will not be counted." *Anderson v. Liberty Lobby, Inc*., 477 U.S. 242, 247-48 (1986). The opposing party "must do more than simply show that there is some metaphysical doubt as to the material facts." *Matsushita Elec. Indus. Co. v. Zenith Radio*, 475 U.S. 574, 588 (1986).

The court must draw all reasonable inferences in favor of the non-moving party, including questions of credibility and of the weight to be accorded particular evidence. *Masson v. New Yorker Magazine, Inc*., 501 U.S. 496 (1991) (*citing Anderson*, 477 U.S. at 255); *Matsushita*, 475 U.S. at 588 (1986). It is the court's responsibility "to determine whether the 'specific facts' set forth by the nonmoving party, coupled with undisputed background or contextual facts, are such that a rational or reasonable jury might return a verdict in its favor based on that evidence." *T.W. Elec. Service v. Pacific Elec. Contractors*, 809 F.2d 626, 631 (9th Cir. 1987). "[S]ummary judgment will not lie if the dispute about a material fact is 'genuine,' that is, if the evidence is such that a reasonable jury could return a verdict for the nonmoving party." *Anderson*, 477 U.S. at 248. However, "[w]here the record taken as a whole could not lead a rational trier of fact to find for the

non-moving party, there is no 'genuine issue for trial.'" *Matsushita*, 475 U.S. at 587.

IV. DISCUSSION

A. Noninfringement

Evaluating infringement is a two-part inquiry: 1) claim construction; and 2) comparison of the properly construed claims to the accused process. *Lockheed Martin Corp. v. Space Sys./Loral, Inc.*, 324 F.3d 1308, 1318 (Fed. Cir. 2003). In the instant case, part one of the inquiry was completed with issuance of the claim construction order on August 4, 2016. Part two is the subject of the present motion.

"[A] determination of infringement, both literal and under the doctrine of equivalents, is a question of fact." *Id.* Because the ultimate burden of proving infringement rests with the patentee, an accused infringer may show that summary judgment of non-infringement is proper either by producing evidence that would preclude a finding of infringement, or by showing that the evidence on file fails to create a material factual dispute as to any essential element of the patentee's case. *See Novartis Corp. v. Ben Venue Labs., Inc.*, 271 F.3d 1043, 1046 (Fed. Cir. 2001). Here, Sandoz can prevail only if no reasonable jury could conclude the accused AEX step infringes claim 7 of the '878 patent either literally or under the doctrine of equivalents.

i. Literal Infringement

To prove literal infringement, a patent holder must establish that every requirement of the claimed method is included in the method accused of infringement. *MicroStrategy Inc. v. Business Objects, S.A.*, 429 F.3d 1344, 1353 (Fed. Cir. 2005). "If . . . even one claim limitation is missing or not met, there is no literal infringement." *Id.* at 1353 (citation omitted).

The overarching thrust of Sandoz's argument is that the claimed protein purification method requires three distinct and sequential steps as well as the application of three distinct solutions. Sandoz's AEX step, by contrast, involves only one step and only one solution. More specifically, Sandoz identifies four requirements of claim 7 it argues are not satisfied by its accused process. First, the eluting step must occur after the washing step. Second, the washing step must occur after direct application of the refold solution. Third and fourth, both the washing

and eluting steps require adding solutions different from the refold solution.

The first ground raised by Sandoz (i.e., that the eluting step must occur after the washing step) is sufficient on its own to support a finding that Sandoz's AEX step does not literally infringe the '878 patent. In construing the phrase "eluting the protein from the separation matrix," the claim construction order noted that the eluting step outlined in 7(g) must occur *after* the washing step described in 7(f). CC Order at 31, 33. This conclusion was reached in heavy reliance on the explicit language of the patent specification:

> The specification teaches, "[a]fter the separation matrix with which the protein has associated has been washed, the protein of interest is eluted using an appropriate solution." '878 Patent at 15:60 62. It further explains that the wash buffer may be comprised of any number of components so long as "[t]he pH range is chosen to optimize the chromatography conditions, preserve protein binding, and to retain the desired characteristics of the protein of interest." '878 Patent at 15:55 57 (emphasis added). Thus, the proteins and separation matrix should remain associated during the washing process. In contrast, elution involves cleaving the protein from the matrix with "a solution that interferes with the binding of the absorbent component of the separation matrix to the protein, for example by disrupting the interactions between Protein A and the Fc region of a protein of interest." '878 Patent at 15:65 16:2 (emphasis added). *Accordingly, the specification discloses a natural, logical order of steps. If the washing and eluting steps occurred simultaneously, the protein captured by the separation matrix could once again comingle with the contaminants and components to be washed away.* In light of the fact Amgen has not offered any reasons to believe the claim does not imply a natural order, the construction of the phrase will make clear the step of "eluting the protein from the separation matrix" occurs *after* the step of "washing the separation matrix."

Id. at 31 (emphasis added).

Nothing has been offered to suggest the above construction needs modification. Based on this construction, the method employed by Sandoz does not have the sequential washing and eluting steps required by claim 7. The AEX step entails continuously pumping a refold solution comprised of filgrastim, a particular detergent ("detergent 1"),[3] and other substances into a column containing a separation matrix. There is no pause in the pumping of the refold solution. Nor is there any point at which Sandoz adds a second solution to the column that is compositionally

[3] This nomenclature is adopted to avoid unnecessarily disclosing confidential aspects of Sandoz's accused process.

different than the refold. There simply is no way to conceive of this continuous pumping process as an eluting step *after* a washing step without straining the language of the patent specification and the claim construction order beyond their reasonable meaning.

Amgen nonetheless argues the washing and eluting steps *do occur* sequentially in Sandoz's process if you look at any given location in the column (e.g., "the leading edge of the refold solution in the downstream end") rather than at the column as a whole. The key, according to Amgen, is recognizing that conditions in the column are changing as the refold solution is applied. When the solution is first applied, conditions are such that filgrastim *is binding* to the separation matrix. While the filgrastim is bound, other contaminants in the solution are flowing over and past it through the column and being discarded (i.e., "washing"). Later, the continued application of refold solution causes conditions to change in the column yet again so that the filgrastim binding is reversed and the protein flows out through the column (i.e., "eluting"). Thus, Amgen argues, Sandoz's description of its AEX step as only one step and one solution is misleading. At any given location in the column where filgrastim binds, the washing step and the eluting step are occurring sequentially consistent with claim 7.

Amgen's attempt to redefine Sandoz's accused process in a way that fits the requirements of claim 7 is unavailing. As the claim construction order noted, the patent specification discloses a natural, logical order of steps. Nowhere is that order of steps more clear than with regard to the requirement that the eluting step in element (g) follow the washing step in element (f).

For similar reasons, Sandoz's argument that the washing and eluting solutions must be distinct is equally compelling and provides an additional ground on which to conclude that Sandoz's process does not literally infringe the claimed method. As previously discussed, Sandoz's AEX step uses only one solution. Yet the patent specification describes a "wash buffer" that is "optimized to preserve protein binding" and an eluting solution that "interferes with the binding." '878 Patent at 15:55-62. *See also* CC Order at 31. The opposite purposes of these two solutions suggests they must indeed be distinct, and cannot be, as Amgen contends, a single solution achieving different ends, due to different conditions, at different points in time.

Sandoz's other arguments—that the washing step must come after the application of the refold solution and that the solutions required for eluting and washing must be separate and distinct from the refold solution—are also strong. Those arguments, however, need not be reached. Eluting must follow washing under the claimed method. The accused AEX step has no such sub-steps. So too, the claimed method requires that the washing and elution solutions be distinct. Yet the accused AEX step involves application of only one solution. Either one of these grounds independently supports a finding that Sandoz's process does not literally infringe.

ii. Doctrine of Equivalents

An accused method that does not literally infringe a patent claim may still be found to be infringing under the doctrine of equivalents if it includes steps that are identical or equivalent to the requirements of the claim. *Warner–Jenkinson Co. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 21 (1997). An accused step is considered equivalent to a claim requirement if a person of ordinary skill in the field would think that the differences between the step and the requirement were not substantial. *See Johnson & Johnston Assocs. Inc. v. R.E. Serv. Co.*, 285 F.3d 1046, 1057 (Fed. Cir. 2002). An accused step may be insufficiently different from a claim requirement if it performs substantially the same function, in substantially the same way, to achieve substantially the same result. *See Warner-Jenkinson Co.*, 520 U.S. at 39-40; *Graver Tank & Mfg. Co. v. Linde Air Products Co.*, 339 U.S. 605, 608 (1950). As the patentee, Amgen bears the burden of establishing equivalency on a limitation-by-limitation basis by particularized testimony and linking argument as to the insubstantiality of the differences between the claimed and accused methods. *Akzo Nobel Coatings, Inc. v. Dow Chem. Co.*, 811 F.3d 1334, 1342 (Fed. Cir. 2016).

Here, the differences between the method claimed by the '878 patent and the accused AEX step are substantial. First, the claimed method and the AEX step do not perform the same function. As explained in the claim construction order, the alleged invention protected by the '878 patent was the discovery that refold solution could be applied directly to a separation matrix without removing components of or diluting the solution. CC Order at 25. The AEX step, by removing an unwanted contaminant ("detergent 1") in advance of capture purification, is in effect doing exactly

what the asserted claims sought to eliminate.

Second, the different functions performed by the two processes are performed in substantially different ways. Sandoz argues this distinction is best illustrated by classifying the claimed method as a "capture purification" process and the accused method as "flow-through." Amgen rejects these classifications as misleading on the grounds that filgrastim actually does bind to at least some portion of the separation matrix during Sandoz's process and is therefore captured. Regardless of how they are labelled, however, the processes are indeed different. The claimed method "discloses a natural, logical order of steps" in which application of the refold solution is followed by a washing step and then an eluting step. The accused method, by contrast, involves only one step: the continuous application of a single solution to a separation matrix.

Lastly, and closely related to the function analysis above, the results produced by the claimed method and the accused method are substantially different. The claimed method, as the patent notes, is a "Capture Purification Process" that produces the protein in question in its purified form. There are no steps beyond the eluting step in element (g). The AEX step, on the other hand, produces a solution that contains the protein to be purified (filgrastim)—and at least one fewer contaminant ("detergent 1") than at the outset of the step—but which requires further purification.

In light of these differences, Amgen cannot prove infringement either literally or under the doctrine of equivalents. Sandoz's motion for summary judgment of noninfringement is granted.

B. Damages

In addition to seeking summary judgment as to noninfringement, Sandoz also moves for summary adjudication of several discrete issues impacting the scope of damages and relief available to Amgen. Specifically, Sandoz asks the Court to find: (1) AML lacks standing to sue for infringement because it is neither an owner nor exclusive licensee of the '878 patent; (2) Amgen Inc. is not entitled to lost profits for Neupogen, because it has never made or sold any Neupogen; (3) Amgen cannot prove the absence of non-infringing alternatives; and (4) the hypothetical negotiation date for determining royalties must be earlier than May 5, 2015. Because Sandoz's

accused method does not infringe the '878 patent, these damages arguments need not be reached.

C. Rule 56(d) Motion

Rule 56(d) of the Federal Rules of Civil Procedure permits denial or continuance of a motion for summary judgment, "[i]f a nonmovant shows by affidavit or declaration that, for specified reasons, it cannot present facts essential to justify its opposition." A party requesting a Rule 56(d) continuance bears the burden of setting forth specific facts he hopes to elicit from further discovery and demonstrating that the facts sought not only exist but also are essential to oppose summary judgment. *Family Home & Fin. Ctr., Inc. v. Fed. Home Loan Mortg. Corp.*, 525 F.3d 822, 827 (9th Cir. 2008). Failing to meet this burden "is grounds for the denial" of a Rule 56(d) motion. *Pfingston v. Ronan Eng. Co.*, 284 F.3d 999, 1005 (9th Cir. 2002).

As discussed previously, Sandoz's accused AEX step involves pumping refold solution into a column containing a separation matrix. The specific matrix Sandoz currently uses, however, will be discontinued in late 2018 or 2019. Sandoz therefore plans to replace its current matrix with a new separation matrix. Amgen argues this change in matrices is significant and moves pursuant to Rule 56(d) to defer a ruling on whether Sandoz's modified process infringes on the claimed method. Such a ruling is not appropriate, Amgen argues, until Sandoz produces more complete documentation regarding how the process will be modified. Specifically, Amgen urges the court to wait until Sandoz submits an application for approval of its modified process to the FDA—which will happen at some point in 2018—and produces that submission and its underlying source documents to Amgen.

The problem with Amgen's request is that the final "process parameters" it hopes to discover (e.g., "column dimensions, flow rate, loading time, and residence time") are not material to the finding of noninfringement. As discussed in the infringement analysis, the method claimed by the '878 patent involves multiple steps and multiple solutions while Sandoz's accused method involves only one continuous step and only one solution. This substantial difference between the methods will not be altered by the replacement of the current matrix with the new matrix. The core function of the new matrix, to capture "detergent 1" as the refold solution moves through the

column, will be materially identical to the function of the current matrix. Sandoz's process will still not contain an eluting step that follows a washing step, as required by claim 7's (f) and (g) elements. It therefore will not infringe. Accordingly, granting Amgen's Rule 56(d) motion would not conserve judicial resources, as Amgen argues, but would instead unnecessarily delay resolution of this already lengthy litigation.

V. CONCLUSION

Sandoz's motion for summary judgment of noninfringement is granted with respect to its accused process as conducted with both the current and new separation matrices. Amgen's Rule 56(d) motion is denied. Sandoz's motion for summary judgment regarding damages is denied as moot. Sandoz is directed to submit a proposed final judgment no later than January 5, 2018.

IT IS SO ORDERED.

Dated: December 19, 2017

RICHARD SEEBORG
United States District Judge

United States District Court
Northern District of California

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

AMGEN INC., et al.,

Plaintiffs,

v.

SANDOZ INC., et al.,

Defendants.

Case No. 14-cv-04741-RS

ORDER CONSTRUING CLAIMS

I. INTRODUCTION

Amgen, Inc. and Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH (collectively "Sandoz") compete to develop, manufacture, promote, and sell biopharmaceutical products, including those used to facilitate stem-cell transplantation. Amgen holds two patents at issue in this action: U.S. Patent Nos. 6,162, 427 ("the '427 Patent") and 8,940,878 ("the '878 Patent"). Amgen accuses Sandoz of infringing those patents. The parties seek construction of ten terms pursuant to *Markman v. Westview Instruments, Inc.*, 52 F.3d 967 (Fed. Cir. 1995) (en banc). For the reasons set forth below, the disputed terms are construed as follows.

II. BACKGROUND

In 2014, Amgen filed claims against Sandoz for infringement of the '427 patent, "Combination of G-CSF with a Chemotherapeutic Agent for Stem Cell Mobilization." Amgen objects to Sandoz's efforts to market and sell ZARXIO®, a drug Amgen contends is biosimilar to its drug, NEUPOGEN®, which is commonly used to "treat[] the side effects of certain forms of

cancer therapy." FAC ¶ 11. The active ingredient in both products is filgrastim, a synthetic version of human granulocyte colony stimulating factor ("G-CSF"). Amgen also accuses Sandoz of violating California's unfair competition law ("UCL"). In response, Sandoz asserts numerous counterclaims for declaratory judgments of compliance with the Biosimilars Price Competition and Innovation Act ("BPCIA"), non-infringement, and patent invalidity. In March 2015, Sandoz obtained partial judgment in its favor with respect to the UCL claim and Sandoz's claim for a declaratory judgment of compliance with the BPCIA pursuant to Federal Rule of Civil Procedure 54(b). The parties jointly requested to stay these proceedings until the issuance of the Federal Circuit's mandate. Dkt. No. 111 at 3.

Amgen then appealed to the Federal Circuit. During the pendency of the appeal, the Federal Circuit entered an injunction to prevent Sandoz from marketing, selling, or importing ZARXIO®. The Federal Circuit affirmed dismissal of the UCL claim, and affirmed in part and reversed in part the order regarding the BPCIA. *See Amgen Inc. v. Sandoz Inc.*, 794 F.3d 1347 (Fed. Cir. 2015). Sandoz filed a petition for en banc review, which is still pending in the Federal Circuit.

Following the issuance of the Federal Circuit's mandate, the parties agreed to lift the stay, and Amgen filed a First Amended Complaint, asserting one additional claim of patent infringement. Amgen now contends Sandoz employs a method of protein capture that infringes the '878 patent, entitled "Capture Purification Processes for Proteins Expressed in a Non-Mammalian System."

III. LEGAL STANDARD

Claim construction is a question of law to be determined by the court. *Markman*, 52 F.3d at 979. "Ultimately, the interpretation to be given a term can only be determined and confirmed with a full understanding of what the inventors actually invented and intended to envelop with the claim." *Renishaw PLC v. Marposs Societa' per Azioni*, 158 F.3d 1243, 1250 (Fed. Cir. 1998). Accordingly, a claim should be construed in a manner that "most naturally aligns with the patent's description of the invention."

The first step in claim construction is to look to the language of the claims themselves. "It is a 'bedrock principle' of patent law that 'the claims of a patent define the invention to which the patentee is entitled the right to exclude.'" *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005) (quoting *Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc.*, 381 F.3d 1111, 1115 (Fed. Cir. 2004)). A disputed claim term should be construed in a manner consistent with its "ordinary and customary meaning," which is "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention, i.e., as of the effective filing date of the patent application." *Phillips*, 415 F.3d at 1312–13. The ordinary and customary meaning of a claim term may be determined solely by viewing the term within the context of the claim's overall language. *See id.* at 1314 ("[T]he use of a term within the claim provides a firm basis for construing the term."). Additionally, the use of the term in other claims may provide guidance regarding its proper construction. *Id.* ("Other claims of the patent in question, both asserted and unasserted, can also be valuable sources of enlightenment as to the meaning of a claim term.").

A claim should also be construed in a manner that is consistent with the patent's specification. *See Markman*, 52 F.3d at 979 ("Claims must be read in view of the specification, of which they are a part."). Typically the specification is the best guide for construing the claims. *See Phillips*, 415 F.3d at 1315 ("The specification is . . . the primary basis for construing the claims."); *see also Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582 (Fed. Cir. 1996) ("[T]he specification is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term."). In limited circumstances, the specification may be used to narrow the meaning of a claim term that otherwise would appear to be susceptible to a broader reading. *See SciMed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc.*, 242 F.3d 1337, 1341 (Fed. Cir. 2001) ("Where the specification makes clear that the invention does not include a particular feature, that feature is deemed to be outside the reach of the claims of the patent, even though the language of the claims, read without reference to the specification, might be considered broad enough to encompass the feature in question."); *Phillips*, 415 F.3d at 1316 ("[T]he specification may reveal an intentional disclaimer,

or disavowal, of claim scope by the inventor. In that instance as well, the inventor has dictated the correct claim scope, and the inventor's intention, as expressed in the specification, is regarded as dispositive."). Precedent forbids, however, a construction of claim terms that imposes limitations not found in the claims or supported by an unambiguous restriction in the specification or prosecution history. *Laitram Corp. v. NEC Corp.*, 163 F.3d 1342, 1347 (Fed. Cir. 1998) ("[A] court may not import limitations from the written description into the claims."); *Comark Commc'ns., Inc. v. Harris Corp.*, 156 F.3d 1182, 1186 (Fed. Cir. 1998) ("[W]hile claims are to be interpreted in light of the specification, it does not follow that limitations from the specification may be read into the claims." (internal quotation marks and alterations omitted)); *SRI Int'l v. Matsushita Elec. Corp. of Am.*, 775 F.2d 1107, 1121 (Fed. Cir. 1985) (en banc) ("It is the *claims* that measure the invention.") (emphasis in original). A final source of intrinsic evidence is the prosecution record and any statements made by the patentee to the United States Patent and Trademark Office ("PTO") regarding the scope of the invention. *See Markman*, 52 F.3d at 980.

Courts may also consider extrinsic evidence, such as expert testimony, dictionaries, or technical treatises, especially if such sources are "helpful in determining 'the true meaning of language used in the patent claims.'" *Phillips*, 415 F.3d at 1318 (quoting *Markman*, 52 F.3d at 980). Ultimately, while extrinsic evidence may aid the claim construction analysis, it cannot be used to contradict the plain and ordinary meaning of a claim term as defined within the intrinsic record. *See Phillips*, 415 F.3d at 1322–23.

IV. DISCUSSION

A. The '427 Patent

Hematopoietic stem cells naturally occur in the human body and are capable of proliferation and differentiation into cells that comprise the blood and immune systems. In other words, they are blood-forming stem cells.[1] These cells self-renew and reside primarily in bone marrow.

[1] In the interest of using plain language, this order uses the term "blood-forming stem cells" instead of "hematopoietic stem cells."

Peripheral stem cell transplantation is a process used to replace damaged blood-forming stem cells—the sort of cellular damage chemotherapy usually causes. Peripheral blood is the blood that circulates through the body. Before peripheral stem cell transplantation can occur, the doctor must collect blood-forming stem cells for later transplantation. Collection requires "mobilizing" stem cells in the bone marrow to move into the peripheral blood stream. Once the stem cells have mobilized, doctors collect them using a process called leukapheresis, which separates the stem cells from other types of blood cells. The collected cells are then set aside for later use. The more blood-forming stem cells in the blood stream, the fewer leukapheresis sessions the patient must undergo to collect enough cells for transplantation.

G-CSF is a protein that naturally occurs in the human body. Filgrastim is a pharmaceutical analog of human G-CSF constructed artificially in *E. coli* bacteria using recombinant DNA technology. Since the early 1990s, doctors and researchers have been using G-CSF in connection with chemotherapy to relieve the side effects of chemotherapy. G-CSF has also been used to facilitate mobilization of blood-forming stem cells from the bone marrow into the peripheral blood.

After the stem-cell collection, the patient undergoes myeloablative radiation (bone marrow destruction) or myelotoxic chemotherapy (bone marrow suppression), which destroy blood-forming stem cells in the process. Once chemotherapy has been administered, the collected stem cells can be reintroduced into the bone marrow to allow for further production of new blood cells.

Both parties agree that the '427 patent describes a method that requires administration of G-CSF before administration of a chemotherapeutic agent. The order of administration (G-CSF first, a chemotherapeutic agent second) is the allegedly novel component of the invention. At the time of the invention, a skilled artisan knew that administration of G-CSF alone, a chemotherapeutic agent alone, or a chemotherapeutic agent followed by G-CSF could mobilize blood-forming stem cells into the blood stream. The patentees purport to have reached the revolutionary conclusion that the structured administration of G-CSF first, followed by administration of a chemotherapeutic agent was the most efficient method of stem cell

mobilization. The patent claims to improve on the process of stem cell collection by following the specified order, which relieves patients of the need to attend multiple of leukapheresis sessions.

1. *"hematopoietic stem cell mobilizing-effective amount of G-CSF"*

The term "hematopoietic stem cell mobilizing-effective amount of G-CSF" appears in only claim 1, but is incorporated by reference into claims 2, 3, 4, and 6. Amgen would have the term construed to mean "an amount of G-CSF effective to mobilize hematopoietic stem cells," whereas Sandoz contends the term is indefinite.

When evaluating whether a term is sufficiently definite, courts must analyze that question "from the viewpoint of a person skilled in the art at the time the patent was filed." *Nautilus, Inc. v. Biosig Instruments, Inc.*, 134 S. Ct. 2120, 2128 (2014) (emphasis, internal quotation marks, and alteration omitted). As noted, the claims "are to be read in light of the patent's specification and prosecution history." *Id.* When examining the definiteness of a term, courts "must take into account the inherent limitations of language," and therefore "[s]ome modicum of uncertainty . . . is the price of ensuring the appropriate incentives for innovation." *Id.* (internal quotation marks omitted). "At the same time, a patent must be precise enough to afford clear notice of what is claimed, thereby apprising the public of what is still open to them." *Id.* at 2129 (internal quotation marks omitted). "Cognizant of the competing concerns," the Supreme Court requires "that a patent's claim, viewed in light of the specification and prosecution history, inform those skilled in the art about the scope of the invention with reasonable certainty." *Id.*

The first step required is to define who is a person skilled in the relevant art. This patent was written for those who practice stem-cell transplantation or study stem-cell biology. A person skilled in the relevant art is therefore one who has obtained a Ph.D. in biological sciences or an M.D. In addition, this person is one with significant experience with stem-cell biology, hematopoiesis, and stem-cell transplantation.

Turning to the question of whether such a skilled artisan understands the phrase "hematopoietic stem cell mobilizing-effective amount of G-CSF," Sandoz takes aim at the word "effective" and offers three arguments for why the term is indefinite. First, it argues neither the

claim language nor the specification inform skilled artisans about how many blood-producing stem cells must mobilize to be considered "effective." In other words, a skilled artisan has no way to discern whether mobilization of one stem cell, ten stem cells, or a thousand stem cells is "effective." Second, Sandoz insists the claim, specification, and prosecution history do not provide information for skilled artisans to tailor the procedure to the species of the patient (human, mouse, dog, horse, etc.). The final argument is that the patent does not explain how artisans should measure the level of stem-cell mobilization. At the time of the invention, practitioners knew of four methods for measuring the extent of stem cell mobilization, all of which varied considerably in terms of accuracy, consistency, and practicality. *See* Sandoz's Expert Negrin Decl. ¶¶ 45-46.

Whether adjectival limitations are indefinite depends on the context of each individual patent. In *Takeda Pharm. Co. v. Mylan Inc.*, No. 13-CV-04001-LHK, 2014 WL 5862134, at *10-11 (N.D. Cal. Nov. 11, 2014), the district court deemed the term "effective amount" definite because the patent described the proper dose of the drug ("about 0.5 to 1,500 mg/day"), and the claim covered treatment of a specific type of disease—reflux esophagitis. *Id.* at *10. In contrast, another district court concluded the term "% identity" was indefinite because "the specification identifie[d] a non-inclusive list of five methods to calculate '% identity' and provide[d] that sequence alignment can be performed using any commercially available or independently developed software." *Butamax Advanced Biofuels LLC v. Gevo, Inc.*, 117 F. Supp. 3d 632, 641 (D. Del. 2015). Similarly, in *Andrulis Pharm. Corp. v. Celgene Corp.*, No. CV 13-1644(RGA), 2015 WL 3978578, at *3-4 (D. Del. June 26, 2015), the term "enhanced therapeutically-effective amounts of thalidomide" was held to be indefinite because "enhanced" could mean "less than additive, additive, or greater than additive." *Id.* at *3.

Here, the claim itself offers little guidance, but the specification provides more direction. It teaches, "[t]he [G-CSF] dosage may depend on various factors such as mode of application, species, age, or individual condition. According to the invention, from 5 to 300 µg/kg/day of G-

CSF sc.[2] is applied." Pai Decl. Ex. 1, '427 Patent 3:4-7. G-CSF administration occurs "once per day over two to three days." '427 Patent 4:5-8. Amgen points to portions of the specification that explain, "[n]umerous substances" are capable of causing mobilization of blood-producing stem cells, such as G-CSF and "[s]ome chemotherapeutic agents." '427 Patent 1:32-37. These passages make clear that, at the time the patent was filed, skilled artisans knew G-CSF caused blood-producing stem cells to mobilize, and that any amount ranging from 5 to 300 µg/kg/day of G-CSF sc. would cause stem cells to mobilize enough to enable collection.

The prosecution history of the '427 patent offers skilled artisans further guidance and additional support for Amgen's proposed construction. Three papers identified in the specification refer to various G-CSF dosage amounts within the range stated in the specification. Two papers examined the efficacy of subcutaneous doses of 10 µg/kg/day. Wu Decl. Ex 4 at 861 (Long et al., *Cancer* 76(5):860-68 (1995)); Wu Decl. Ex. 6 at 146 (Pierelli et al., *J. Hematotherapy* 2:145-53 (1993)). Another study tested the comparative potency of subcutaneous or intravenous doses of 10 µg/kg/day or 5 µg/kg/day. Wu Decl. Ex. 5 at 2177 (Nademanee et al., *J. Clinical Oncology* 12(10):2176-86 (1994)). These three studies supply a person skilled in the art with more information to determine with a reasonable degree of certainty how much G-CSF to administer to achieve more than a *de minimus* level of stem-cell mobilization.

While the range of the amounts of G-CSF to administer is admittedly wide and variable depending on the size or species of the subject, a skilled artisan is not without any guidance to figure out how much G-CSF to administer. After all, "breadth is not indefiniteness." *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1341 (Fed. Cir. 2005). To the extent a skilled artisan may have difficulty adjusting the amount of G-CSF to administer depending on the species of the subject, the lack of precision in the claim and specification impacts only his or her ability to practice all embodiments of the claim—a question of enablement, not indefiniteness. *See Takeda*, 2014 WL 5862134, at *10 (citing *Exxon Research & Eng'g Co. v. United States*, 265 F.3d 1371,

[2] "sc." stands for "subcutaneous." Negrin Decl. ¶ 40.

1382 (Fed. Cir. 2001) (noting that "'inoperable embodiments' raise 'an issue of enablement, and not indefiniteness")). Overall, the patent communicates the purpose of G-CSF administration: to cause more than a *de minimus* number of blood-producing stem cells to enter the peripheral blood. While the claim and specification could have offered more precise guideposts, the disclosures provide those skilled in the art with sufficient information to figure out how to accomplish that goal. Accordingly, the phrase "hematopoietic stem cell mobilizing-effective amount of G-CSF" is not indefinite. It will be construed as follows: "an amount of G-CSF effective to mobilize hematopoietic stem cells."

2. *"A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment"*

The second phrase to construe is the preamble to claim 1. Although it appears in only claim 1, claims 2, 3, 4, and 6 incorporate the preamble by reference. Both parties agree the preamble limits the scope of the claim, but they disagree about how to construe it. The crux of the dispute is about the phrase "a method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment." Amgen construes the phrase as follows: "In the practice of the method, a patient in need of a stem cell transplant receives a transplant of peripheral stem cells." Sandoz offers the following construction: "In the practice of the method of treating a disease, a patient receives a transplant of peripheral stem cells." The fight boils down to whether peripheral stem cell transplantation is itself disease treatment, or whether it is a component of disease treatment to alleviate the side effects of treatment (namely chemotherapy). The text of the claim itself and the intrinsic record support Sandoz's construction.

To begin, the phrase "such treatment" must have an antecedent. *See Rapoport v. Dement*, 254 F.3d 1053, 1059 (Fed. Cir. 2001) (noting the phrase "to a patient in need of such treatment" must have an antecedent basis). Sandoz argues the antecedent is "a method of treating a disease," whereas Amgen insists it refers back to "peripheral stem cell transplantation." Under Sandoz's construction, the treatment (usually chemotherapy) necessitates stem-cell transplantation. To practice the treatment method, the doctor mobilizes, collects, and transplants blood-producing

stem cells into the patient. In contrast, under Amgen's reading it is the disease (primarily cancer) that requires peripheral stem cell transplantation. While "such treatment" surely requires an antecedent, both proposed constructions are grammatically correct, and therefore to construe the terms requires a more searching inquiry.

The text of the whole claim lends support to Sandoz's construction. The method claim 1 includes two steps: (1) administration of G-CSF, and (2) administration of a chemotherapeutic agent. The claim describes the quantity of G-CSF to be administered as "stem cell mobilizing," whereas the chemotherapeutic agent is described as "disease treating." *See* '427 Patent at 10:26-29. In other words, one substance mobilizes stem cells, while the other treats a disease. The claim suggests the transplantation itself does not treat disease.

The specification bears out this interpretation. It explains the purpose of the claimed method: "The use of high-dosage chemotherapy or bone marrow ablation by irradiation requires subsequent incorporation of hematopoietic stem cells into the patient, in which case recovery of such cells is required." '427 Patent at 1:18-21. Mobilization of blood-forming stem cells "has a crucial influence on the efficiency of" peripheral stem cell recovery. '427 Patent at 1:22-24. The method claimed by the patent improves upon the process of collecting stem cells by increasing the number of stem cells in the peripheral blood, thereby reducing the number of leukaphereses required. *See* '427 Patent at 1:24-27 ("At present, 2-3 leukaphereses are required for successful peripheral stem cell transplantation, resulting in considerable stress for the patients."); *id.* at 1:55-61 ("As the required number of leukaphereses is extremely stressing for the patient in the run-up to the treatment of particular diseases, e.g., in preparing myeloablative or myelotoxic therapy, the invention was based on the object of achieving a superior yield of stem cells or a decrease in the number of leukophereses via enhanced mobilization of stem cells."). Finally, the specification teaches that administration of G-CSF followed by administration of a chemotherapeutic agent is part of the "run-up to a, e.g. antitumor therapy," and therefore is not the disease treatment itself. '427 Patent at 4:24-25.

Thus, the specification clarifies any ambiguity in the text of the claim about whether peripheral stem-cell transplantation is a treatment for disease or a component of disease treatment.[3] It is the latter. Accordingly, Sandoz has the better construction, and it is adopted.

3. *"disease treating-effective amount of at least one chemotherapeutic agent"*

The next phrase up for construction is "disease treating-effective amount of at least one chemotherapeutic agent."[4] It appears in claim 1, and the patentee also incorporated the term by reference into claims 2, 3, 4, and 6. Amgen proposes defining the phrase as "an amount of at least one chemotherapeutic agent sufficient to enhance the mobilization of stem cells for recovery from the blood for subsequent peripheral transplantation." Sandoz offers the following construction: "an amount sufficient to treat a disease for which at least one chemotherapeutic agent is prescribed." The crux of the dispute revolves around whether the chemotherapeutic agent treats a disease such as cancer (Sandoz's construction), or whether the chemotherapeutic agent's purpose is to mobilize blood-producing stem cells for collection and subsequent peripheral transplantation (Amgen's construction). The text of the claim and the specification compel adoption of Sandoz's proposal.

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[3] This is not to say all forms of peripheral stem-cell transplantation are not treatments. Sandoz's expert witness, Robert S. Negrin, M.D., has explained the difference between two types of stem-cell transplants: allogeneic transplants and autologous transplants. Allogeneic transplants involve transplantation of a healthy donor's stem cells, and are used to treat certain cancers. *See* Negrin Sur-Reply Decl. ¶ 12. In contrast, autologous transplants involve using the patient's own stem cells. *Id.* ¶ 11. Autologous transplants do not treat diseases; they counteract the negative side effects of disease treatments such as myelotoxic chemotherapy or radiation. *Id.* The '427 Patent obviously addresses autologous transplants, not allogeneic transplants.

[4] In *Amgen, Inc. v. Apotex Inc.*, No. 15-61631-CIV, 2016 WL 1375566, at *5-6 (S.D. Fla. Apr. 7, 2016), the district court construed this very phrase. The court adopted Amgen's proposed construction, concluding a "disease treating-effective amount" of the chemotherapeutic agent is an amount "needed to achieve the goal of enhancing stem cell mobilization for recovery from blood and subsequent transplantation." *Id.* at *6. Prior claim construction orders are not binding or dispositive unless "an earlier suit . . . trigger[s] application of the doctrine of collateral estoppel." *W. v. Quality Gold, Inc.*, No. 5:10-CV-03124-JF HRL, 2011 WL 6055424, at *2 (N.D. Cal. Sept. 16, 2011). Sandoz was not a party to the Florida action, and therefore the doctrine of collateral estoppel is wholly inapplicable.

In *Apotex*, rather than proposing a construction, the defendant argued the term "disease treating-effective amount" is indefinite. *See Apotex*, 2016 WL 1375566, at *5. The district court did not weigh in on the question presented here, and therefore its construction is of limited weight.

"[T]he context in which a term is used in the asserted claim can be highly instructive." *Phillips*, 415 F.3d at 1314. There are three parts to claim 1—the preamble and two limitations: the first limitation is a description of step one (administration of G-CSF); the second limitation is a description of step two (administration of the chemotherapeutic agent). Rather than referring to the two steps of the claimed process as "stem-cell mobilizing," the patentee chose to use different descriptors for G-CSF and chemotherapeutic agents. G-CSF is "hematopoietic stem cell mobilizing," whereas the chemotherapeutic agent is "disease treating." *See* '427 Patent at 10:27-29. "Different claim terms are presumed to have different meanings." *Bd. of Regents v. BENQ Am. Corp.*, 533 F.3d 1362, 1371 (Fed. Cir. 2008). Here, the patentee chose to use two different words, and thus the two terms presumably carry different meanings.

A natural reading of these two terms suggests they are not synonyms. Nevertheless, claims "do not stand alone. Rather they are part of a fully integrated written instrument, consisting principally of a specification that concludes with the claims." *Phillips*, 811 F.3d at 1315 (internal quotation marks and citation omitted). Thus, if the specification suggests the two phrases describe similar functions, then the claim must be construed accordingly. Indeed, there is some evidence in the specification that the purpose of administering a chemotherapeutic agent is the same as that for G-CSF administration. The specification teaches about stem-cell mobilizing characteristics of chemotherapeutic agents. *See, e.g.*, '427 1:35-36 (citing Richman et al., Blood, Vol. 47, No. 6 1031 (1976)) ("Some chemotherapeutic agents are also known to possess the ability of mobilizing bone marrow stem cells . . . ."); '427 Patent at 1:5-9 ("The present invention relates to the novel use of G-CSF and a chemotherapeutic agent or a combination of chemotherapeutic agents to produce a pharmaceutical preparation for enhanced mobilization of hematopoietic stem cells in the treatment of diseases requiring peripheral stem cell transplantation."); '427 Patent at 3:13-17 ("Surprisingly, it was determined that administration of G-CSF prior to opening of the endothelial barrier induced by chemotherapeutic agents significantly increases the stem cell mobilization and thus, can improve leukapheresis efficiency."). Yet, these references to the chemotherapeutic agent's ability to open the endothelial barrier cannot supplant language of the claim itself. In

claim 4, the patentee chose to describe one function of a chemotherapeutic agent: its ability to "open[] the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells." '427 Patent at 10:36-38. Claim 4 demonstrates the patentee's ability to differentiate between two of chemotherapeutic agents' known functions: opening the endothelial barrier and treating disease (typically cancer). Thus, while the specification provides critical context for understanding the claim language, it cannot "be used to rewrite, the chosen claim language. Specifications teach. Claims claim." *SuperGuide Corp. v. DirecTV Enterprises, Inc.*, 358 F.3d 870, 875 (Fed. Cir. 2004) (internal quotation marks omitted).

Here, in claim 1, the patentee claimed the disease-treating function of chemotherapeutic agents. It shall therefore be construed as follows: "an amount sufficient to treat a disease for which at least one chemotherapeutic agent is prescribed."

4. "chemotherapeutic agent"

The term "chemotherapeutic agent" appears in claims 1, 4, 5, and 6, and claims 2 and 3 incorporate the term by reference to claim 1. On the one hand, Amgen would construe the term as an "exogenous substance that is capable of damaging or destroying microorganisms, parasites or tumor cells and that may open the endothelial barrier." On the other hand, Sandoz would prefer to construe the phrase as an "exogenous substance suited and used to damage or destroy microorganisms, parasites or tumor cells." While the two constructions are similar, there are two points of dispute. First, they disagree about whether chemotherapeutic agents perform two functions (damaging and destroying microorganisms *and* opening the endothelial barrier), or just one (damaging and destroying microorganisms). Second, they part company over whether the chemotherapeutic agent must be "capable of" those functions, or "suited and used" for a certain purpose. At the *Markman* hearing, Amgen agreed to drop any reference to opening the endothelial barrier from its construction. Dkt. No. 199, Hr'g Tr. 118:23-119:1. Thus, the only remaining dispute is whether to use the words "capable of" or "suited and used for."

Sandoz generated its construction directly from the specification, which defines "chemotherapeutic agents." '427 Patent 2:37-39 ("[C]hemotherapeutic agents are understood to

be exogenous substances suited and used to damage or destroy microorganisms, parasites or tumor cells."). "[T]he specification may reveal a special definition given to a claim term by the patentee that differs from the meaning it would otherwise possess." *Phillips*, 415 F.3d at 1316. It may also "reveal an intentional disclaimer, or disavowal, of claim scope by the inventor." *Id.* When the inventor provides a definition, his or her chosen lexicography is dispositive. *Id.* Amgen must therefore mount a strong case in order to change the definition the patentee included in the specification.

Amgen tries to do so by arguing the definition from the specification might be read to limit the scope of the claim to those chemotherapeutic agents known and used at the time of the invention. As a general matter, patentees need not "describe in [their] specification every conceivable and possible future embodiment of [their] invention." *SRI Int'l*, 775 F.2d at 1121. The specification lists various types of cytostatic agents (alkylating agents, metal complex cytostatic agents, antimetabolites, natural substances, antibiotic agents, hormones and hormone antagonists, and "other compounds") and offers examples of each group. *See* '427 Patent at 2:40-54. This list of examples suggests the patentee did not intend the claim to be limited to those chemotherapeutic agents known and used at the time of the invention.

This fact alone does not necessarily militate in favor of deviating from the definition provided in the specification or Amgen's proposed construction. The concern is that there are many agents, like battery acid, which are technically capable of damaging or destroying microorganisms, parasites, and tumors, but are not used or suited for that purpose.[5]

[5] In *Apotex*, the district court adopted the construction of "chemotherapeutic agent" Amgen now offers. *See* 2016 WL 1375566, at *5. Once again, the *Apotex* court's constructions are of limited persuasive value because it confronted different proposed constructions than those at issue here. Apotex suggested the following construction, which limited chemotherapeutic agents to those that open the endothelial barrier: "Therapeutic agents which open the endothelial barrier, rendering it permeable for stem cells and/or exogenous substances suited and used to damage or destroy microorganisms, parasites or tumors." *Id.* Thus, the district court did not address or consider whether "capable" is a synonym for "suited and used for," or whether the construction of this term should mention anything about opening the endothelial barrier.

At the hearing, Amgen offered two alternative constructions: "an exogenous substance that is *suitable for use to* damage or destroy microorganisms, parasites or tumor cells and that may open the endothelial barrier" or "an exogenous substance that is suited to damage or destroy . . . ." *See* Tr. Hr'g 121:1-4; 122:14-22. Sandoz did not agree to either proposal for the simple reason that the patentee chose a definition and cannot change that definition at a later time, and its position is certainly correct as a matter of law. *Phillips*, 415 F.3d at 1316.

That there may be factual disputes as this case progresses does not counsel in favor of adopting Amgen's construction. In the absence of any reason to deviate from the patentee's definition of "chemotherapeutic agent," it shall be adopted for the purposes of this litigation. Accordingly, the term "chemotherapeutic agent" shall mean "exogenous substance suited and used to damage or destroy microorganisms, parasites, or tumor cells."

5. "comprising administering . . . G-CSF; and thereafter administering . . . at least one chemotherapeutic agent"

The fifth phrase requiring construction appears or is incorporated into claims 1, 2, 3, 4, and 6: "comprising administering . . . G-CSF; and thereafter administering . . . at least one chemotherapeutic agent." Amgen proposes the following construction: "G-CSF and the at least one chemotherapeutic agent are given in combination for purposes of stem cell mobilization, and the order in which G-CSF and the chemotherapeutic agent(s) are administered for that purpose is G-CSF first followed by the chemotherapeutic agent(s)."[6] Sandoz contends the word "comprising" means "including but not limited to," and allows for additional steps before, in

[6] Initially, Amgen proposed a lengthier construction of the phrase: "G-CSF and the at least one chemotherapeutic agent are given in combination for purposes of stem cell mobilization, and the order in which G-CSF and the chemotherapeutic agent(s) are administered for that purpose is G-CSF first followed by the chemotherapeutic agent(s). Other than the foregoing stem cell mobilization step, the method for treating a disease requiring peripheral stem cell transplantation involves additional steps such as collection of cells by leukapheresis, myeloablative and/or myelotoxic therapy, and transplanting the collected peripheral stem cells back into the patient. The term 'comprising' allows for these additional steps." At the *Markman* hearing, Amgen withdrew the second sentence, and so this order will focus on only the first. *See* Tr. Hr'g 137:3-138:6 ("[W]e would be perfectly happy to just go with the first sentence of the proposal.").

between, and after the steps recited in the claim. It also offers the following construction of the remainder of the phrase: "In the practice of the method, at least one administration of G-CSF must occur before at least one administration of a chemotherapeutic agent." Thus, there are two disputes to resolve: (1) whether to construe the word "comprising" (and how), and (2) whether to include some explanation about the purpose of each step. For the reasons discussed below, the construction Sandoz advances must be adopted.

"Comprising" is a term of art, which means "including but not limited to." *Exergen Corp. v. Wal-Mart Stores, Inc.*, 575 F.3d 1312, 1319 (Fed. Cir. 2009). Sandoz's thus accords with longstanding rules of patent interpretation. Amgen urges declining to adopt the traditional construction of the word "comprising" out of fear that such construction would obfuscate the novelty of the invention, i.e. the precise order of administration (G-CSF first, and chemotherapeutic agent(s) second).[7] The trouble with this position is the simple fact the word "comprising" appears in the claim, and therefore must be construed. Amgen has not adequately explained how Sandoz's construction fails to convey the essence of the method claimed: the order of administration. Accordingly, the word "comprising" must be construed as usual to mean "including but not limited to." This construction naturally implies there may be steps before, in between, and after the steps recited in the claim.

The crux of the second dispute is whether Amgen's proposed construction improperly imports a purpose limitation into the claim. Critically, Amgen's suggested reading emphasizes that the purpose of administering a chemotherapeutic agent is to mobilize blood-forming stem

[7] Indeed, numerous portions of the specification make clear the invention relates to the timing of the administration of G-CSF and the chemotherapeutic agent—G-CSF first, the chemotherapeutic agent second. *See* '427 Patent at 3:18-23 ("By administering G-CSF prior to administration of the chemotherapeutic agent(s) . . ."); *id.* at 1:66-21:11 ("The G-CSF and chemotherapeutic agent administration forms can be taken out separately and administered successively according to the optimum application regimen."); *id.* at 3:5-17 ("The administration of the chemotherapeutic agent(s) is initiated either immediately after the second or third G-CSF injection or on the fourth day . . . . [I]t was determined that administration of G-CSF prior to opening of the endothelial barrier induced by chemotherapeutic agents significantly increases the stem cell mobilization . . . ."); *id.* at 3:31-41 ("[T]he administration of G-CSF prior to administration of the chemotherapeutic agent . . . for enhanced mobilization of hematopoietic stem cells.").

cells, and not to treat disease. As addressed above, Sandoz hotly contests this point. No reiteration of the arguments about whether the purpose of administration of the chemotherapeutic agent is necessary for there is no textual basis to import a purpose limitation into the claim. The text of the claim and the specification make clear the method encompasses a specific order of administration (G-CSF, followed by a chemotherapeutic agent). Sandoz's proposed construction aligns with both, and therefore must be adopted in full. Accordingly, the word "comprising" means "including but not limited to," and allows for additional steps before, in between, and after the steps recited in the claim. In the practice of the method, at least one administration of G-CSF must occur before at least one administration of a chemotherapeutic agent.

6. "opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells"

The final term in the '427 Patent to construe pertains to only claim 4: "opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells." Amgen proposes construing the claim to mean "enhances the transit of stem cells from the bone marrow to the peripheral blood," whereas Sandoz contends it should mean "disrupts the bone marrow endothelial barrier." This dispute boils down to whether the phrase encompasses all mechanisms by which the chemotherapeutic agent allows stem cells to travel from bone marrow into the peripheral blood or whether the claim is limited to one mechanism, namely breaking down the barrier altogether.

The text of the claim does not resolve this dispute, but the specification offers some guidance. At the time of the alleged invention, skilled artisans were aware that administration of cytotoxic agents caused the number of stem cells to increase in the peripheral blood. What was unknown at the time was how exactly blood cells moved from bone marrow into the peripheral blood. One article described the process as an "[i]njury to the supporting structure of the marrow." Wu Decl. Ex. 10 at 1037, Richman et al., *Blood* 47(6): 1031-39 (1976) (cited at '427 Patent at 1:35-37). Another researcher explained, "[A] cytotoxic conditioning regimen can induce membrane instability leading to massive loss of the endothelial membrane." Wu Decl. Ex. 11 at

373, Shirota et al., *Exp. Hematol* 19:369-73 (1991) (cited by '427 Patent at 1:50-54). Yet another researcher described the process as a "disrupt[ion] [of] normal marrow endothelial cell barriers." Wu Decl. Ex. 12 at 1965 (Nebren et al., *Blood* 81(7):1960-67 (1993) (cited by '427 Patent cover). Researchers had observed disruption of the endothelial barrier of some kind, which then caused the stem cells to enter the peripheral blood stream. While researchers generally abstained from identifying this disruption as the only reason blood-producing stem cells are released into the blood stream, the available articles would inform a skilled artisan that the probable method involved destruction of the endothelial barrier. Amgen believes its construction captures this state of affairs.

Sandoz for its part has derived its construction from the prosecution history. During the patent prosecution, the PTO rejected the claim because the specification did not adequately disclose information about how to use chemotherapeutic agents that increase the permeability of the endothelial barrier. Pai Decl. Ex. 7, June 30, 2000 Resp. to Office Action at 3. In response to this inquiry, the patentee stated, "cyclophosphamide is one of the examples of cytotoxic agents that disrupt normal bone marrow endothelial cell barriers." *Id.* Sandoz argues that this history suggests the clarification about the meaning of the word "open" and using the term "disrupt" as its synonym was an essential precursor for approval.

Ultimately, Sandoz's position boils down to two concerns. The first issue involves whether jurors might believe "opening" the endothelial barrier leaves the barrier intact, which implies a temporary removal, like a door or a curtain. "Disrupt" on the other hand connotes a more damaging process, whereby the barrier may repair over time, but not immediately. Amgen maintains there may be mechanisms for opening the endothelial barrier that do not involve disruption. The trouble with Amgen's proposed construction is the fact it untethers the claim from the specification and the prosecution history. That the patentee chose to use "disrupt" as a synonym for "open" with the PTO militates in favor of using "disrupt" in the construction of this phrase.

Second, Sandoz contends the articles referenced in the specification merely hypothesize about how opening the endothelial barrier facilitates stem-cell transit from bone marrow into the peripheral drug. An open door certainly facilitates movement from the outside to the inside, but it does not cause such movement. During the *Markman* hearing, Sandoz agreed to amend its proposed construction to include a purpose limitation: to disrupt the bone marrow endothelial barrier to facilitate the permeability of the endothelial barrier for stem cells. Tr. Hr'g 156:9–157:4. The words "facilitate the permeability of the endothelial barrier for stem cells" appears in the specification, '427 Patent at 1:53–55, and therefore resolves at least one of Amgen's concerns, namely that the construction must communicate the purpose of the opening, *see* Tr. Hr'g 158:9 – 159:12 (Amgen counsel: "I don't think we have any difficulty with the language that's in the specification. . . . If we building the idea that its' facilitating permeability, maybe we have captured that.").

All in all, the following construction aligns with the specification and prosecution history, and is therefore adopted: "disrupts the bone marrow endothelial barrier to facilitate permeability of the endothelial barrier for stem cells."

B. The '878 Patent

Recombinant proteins are genetically engineered proteins. Scientists introduce human DNA encoding into a host cell of a different species, such as *E. Coli*, to create recombinant proteins. Introduction of human DNA into the host cell causes the bacteria to produce human proteins even though the bacteria would not produce such proteins naturally. This process has been used to engineer various human proteins since the 1980s.

To be therapeutically useful, a recombinant protein must attain a three-dimensional shape. Trouble arises when the host cells produce the recombinant proteins "unfolded," meaning the proteins do not have the proper three-dimensional shape. These unfolded recombinant proteins accumulate in the host cell and form insoluble aggregates called "inclusion bodies." To remedy this problem, scientists break open (lyse) the host cell to release the inclusion bodies. Next, the scientists solubilize the inclusion bodies, which is a process of mixing the protein with various

chemicals to create a solution. That solution is then combined with a "refold buffer" to cause the protein to take a workable, three-dimensional shape.

Once the protein has refolded, the scientists must then separate the refolded recombinant protein from the chemicals used to solubilize and to refold the protein. This step is called purification and typically involves a "separation matrix." The separation matrix utilizes characteristics of the protein to be isolated to trap the protein in the matrix. The unwanted chemicals and proteins that do not have the targeted properties will not associate with the separation matrix and can be discarded.

There are two types of purification: flow-through purification and capture purification. The process of flow-through purification involves applying the solution containing the refolded protein to a resin. Resin attracts the chemicals used to solubilize and to refold the protein. The refolded proteins do not attach to the resin, and therefore they "flow through" the resin and remain in the solution.

In contrast, capture purification utilizes a resin designed to trap protein. The unwanted substances and chemicals stay in the solution and flow over the resin. Scientists discard the solution containing the unwanted contaminants and chemicals, leaving only the resin with the protein to be purified. The process of elation causes the resin to release the purified protein.

At the time of the alleged invention, skilled artisans believed the solution containing the solubilized and refolded protein had to be diluted to remove certain components of the refold solution before they could apply the separation matrix to it. Pai Decl. Ex. 2, Patent '878 at 1:44-46. Skilled artisans believed these contaminants would interfere with the protein's ability to affiliate with the separation matrix. The patentees allegedly discovered that this dilution step was unnecessary; scientists can apply the separation matrix to the refolding solution without diluting the solution first. '878 Patent at 15:33-37. The method disclosed in the patent removes a costly, time-consuming step in the purification process. '878 Patent at 4:55-60.

1. "directly applying the refold solution to a separation matrix"

The first phrase of the '878 Patent to construe appears in claims 7, 8, 11, 13, 14, 15, 16,

and 17. Amgen construes the phrase "directly applying the refold solution to a separation matrix" to mean "applying the refold solution to a column that contains the separation matrix without removing components of or diluting the refold solution." Sandoz offers a slightly different construction of the phrase: "applying the refold solution to a separation matrix without diluting the refold solution or removing one or more of a denaturant, a reductant, a surfactant, an aggregation suppressor, a protein stabilizer, and a redox component." There are two points of disagreement between the parties. First, Amgen does not wish to list the components of the refold solution, whereas Sandoz believes such components should be specified. The crux of the dispute is whether the claim allows for steps between the refolding process and the purification process, which remove components of the refold solution that are *not* required for refolding. Second, they part company over whether the refold solution is applied to a column or whether the claim covers other methods of applying separation matrices, such as batch processes. For the reasons discussed below the phrase will be construed as follows: "applying the refold solution to a separation matrix without removing components of or diluting the refold solution."

a. "Directly Applying"[8]

Amgen and Sandoz agree the patent teaches a method of purification that does not require dilution of the refold solution. Sandoz's construction is drawn from the claim itself, which lists the components "comprising" a solubilization solution: one or more of a denaturant, a reductant, and a surfactant. '878 Patent at 22:9-13. Claim 7 further defines a "refold solution" as "comprising the solubilization solution and a refold buffer." '878 Patent at 22:14-15. The "refold buffer" "compris[es] one or more of" a denaturant, an aggregation suppressor, a protein stabilizer, and a redox component. '878 Patent at 22:15-20. Thus, according to Sandoz's expert, Nigel J.

[8] Along with the reply brief, Amgen submitted a declaration to rebut the extrinsic evidence Sandoz submitted. Sandoz sought to strike the declaration or, in the alternative, to file a sur-reply brief. Because the submission of new evidence and new argument in reply was improper, Sandoz received leave to file a sur-reply. One of the sur-reply declarations submitted included an interrogatory response. Amgen objected to the admission of this exhibit, but really used the objection as an opportunity to argue why the submitted exhibit did not actually support Sandoz's argument. Accordingly, the objection is overruled.

Titchener-Hooker, Ph.D., a skilled artisan would understand the "refold solution," which is applied to the separation matrix includes the listed components. Sandoz's Expert Titchener-Hooker Decl. ¶¶ 31-32.

Amgen contends the word "directly" means there are no intermediary steps of any kind between refolding and purification. The dispute about proper construction of the word "directly revolves around whether the claim allows for removal of components of the refold solution that are *not* required for refolding.

The proper starting point is, of course, the text of the claim. A person skilled in the art could read the claim and reasonably conclude no dilution steps of any kind are allowed between refolding and washing. Yet, "directly" could also mean the refold solution need not pass through something to come into contact with the separation matrix. Accordingly, the text of the claim itself does not resolve the dispute.

The specification teaches the method claimed involves applying "the refold solution comprising the refolded protein of interest" "directly to the separation matrix, without the need for diluting or removing *the components of the solution required for refolding the protein*." '878 Patent at 15:25-29 (emphasis added). Similarly, in the prosecution history, the patentee used related language after the PTO rejected the proposed claims because it believed U.S. Patent No. 7,138,370 anticipated the claimed method. Patent '370 disclosed a method of protein purification requiring three processing steps before the refold solution could be applied to the separation matrix: dialysis, precipitation, and centrifugation. To remedy this problem, the patentee added the word "directly" to the claim to emphasize that the disclosed method did not require removal of "the components of the solution required for refolding the protein." Wu Decl. Ex. 13 at 3. Amgen insists these statements in the specification and prosecution history make clear no dilution whatsoever is required.

The trouble with this position is the fact claim 7's steps (c) and (d) and preamble recites the components that comprise the refold solution: one or more of a denaturant, a reductant, a surfactant, an aggregation suppressor, a protein stabilizer, and a redox component—the very

components listed in Sandoz's construction. That said, Sandoz's construction does not fully capture the claim because, in the world of patents, the word "comprising" means "including but not limited to." *Exergen*, 575 F.3d at 1319. The six components listed in the claim are not necessarily the only components of the refold solution. Moreover, the patentee's attempt to distinguish the claimed method from the prior art, and the '370 Patent, in particular, clarify that the patentee believed there should not be any intermediary steps between the refolding process and application of such solution to the separation matrix.

b. "Column"

The second point of conflict is whether the refold solution must be applied to a column containing the separation matrix or whether the claim contemplates other methods of bringing the separation matrix in contact with the refold solution. Amgen and Sandoz agree on at least one point: that the claim, specification, and prosecution history all contemplate that scientists could load the refold solution into a column containing the separation matrix. Conflict has arisen, however, because there are various methods of chromatography used to bring into contact separation matrices and refold solution. The column method involves loading the refold solution into a column containing a separation matrix. As the solution flows down the column, it flows past the separation matrix and down into a collection vessel, where either the contaminants or protein collect. There are, however, other methods scientists used to accomplish the same goal, such as batch processing and filtration systems. '878 Patent at 16:47-54. The batch method employs resin beads with the separation matrices. Scientists pour the refold solution into a container containing these resin beads, and then they discard the excess solution. Despite the fact that there are multiple methods of chromatography, Amgen contends claim 7 is limited to the column method even though the claim does not specify the chromatography method used.

The most significant problem with Amgen's proposal is that the word "column" does not appear in the claim, and thus there is no reasonable argument for the proposition "column" is a synonym for any word appearing therein. "[A] bedrock principle of patent law [is] that the claims of a patent define the invention to which the patentee is entitled the right to exclude." *Phillips*,

415 F.3d at 1312 (internal quotation marks omitted). Amgen therefore faces an uphill battle to show its construction including the word "column" is proper.

Amgen first turns to the specification, which describes three embodiments of the method—all of which describe column chromatography. Sandoz correctly notes the first two examples describe affinity separation matrices, which do not pertain to claim 7. Nevertheless, a skilled artisan reading the specification would read about only column processes—a fact suggesting, but not establishing, that the method involves column application. Yet, the specification also teaches:

> [A]ny or all steps of the invention can be carried out *by any mechanical means.* As noted, the separation matrix can be disposed in a column. The column can be run with or without pressure and from top to bottom or bottom to top. The direction of the flow of fluid in the column can be reversed during the purification process. Purifications can also be carried out using a batch process in which the solid support is separated from the liquid used to load, wash, and elute the sample by any suitable means, including gravity, centrifugation, or filtration. Moreover, purifications can also be carried out by contacting the sample with a filter that adsorbs or retains some molecules in the sample more strongly than others.

'878 Patent at 16:42-54 (emphasis added). The specification thus makes clear the method is not limited to column chromatography alone and even offers additional methods. In light of the fact the Federal Circuit has rejected the notion "that if a patent describes a single embodiment, the claims of the patent must be construed as being limited to that embodiment," *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d 898, 906 (Fed. Cir. 2004), the simple fact most examples disclosed in the specification involve column chromatography is not dispositive.

The second problem with Amgen's "column" proposal is that the specification describes the process of putting the refold mixture into the column as "loading," whereas the word "applying" implies a broader range of mechanisms. *See* Titchener-Hooker Decl. ¶35. For that reason, Titchener-Hooker contends that had the patentee wished to limit the method claimed to the column process, it should have used the word "loading." *Id.* (citing '878 Patent at 18:7-17, 19:4-17).

In sum, the text of the claim and intrinsic record do not support Amgen's proposal to limit the claim to the column process. At the same time, neither the intrinsic record nor the extrinsic record support Sandoz's attempt to list the components of the refold solution that need not be removed before the solution is applied to the separation matrix. Accordingly the phrase "directly applying the refold solution to a separation matrix must be construed as follows: "applying the refold solution to a separation matrix without removing components of or diluting the refold solution."

2. "under conditions suitable for the protein to associate with the matrix"

The phrase "under conditions suitable for the protein to associate with the matrix" relates to claims 7, 8, 11, 13, 14, 15, 16, and 17. There are two points of disagreement about how properly to construe this phrase: the construction of the words "protein" and "associate." For the reasons discussed below, the phrase shall be construed as follows: "under conditions suitable for the protein to be purified to bind to the matrix."

a. Protein

Amgen believes the word "protein" refers to any protein expressed by the non-mammalian expression system, not just the protein of interest, i.e., the recombinant protein expressed by the host cell. In contrast, Sandoz argues "protein" refers to a specific protein the scientists intended the non-mammalian organism to express (G-CSF, for example).

Both parties argue the text of the claim supports their respective constructions. Amgen points to the preamble of claim 7: "A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system . . . ." '878 Patent at 22:3-5. The patentee chose to use "a protein" instead of "the protein" to make clear the method could be used to capture any protein expressed by a non-mammalian organism.

More importantly, the patentee defined the word "protein" in the specification as follows: "the terms 'protein' and 'polypeptide' are used interchangeably and mean any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds." '878 Patent at 5:62-65. When an inventor has expressly defined a term in the specification, it controls for

construction purposes. *See Phillips*, 415 F.3d at 1316. In contrast, the patentee refers to the "protein of interest," meaning the protein the scientists caused bacteria to express, throughout the disclosure. *See e.g.*, '878 Patent at 4:8-9 ("[T]he present invention relates to a method of isolating a protein of interest . . . ."); '878 Patent at 4:31-32 (same). According to Amgen, the patentee's conscious decision to use the word "protein" instead of "protein of interest" in claim 7's text is significant and counsels in favor of using the specification's definition of "protein."

Sandoz begins with the text and structure of the claim. Step (a) of Claim 7 involves "expressing a protein," whereas steps (c), (e), and (g) involve doing something to "the protein." '878 Patent at 22:3-6, 22:9-25. The Federal Circuit has explained "[s]ubsequent use of the definite articles 'the' or 'said' in a claim refers back to the same term recited earlier in the claim." *Wi-Lan, Inc. v. Apple, Inc.*, 811 F.3d 455, 462 (Fed. Cir. 2016). Thus, Sandoz contends the steps refer back to the antecedent basis: the protein expressed in a non-native expression system is the protein to be purified.

In addition, Sandoz correctly points out that the method claimed is one of protein purification, and therefore all steps listed in the claim drive towards the purification of one specific protein. Indeed, the specification teaches, "[a]fter the protein of interest has associated with the separation matrix the separation matrix is washed to remove unbound protein, lysate, impurities and unwanted components of the refold solution." '878 Patent at 15:43-46. The process of washing removes unwanted protein from the refold mixture, leaving only the sought-after protein stuck to the separation matrix. Once all unwanted materials have been washed away, the final step of the claimed process is elution, whereby the protein disassociates from the matrix. The end result is a clean protein ready for future use. All these steps lead to the electable conclusion the method claimed and the steps claim 7 describes target a specific protein.

That the clean protein emerging from the process is the expressed protein does not, however, necessarily follow from the text of the claim. Accordingly, Sandoz's construction must be rejected for that reason. Nevertheless, the method claimed is also more targeted than Amgen suggested. The targeted protein is the protein to be purified. Thus, in the context of claim 7 (and

all derivative claims), the word "protein" means "the protein to be purified."[9]

b. Associate

The parties also dispute whether "associate" is a synonym for "bind." Amgen insists binding is merely one of the mechanisms by which proteins associate with separation matrices. Sandoz believes binding is the only mechanism by which the proteins interact with the separation matrix.

Amgen derives its construction from the specification and its definition of "separation matrix":

> As used herein, the term "separation matrix" means any absorbent material that utilizes *specific, reversible interactions* between synthetic and/or biomolecules, e.g., the property of Protein A to bind to an Fc region of an IgG antibody or other Fc-containing protein, in order to effect the separation of the protein from its environment. In other embodiments the specific, reversible interactions can be base[d] on a property such as isoelectric point, hydrophobicity, or size.

'878 Patent at 14:65-15:5 (emphasis added). Amgen reads this section to mean binding is just an example of the type of reversible interactions the process involves, whereas other embodiments of the method involve resins that retard the flow of the refold solution through the column or which trap large proteins and permit smaller proteins to flow through. While there is a temptation to treat the specification's definition of "separation matrix" as a definition for associate, it is not. The specification defines "separation matrix," and not "associate." Accordingly, the specification offers some, but not definitive, support for Amgen's proposed construction.

Sandoz has identified portions of the specification that support its position, where the patentee used the words "associate" and "bind" interchangeably. For example: "After the protein of interest has *associated* with the separation matrix, the separation matrix is washed to remove *unbound* protein, lysate, impurities and unwanted components of the refold solution." '878 Patent

[9] Recently, the *Apotex* court construed the word "protein" in accord with Amgen's proposed construction. *See* Dkt. 195-1 at 14-17. While the court's opinion is persuasive authority, its value goes only so far. First, the court was construing a different patent. Second, in *Apotex*, the defendant argued the word "protein" should be construed to mean "protein of interest," whereas Sandoz has not proposed such a construction.

at 15:43-46 (emphasis added). The patentee differentiated between proteins "associated with" the separation matrices and those components and proteins "unbound" from the matrices—a telling choice of words which implies the words are synonyms for the same process. In addition, when describing the elution process, the specification teaches, "[t]he protein of interest can be eluted using a solution that interferes with the *binding* of the absorbent component of the separation matrix to the protein." '878 Patent at 15:65-67. Thus, the specification equates binding with associating.

Sandoz's final argument is that the other steps listed in the claim clarify that "associate" means "bind." Step (g) of claim 7 states, "the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin." '878 Patent at 22:25-28. Figure 4 of the patent, titled "demonstration of Dynamic Binding Capacity for Ion Exchangers and Mixed mode Resins," describes "a plot demonstrating the binding profiles of a refolded, non-mammalian non-native limited solubility complex protein to six different ion exchange resins." '878 Patent at 3:22-29, Figure 4. This figure, Sandoz argues, demonstrates the ion exchanges and mixed mode resins operate by binding to proteins of interest.

Ultimately, Amgen's proposed construction does not make sense in the context of the claim as a whole. There are some interactions between resin and protein, which do not facilitate protein capture. For example, the proteins and resins may repel one another, but the repellence does not facilitate protein capture or purification. Although Amgen has provided examples of how proteins interact with separation matrices that do not involve a binding mechanism, they do not lend support for its construction. Sizing resins that trap proteins of a certain size while allowing smaller components to pass through are not non-affinity resins. Claim 7 discloses a capture method involving a non-affinity resin, *see* '878 Patent at 22:26, and therefore sheds no light on the question of whether the claimed method covers interactions other than binding interactions. Similarly, isocratic protein separations, which retard the transit of some proteins moving through a column containing a separation matrix, do not employ "reversible interactions." The interaction between the resin and the protein never reverses; the protein simply takes longer to pass through

the column. *See* Titchener-Hooker Sur-Reply Decl. ¶ 40.

Ultimately, most problematic aspect of Amgen's proposed construction is that it is confusing and no clearer than the text of the claim itself. Accordingly, the word "associate" will be construed to mean "bind."

c. *"washing the separation matrix"*

The phrase "washing the separation matrix" must be construed to shed light on the meaning of claims 7, 8, 11, 13, 14, 15, 16, and 17. Amgen proposes construing the phrase as "adding a solution into the column that contains the separation matrix, to remove materials in the refold solution that do not interact with the separation matrix." Sandoz on the other hand proposes the following construction: "applying a solution to remove unbound protein, lysate, impurities, and unwanted components of the refold solution from the separation matrix while preserving binding of the expressed protein."

The parties' disagreements are familiar and involve the meaning of "associate," whether the chromatography method described is the column method, and whether the protein captured is the expressed protein. Each of these issues has been previously addressed and resolved. The claim shall not be limited to the column method of chromatography. The claim covers the capture of proteins other than the protein of interest. Finally, the proteins bind to the separation matrix when they "associate" with it.

Nevertheless, there remains one material difference between the two proposed constructions about which the parties offer no argument for their disagreement: whether to list the components to be washed away. Sandoz lists those components (lysate, unbound protein, impurities, etc.), whereas Amgen suggests anything that "do[es] not interact with the separation matrix" will be removed. Sandoz offers no reason to list (or to limit) the components to be washed away. Amgen's proposal is therefore not only simpler, but seems accurately to describe the process set forth in claim 7.

In sum, the phrase must be construed as a hybrid of the two proposals. "Washing the separation matrix" shall mean "adding a solution to the separation matrix to remove materials in

the refold solution while preserving binding of the protein to be purified."

d. *"eluting the protein from the separation matrix"*

The final phrase to construe—"eluting the protein from the separation matrix"— informs the scope of claims 7, 8, 11, 13, 14, 15, 16, and 17. Amgen would construe the phrase to mean "adding a solution into the column that contains the separation matrix, which as the effect of reversing the interactions between protein and the separation matrix." Sandoz proposes to construe the phrase to mean "applying a solution that reverses the binding of the expressed protein to the separation matrix." Under Sandoz's proposed construction, "this step must occur after the step of 'washing the separation matrix.'"

The disputes about how properly to construe the phrase are linked to the parties' disagreement about the meaning of "associate," "protein," and "separation matrix," and have been resolved. There is, however, one unique feature of this phrase: whether the eluting step must occur after the washing step. Amgen believes this claim does not properly present the issue of the order of the steps because Sandoz did not seek to construe the word "and" (as in "washing . . . and eluting"). Indeed, Amgen is so confident of this point, it did not even respond to Sandoz's argument.

As an initial matter, Sandoz has not waived its right to seek construction of this phrase or to argue the claim has an implied order of steps. "As a general rule, unless the steps of a method claim actually recite an order, the steps are not ordinarily construed to require one." *Mformation Techs., Inc. v. Research in Motion Ltd.*, 764 F.3d 1392, 1398 (Fed. Cir. 2014) (internal quotation marks omitted). A claim may have a required order of steps, however, when "as a matter of logic or grammar, [the claim] requires that the steps be performed in the order written, or the specification directly or implicitly requires an order of steps." *Id.* (internal quotation marks omitted). Thus, by designating "eluting the protein from the separation matrix" for construction, Sandoz adequately notified Amgen of its intent to seek construction and limited the number of terms to be construed to ten, as required by the local patent rules. *See* Local Patent Rule 4-1(b).

The specification teaches, "[a]fter the separation matrix with which the protein has associated has been washed, the protein of interest is eluted using an appropriate solution." '878 Patent at 15:60-62. It further explains that the wash buffer may be comprised of any number of components so long as "[t]he pH range is chosen to optimize the chromatography conditions, *preserve protein binding*, and to retain the desired characteristics of the protein of interest." '878 Patent at 15:55-57 (emphasis added). Thus, the proteins and separation matrix should remain associated during the washing process. In contrast, elution involves cleaving the protein from the matrix with "a solution *that interferes with the binding* of the absorbent component of the separation matrix to the protein, for example by *disrupting the interactions* between Protein A and the Fc region of a protein of interest." '878 Patent at 15:65-16:2 (emphasis added). Accordingly, the specification discloses a natural, logical order of steps. If the washing and eluting steps occurred simultaneously, the protein captured by the separation matrix could once again comingle with the contaminants and components to be washed away. In light of the fact, Amgen has not offered any reasons to believe the claim does not imply a natural order, the construction of the phrase will make clear the step of "eluting the protein from the separation matrix" occurs after the step of "washing the separation matrix."

As discussed above, the method claim 7 describes is not limited to the "expressed protein" or the "protein of interest." Accordingly, the protein eluted from the separation matrix is "the purified protein." After all, if elution is the final step of the purification process, the resulting protein is "purified." Thus, the phrase "eluting the protein from the separation matrix" shall mean "applying a solution that reverses the binding of the purified protein to the separation matrix."

V. CONCLUSION

The disputed claim terms of the patents-in-suit are construed as set forth as follows:

	’427 Claim Term	Construction
1.	“hematopoietic stem cell mobilizing-effective amount of G-CSF”	An amount of G-CSF effective to mobilize hematopoietic stem cells.
2.	“A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment”	The preamble limits the scope of the claim. In the practice of the method of treating a disease, a patient receives a transplant of peripheral stem cells.
3.	“disease treating-effective amount of at least one chemotherapeutic agent”	An amount sufficient to treat a disease for which at least one chemotherapeutic agent is prescribed.
4.	“chemotherapeutic agent”	Exogenous substance suited and used to damage or destroy microorganisms, parasites, or tumor cells.
5.	“comprising administering . . . G-CSF; and thereafter administering . . . at least one chemotherapeutic agent”	The word “comprising” means “including but not limited to,” and allows for additional steps before, in between, and after the steps recited in the claim. In the practice of the method, at least one administration of G-CSF must occur before at least one administration of a chemotherapeutic agent.
6.	“opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells”	Disrupts the bone marrow endothelial barrier to facilitate permeability of the endothelial barrier for stem cells.

	’878 Claim Term	Construction
7.	“directly applying the refold solution to a separation matrix”	Applying the refold solution to a separation matrix without removing components of or diluting the refold solution.
8.	“under conditions suitable for the protein to associate with the matrix”	Under conditions suitable for the protein to be purified to bind to the matrix.
9.	“washing the separation matrix”	Applying a solution to remove unbound protein, lysate, impurities, and unwanted components of the refold solution from the separation matrix while preserving binding of the expressed protein.
10.	“eluting the protein from the separation matrix”	Applying a solution that reverses the binding of the purified protein to the separation matrix. This step must occur after the step of “washing the separation matrix.”

A further Case Management Conference shall be held on September 15, 2016, at 10:00 a.m. in Courtroom 3, 17th Floor, United States Courthouse, 450 Golden Gate Avenue, San Francisco, California. The parties shall file a Joint Case Management Statement at least one week prior to the Conference.

IT IS SO ORDERED.

Dated: August 4, 2016

RICHARD SEEBORG
United States District Judge

United States District Court
Northern District of California

Vernon M. Winters (SBN 130128)
SIDLEY AUSTIN LLP
555 California Street, Suite 2000
San Francisco, CA 94104-1503
Telephone: (415) 772-1200
Facsimile: (415) 772-7400
vwinters@sidley.com

Nicholas Groombridge (*pro hac vice*)
1285 Avenue of the Americas
New York, NY 10019-6064
Telephone: (212) 373-3000
Facsimile: (212) 757-3990
ngroombridge@paulweiss.com
Additional counsel listed below

Attorneys for Plaintiffs Amgen Inc. and Amgen Manufacturing, Limited

Erik J. Olson (SBN 175815)
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304
Telephone: 650.813.5600
Facsimile: 650.494.0792
ejolson@mofo.com
Additional counsel listed below

Attorneys for Defendants Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA
SAN FRANCISCO DIVISION

AMGEN INC. and AMGEN MANUFACTURING, LIMITED,

Plaintiffs,

v.

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, and SANDOZ GMBH,

Defendants.

Case No. 3:14-cv-04741-RS

STIPULATION AND [~~PROPOSED~~] ORDER FOR ENTRY OF JUDGMENT REGARDING U.S. PATENT NO. 6,162,427

WHEREAS Amgen Inc. and Amgen Manufacturing, Limited (collectively, "Amgen") filed a complaint against Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH (collectively, "Sandoz") in the Northern District of California (the "Court") on October 24, 2014 (Docket No. 1), and a first amended and supplemental complaint on October 15, 2015 (Docket No. 145), alleging, among other things, infringement of United States Patent Number 6,162,427 ('427 patent);

WHEREAS Sandoz has appeared and denied infringement, and Sandoz Inc. has counterclaimed for declaratory judgment of invalidity of the '427 patent (Docket Nos. 22, 149);

WHEREAS the Court construed certain disputed claim terms associated with claims 1-4, and 6 of the '427 patent ("Asserted Claims") in an order dated August 4, 2016 (Docket No. 205);

WHEREAS the parties have completed fact discovery regarding the '427 patent;

WHEREAS Amgen has provided an expert report regarding the alleged infringement of the Asserted Claims of the '427 patent, and Sandoz has provided an expert report regarding the alleged invalidity of the Asserted Claims of the '427 patent;

WHEREAS the time to add or amend infringement and invalidity contentions or add or amend the Asserted Claims has passed;

WHEREAS the parties agree that Amgen may preserve its right to appeal the claim construction order after a final judgment is entered pursuant to 28 U.S.C. §§ 1291 & 1292(c)(2);

THEREFORE Amgen and Sandoz agree that:

1. Amgen and Sandoz stipulate that Sandoz does not infringe the Asserted Claims of the '427 patent within the meaning of any provision of 35 U.S.C. § 271 in light of the claim constructions included in the August 4, 2016 order, Docket No. 205.

2. Amgen and Sandoz stipulate that the Court may enter a judgment of non-infringement in favor of Sandoz and against Amgen for Amgen's Third Cause of Action of its First Amended and Supplemental Complaint filed on October 15, 2015 (Docket No. 145) and

Sandoz's Sixth Counterclaim of Sandoz Inc.'s Answer to Amended Complaint filed November 2, 2015 (Docket No. 149).

3. Amgen and Sandoz stipulate that Sandoz Seventh Counterclaim of Sandoz Inc.'s Answer to Amended Complaint filed November 2, 2015 (Docket No. 149) for a declaration of invalidity for the '427 Patent will be dismissed without prejudice and that Sandoz will be allowed to assert the Seventh Counterclaim in the event this matter is remanded for further consideration following any appeal.

4. This Stipulation and [Proposed] Order are without prejudice to Amgen's right to appeal the Claim Construction Order (Docket No. 205), and any final judgment based thereon pursuant to 28 U.S.C. §§ 1291 & 1292(c)(2).

5. No party will conduct any further discovery or pretrial activities related to allegations of liability or damages regarding the '427 patent, including any activity related to Sandoz's alleged defense and counterclaim that the '427 patent is invalid.

6. Neither party shall be obligated to pay the opposing party any money in connection with this stipulation or resolution, and Sandoz agrees not to seek its costs with respect to the '427 patent. Neither party shall use as evidence or rely on the fact of this stipulation or the judgment in favor of Sandoz and against Amgen directed to the '427 patent to argue that this case is exceptional.

7. (i) Neither party shall use as evidence or rely on the fact of this stipulation or the judgment in favor of Sandoz and against Amgen directed to the '427 patent in connection with the continuing litigation involving United States Patent Number 8,940,878, (ii) neither party shall assert in any forum that this stipulation or the judgment in favor of Sandoz and against Amgen directed to the '427 patent is inconsistent with positions regarding infringement taken by any party or its experts prior to the date of this stipulation, and (iii) neither party shall use as evidence or rely on the contents of this stipulation or the judgment in favor of Sandoz and against Amgen directed to the '427 patent in continuing litigation relating to Amgen's unfair

competition and conversion claims except to note the fact that judgment has entered with respect to the '427 patent and that the '427 patent was the only patent asserted against Sandoz by Amgen prior to October 15, 2015. For the avoidance of doubt, this stipulation has no impact on the claims, defenses, or prayer for relief of either party related to the validity of, infringement of, or relief available for the '878 patent.

8. Neither party shall issue a press release or make an affirmative press statement regarding this stipulation.

Respectfully submitted,

Dated: September 13, 2017

By: */s/ Nicholas Groombridge*
Nicholas Groombridge
PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
1285 Avenue of the Americas
New York, NY 10019-6064
Telephone: (212) 373-3000
Facsimile: (212) 757-3990
ngroombridge@paulweiss.com

Vernon M. Winters (SBN 130128)
SIDLEY AUSTIN LLP
555 California Street, Suite 2000
San Francisco, CA 94104-1503
Telephone: (415) 772-1200
Facsimile: (415) 772-7400
vwinters@sidley.com

Attorneys for Amgen Inc. and Amgen Manufacturing, Ltd.

Dated: September 13, 2017

By: */s/ Erik J. Olson*
Erik J. Olson (SBN 175815)
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304
Telephone: 650.813.5600
Facsimile: 650.494.0792
ejolson@mofo.com

Attorneys for Defendants Sandoz Inc., Sandoz International GmbH, and Sandoz GmbH

SIGNATURE ATTESTATION

Pursuant to Civil Local Rule 5-1(i)(3), I hereby certify that concurrence in the filing of this document has been obtained from each of the other Signatories shown above.

Dated: September 13, 2017

By: /s/ Sue Wang
Sue Wang

PURSUANT TO STIPULATION, IT IS SO ORDERED.

Dated: 9/13, 2017

THE HONORABLE RICHARD SEEBORG
UNITED STATES DISTRICT COURT JUDGE

ADDITIONAL COUNSEL

SIDLEY AUSTIN LLP
Sue Wang (SBN 286247)
555 California Street, Suite 2000
San Francisco, CA 94104-1503
Telephone: (415) 772-1200
Facsimile: (415) 772-7400
Email: abaxter@sidley.com

PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
Eric Alan Stone (*pro hac vice*)
Jennifer H. Wu (*pro hac vice*)
Jennifer Gordon
Peter Sandel (*pro hac vice*)
Stephen A. Maniscalco (*pro hac vice*)
1285 Avenue of the Americas
New York, NY 10019-6064
Telephone: (212) 373-3000
Facsimile: (212) 757-3990
Email: estone@paulweiss.com
jwu@paulweiss.com
psandel@paulweiss.com
jgordon@paulweiss.com
smaniscalco@paulweiss.com

AMGEN INC.
Wendy A. Whiteford (SBN 150283)
Lois M. Kwasigroch (SBN 130159)
One Amgen Center Drive
Thousand Oaks, CA 91320-1789
Telephone: (805) 447-1000
Facsimile: (805) 447-1010
Email: wendy@amgen.com

Attorneys for Plaintiffs Amgen Inc. and Amgen Manufacturing Limited

MORRISON & FOERSTER LLP
Eric C. Pai (SBN 247604)
755 Page Mill Road
Palo Alto, California 94304
Telephone: 650.813.5600
Facsimile: 650.494.0792
Email: epai@mofo.com

Stephen David Keane (S.B.N. 247588)
12531 High Bluff Drive, Ste. 100
San Diego, California 92130
Telephone: (858) 720-5100
Facsimile: (858) 720-5125
Email: skeane@mofo.com

Attorneys for Defendants Sandoz Inc., Sandoz GmbH, Sandoz International GmbH, and Lek Pharmaceuticals, d.d.

SIDLEY AUSTIN LLP
Vernon M. Winters (SBN 130128)
Alexander D. Baxter (SBN 281569)
555 California Street, Suite 2000
San Francisco, CA 94104-1503
Telephone: (415) 772-1200
Facsimile: (415) 772-7400
vwinters@sidley.com

PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
Nicholas Groombridge (*pro hac vice*)
Eric Alan Stone (*pro hac vice)*
Jennifer H. Wu (*pro hac vice*)
Jennifer Gordon
Peter Sandel (*pro hac vice*)
Ana Friedman (*pro hac vice*)
Arielle K. Linsey (*pro hac vice*)
Stephen A. Maniscalco (*pro hac vice*)
1285 Avenue of the Americas
New York, NY 10019-6064
Telephone: (212) 373-3000
Facsimile: (212) 757-3990
ngroombridge@paulweiss.com

AMGEN INC.
Wendy A. Whiteford (SBN 150283)
Lois M. Kwasigroch (SBN 130159)
One Amgen Center Drive
Thousand Oaks, CA 91320-1789
Telephone: (805) 447-1000
Facsimile: (805) 447-1010
wendy@amgen.com

Attorneys for Plaintiffs Amgen Inc. and Amgen Manufacturing Limited

KIRKLAND & ELLIS LLP
James Beard (SBN 267242)
555 California Street, Suite 2000
San Francisco, CA 94104
Telephone: (415) 439-1689
james.beard@kirkland.

James F. Hurst (*pro hac vice*)
Cristina Q. Almendarez (*pro hac vice*)
300 North LaSalle
Chicago, IL 60654
Telephone: (312) 862-2000
james.hurst@kirkland.com
cristina.almendarez@kirkland.com

Jeanna M. Wacker (*pro hac vice*)
601 Lexington Avenue
New York, NY 10022
Telephone: (212) 446-4800
jeanna.wacker@kirkland.com

Attorneys for Defendants Sandoz Inc.

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA
SAN FRANCISCO DIVISION

AMGEN INC. and AMGEN MANUFACTURING LIMITED,

Plaintiffs,

vs.

SANDOZ INC., SANDOZ INTERNATIONAL GMBH, and SANDOZ GMBH, LEK PHARMACEUTICALS, D.D.

Defendants.

Case No. 3:16-CV-02581-RS

JOINT STIPULATION OF DISMISSAL OF ALL CLAIMS AND COUNTERCLAIMS RELATED TO U.S. PATENT NO. 5,824,784, AND ~~PROPOSED~~ ORDER

Pursuant to Civil Local Rule 7-12, Plaintiffs Amgen Inc. and Amgen Manufacturing Limited (collectively, "Amgen") and Defendant Sandoz Inc., by and through their counsel, jointly stipulate to the dismissal, without prejudice, of all claims and counterclaims related to U.S. Patent No. 5,824,784 ("the '784 Patent") on the terms set forth herein:

1. Amgen's cause of action directed solely to the '784 Patent, specifically the Third Cause of Action of its Complaint filed May 12, 2016 [Dkt. No. 1], is hereby dismissed without prejudice.

2. Sandoz Inc.'s counterclaims directed solely to the '784 Patent, specifically the Third Counterclaim and the Fourth Counterclaim of Sandoz Inc.'s Answer and Affirmative Defenses and Counterclaims filed June 23, 2016 [Dkt. No. 18], are hereby dismissed without prejudice.

3. The parties agree that neither party is a prevailing party with respect to the '784 Patent, and accordingly no party shall be entitled to attorneys' fees or costs with respect to the '784 Patent, either now or at any future point in the case. To avoid any doubt, this stipulated dismissal of the '784 Patent shall play no role in any argument for or determination of attorneys' fees and costs in this litigation.

Dated: December 1, 2016
Respectfully submitted,

By: */s/ Vernon M. Winters*
Vernon M. Winters (SBN 130128)
Alexander D. Baxter (SBN 281569)
SIDLEY AUSTIN LLP
555 California Street, Suite 2000
San Francisco, CA 94104-1503
Telephone: (415) 772-1200
Facsimile: (415) 772-7400
vwinters@sidley.com

Nicholas Groombridge (*pro hac vice*)
Eric Alan Stone (*pro hac vice)*
Jennifer H. Wu (*pro hac vice*)
Jennifer Gordon
Peter Sandel (*pro hac vice*)
Ana Friedman (*pro hac vice*)
Arielle K. Linsey (*pro hac vice*)
Stephen A. Maniscalco (*pro hac vice*)
PAUL, WEISS, RIFKIND, WHARTON & GARRISON LLP
1285 Avenue of the Americas
New York, NY 10019-6064
Telephone: (212) 373-3000
Facsimile: (212) 757-3990
ngroombridge@paulweiss.com

Wendy A. Whiteford (SBN 150283)
Lois M. Kwasigroch (SBN 130159)
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, CA 91320-1789
Telephone: (805) 447-1000
Facsimile: (805) 447-1010
wendy@amgen.com

Attorneys for Plaintiffs Amgen Inc. and Amgen Manufacturing Limited

By: */s/ James Beard*
James Beard (SBN 267242)
555 California Street, Suite 2000
San Francisco, CA 94104
Telephone: (415) 439-1689
james.beard@kirkland.

James F. Hurst (*pro hac vice*)
Cristina Q. Almendarez (*pro hac vice*)
300 North LaSalle
Chicago, IL 60654
Telephone: (312) 862-2000
james.hurst@kirkland.com
cristina.almendarez@kirkland.com

Jeanna M. Wacker (*pro hac vice*)
601 Lexington Avenue
New York, NY 10022
Telephone: (212) 446-4800
jeanna.wacker@kirkland.com

Attorneys for Defendants Sandoz Inc.

ECF ATTESTATION

I, Vernon M. Winters, am the ECF User whose ID and Password are being used to file this document. I attest that concurrence in the filing of this document has been obtained from the above signatories.

Dated: December 1, 2016

SIDLEY AUSTIN LLP

By: */s/ Vernon M. Winters*

PURSUANT TO STIPULATION, IT IS SO ORDERED.

Dated: 12/7, 2016

THE HONORABLE RICHARD SEEBORG
UNITED STATES DISTRICT COURT JUDGE

US008940878B2

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

(10) **Patent No.: US 8,940,878 B2**
(45) **Date of Patent: *Jan. 27, 2015**

(54) **CAPTURE PURIFICATION PROCESSES FOR PROTEINS EXPRESSED IN A NON-MAMMALIAN SYSTEM**

(75) Inventors: **Joseph Edward Shultz**, Santa Rosa Valley, CA (US); **Roger Hart**, Loveland, CO (US)

(73) Assignee: **Amgen Inc.**, Thousand Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 471 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/822,990**

(22) Filed: **Jun. 24, 2010**

(65) **Prior Publication Data**

US 2010/0331526 A1 Dec. 30, 2010

Related U.S. Application Data

(60) Provisional application No. 61/220,477, filed on Jun. 25, 2009.

(51) **Int. Cl.**

C07K 1/22	(2006.01)
C07K 1/14	(2006.01)
C07K 1/18	(2006.01)
C07K 1/32	(2006.01)

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

CPC . ***C07K 1/22*** (2013.01); ***C07K 1/145*** (2013.01); ***C07K 1/18*** (2013.01); ***C07K 1/32*** (2013.01)

USPC **530/413**

(58) **Field of Classification Search**

CPC C07K 1/22; C07K 1/18; C07K 1/32; C07K 1/145

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,466,377	A	11/1995	Grandics et al.	
6,660,843	B1	12/2003	Feige et al.	
6,972,327	B1 *	12/2005	Madani et al.	530/413
7,138,370	B2	11/2006	Oliner et al.	
7,511,012	B2	3/2009	Han et al.	

FOREIGN PATENT DOCUMENTS

WO	WO97/28272	8/1997
WO	WO2009/023270	2/2009

OTHER PUBLICATIONS

Pavlinkova et al. (Nuclear Med. Biol., 26:27-34, 1999).*

Ronnmark et al. (J. Immunologic. Meth., 261:199-211, 2002).*

Tengliden (Development of Cleaning-In-Place Procedures for Protein a Chromatography Resins Using Design of Experiments and High Throughput Screening Technologies, Masters Thesis, Linkoping University, Feb. 2008).*

Amersham Biosciences (Amersham Biosciences Sepharose instructions, Edition AE, p. 1-12, 2002).*

Arvidsson, P. et al., "Direct chromatographic capture of enzyme from crude homogenate using immobilized metal affinity chromatography on a continuous supermacroporous adsorbent," J. Chromatography 986 (2): 276-290 (2003).

Ling et al., "Integration of mechanical cell disruption and fluidised bed recovery of G3PHD from unclarified disrupted yeast: a comparative study of the performance of unshielded and polymer shielded dye-ligand chromatography systems," J. Biotech. 119(4): 436-448 (2005).

Fischer, B. et al., "Isolation renaturation and formation of disulfide bonds of eukaryotic proteins expressed in *escherichia coli* as inclusion bodies," Biotech. and Bioengineering, 41(1): 3-13 (1993).

Ford et al., "Affinity purification of novel bispecific antibodies recognising carcinoembryonic antigen and doxorubicin," J. Chromatogr. B, 754: 427-435 (2001).

Shukla et al., "Downstream processing of monoclonal antibodies—Application of platform approaches," Journal of Chromatography B, 848(1):28-39 (2007).

Wang et al., "Perturbation of the antigen-binding site and staphylococcal protein A-binding site of IgG before significant changes in global conformation during denaturation: an equilibrium study," Biochem. J. 325(Part 3):707-710 (1997).

Hasemann & Capra, "Immunoglobulins: Structure and Function," in William E. Paul, ed., Fundamental Immunology, Second Edition, 209, 210-218 (1989).

Ostrove, "Affinity Chromatography: General Methods," Guide to Protein Purification, Methods in Enzymology 182: 371-379 (1990).

Devereux et al., "A comprehensive set of sequence analysis programs for the VAX," Nucleosides, nucleotides; & nucleic acids: Nucl. Acids Res. 12: 387-389 (1984).

Gribskov and Burgess, "Sigma factors from *E. coli, B. subtilis*; phage SP01, and phage T4 are homologous proteins," Nucl. Acids Res. 14: 6745 (1986).

Gulich, Susanne, et al., "Protein engineering of an IgG-binding domain allows milder elution conditions during affinity chromatography," J. Biol. 76, Issues 2-3, pp. 233-244 (2000).

Ostrove "Affinity Chromatography: General Methods," Guide to Protein Purification, Methods in Enzymology 182: 357-371 (1990).

Stoscheck, C., "Quantitation of Protein," Guide to Protein Purification, Methods in Enzymology 182: 50-68 (1990).

Vola et al., "Recombinant proteins L and LG. Two new tools for purification of murine antibody fragments," Cell Biophys. 24-25: 27-36 (1994).

Aybay and Imir "Development of a rapid, single-step procedure using protein G affinity chromatography to deplete fetal calf serum of its IgG and to isolate murine IgG1 monoclonal antibodies from supernatants of hybridoma cells," Int'l. J. Immunol. Methods 233(1-2): 77-81 (2000).

Ejima, Daisuke, et al., Effective elution of antibodies by arginine and arginine derivatives in affinity column chromatography, Analytical Biochem. 345 250-257 (2005).

(Continued)

Primary Examiner — Brian J Gangle
(74) *Attorney, Agent, or Firm* — David B. Ran

(57) **ABSTRACT**

Methods of purifying proteins expressed in non-mammalian expression systems in a non-native soluble form directly from cell lysate are disclosed. Methods of purifying proteins expressed in non-mammalian expression systems in a non-native limited solubility form directly from a refold solution are also disclosed. Resin regeneration methods are also provided.

25 Claims, 5 Drawing Sheets

(56) **References Cited**

OTHER PUBLICATIONS

Arakawa, Tsutomu et al., "Elution of antibodies from a Protein-A column by acqueous arginine solutions," Protein Express & Purif., 36: 244-248 (2004).

Miller, Timothy et al., The rapid isolation of ribonuclease-free immunoglobulin G by protein A-sepharose affinity chromatography, J. Immun. Methods 24: 111-125 (1978).

Snyder et al., "Characterization of DC-SIGN/R Interaction with Human Immunodeficiency Virus Type 1 gp 120 and ICAM Molecules favors the receptor's role as an antigen-capturing rather than an adhesion receptor," *J. Virology* 79(8): 4589-4598, Apr. 2005.

GE Healthcare Instructions 71-7089-00AE: Affinity media, Protein A Sepharose CL-4B, p. 1-8, Mar. 2006. (Cited in JP Office action as D5 http://eclub.biomart.cn/sites/eclub.biomart.cn/themes/aktaclub/Files/71708900AE__UM__Protein__A__Sepharose__CL-4B.pdf, Mar. 2006).

GE Healthcare Instructions 71-5002-60 AE: Ion exchange chromatography; Q Sepharose XL, XL virus licensed, SP Sepharose XL, pp. 1-16, Feb. 2006 (cited in JP Office action as D6https://www.gelifesciences.com/gchels__images/GELS/Related%20Content/Files/1314723116657/litdoc71500260AE__20110830185637.pdf, Feb. 2006).

* cited by examiner

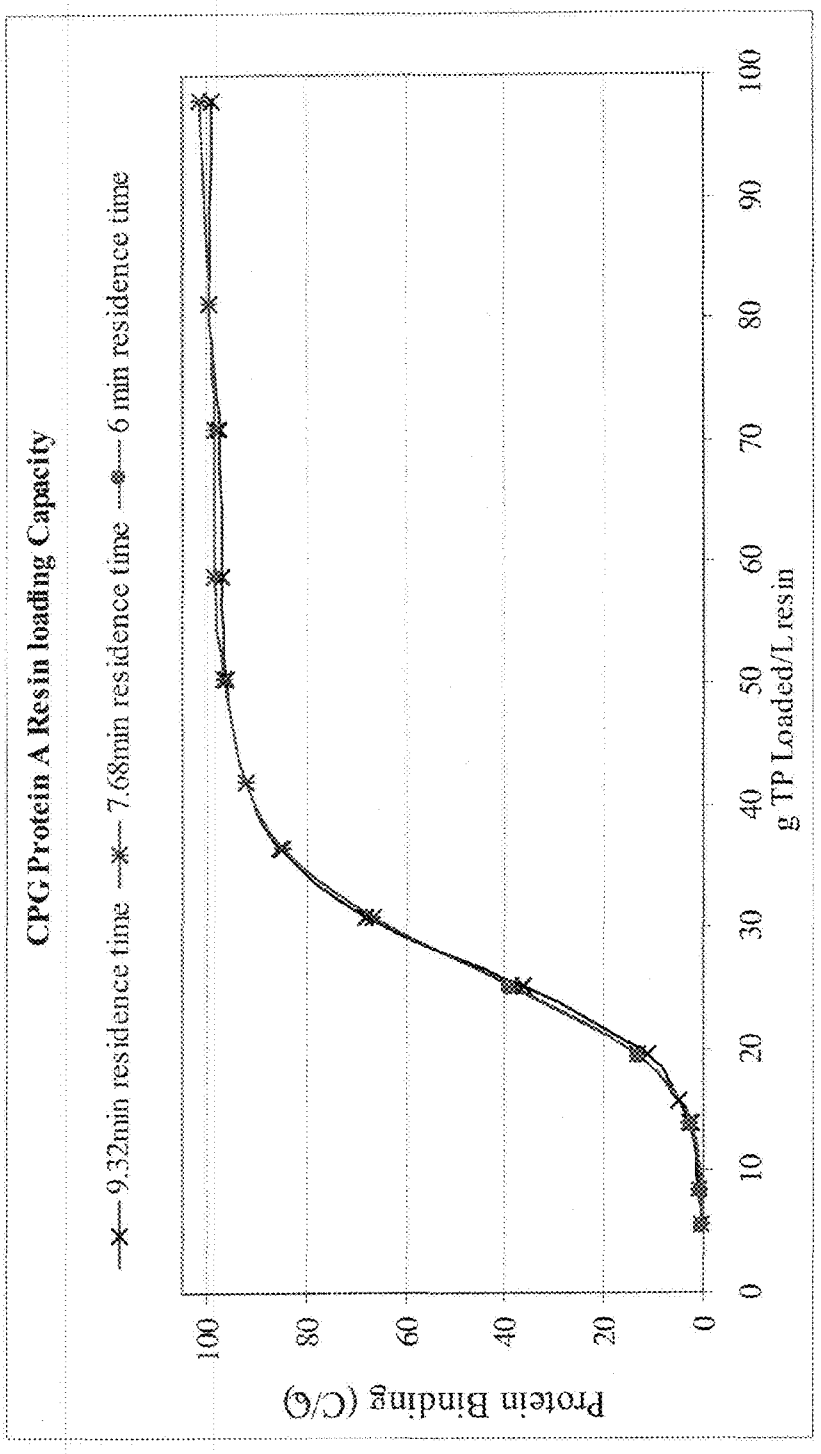
CPG Protein A Resin loading Capacity
9.32min residence time
7.68min residence time
6 min residence time
Protein Binding (C/C0)
0
20
40
60
80
100
0
10
20
30
40
50
60
70
80
90
100
g TP Loaded/L resin

Figure 1

		Average Purity					
		RP-HPLC Main Peak Purity (%)	SE-HPLC Main Peak Purity (%)	CE-SDS Main Peak Purity (%)	Host Protein Level (ppm)	DNA Level (pg/mg protein)	Average Yield (%)
Load	Average (n=13)	34.5	74.5	79.2	9100.0	>70000	-
	Std. Dev (n=13)	2.4	2.7	4.4	424.3	*	-
Purified Pool	Average (n=17)	41.3	68.8	84.7	41.0	215.2	81.7
	Std. Dev (n=17)	1.5	3.8	4.0	5.7	301.2	12.3

* Data limited to N=1

Figure 2

		Average Purity					
		RP-HPLC Main Peak Purity (%)	SE-HPLC Main Peak Purity (%)	CE-SDS Main Peak Purity (%)	Host Protein Level (ppm)	DNA Level (pg/mg protein)	Average Yield (%)
Load	Average (n=5)	36.0	76.1	75.5	1400.0	>70000	-
	Std. Dev (n=5)	0.9	1.9	1.5	*	*	-
Purified Pool	Average (150 cycles)	40.2	75.0	82.4	71.4	89.2	84.3
	Std. Dev (150 cycles)	2.5	8.7	4.6	23.0	175.0	18.8

* Data limited to N=1

Figure 3

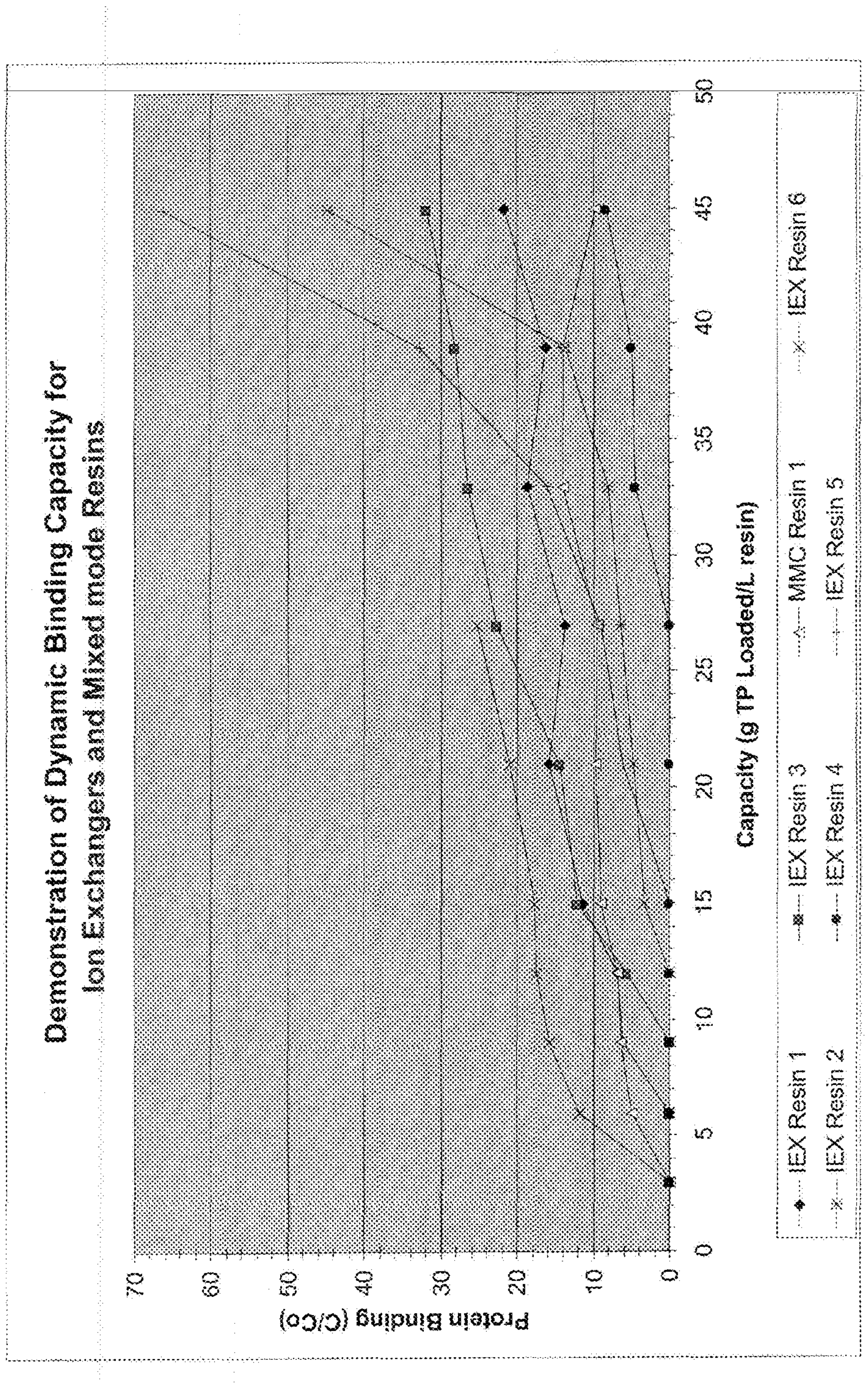
Demonstration of Dynamic Binding Capacity for
Ion Exchangers and Mixed mode Resins
Protein Binding (C/Co)
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
Capacity (g TP Loaded/L resin)
IEX Resin 1
IEX Resin 3
MMC Resin 1
IEX Resin 6
IEX Resin 2
IEX Resin 4
IEX Resin 5

Figure 4

	RP-HPLC Main Peak Purity (%)	SE-HPLC Main Peak Purity (%)	Average Yield (%)
Load	29.8	64.6	-
CEX	46.0	80.3	62.0
AEX	30.9	75.7	85.0

Figure 5

CAPTURE PURIFICATION PROCESSES FOR PROTEINS EXPRESSED IN A NON-MAMMALIAN SYSTEM

This application claims the benefit of U.S. Provisional Application No. 61/220,477 filed Jun. 25, 2009, which is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates generally to processes for purifying proteins expressed in non-mammalian systems in both non-native soluble and non-native insoluble forms, and more particularly to the direct capture of such proteins from a refold mixture or a cell lysate pool by a separation matrix.

BACKGROUND OF THE INVENTION

Fc-containing proteins are typically expressed in mammalian cells, such as CHO cells. The use of affinity chromatography to purify Fc-containing proteins is documented (see, e.g., Shukla et al., (2007) *Journal of Chromatography B* 848 (1):28-39) and is successful, in part, due to the degree of Fc structure observed in proteins expressed in such systems. Fc-containing proteins expressed in non-mammalian cells, however, are often deposited in the expressing cells in limited solubility forms, such as inclusion bodies, that require refolding, and this has been a limiting factor in selecting non-mammalian systems for expressing Fc-containing proteins.

A drawback to the use of Protein A, Protein G and other chemistries is that in order for a protein comprising an Fc region to associate with the Protein A or Protein G molecule, the protein needs to have a minimum amount of structure. Often, the requisite amount of structure is absent from proteins expressed recombinantly in a soluble, but non-native, form and consequently Protein A chromatography is not performed in a purification process.

In the case of a protein expressed in an insoluble non-native form, Protein A chromatography is typically not performed in a purification process until after the protein has been refolded to a degree that it can associate with the Protein A molecule and has been subsequently diluted out of its refold solution. This is because it was believed that after a protein has been refolded it was necessary to dilute or remove the components of the refold mixture in a wash step, due to the tendency of the components that typically make up a refold solution to disrupt interactions between the target protein and the Protein A molecules (Wang et al., (1997). *Biochem. J.* 325 (Part 3):707-710). This dilution step can consume time and resources which, when working at a manufacturing scale of thousands of liters of culture, can be costly.

The present disclosure addresses these issues by providing simplified methods of purifying proteins comprising Fc regions that are expressed in non-mammalian expression systems in a non-native soluble form or in a non-native insoluble form.

SUMMARY OF THE INVENTION

A method of purifying a protein expressed in a non-native soluble form in a non-mammalian expression system is provided. In one embodiment the method comprises (a) lysing a non-mammalian cell in which the protein is expressed in a non-native soluble form to generate a cell lysate; (b) contacting the cell lysate with an separation matrix under conditions suitable for the protein to associate with the separation matrix; (c) washing the separation matrix; and (d) eluting the protein from the separation matrix.

The protein can be a complex protein, such as a protein is selected from the group consisting of a multimeric protein, an antibody and an Fc fusion protein. The non-mammalian expression system can comprise bacteria or yeast cells. The separation matrix can be an affinity resin, such as an affinity resin selected from the group consisting of Protein A, Protein G and a synthetic mimetic affinity resin, or it can be a non-affinity resin, such as a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin. The cell lysate can be filtered before it is contacted with the separation matrix. Although not required, the method can further comprise refolding the protein to its native form after it is eluted from the separation matrix.

A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system is provided. In one embodiment that method comprises (a) expressing a protein in a non-native limited solubility form in a non-mammalian cell; (b) lysing a non-mammalian cell; (c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following: (i) a denaturant; (ii) a reductant; and (iii) a surfactant; (d) forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of the following: (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer; and (iv) a redox component; (e) applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix; (f) washing the separation matrix; and (g) eluting the protein from the separation matrix.

The non-native limited solubility form can be a component of an inclusion body. The protein can be a complex protein, such as a complex protein selected from the group consisting of a multimeric protein, an antibody, a peptibody, and an Fc fusion protein. The non-mammalian expression system can be bacteria or yeast cells. The denaturant can comprise one or more of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea, the reductant can comprise one or more of cysteine, DTT, beta-mercaptoethanol and glutathione, the surfactant can comprise one or more of sarcosyl and sodium dodecylsulfate, the aggregation suppressor can be selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, tris, sodium sulfate, potassium sulfate and osmolytes, the protein stabilizer can comprise one or more of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, tris, sodium sulfate, potassium sulfate and osmolytes, and the redox component can comprise one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol. The separation matrix can be an affinity resin such as an affinity resin selected from the group consisting of Protein A, Protein G, and synthetic mimetic affinity resin or the separation matrix can be a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin.

In other embodiments, the disclosed methods can further comprise the steps of (a) washing the separation matrix with a regeneration reagent; and (b) regenerating the separation matrix. The regeneration reagent can be one of a strong base, such as sodium hydroxide or a strong acid, such as phosphoric acid. The regenerating can comprise washing the separation matrix with a solution comprising one or both of a chaotrope present at a concentration of 4-6 M and a reductant. The

chaotrope can be one of urea, dimethyl urea, methylurea, ethylurea, and guanidinium, and the reductant can be one of cysteine, DTT, beta-mercaptoethanol and glutathione. In a particular embodiment the regenerating comprises washing the separation matrix with a solution comprising 50 mM Tris, 10 mM citrate, 6M urea, 50 mM DTT at pH 7.4.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a plot demonstrating the binding of refolded, non-mammalian non-native limited solubility fraction complex protein, to Protein A media; in the figure the X denotes resin loading at a 9.32 min residence time, star denotes resin loading at a 7.68 min residence time and solid circles denote resin loading at a 6 min residence time.

FIG. **2** is a table demonstrating purification of a complex protein comprising an Fc domain using Protein A resin.

FIG. **3** is a table demonstrating the reusability of Protein A resin when used to capture a non-mammalian non-native limited solubility complex protein over 150 cycles using the disclosed methods.

FIG. **4** is a plot demonstrating the binding profiles of a refolded, non-mammalian non-native limited solubility complex protein to six different ion exchange resins (IEX Resins 1, 2, 3, 4, 5, 6, corresponding to Toyopearl SP550C™, Toyopearl SP650M™, GigaCAP S™, POROS HS50™, Toyopearl SP650C™ and GE Healthcare SPxL™, respectively) and a mixed-mode resin (MMC Resin 1, GE Healthcare MMC™) following capture using the disclosed methods.

FIG. **5** is a table demonstrating purification levels achieved for a protein comprising an Fc domain using one anion exhange resin (Fractogel TMAE™) and one cation exchange resin (Fractogel SO_3^-™).

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides methods of capturing on a separation matrix non-native proteins produced in microbial cells. In the case of the direct capture of a protein expressed in a non-native soluble form the advantages of the present invention over typical processes include enhanced protein concentration, volume reduction, and increased recovery over traditional methods, improved protein stability, and ultimately process cost savings.

In the case of the direct capture of a protein expressed in a non-native limited solubility form, the advantages of the present invention over typical processes include the elimination of the need to dilute the protein out of a refold solution prior to capturing it on a separation matrix.

Another advantage of the disclosed methods is that they may be performed at a range of scales, from laboratory scale (typically milliliter or liter scale), a pilot plant scale (typically hundreds of liters) or on an industrial scale (typically thousands of liters). The application of the disclosed methods on large scales may be particularly desirable, due to the potential savings in time and resources.

Non-mammalian, e.g., microbial, cells can naturally produce, or can be engineered to produce, proteins that are expressed in either a soluble or a limited solubility form. Most often, engineered non-mammalian cells will deposit the recombinant proteins into large limited solubility aggregates called inclusion bodies. However, certain cell growth conditions (e.g., temperature or pH) can be modified to drive the recombinant proteins to be expressed as intracellular, soluble monomers. As an alternative to producing a protein of interest in cells in which the protein is expressed in the form of limited solubility inclusion bodies, cell growth conditions can be modified such that proteins are expressed in a non-native yet soluble form. The cells can then be lysed and the protein can be isolated by capturing it directly from cell lysate using ion exchange chromatography, affinity chromatography or mixed mode chromatography, as described herein. The method can be particularly useful for purifying proteins comprising an Fc region.

In one aspect, therefore, the present disclosure relates to a method of isolating a protein of interest comprising an Fc region that is expressed in a non-mammalian cell in a non-native, yet soluble form, from a pool of lysate generated from the cell in which the protein was expressed. The method employs a separation matrix, such as Protein A. One beneficial aspect of the disclosed method is that it eliminates the need for a refolding step before the protein is applied to the separation matrix. That is, non-mammalian cells expressing the protein of interest in a non-native soluble form can be lysed, the lysate applied directly to the separation matrix and the protein subsequently eluted from the separation matrix. This process allows the separation of proteins from cell cultures in highly concentrated pools that can be subsequently refolded at high concentrations and can be of benefit when producing large quantities of protein, particularly since the method is scalable from bench scale, which involves cultures on the order of several liters, up to production scale, which involves cultures of thousands of liters.

Following isolation by the separation matrix, the protein of interest can optionally be subsequently refolded using any technique known or suspected to work well for the protein of interest.

In another aspect, the present invention relates to a method of isolating a protein of interest comprising an Fc region that is expressed in a non-native limited solubility form, for example in inclusion bodies, that needs to be refolded and isolated from the refold mixture. Commonly, a refold solution contains a denaturant (e.g., urea or other chaotrope, organic solvent or strong detergent), an aggregation suppressor (e.g., a mild detergent, arginine or low concentrations of PEG), a protein stabilizer (e.g., glycerol, sucrose or other osmolyte, salts) and/or a redox component (e.g., cysteine, cystine, cystamine, cysteamine, glutathione). While often beneficial for refolding proteins, these components can inhibit purification (see, e.g., Wang et al., (1997) *Biochemical Journal* 325 (Part 3):707-710) and it is necessary to isolate or dilute the protein from these components for further processing, particularly before applying the protein to a separation matrix.

In one embodiment of the disclosed method, purification is achieved by directly applying a protein of interest, which is present in a refold mixture, to a separation matrix. In this approach, following a refold step the entire refold mixture, including the protein of interest, is applied directly to a separation matrix, such as a Protein A or G resin. The protein of interest associates with the matrix in the presence of the components of refold buffer, impurities are washed away and the protein is eluted. Since the method omits the need for removing any components of the refold mixture before the refold mixture is applied to a separation matrix, the method can have the effect of saving steps, time and resources that are typically expended on removing the protein from refolding and dilution buffers in purification processes. In some cases, the method can also reduce or eliminate the need for subsequent purification steps.

The disclosed methods can also be employed to purify proteins expressed in a non-native soluble and non-native limited solubility forms in a non-mammalian expression system that have subsequently been derivatized. For example, following expression a protein comprising an Fc region can

be associated with a small molecule, such as a toxin. Such conjugates can be purified using the methods described herein.

I. DEFINITIONS

As used herein, the terms "a" and "an" mean one or more unless specifically indicated otherwise.

As used herein, the term "non-mammalian expression system" means a system for expressing proteins in cells derived from an organism other than a mammal, including but not limited to, prokaryotes, including bacteria such as *E. coli*, and yeast. Often a non-mammalian expression system is employed to express a recombinant protein of interest, while in other instances a protein of interest is an endogenous protein that is expressed by a non-mammalian cell. For purposes of the present disclosure, regardless of whether a protein of interest is endogenous or recombinant, if the protein is expressed in a non-mammalian cell then that cell is a "non-mammalian expression system." Similarly, a "non-mammalian cell" is a cell derived from an organism other than a mammal, examples of which include bacteria or yeast.

As used herein, the term "denaturant" means any compound having the ability to remove some or all of a protein's secondary and tertiary structure when placed in contact with the protein. The term denaturant refers to particular chemical compounds that affect denaturation, as well as solutions comprising a particular compound that affect denaturation. Examples of denaturants that can be employed in the disclosed method include, but are not limited to urea, guanidinium salts, dimethyl urea, methylurea, ethylurea and combinations thereof.

As used herein, the term "aggregation suppressor" means any compound having the ability to disrupt and decrease or eliminate interactions between two or more proteins. Examples of aggregation suppressors can include, but are not limited to, amino acids such as arginine, proline, and glycine; polyols and sugars such as glycerol, sorbitol, sucrose, and trehalose; surfactants such as, polysorbate-20, CHAPS, Triton X-100, and dodecyl maltoside; and combinations thereof.

As used herein, the term "protein stabilizer" means any compound having the ability to change a protein's reaction equilibrium state, such that the native state of the protein is improved or favored. Examples of protein stabilizers can include, but are not limited to, sugars and polyhedric alcohols such as glycerol or sorbitol; polymers such as polyethylene glycol (PEG) and α-cyclodextrin; amino acids salts such as arginine, proline, and glycine; osmolytes and certain Hoffmeister salts such as Tris, sodium sulfate and potassium sulfate; and combinations thereof.

As used herein, the terms "Fc" and "Fc region" are used interchangeably and mean a fragment of an antibody that comprises human or non-human (e.g., murine) C_{H2} and C_{H3} immunoglobulin domains, or which comprises two contiguous regions which are at least 90% identical to human or non-human C_{H2} and C_{H3} immunoglobulin domains. An Fc can but need not have the ability to interact with an Fc receptor. See, e.g., Hasemann & Capra, "Immunoglobulins: Structure and Function," in William E. Paul, ed., *Fundamental Immunology*, Second Edition, 209, 210-218 (1989), which is incorporated by reference herein in its entirety.

As used herein, the terms "protein" and "polypeptide" are used interchangeably and mean any chain of at least five naturally or non-naturally occurring amino acids linked by peptide bonds.

As used herein, the term "complex molecule" means any protein that is (a) larger than 20,000 MW, or comprises greater than 250 amino acid residues, and (b) comprises two or more disulfide bonds in its native form. A complex molecule can, but need not, form multimers. Examples of complex molecules include but are not limited to, antibodies, peptibodies and polypeptides comprising an Fc domain and other large proteins. Peptibodies are described in U.S. Pat. No. 6,660,843, U.S. Pat. No. 7,138,370 and U.S. Pat. No. 7,511,012.

As used herein, the term "peptibody" refers to a polypeptide comprising one or more bioactive peptides joined together, optionally via linkers, with an Fc domain. See U.S. Pat. No. 6,660,843, U.S. Pat. No. 7,138,370 and U.S. Pat. No. 7,511,012 for examples of peptibodies.

As used herein, the terms "Fc fusion" and "Fc fusion protein" are used interchangeably and refer to a peptide or polypeptide covalently attached to an Fc domain.

As used herein the term "Protein A" means any protein identical or substantially similar to Staphylococcal Protein A, including commercially available and/or recombinant forms of Protein A. For the purposes of this invention, Protein A specifically includes engineered Protein A derived media, such as Mab Select SuRe™ media (GE Healthcare), in which a single subunit (e.g., the B subunit) is replicated two or more times and joined in a contiguous sequence to form a recombinant Protein A molecule, and other non-naturally occurring Protein A molecules.

As used herein, the term "Protein G" means any protein identical or substantially similar to Streptococcal Protein G, including commercially available and/or recombinant forms of Protein G.

As used herein, the term "substantially similar," when used in the context of a protein, including Protein A, means proteins that are at least 80%, preferably at least 90% identical to each other in amino acid sequence and maintain or alter in a desirable manner the biological activity of the unaltered protein. Included in amino acids considered identical for the purpose of determining whether proteins are substantially similar are amino acids that are conservative substitutions, unlikely to affect biological activity, including the following: Ala for Ser, Val for Ile, Asp for Glu, Thr for Ser, Ala for Gly, Ala for Thr, Ser for Asn, Ala for Val, Ser for Gly, Tyr for Phe, Ala for Pro, Lys for Arg, Asp for Asn, Leu for Ile, Leu for Val, Ala for Glu, Asp for Gly, and these changes in the reverse. See, e.g., Neurath et al., *The Proteins*, Academic Press, New York (1979). The percent identity of two amino sequences can be determined by visual inspection and mathematical calculation, or more preferably, the comparison is done by comparing sequence information using a computer program such as the Genetics Computer Group (GCG; Madison, Wis.) Wisconsin package version 10.0 program, "GAP" (Devereux et al., 1984, *Nucl. Acids Res.* 12: 387) or other comparable computer programs. The preferred default parameters for the "GAP" program includes: (1) the weighted amino acid comparison matrix of Gribskov and Burgess ((1986), *Nucl. Acids Res.* 14: 6745), as described by Schwartz and Dayhoff, eds., *Atlas of Polypeptide Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358 (1979), or other comparable comparison matrices; (2) a penalty of 30 for each gap and an additional penalty of 1 for each symbol in each gap for amino acid sequences; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by those skilled in the art of sequence comparison can also be used.

As used herein, the terms "isolate" and "purify" are used interchangeably and mean to reduce by 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95%, or more, the

amount of heterogenous elements, for example biological macromolecules such as proteins or DNA, that may be present in a sample comprising a protein of interest. The presence of heterogenous proteins can be assayed by any appropriate method including High-performance Liquid Chromatography (HPLC), gel electrophoresis and staining and/or ELISA assay. The presence of DNA and other nucleic acids can be assayed by any appropriate method including gel electrophoresis and staining and/or assays employing polymerase chain reaction.

As used herein, the term "separation matrix" means any adsorbent material that utilizes specific, reversible interactions between synthetic and/or biomolecules, e.g., the property of Protein A to bind to an Fc region of an IgG antibody or other Fc-containing protein, in order to effect the separation of the protein from its environment. In other embodiments the specific, reversible interactions can be base on a property such as isoelectric point, hydrophobicity, or size. In one particular embodiment, a separation matrix comprises an adsorbent, such as Protein A, affixed to a solid support. See, e.g., Ostrove (1990) in "Guide to Protein Purification," *Methods in Enzymology* 182: 357-379, which is incorporated herein in its entirety.

As used herein, the terms "non-native" and "non-native form" are used interchangeably and when used in the context of a protein of interest, such as a protein comprising a Fc domain, mean that the protein lacks at least one formed structure attribute found in a form of the protein that is biologically active in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity. Examples of structural features that can be lacking in a non-native form of a protein can include, but are not limited to, a disulfide bond, quaternary structure, disrupted secondary or tertiary structure or a state that makes the protein biologically inactive in an appropriate assay. A protein in a non-native form can but need not form aggregates.

As used herein, the term "non-native soluble form" when used in the context of a protein of interest, such as a protein comprising a Fc domain, means that the protein lacks at least one formed structure attribute found in a form of the protein that is biologically active in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity, but in which the protein is expressed in a form or state that is soluble intracellularly (for example in the cell's cytoplasm) or extracellularly (for example, in a lysate pool).

As used herein, the term "non-native limited solubility form" when used in the context of a protein of interest, such as a protein comprising a Fc domain, means any form or state in which the protein lacks at least one formed structural feature found in a form of the protein that (a) is biologically active in an appropriate in vivo or in vitro assay designed to assess the protein's biological activity and/or (b) forms aggregates that require treatment, such as chemical treatment, to become soluble. The term specifically includes proteins existing in inclusion bodies, such as those sometimes found when a recombinant protein is expressed in a non-mammalian expression system.

As used herein, the term "soluble form" when used in the context of a protein of interest, such as a protein comprising a Fc domain, broadly refers to a form or state in which the protein is expressed in a form that is soluble in a intracellularly (for example in the cell's cytoplasm) or extracellularly (for example, in a cell lysate pool).

II. DIRECT CAPTURE OF A PROTEIN EXPRESSED IN A NON-NATIVE SOLUBLE FORM IN A NON-MAMMALIAN EXPRESSION SYSTEM

One advantage of the disclosed method over typical purification methods is the elimination of the need for a refolding step before the soluble protein is applied to the separation matrix. That is, a protein solublized in cell lysate can be directly applied to the separation matrix. This is advantageous because the method does not require any initial purification efforts, although an initial filtration step may be desirable in some cases.

In the case of a protein comprising a Fc domain, the Fc region must have a certain level of structure to be bound by protein A, (Wang et al., (1997) *Biochem. J.* 325 (Part 3):707-710). This fact has limited the application of separation matrices for purifying proteins that are expressed in a non-native soluble form, particularly proteins comprising an Fc region, because it is commonly believed that a soluble non-native Fc-containing protein would not have the requisite structural elements required to associate with a separation matrix. Furthermore, the Fc region of an antibody spontaneously forms a homodimer under non-reducing conditions and prior to the instant disclosure it was unexpected to observe that even in the reductive environment of the cell, the Fc-conjugated proteins and peptides not only form enough structure for protein to bind to the affinity resin, but that the individual peptide chains readily formed non-covalent dimers, even though the proteins had not yet been completely refolded to native form.

In view of prevailing beliefs, the success of the disclosed method was surprising and unanticipated because it was not expected that a non-mammalian, microbial cell fermentation could be induced to produce a protein that was soluble, yet still had enough structure to associate with the affinity separation matrix.

The disclosed method can be employed to purify a protein of interest that is expressed in a non-native soluble form in a non-mammalian cell expression system. The protein of interest can be produced by living host cells that either naturally produce the protein or that have been genetically engineered to produce the protein. Methods of genetically engineering cells to produce proteins are known in the art. See, e.g., Ausabel et al., eds. (1990), *Current Protocols in Molecular Biology* (Wiley, New York). Such methods include introducing nucleic acids that encode and allow expression of the protein into living host cells. In the context of the present disclosure, a host cell will be a non-mammalian cell, such as bacterial cells, fungal cells, yeast cells, and insect cells. Bacterial host cells include, but are not limited to, *Escherichia coli* cells. Examples of suitable *E. coli* strains include: HB101, DH5α, GM2929, JM109, KW251, NM538, NM539, and any *E. coli* strain that fails to cleave foreign DNA. Fungal host cells that can be used include, but are not limited to, *Saccharomyces cerevisiae, Pichia pastoris* and *Aspergillus* cells. New cell lines can be established using methods known to those skilled in the art (e.g., by transformation, viral infection, and/or selection). It is noted that the method can be performed on proteins that are endogenously expressed by the non-mammalian cell as well.

During the production of a non-mammalian culture, growth conditions can be identified and employed so as to favor the production of a protein of interest in an intracellular soluble form. Such conditions can be identified by systematic empirical optimization of the culture condition parameters, such as temperature or pH. This optimization can be achieved using analysis of multifactorial matrices. For example, a matrix or series of multifactorial matrices can be evaluated to optimize temperature and pH conditions favor production of a desired species (i.e., a non-native soluble form). An optimization screen can be set up to systematically evaluate temperature and pH in a full or partial factorial matrix, with each component varied over a range of at least three temperature or pH levels with all other parameters kept constant. The protein

can be expressed and the yield and quality of protein expressed in the desired form can be evaluated using standard multivariate statistical tools.

Initially, non-mammalian cells that express a particular protein of interest are grown to a desired target density under conditions designed to induce expression of the protein in a soluble form. In one embodiment, the cells express a wild type protein of interest. In another embodiment, the cells can be engineered using standard molecular biology techniques to recombinantly express a protein of interest, and induced to produce the protein of interest. The protein of interest can be any protein, for example a protein that comprises an Fc moiety. Such a protein can be, for example, an antibody, a peptibody or an Fc fusion protein, any of which can be joined to an Fc moiety via a linker.

Once the desired target density is reached, the non-mammalian cells are separated from the growth media. One convenient way of achieving separation is by centrifugation, however filtration and other clarification methods can also be used.

The cells are then collected and are resuspended to an appropriate volume in a resuspension solution. Examples of resuspension solutions that can be used in the disclosed methods include phosphate buffered saline, Tris buffered saline, or water. The selection of an appropriate buffer will be determined, in part, by the properties of the molecule of interest as well as any volume or concentration constraints.

Following resuspension, the non-mammalian cells are lysed to release the protein, which will be present in the cell lysate in a non-native soluble form to generate a cell lysate. The lysis can be performed using any convenient means, such as feeding the cell suspension through a high pressure homogenizer or by employing a chemical lysis process. Whichever lytic process is selected, the function of the lysis step is to break open the cells and to break down DNA. The lysis can be performed in multiple cycles to achieve a more complete lysis or to accommodate large volumes of cell suspension. For example, the cell suspension can be fed through a mechanical homogenizer several times. This process releases the intracellular contents, including the protein of interest, and forms a pool of cell lysate.

Following the lysis procedure, the cell lysate can optionally be filtered. Filtration can remove particulate matter and/or impurities, such as nucleic acids and lipids, and may be desirable in some cases, such as when one suspects that direct application of the cell lysate to the chromatography equipment or media may lead to fouling or clogging, or when the separation matrix is sensitive to fouling or difficult to clean in-place. The benefit of filtering the cell lysate prior to contacting it with the separation matrix can be determined on a case-by-case basis.

After the lysis procedure, the cell lysate can optionally be incubated for an appropriate amount of time in the presence of air or oxygen, or exposed to a redox component or redox thiol-pair. The incubation can facilitate and/or ensure the formation of the minimal secondary structure required to facilitate an association with a separation matrix. The particular length of the incubation can vary with the protein but is typically less than 72 hours (e.g., 0, 0.5, 1, 2, 3, 5, 7, 10, 12, 18, 24, 36, 48 or 72 hours). When an incubation is performed, the length of incubation time can be determined by empirical analysis for each protein, which in some cases will be shorter (or omitted) and other cases longer.

Following the incubation period the cell lysate, which comprises the released protein of interest, is contacted with a separation matrix under conditions suitable for the protein to associate with a binding element of the separation matrix. Representative conditions conducive to the association of a protein with an affinity matrix are provided in the Examples. The separation matrix can be any media by which the protein of interest can be separated from the components of the resuspension and/or lysis buffer, including impurities such as host cell proteins, DNA, lipids and chemical impurities introduced by the components of the resuspension and/or lysis buffer.

Proteins A and G are often employed to purify antibodies, peptibodies and other fusion proteins comprising a Fc region by affinity chromatography. See, e.g., Vola et al. (1994), *Cell Biophys.* 24-25: 27-36; Aybay and Imir (2000), *J. Immunol. Methods* 233(1-2): 77-81; Ford et al. (2001), *J. Chromatogr. B* 754: 427-435. Proteins A and G are useful in this regard because they bind to the Fc region of these types of proteins. Recombinant fusion proteins comprising an Fc region of an IgG antibody can be purified using similar methods. Proteins A and G can be employed in the disclosed methods as an adsorbent component of a separation matrix.

Thus, examples of separation matrices that can be employed in the present invention include Protein A resin, which is known to be, and is commonly employed as, an effective agent for purifying molecules comprising an Fc moiety, as well as Protein G and synthetic mimetic affinity resins, such as MEP HyperCel® chromatography resin.

After the protein of interest has been associated with the separation matrix by contacting the cell lysate containing the protein with the separation matrix, thereby allowing the protein to associate with the adsorbent component of the separation matrix, the separation matrix is washed to remove unbound lysate and impurities.

The wash buffer can be of any composition, as long as the composition and pH of the wash buffer is compatible with both the protein and the matrix, and maintains the interaction between the protein and the matrix. Examples of suitable wash buffers that can be employed include solutions containing glycine, Tris, citrate, or phosphate; typically at levels of 5-100 mM (e.g., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75 or 100 mM). These solutions can also contain an appropriate salt ion, such as chloride, sulfate or acetate at levels of 5-500 mM (e.g., 5, 10, 12, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mM). The resin can be washed once or any number of times. The exact composition of a wash buffer will vary with the protein being purified.

After the separation matrix with which the protein has associated has been washed, the protein of interest is eluted from the matrix using an appropriate solution. The protein of interest can be eluted using a solution that interferes with the binding of the adsorbent component of the separation matrix to the protein, for example by disrupting the interactions between the separation matrix and the protein of interest. This solution can include an agent that can either increase or decrease pH, and/or a salt. For example, the pH can be lowered to about 4.5 or less, for example to between about 3.3 and about 4.0, e.g., 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4 or 4.5. A solution comprising citrate or acetate, for example, can be employed to lower the pH. Other methods of elution are also known, such as via the use of chaotropes (see, e.g., Ejima et al. (2005) *Analytical Biochemistry* 345(2):250-257) or amino acid salts (see, e.g., Arakawa et al. (2004) *Protein Expression & Purification* 36(2):244-248). Protocols for such affinity chromatography are well known in the art. See, e.g., Miller and Stone (1978), *J. Immunol. Methods* 24(1-2): 111-125. Conditions for binding and eluting can be readily optimized by those skilled in the art. The exact composition of an elution buffer will vary with the protein being purified. The protein can then optionally be further purified from the elution pool and refolded as necessary. In other

situations the protein need not be further purified and instead can be refolded directly from the elution pool. Refolding directly from the elution pool may or may not require denaturation or reduction of the protein prior to incubation in a refolding solution and will depend in part on the properties of the protein.

In some cases it will be desirable to provide the separation matrix in a column format. In such cases a chromatography column can be prepared and then equilibrated before the cell suspension is loaded. Techniques for generating a chromatography column are well known and can be employed. An optional preparation and equilibration step can comprise washing the column with a buffer having an appropriate pH and salt condition that is conducive to protein-matrix interactions. This step can provide the benefit of removing impurities present in the separation matrix and can enhance the binding of the protein to be isolated to the adsorbent component of a separation matrix.

As noted, the separation matrix can be disposed in a column. The column can be run with or without pressure and from top to bottom or bottom to top. The direction of the flow of fluid in the column can be reversed during the purification process. Purifications can also be carried out using a batch process in which the solid support is separated from the liquid used to load, wash, and elute the sample by any suitable means, including gravity, centrifugation, or filtration. Moreover, purifications can also be carried out by contacting the sample with a filter that adsorbs or retains some molecules in the sample more strongly than others, such as anion exchange membrane chromatography.

If desired, the protein concentration of a sample at any given step of the disclosed method can be determined, and any suitable method can be employed. Such methods are well known in the art and include: 1) colorimetric methods such as the Lowry assay, the Bradford assay, the Smith assay, and the colloidal gold assay; 2) methods utilizing the UV absorption properties of proteins; and 3) visual estimation based on stained protein bands on gels relying on comparison with protein standards of known quantity on the same gel. See, e.g., Stoschek (1990), "Quantitation of Protein," in "Guide to Protein Purification," *Methods in Enzymology* 182: 50-68. Periodic determinations of protein concentration can be useful for monitoring the progress of the method as it is performed.

It is noted that any or all steps of the disclosed methods can be carried out manually or by any convenient automated means, such as by employing automated or computer-controlled systems.

III. DIRECT CAPTURE OF NON-NATIVE LIMITED SOLUBILITY PROTEIN FORMS FROM A REFOLD SOLUTION FOLLOWING EXPRESSION IN NON-MAMMALIAN CELLS

In another aspect of the present disclosure, a method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system is disclosed. An advantage of the disclosed method is that the method eliminates the need for removing or diluting the refold solution before applying the protein to a separation matrix, thereby saving the time and resources associated with what is a typical step in a purification process for isolating proteins expressed in a non-native limited solubility form.

Non-mammalian cells, e.g., microbial cells, can produce recombinant proteins that are expressed intracellularly in either a soluble or a limited solubility form. When the growth conditions are not directed to force expression of the protein in a soluble form, the cells may deposit the recombinant proteins into large relatively insoluble aggregates, such as inclusion bodies. These aggregates comprise protein that is typically not biologically active or less active than the completely folded native form of the protein. In order to produce a functional protein, these inclusion bodies often need to be carefully denatured so that the protein of interest can be extracted and refolded into a biologically active form.

In typical approaches, the inclusion bodies need to be captured, washed, exposed to a denaturing and/or reducing solubilization solution and the denaturing solution is then diluted with a solution to generate a condition that allows the protein to refold into an active form and form a structure that is found in the native protein. Subsequently, it is necessary to remove the components of the diluted denaturing solution from the immediate location of the protein. In order to do this, the refold solution comprising the solubilization solution and the refolded protein is typically diluted with a buffered solution before it is applied to a separation matrix, such as a Protein A ion exchange or other mixed-mode adsorbents. This process can be time-consuming and resource-intensive. It also significantly increases the volumes that need to be handled, as well as the associated tankage requirements, which can become limiting when working on large scales. The disclosed method eliminates the need for such a dilution step

The disclosed method is particularly useful for purifying a protein of interest that is expressed in a non-native limited solubility form in a non-mammalian cell expression system. The protein of interest can be produced by living host cells that either naturally produce the protein or that have been genetically engineered to produce the protein. Methods of genetically engineering cells to produce proteins are well known in the art. See, e.g., Ausabel et al., eds. (1990), *Current Protocols in Molecular Biology* (Wiley, New York). Such methods include introducing nucleic acids that encode and allow expression of the protein into living host cells. In the context of the present disclosure, these host cells will be non-mammalian cells, such as bacterial cells, fungal cells. Bacterial host cells include, but are not limited to *Escherichia coli* cells. Examples of suitable *E. coli* strains include: HB101, DH5α, GM2929, JM109, KW251, NM538, NM539, and any *E. coli* strain that fails to cleave foreign DNA. Fungal host cells that can be used include, but are not limited to, *Saccharomyces cerevisiae, Pichia pastoris* and *Aspergillus* cells. New cell lines can be established using methods well know by those skilled in the art (e.g., by transformation, viral infection, and/or selection). It is noted that the method can be performed on endogenous proteins that are naturally expressed by the non-mammalian cell as well.

Initially, non-mammalian cells that express a particular protein of interest are grown to a desired target density. In one embodiment, the cells can be expressing a particular wild type microbial protein of interest. In another embodiment, the cells can be engineered using standard molecular biology techniques to recombinantly express a protein of interest, and in this context they can be induced to overproduce the protein of interest. The protein of interest can be any protein, for example a protein that comprises an Fc moiety. Such a protein can be, for example, an antibody, a peptibody or an Fc fusion protein, any of which can be joined to an Fc moiety via a linker.

Once the desired target density is reached, the non-mammalian cells can be separated from the growth media. One convenient way of achieving separation is by centrifugation, however filtration and other clarification methods can also be used.

The cells are then collected and are resuspended to an appropriate volume in a resuspension solution. Examples of resuspension solutions that can be used in the present invention include phosphate-buffered saline, Tris-buffered saline, or water. The selection of an appropriate buffer will be determined, in part, by the properties of the molecule of interest as well as any volume or concentration constraints.

In order to release the limited solubility non-native protein from the cells, the non-mammalian cells are lysed to form a cell lysate comprising the released the limited solubility non-native protein. The lysis can be performed in any convenient way, such as feeding the cell suspension through a high pressure homogenizer or by employing a chemical lysis process. Whichever lysis process is selected, the function of the lysis step is to break open the cells and to break down DNA. The lysis can be performed in multiple cycles to achieve a more complete lysis or to accommodate large volumes of cell suspension. For example, the cell suspension can be fed through a mechanical homogenizer several times. This process releases the intracellular contents, including the naturally-occurring or recombinant protein of interest, and forms a pool of cell lysate.

Next, the limited solubility non-native protein is separated from the rest of the lysis pool. This can be done, for example, by centrifugation. Representative conditions for a centrifuge-mediated separation or washing typically include removal of excess water from the cell lysate, resuspension of the resulting slurry in a resuspension solution. This washing process may be performed once or multiple times. Examples of typical centrifuge types include, but are not limited to, disk-stack, continuous discharge, and tube bowl. Examples of resuspension solutions that can be used in the present invention include phosphate-buffered saline, Tris-buffered saline, or water and can include other agents, such as ETDA or other salts. The selection of an appropriate buffer will be determined, in part, by the properties of the molecule of interest as well as any volume or concentration constraints. The exact composition of an resuspension buffer will vary with the protein being purified.

The expressed protein is then solubilized in a solubilization solution comprising one or more of (i) a denaturant, (ii) a reductant and (iii) a surfactant. The denaturant can be included as a means of unfolding the limited solubility protein, thereby removing any existing structure, exposing buried residues and making the protein more soluble.

Any denaturant can be employed in the solubilization solution. Examples of some common denaturants that can be employed in the refold buffer include urea, guanidinium, dimethyl urea, methylurea, or ethylurea. The specific concentration of the denaturant can be determined by routine optimization.

The reductant can be included as a means to reduce exposed residues that have a propensity to form covalent intra or intermolecular-protein bonds and minimize non-specific bond formation. Examples of suitable reductants include, but are not limited to, cysteine, DTT, beta-mercaptoethanol and glutathione. The specific concentration of the reductant can be determined by routine optimization.

A surfactant can be included as a means of unfolding the limited solubility non-native protein, thereby exposing buried residues and making the protein more soluble. Examples of suitable surfactants include, but are not limited to, sarcosyl and sodium dodecylsulfate. The specific concentration of the surfactant can be determined by routine optimization.

Although the composition of a solubilization solution will vary with the protein being purified, in one particular embodiment the solubilization solution comprises 4-6 M guanidine, 50 mM DTT.

Continuing, a refold solution comprising the solubilization solution (which comprises the protein), and a refold buffer is formed. The refold buffer comprises one or more of (i) a denaturant; (ii) an aggregation suppressor; (iii) a protein stabilizer; and (iv) a redox component. The denaturant can be included as a means of modifying the thermodynamics of the solution, thereby shifting the equilibrium towards an optimal balance of native form. The aggregation suppressor can be included as a means of preventing non-specific association of one protein with another, or with one region of a protein with another region of the same protein. The protein stabilizer can be included as a means of promoting stable native protein structure and may also suppress aggregation.

In various embodiments, the denaturant in the refold buffer can be selected from the group consisting of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea.

In various embodiments, the protein stabilizer in the refold buffer can be selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

In various embodiments, the aggregation suppressor can be selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

In various embodiments, the thiol-pairs can comprise at least one component selected from the group consisting of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

The specific concentrations of the components of a refold buffer can be determined by routine optimization. For example, a matrix or series of multifactorial matrices can be evaluated to optimize the refolding buffer for conditions that optimize yield and distributions of desired species. An optimization screen can be set up to systematically evaluate denaturant, aggregation suppressor, protein stabilizer and redox component concentrations and proportions in a full or partial factorial matrix, with each component varied over a range of concentrations with all other parameters kept constant. The completed reactions can be evaluated by RP-HPLC and SE-HPLC analysis for yield and product quality using standard multivariate statistical tools.

The function of the buffer component of the refold solution is to maintain the pH of the refold solution and can comprise any buffer that buffers in the appropriate pH range. Examples of the buffering component of a refold buffer that can be employed in the method include, but are not limited to, phosphate buffers, citrate buffers, tris buffer, glycine buffer, CHAPS, CHES, and arginine-based buffers, typically at levels of 5-100 mM (e.g., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 80, 85, 90, 95 or 100, mM).

Although the composition of an refold buffer will vary with the protein being purified, in one embodiment a refold buffer comprises arginine, urea, glycerol, cysteine and cystamine.

The refold solution can then be incubated for a desired period of time. The incubation period can be of any length but is typically between 0 and 72 hours (e.g., 0, 0.5, 1, 2, 3, 5, 7, 10, 12, 18, 24, 36, 48 or 72 hours).

After an appropriate incubation time, the refold solution is then applied to a separation matrix under conditions suitable for the protein to associate with the matrix. The separation

matrix can be any media by which the protein of interest can be separated from the components of the resuspension and/or lysis buffer, including impurities such as host cell proteins, DNA and chemical impurities introduced by the components of the solubilization and/or lysis buffer.

Proteins A and G are often employed to purify antibodies, peptibodies and other fusion proteins comprising a Fc region by affinity chromatography. See, e.g., Vola et al. (1994), *Cell Biophys.* 24-25: 27-36; Aybay and Imir (2000), *J. Immunol. Methods* 233(1-2): 77-81; Ford et al. (2001), *J. Chromatogr. B* 754: 427-435. Proteins A and G are useful in this regard because they bind to the Fc region of these types of proteins. Recombinant fusion proteins comprising an Fc region of an IgG antibody can be purified using similar methods. Proteins A and G can be employed in the disclosed methods as an adsorbent component of a separation matrix.

Thus, examples of affinity separation matrices that can be employed in the present invention include Protein A resin, which is know to be, and is commonly employed as, an effective agent for purifying molecules comprising an Fc moiety, as well as Protein G and synthetic mimetic affinity resins. Other materials that can be employed include HIC and ion exchange resins (see Example 4), depending on the properties of the protein to be purified.

It is noted that when performing the method, the refold solution comprising the refolded protein of interest is applied directly to the separation matrix, without the need for diluting or removing the components of the solution required for refolding the protein. This is an advantage of the disclosed method. Initially, it was expected that the highly ionic and/or chaotropic compounds and various other components of the refold solution would inhibit the association of the protein with the separation matrix. However, in contrast to reports in the literature (e.g., Wang et al. (1997) *Biochemical Journal.* 325 (Part 3):707-710), it was surprising to observe that the protein was in fact able to associate with the separation matrix in the presence of the components of the refold solution. The unexpected finding that the protein could associate with the separation matrix in the presence of the components of the refold solution facilitates the elimination of a dilution step or buffer exchange operation, providing a savings of time and resources.

After the protein of interest has associated with the separation matrix the separation matrix is washed to remove unbound protein, lysate, impurities and unwanted components of the refold solution.

The wash buffer can be of any composition, as long as the composition and pH of the wash buffer is compatible with both the protein and the matrix. Examples of suitable wash buffers that can include, but are limited to, solutions containing glycine, tris, citrate, or phosphate. These solutions may also contain an appropriate salt. Suitable salts include, but are not limited to, sodium, potassium, ammonium, magnesium, calcium, chloride, fluoride, acetate, phosphate, and/or citrate. The pH range is chosen to optimize the chromatography conditions, preserve protein binding, and to retain the desired characteristics of the protein of interest. The resin can be washed once or any number of times. The exact composition of a wash buffer will vary with the protein being purified.

After the separation matrix with which the protein has associated has been washed, the protein of interest is eluted using an appropriate solution (e.g., a low pH buffered solution or a salt solution) to form an elution pool comprising the protein of interest.

The protein of interest can be eluted using a solution that interferes with the binding of the adsorbent component of the separation matrix to the protein, for example by disrupting the interactions between Protein A and the Fc region of a protein of interest. This solution may include an agent that can either increase or decrease pH, and/or a salt. In various embodiments, the elution solution can comprise acetic acid, glycine, or citric acid. Elution can be achieved by lowering the pH. For example, the pH can be lowered to about 4.5 or less, for example to between about 3.3 to about 4.2 (e.g., 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1 or 4.2, using a solution comprising citrate or acetate, among other possibilities.

In some situations, the protein can then be further purified from the elution pool and can be further refolded, if necessary. In other situations the protein need not be further purified and instead can be further refolded directly in the elution pool, if necessary.

Protocols for such affinity chromatography are known in the art. See, e.g., Miller and Stone (1978), *J. Immunol. Methods* 24(1-2): 111-125. In the cases that utilize ion exchange, mixed-mode, or hydrophobic interaction chromatography, the concentration of salt can be increased or decreased to disrupt ionic interaction between bound protein and a separation matrix. Solutions appropriate to effect such elutions can include, but are not limited to, sodium, potassium, ammonium, magnesium, calcium, chloride, fluoride, acetate, phosphate, and/or citrate. Other methods of elution are also known. Conditions for binding and eluting can be readily optimized by those skilled in the art.

The exact composition of an elution buffer will vary with the protein being purified and the separation matrix being employed.

In some cases it will be desirable to situate the separation matrix in a column format. In such cases a column can be prepared and then equilibrated before the cell suspension is loaded. Techniques for generating a chromatography column are well known and can be employed. The optional preparation and equilibration step can comprise washing the column with a buffer having an appropriate pH and composition that will prepare the media to bind a protein of interest. This step has the benefit of removing impurities present in the separation matrix and can enhance the binding of the protein to be isolated to the adsorbent component of a separation matrix.

It is noted that any or all steps of the invention can be carried out by any mechanical means. As noted, the separation matrix can be disposed in a column. The column can be run with or without pressure and from top to bottom or bottom to top. The direction of the flow of fluid in the column can be reversed during the purification process. Purifications can also be carried out using a batch process in which the solid support is separated from the liquid used to load, wash, and elute the sample by any suitable means, including gravity, centrifugation, or filtration. Moreover, purifications can also be carried out by contacting the sample with a filter that adsorbs or retains some molecules in the sample more strongly than others.

If desired, the protein concentration of a sample at any given step of the disclosed method can be determined by any suitable method. Such methods are well known in the art and include: 1) colorimetric methods such as the Lowry assay, the Bradford assay, the Smith assay, and the colloidal gold assay; 2) methods utilizing the UV absorption properties of proteins; and 3) visual estimation based on stained protein bands on gels relying on comparison with protein standards of known quantity on the same gel. See, e.g., Stoschek (1990), "Quantitation of Protein," in "Guide to Protein Purification," *Methods in Enzymology* 182: 50-68. Periodic determinations of protein concentration can be useful for monitoring the progress of the method as it is performed.

It is noted that any or all steps of the disclosed methods can be carried out manually or by any convenient automated means, such as by employing automated or computer-controlled systems.

IV. COLUMN CLEANING

In another aspect the present disclosure relates to the observation that in many cases the separation matrix employed in the methods provided herein can be cleaned after multiple separations and reused. This unexpected property of the method provides a significant cost and resource savings, particularly on the manufacturing scale, since the separation matrix need not be discarded after a separation is complete.

Common wisdom in the industry suggests that after a separation matrix, such as Protein A, is repeatedly exposed to highly heterogenous feedstocks comprising high lipid and host protein content it becomes irreversibly contaminated and unusable when treated with the mild regeneration solutions commonly utilized for protein-based affinity resins. The disclosed methods, however, avoid this situation and extend the usable lifetime of a separation matrix. In the context of a large scale manufacturing process this can translate into a measurable savings of time and money. Moreover, the cleaning step can be performed, as disclosed in the Examples, in-place and with no need to extract the separation matrix from a column or other matrix retaining device for cleaning, thus saving time and resources.

In one embodiment of a cleaning operation of a separation matrix, following a separation employing the disclosed method the separation matrix is washed with a regeneration reagent, such as sodium hydroxide, or an acidic reagent, such as phosphoric acid.

In one particular embodiment of a cleaning operation, Protein A is the separation matrix and a column containing Protein A resin is washed with 5 column volumes of 150 mM phosphoric acid and held for >15 minutes over the column. Following the wash with the acid, the column can be flushed with water, regenerated with 5 column volumes of 50 mM Tris, 10 mM citrate, 6M urea, 50 mM DTT; pH 7.4, subsequently washed with water, and then flushed with 3 column volumes of 150 mM phosphoric acid. This cleaning protocol has been utilized to achieve over 200 cycles of protein A resin. FIG. **3** highlights the results achievable using the disclosed cleaning methods.

EXAMPLES

The following examples demonstrate embodiments and aspects of the present invention and are not intended to be limiting.

Example 1

Direct Capture of Proteins Expressed in a Soluble Form Using Protein A Affinity Chromatography

The following experiment demonstrates that a protein comprising a plurality of polypeptides joined to an Fc moiety can be separated from an *E. coli* cell lysate slurry using a Protein A affinity media.

A protein comprising a plurality of polypeptides joined to an Fc moiety was expressed in an *E. coli* fermentation induced at 30° C. and driven to express soluble-form protein product. The fermentation broth was centrifuged, the liquid fraction removed, and the cell paste was collected. The cells were resuspended in a 10 mM potassium phosphate, 5 mM EDTA; pH 6.8 buffer solution, to approximately 100% of the original volume. The cells were then lysed by means of three passes through a high pressure homogenizer. After the cells were lysed, the cell lysate was filtered through a 0.1 μm filter to reduce particulate levels. The material was then stored in a closed bottle for ~24 hours at approximately 5° C.

In a separate operation, a packed column comprising GE Healthcare Mab Select™ Protein A affinity resin was prepared and equilibrated with 5 column volumes (CV) of 10 mM Tris; pH 8.0.

An aliquot of a protein comprising an Fc moiety was sampled directly from a lysate. The protein mixture was loaded to approximately 0.02 millimoles total protein/L resin at a 6-10 minute residence time. See FIG. **1**, which correlates protein bound and protein loaded as a function of residence time.

After loading, the column was washed with 10 mM Tris; pH 8.0, for 5 CV at up to 220 cm/hr. The protein of interest was recovered from the resin by elution with 50 mM sodium acetate, pH 3.1 at up to 220 cm/hr. The elution pool yielded greater than 90% recovery of the soluble material in the initial cell broth. The collected protein in the elution pool was stored at 2-8° C. until the next purification step was carried out.

Following the separation, the resin media was cleaned in-place by flowing 5 CV of 6 M Guanidine, pH 8.0 at 220 cm/hr.

The results of this separation demonstrated that a soluble protein expressed in a non-mammalian system can be captured and purified, with high yield, directly from cell lysate broth without having to refold the protein prior to application to a separation matrix.

Example 2

Capture of a Fc-Containing Protein Expressed in a Limited Solubility Form from a Refold Mixture Using Protein A Affinity Chromatography

The following experiments demonstrate that an Fc-containing protein can be separated from a refold mixture comprising glycerol, guanidine, urea, and arginine using Protein A affinity media.

In one experiment, a recombinant protein comprising a biologically active peptide linked to the C-terminus of the Fc moiety of an IgG1 molecule via a linker and having a molecular weight of about 57 kDa and comprising 8 disulfide bonds, in a non-mammalian expression system, namely *E. coli*, harvested, refolded under appropriate conditions, and captured using Protein A affinity media.

The growth media in which the cells were growing was centrifuged and the liquid fraction removed, leaving the cells as a paste. The cells were resuspended in water to approximately 60% of the original volume. The cells were lysed by means of three passes through a high pressure homogenizer.

After the cells were lysed, the lysate was centrifuged in a disc-stack centrifuge to collect the protein in the solid fraction, which was expressed in a limited solubility non-native form, namely as inclusion bodies.

The protein slurry was washed multiple times by resuspending the slurry in water to between 50 and 80% of the original fermentation broth volume, mixing, and centrifugation to collect the protein in the solid fraction.

The concentrated protein was then combined in a solubilization solution containing the protein, guanidine, urea, and DTT.

After incubation for one hour, the protein solution was diluted in to a refold buffer containing appropriate levels of arginine, urea, glycerol, cysteine, and cystamine.

In a separate operation, a packed column comprising ProSep VA Ultra™ Protein A affinity resin with dimensions of 1.1 cm internal diameter and ~25 cm height, was prepared and equilibrated with 5 column volumes (CV) of 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution.

An aliquot of a protein comprising an Fc moiety from the refold solution was filtered through a series of depth and/or membrane filter to remove particulates. The conditioned and filtered protein mixture was loaded to approximately 0.35 millimoles total protein/L resin at a 6-10 minute residence time. See FIG. **1**, which correlates protein bound and protein loaded as a function of residence time.

After loading, the column was washed with 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution, for 4.5 CV at up to 400 cm/hr. The Fc-contiaing protein was recovered from the resin by elution with 100 mM sodium acetate, pH 3.7 at up to 300 cm/hr. The average level of purity achieved is shown in FIG. **3**.

Following the separation, the resin media was cleaned in-place by flowing 5 CV of 150 mM phosphoric acid. The column was regenerated with 5 CV of 50 mM Tris, 10 mM citrate, 6M urea and 50 mM DTT; pH 7.4, washed with water, and then flushed with 3 CV of 150 mM phosphoric acid.

The results of this separation demonstrate that an insoluble protein expressed in a non-mammalian system can be purified directly from a refold buffer without having to dilute the refold buffer prior to application to a separation matrix for more than 150 cycles, as indicated by the table presented in FIG. **3**.

In another separation, the Protein A column was cycled with the above procedure 8-10 times and then the final cycle was run as follows: The media was equilibrated with 5 column volumes (CV) of 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution. An aliquot of protein sampled directly from a refold buffer was filtered through a series of depth and/or membrane filter to remove particulates. The conditioned and filtered protein mixture was then loaded on the column to 0.35 millimoles total protein/L resin at a 6-10 minute residence time. See FIG. **1**, which correlates protein bound and protein loaded as a function of residence time.

After loading, the column was washed with 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution, for 4.5 CV at up to 400 cm/hr. The protein of interest was recovered from the resin by eluting with 100 mM sodium acetate, pH 3.7 at up to 300 cm/hr. The resin media was cleaned in-place by flowing 5 CV of 150 mM phosphoric acid over it. Finally, the column was flushed with water, regenerated with 5 CV of 50 mM Tris, 10 mM citrate, 6M urea, and 50 mM DTT; pH 7.4, washed with water, and then flushed with 3 CV of 150 mM phosphoric acid. Subsequent analysis of the resin showed no protein carry-over between cycles, demonstrating the ability to re-use the resin after both cleaning methods.

Example 3

Separation of an Fc-Containing Protein from a Refold Mixture Using Cation Exchange Chromatography

The following experiments demonstrate that an Fc-containing protein can be separated from a refold mixture comprising glycerol, guanidine, urea, and arginine using cation exchange media.

In one experiment, a recombinant protein comprising a biologically active peptide linked to the C-terminus of the Fc moiety of an IgG1 molecule via a linker and having a molecular weight of about 57 kDa and comprising 8 disulfide bonds, was expressed in a non-mammalian expression system, namely *E. coli*, harvested, refolded under appropriate conditions, and captured using cation exchange media.

The growth media in which the cells were growing was centrifuged and the liquid fraction removed, leaving the cells as a paste. The cells were resuspended in water. The cells were lysed by means of multiple passes through a high pressure homogenizer. After the cells were lysed, the lysate was centrifuged to collect the protein, which was expressed in a limited solubility non-native form, namely as inclusion bodies. The protein slurry was washed multiple times by resuspending the slurry in water, mixing, and centrifugation to collect the protein. The concentrated protein was then transferred to a solubilization buffer containing guanidine and DTT. After incubation for one hour, the protein solution was diluted in to a refold buffer containing appropriate levels of arginine, urea, glycerol, cysteine, and cystamine.

In a separate operation, a packed column comprising EMD Fractogel $S0_3^-$ cation exchange resin with dimensions of 1.1 cm internal diameter and 20 cm height, was prepared and equilibrated with 5 column volumes of 30 mM MES; pH 4.5 buffered solution.

An aliquot of a protein comprising an Fc moiety was sampled directly from a refold solution, was diluted 3-fold with water, titrated with 50% hydrochloric acid to ~pH 4.5 and was filtered through a series of depth and/or membrane filter to remove particulates. The conditioned and filtered protein mixture was loaded to approximately 0.96 millimoles total protein/L resin at 60 cm/hr.

After loading, the column was washed with 30 mM MES; pH 4.5, for 3 CV at 60 cm/hr, then washed with an additional 3 CV of 30 mM MES; pH 6.0. The protein of interest was recovered from the resin by gradient elution over 25 CV between 30 mM MES; pH 6.0 and 30 mM MES, 500 mM NaCl; pH 6.0 at 60 cm/hr. The collected protein in the elution pool was stored at 2-8° C. until the next purification step was carried out.

Purity levels achieved, as determined by SEC and RP-HPLC are shown in FIG. **5**.

Following the separation, the resin media was cleaned in-place by flowing 3 CV of 1 M sodium hydroxide, at 120 cm/hr and held for 60 minutes prior an additional 3 CV wash with 1 m sodium hydroxide.

The results of this separation demonstrate that an insoluble protein expressed in a non-mammalian system can be captured and purified from a refold buffer with a variety of separation matrices, including an ion-exchange separation matrix.

Example 4

Re-Usability of Protein A Affinity Resin Used to Isolate a Fc-Containing Protein Directly from a Refold Buffer by Affinity Chromatography

In another aspect of the method, a range of column cleaning methods can be employed in conjunction with the methods described herein, allowing the chromatography resins to be reused to an extent that make the method economically feasible. As described in Examples 2 and 3 for the case of Protein A affinity resins, cleaning protocols have been developed and demonstrated to remove product and non-product contaminants from the resin to allow reuse. The cleaning

agents include caustic (e.g. sodium or potassium hydroxide), detergents (e.g. SDS or Triton X-100), denaturants (e.g. urea or guanidine-derivatives), and reductants (e.g. DTT, or thioglycolates). These agents can be used in combination or alone.

In order to demonstrate the reusability of column resins following application of the direct capture methods described, an aliquot of pH adjusted and filtered Fc-containing protein was loaded on new, unused resin and resin that had been previously cycled 94 times to evaluate the cleaning of the Protein A resin and the effect on purification binding and separation of an Fc-containing protein with regard to resin history.

The media was equilibrated with 5 column volumes (CV) of 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution. An aliquot of protein sampled directly from a refold buffer was filtered through a series of depth and/or membrane filter to remove particulates. The conditioned and filtered protein mixture was then loaded on the column to approximately 0.35 millimoles total protein/mL resin at a 6-10 minute residence time. See FIG. **1**, which correlates protein bound and protein loaded as a function of residence time.

After loading, the column was washed with 25 mM Tris, 100 mM sodium chloride; pH 7.4, or similar buffered solution, for 4.5 CV at up to 400 cm/hr. The protein of interest was recovered from the resin by eluting with 100 mM sodium acetate, pH 3.7 at up to 300 cm/hr. Each column was regenerated using 5 CV phosphoric acid and 5 CV of an acidic buffered solution containing 50 mM Tris, 10 mM citrate, 6M urea, and 50 mM DTT; pH 7.4.

This procedure was repeated for greater than 100 cycles. Selected samples from this reuse study were submitted for SEC-HPLC analysis. The goal was to track the % MP purity, % HMW and % dimer species from the pools as well as to understand the change of purity level from the load. No major differences were observed between the used columns and new columns.

This Example demonstrates that not only can a complex protein be captured from a complex chemical solution, but that the resin can be cycled repeatedly and cleaned and reused reproducibly over a number of industrially-relevant cycles.

What is claimed is:

1. A method of purifying a protein expressed in a non-native soluble form in a non-mammalian expression system comprising:

(a) lysing a non-mammalian cell in which the protein is expressed in a non-native soluble form to generate a cell lysate;

(b) contacting the cell lysate with a separation matrix under conditions suitable for the protein to associate with the separation matrix;

(c) washing the separation matrix; and

(d) eluting the protein from the separation matrix, wherein the separation matrix is an affinity resin selected from the group consisting of Protein A, Protein G and a synthetic mimetic affinity resin.

2. The method of claim **1**, wherein the protein is a complex protein.

3. The method of claim **2**, wherein the complex protein is selected from the group consisting of a multimeric protein, an antibody and an Fc fusion protein.

4. The method of claim **1**, wherein the non-mammalian expression system comprises bacteria or yeast cells.

5. The method of claim **1**, wherein the cell lysate is filtered before it is contacted with the separation matrix.

6. The method of claim **1**, further comprising refolding the protein to its native form after it is eluted.

7. A method of purifying a protein expressed in a non-native limited solubility form in a non-mammalian expression system comprising:

(a) expressing a protein in a non-native limited solubility form in a non-mammalian cell;

(b) lysing a non-mammalian cell;

(c) solubilizing the expressed protein in a solubilization solution comprising one or more of the following:

(i) a denaturant;

(ii) a reductant; and

(iii) a surfactant;

(d) forming a refold solution comprising the solubilization solution and a refold buffer, the refold buffer comprising one or more of the following:

(i) a denaturant;

(ii) an aggregation suppressor;

(iii) a protein stabilizer; and

(iv) a redox component;

(e) directly applying the refold solution to a separation matrix under conditions suitable for the protein to associate with the matrix;

(f) washing the separation matrix; and

(g) eluting the protein from the separation matrix, wherein the separation matrix is a non-affinity resin selected from the group consisting of ion exchange, mixed mode, and a hydrophobic interaction resin.

8. The method of claim **7**, wherein the non-native limited solubility form is a component of an inclusion body.

9. The method of claim **7**, wherein the protein is a complex protein.

10. The method of claim **7**, wherein the complex protein is selected from the group consisting of a multimeric protein, an antibody, a peptibody, and an Fc fusion protein.

11. The method of claim **7**, wherein the non-mammalian expression system is bacteria or yeast cells.

12. The method of claim **7**, wherein the denaturant of the solubilization solution or the refold buffer comprises one or more of urea, guanidinium salts, dimethyl urea, methylurea and ethylurea.

13. The method of claim **7**, wherein the reductant comprises one or more of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione.

14. The method of claim **7**, wherein the surfactant comprises one or more of sarcosyl and sodium dodecylsulfate.

15. The method of claim **7**, wherein the aggregation suppressor is selected from the group consisting of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, Tris, sodium sulfate, potassium sulfate and osmolytes.

16. The method of claim **7**, wherein the protein stabilizer comprises one or more of arginine, proline, polyethylene glycols, non-ionic surfactants, ionic surfactants, polyhydric alcohols, glycerol, sucrose, sorbitol, glucose, tris, sodium sulfate, potassium sulfate and osmolytes.

17. The method of claim **7**, wherein the redox component comprises one or more of glutathione-reduced, glutathione-oxidized, cysteine, cystine, cysteamine, cystamine and beta-mercaptoethanol.

18. The method of claim **1** or **7**, further comprising the step of washing the separation matrix with a regeneration reagent.

19. The method of claim **18**, wherein the regeneration reagent is one of a strong base or a strong acid.

20. The method of claim **19**, wherein the strong acid is phosphoric acid.

21. The method of claim **19**, wherein the strong base is sodium hydroxide.

22. The method of claim **18**, wherein the regenerating comprises washing the separation matrix with a solution comprising one or both of a chaotrope present at a concentration of 4-6 M and a reductant.

23. The method of claim **22**, wherein the chaotrope is one of urea, dimethyl urea, methylurea, ethylurea, and guanidinium.

24. The method of claim **22**, wherein the reductant is one of cysteine, dithiothreitol (DTT), beta-mercaptoethanol and glutathione.

25. The method of claim **18**, wherein the regenerating comprises washing the separation matrix with a solution comprising 50 mM Tris, 10 mM citrate, 6 M urea, 50 mM dithiothreitol (DTT) at pH 7.4.

* * * * *

US006162427A

United States Patent [19]

[11] **Patent Number: 6,162,427**
[45] **Date of Patent: *Dec. 19, 2000**

Baumann et al.

[54] **COMBINATION OF G-CSF WITH A CHEMOTHERAPEUTIC AGENT FOR STEM CELL MOBILIZATION**

[75] Inventors: **Matthias Baumann**, Karl-Ludwig-Strasse; **Peter-Paul Ochlich**, Wallonenstrasse, both of Germany

[73] Assignee: **Roche Diagnostics GmbH**, Mannheim, Germany

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **09/091,401**

[22] PCT Filed: **Dec. 12, 1996**

[86] PCT No.: **PCT/EP96/05568**

§ 371 Date: **Nov. 12, 1998**

§ 102(e) Date: **Nov. 12, 1998**

[87] PCT Pub. No.: **WO97/22359**

PCT Pub. Date: **Jun. 26, 1997**

[30] **Foreign Application Priority Data**

Dec. 20, 1995 [DE] Germany 195 49 232

[51] **Int. Cl.**[7] **A61K 38/19**; A61K 31/675

[52] **U.S. Cl.** **424/85.1**; 514/2; 514/8; 514/76; 514/54; 514/110; 514/114

[58] **Field of Search** 424/85.1; 514/2, 514/8, 76, 54, 110, 114

[56] **References Cited**

PUBLICATIONS

Neben et al. Blood, vol. 81, No. 7, 1993, pp. 1960–1967, Apr. 1993.

Primary Examiner—Prema Mertz
Assistant Examiner—Fozia Hamud
Attorney, Agent, or Firm—Arent Fox Kintner; Plotkin & Kahn, PLLC

[57] **ABSTRACT**

The invention relates to the use of G-CSF in combination with a chemotherapeutic agent (in particular, cyclophosphamide) to produce a pharmaceutical preparation for boosting the mobilization of hematopoietic stem cells from bone marrow in the treatment of diseases requiring peripheral stem cell transplantation. The claimed combination results in more efficient leukapheresis, e.g. before myeloblative or myelotoxic therapy.

7 Claims, No Drawings

COMBINATION OF G-CSF WITH A CHEMOTHERAPEUTIC AGENT FOR STEM CELL MOBILIZATION

The present invention relates to the novel use of G-CSF and a chemotherapeutic agent or a combination of chemotherapeutic agents to produce a pharmaceutical preparation for enhanced mobilization of hematopoietic stem cells in the treatment of diseases requiring peripheral stem cell transplantation as is the case, e.g., in high-dosage chemotherapy or bone marrow ablation by irradiation. In addition, the invention is directed to a pharmaceutical packaging unit containing G-CSF, chemotherapeutic agent(s) and informational instructions regarding the application of the G-CSF and the chemotherapeutic agent or the combination of chemotherapeutic agents for enhanced mobilization of hematopoietic stem cells prior to the onset of a corresponding therapy.

The use of high-dosage chemotherapy or bone marrow ablation by irradiation requires subsequent incorporation of hematopoietic stem cells into the patient, in which case recovery of such cells is required.

In the methods of peripheral stem cell recovery (e.g., in leukopheresis), the mobilization of bone marrow stem cells has a crucial influence on the efficiency of these methods. At present, 2–3 leukophereses are required for successful peripheral stem cell transplantation, resulting in considerable stress for the patients.

The success of treatment crucially depends on the mobilization of the bone marrow stem cells, the subsequent return of which permitting to achieve reconstitution of a functioning hematopoietic system.

Numerous substances capable of effecting such a mobilization are known, e.g., G-CSF (granulocyte colony stimulating factor).

Some chemotherapeutic agents are also known to possess the ability of mobilizing bone marrow stem cells (Richman et al., Blood, Vol. 47, No. 6. 1031 (1976)).

Various documents also describe the combination of G-CSF with other active substances. Thus, combined treatments using antibiotics are known from EP-A-0,648,501 and WO-A-95/28178. The U.S. Pat. No. 5,422,105 reports the combination with one or more antimicrobial substances such as antiviral, antifungal or antibacterial agents in order to enhance the effect of a CSF-1 therapy. In addition, there have been investigations on the use of G-CSF in association with high-dosage chemotherapies in autologous bone marrow transplantations (Lymphokine Cytokine Res. (1994), 13(6), 383–90; and Leukemia and Lymphoma (1995), 19(5–6), 479–84).

In other investigations related to bone marrow transplantations, Shirota et al. have determined that cyclophosphamide which is known as cytostatic agent facilitates the permeability of the endothelial barrier for stem cells (Exp. Hematol. 19, 369–373 (1991)).

As the required number of leukophereses is extremely stressing for the patient in the run-up to the treatment of particular diseases, e.g., in preparing a myeloablative or myelotoxic therapy, the invention was based on the object of achieving a superior yield of stem cells or a decrease in the number of leukophereses via enhanced mobilization of stem cells.

Surprisingly, it has now been found that an unexpectedly high stem cell concentration in blood can be achieved when administering G-CSF in combination with a chemotherapeutic agent (chemotherapeutic agents).

Therefore, the invention is directed to the use of G-CSF and a chemotherapeutic agent or a combination of chemotherapeutic agents to produce a pharmaceutical preparation for enhanced mobilization of hematopoietic stem cells in the treatment of diseases requiring peripheral stem cell transplantation, wherein G-CSF and the chemotherapeutic agent are present formulated in separate administration forms, so that they can be taken out separately and administered successively according to the optimum application regimen. According to the invention, it is preferred to apply the G-CSF prior to the onset of the administration of chemotherapeutic agents in order to enhance the mobilization of hematopoietic stem cells.

The combined use according to the invention of G-CSF and chemotherapeutic agent relates to all those diseases requiring recovery of stem cells from the blood for subsequent peripheral transplantation, particularly tumor diseases.

According to the present invention, G-CSF prepared using recombinant methods and variants thereof may be used. The term G-CSF or G-CSF variant according to the present invention encompasses all naturally occurring variants of G-CSF, as well as G-CSF proteins derived therefrom, modified by recombinant DNA technology, particularly fused proteins containing other protein sequences in addition to the G-CSF portion. Particularly preferred in this meaning is a G-CSF mutein having an N-terminal Met residue at position 1, which is suited for expression in prokaryotic cells. Similarly suitable is a recombinant G-CSF variant free of methionine which may be prepared according to WO-A-91/11520. The term "G-CSF variant" is understood to comprise those G-CSF molecules wherein one or more amino acids may be deleted or replaced by other amino acids, with the essential properties of G-CSF, particularly the ability to mobilize bone marrow cells, being largely retained. Suitable G-CSF muteins are described in EP-A-0,456,200, for example.

As chemotherapeutic agents in the meaning of the invention those therapeutic agents may be used which open the endothelial barrier, rendering it permeable for stem cells. Hereinbelow, chemotherapeutic agents are understood to be exogenous substances suited and used to damage or destroy microorganisms, parasites or tumor cells. Here, in particular, cytostatic agents or derivatives thereof from the following group of cytostatic agents may be mentioned: alkylating agents such as, e.g., cyclophosphamide, chlorambucil, melphalan, busulfan, N-mustard compounds, mustargen; metal complex cytostatic agents such as metal complexes of platinum, palladium or ruthenium; antimetabolites such as methotrexate, 5-fluorouracil, cytorabin; natural substances such as vinblastine, vincristine, vindesine, etc.; antibiotic agents such as dactinomycin, daunorubicin, doxorubicin, bleomycin, mitomycin, etc.; hormones and hormone antagonists such as diethylstilbestrol, testolactone, tamoxifen, aminoglutethimide, and other compounds such as, e.g., hydroxyurea or procarbacin, as well as corticoids such as prednisolone, with cyclophosphamide being particularly preferred.

G-CSF may be administered using standard administration forms, with injection solutions being preferred. Water is preferably used as injection medium which includes adjuvants common in injection solutions, such as stabilizers, solubilizers and buffers. For example, such adjuvants are tartrate and citrate buffers, ethanol, complexing agents such as ethylenediaminetetraacetic acid and the non-toxic salts thereof, high molecular weight polymers such as liquid polyethylene oxide for viscosity control. Liquid vehicles for injection solutions must be sterile and are preferably filled into ampoules.

The chemotherapeutic agents may be applied in liquid or solid form on the enteral or parenteral route. Here, the

standard administration forms such as tablets, capsules, coated tablets, syrups, solutions and suspensions are possible.

The dosage may depend on various factors such as mode of application, species, age, or individual condition. According to the invention, from 5 to 300 μg/kg/day of G-CSF sc. is applied. The administration of G-CSF is effected once per day over two to three days. The administration of chemotherapeutic agent(s) is initiated either immediately after the second or third G-CSF injection or on the fourth day. According to the invention, from 0.05–100 mg/kg/day of chemotherapeutic agent(s) is/are administered.

Surprisingly, it was determined that administration of G-CSF prior to opening of the endothelial barrier induced by chemotherapeutic agents significantly increases the stem cell mobilization and thus, can improve leukopheresis efficiency.

By administering G-CSF prior to administration of the chemotherapeutic agent(s), a massive granulopoiesis in the spleen and a substantial increase of the spleen weight could be observed which, according to Bungart et al., Brit. J. Haem. 76, 174–179, 1990, is attributable to the stem cell mobilization.

In addition, administration of G-CSF and a chemotherapeutic agent in the run-up to a, e.g., antitumor therapy offers the opportunity of recovering the stem cells mobilized in large amounts from the blood with higher efficiency (e.g., using leukopheresis), then performing the antitumor therapy using a cytostatic agent or irradiation and subsequently, conducting the peripheral stem cell transplantation.

The invention is also directed to a pharmaceutical packaging unit including at least three spatially separated components, the first component being a standard administration form of G-CSF, the second component representing a standard pharmaceutical administration form of a chemotherapeutic agent or a combination of chemotherapeutic agents, and the third component comprising informational instructions for the administration of G-CSF prior to administration of the chemotherapeutic agent (chemotherapeutic agents) for enhanced mobilization of hematopoietic stem cells.

Where G-CSF is administered in combination with, e.g., two chemotherapeutic agents, these chemotherapeutic agents may be formulated separately or together, so that the packaging unit consists of either three or four spatially separated components.

Without intending to be limiting, the invention will be illustrated in more detail in the following embodiment.

Embodiment

Using mice, the in vivo interactions between rh G-CSF and cyclophosphamide (CY) applications regarding the effects of various schemes of treatment on

the hematopoietic capacity of femoral cells,

the femoral bone marrow and spleen histologies, and

the leukocyte number (WBC) were examined.

The following test groups were examined:

G-CSF/CY group: G-CSF application was effected on three successive days prior to cyclophosphamide (CY) administration; the third injection was effected immediately before CY administration.

CY/G-CSF group: corresponding to the present clinical practice, G-CSF was applied beginning 24 hours after CY injection.

CY group: treatment was effected using CY alone.

Control group.

1. Materials

a) Animals

Female NMRI mice were purchased. Initially, their body weight was approximately between 26 and 28 g. The animals were fed on pellets and had ad libitum access to feed and drinking water.

They were kept separately at room temperature (23±1° C.) and a relative humidity of 55% (50–70%). The room air was exchanged approximately 10 times per hour. The day/night rhythm was held constant, with light/dark periods of 12 hours each, beginning at 6 a.m. A light intensity of about 60 lux was provided throughout the room during the light period. The health condition of the animals was recorded daily, and cleaning was effected at regular intervals. Categorizing of the animals into the individual test groups can be inferred from Table 1.

b) Reagents

Recombinant human (rh) G-CSF, cyclophosphamide

2. Methods

a) Peripheral Leukocyte Number (WBC)

The measurements were conducted using an analyzer. Under anesthesia, 25 μl of native whole blood was withdrawn from the postorbital plexus using heparinized glass capillaries, diluted with 3.75 ml of an isosmotic solution, and analyzed with respect to WBC.

b) Femoral Bone Marrow Cell Number (BMC)

After 4 weeks of treatment or after a two weeks period free of treatment, respectively, the femora of 5 animals (n=8, G-CSF/CY group) from the various test groups were collected. They were opened aseptically at the proximal and distal ends. Rinsing the bone marrow cavities with 1.5 ml of MEM (supplemented with penicillin/streptomycin and L-glutamine), the bone marrow cells were recovered using syringes equipped with adapters. Except for the G-CSF/CY test group wherein both femora of from 3 to 8 animals were analyzed, one femur of each animal was examined. The cells were counted in an autolyzer system.

c) CFU-C Test (Colony-Forming Units Culture)

The femoral bone marrow cell number was adjusted to 2.5×10^{-6} cells/ml in MEM (flow). 0.2 ml of this suspension was mixed with 0.5 ml of horse serum, 0.1 ml of thioglycerol (20 mM, diluted 1:4 with MEM), 1.0 ml of methylcellulose (2% in MEM), 0.6 ml of MEM (flow), and 0.1 ml of either additional medium or standardized stimulated mouse serum (1:200 dilution of serum, withdrawn 3 hours after ip. administration of 2.5 mg/kg lipopolysaccharide (LPS)) or 5 ng/ml rhG-CSF. The well-mixed semi-solid suspension was pipetted into Petri dishes 4 cm in diameter and incubated for 6 days at 37° C., 5% CO_2 and 95% r.h.. After addition of 0.5 ml of p-iodonitrotetrazolium violet solution (0.5 mg/ml PBS), the dishes were incubated for another 24 hours. The colonies were counted using a colony counter and standardized to 10^6 bone marrow cells.

d) Tissue Preparation and Histological Test

The animals were sacrificed on the day of final administration of the compounds, and spleen tissue as well as one femoral bone of each animal were fixed in 10% neutral-buffered formaldehyde solution. The bone samples were decalcified over two weeks in 5% formic acid, dissolved in formaldehyde/distilled water. Spleen and bones were stored routinely in paraffine, cut to 4 μm thickness, and stained with hematoxylin-eosin (HE), as well as with PAS. Bone marrow and spleen were semi-quantitatively evaluated with respect to cell quality, myelofibrosis and cellular necrosis using a light microscope.

e) Statistics

The various test groups were compared with control animals with respect to the end points of BMC, CFU-C response to G-CSF, CFU-C response to serum, and spleen weight. Repeated measurements of WBC (basis: 1, 2, 3, and 4 weeks) were transformed into an end point, based on the individual AUC approximation according to Zerbe et al., Biometrics 33, 653, 1992. Investigations for approximate normal distribution of WBC and spleen weight were analyzed according to the Welch T Test (Welch, Biometrika 34, 28, 1947) because a notable variance in the heterogeneity was observed. Due to the absence of an approximately normal distribution for the other end points, a permutational U test according to Mehta et al., CYTEL Software Corp. Turbo Version, Cambridge, U.S.A., 1992, was conducted. The method of multiple end point analysis was carried out for the end points of CFU-C, when administering G-CSF or serum, and the spleen weight. The end points after the period with no treatment (week 6) were analyzed for reversibility using a method according to Dunnett, JASA 50, 1096, 1955, which may be used for comparing with the controls. The calculations were performed using SAS, Version 6.10 (SAS/STAT: Changes and enhancements, Release 6.10, SAS Institute, 1994) and Statxact (Mehta et al., see above).

3. Results

a) Effects Regarding the Femoral Bone Marrow Cell Number (BMC)

The effects of various treatment regimens are included in Table 2. CY alone reduced the bone marrow cell number to about 60% of the control. Both combinations of CY and G-CSF reduced the number to about 30% of the control. Two weeks after the treatment was completed, however, the animals from the CY/G-CSF test group again showed increasing bone marrow cell numbers compared to the number immediately after treatment. At the end, they reached about 50% of the control. The other three test groups did not show any relevant changes during the follow-up period with no treatment.

b) Effects in the CFU-C Test

The response to serum of LPS-treated mice and to G-CSF was massively decreased in the CY group, compared to the bone marrow cells of the controls. A marked decrease was observed in G-CSF/CY treatment, while the CY/G-CSF test group showed increased colony formation in the presence of serum of LPS-treated mice and in the presence of G-CSF.

After a 2 weeks period with no treatment, the differences between both G-CSF groups and the controls became smaller. The proliferative response to serum of LPS-treated mice in the CY group showed after this period an extraordinary elevation compared to the marked decrease at the end of the treatment period.

c) Bone Marrow Histology

At the end of the treatment period: The granulopoietic cell density in the hematopoietically active areas of the femoral bone marrow markedly increased in both groups that had been treated with G-CSF and CY compared to the control animals and the CY test group (Table 3). The effect is particularly apparent in the G-CSF/CY test group. However, it can be seen that a clearly perceptible decrease of the hematopoietic areas as a result of fibrosis and ossification occurred in the various treatment groups. The CY group did not show any signs of increased granulopoiesis.

The stimulation of granulopoiesis due to administration of G-CSF prior to CY gave rise to all stages of maturity, whereas maturity stages were observed with less abundance upon administration of CY/G-CSF. The occurrence of single cell necroses was moderate in the G-CSF/CY and CY test groups and low in the CY/G-CSF group.

d) Effects on the Spleen

Histology at the end of treatment: Light microscopy of spleen tissue revealed a marked increase of granulopoietic cells after 4 weeks of treatment in both groups which had received G-CSF in combination with CY (Table 4). Granulopoiesis comprised all stages of granulopoietic cell maturation most markedly in the G-CSF/CY group. The granulopoietic cell proliferation in the CY/G-CSF group mainly consisted of myeloblastic cells, maturity stages were barely observable. The considerable increase of granulopoietic cells occurred in association with a considerable rearrangement of the spleen organic structure. Single cell necroses were observed in the G-CSF/CY group and to a lesser extent, in the animals of the CY/G-CSF test group. The spleen of animals that had been treated with CY alone showed a slight cellular decline in the follicles and the reticulum. During a 2 weeks period with no treatment, the changes returned to normal. In those groups, however, where G-CSF and CY had been administered, there were signs of a slightly increased hematopoietic stimulation in the form of elevated granulopoiesis, erythropoiesis and megakaryopoiesis.

e) Effects on Spleen Weight

An enormous increase (more than 3.3 fold of the control) was determined at the end of the treatment period in those animals that had been treated with G-CSF/CY (Table 5), and an 1.8 fold increase compared to the control was observed in the CY/G-CSF test group. There were no relevant effects on the spleen weight in the CY group.

f) WBC

Blood samples were taken prior to the first treatment and then once per week immediately before administering CY (or placebo). Thus, the blood samples in the G-CSF/CY test group were taken after the administration of G-CSF. Additional blood samples were collected at the end of the treatment period (after 4 weeks) and after the two weeks period with no treatment, respectively.

As is apparent from Table 6, there were no relevant differences in the WBC between the controls and the CY and CY/G-CSF groups.

During week 1, the G-CSF/CY test group showed a WBC slightly elevated above the upper limit of normal; during the following weeks 2, 3 and 4, there was a substantial WBC increase (from 7- to 8 fold of the control); complete reversal of this effect could be observed after the two weeks period with no treatment.

On the whole, it can be seen that the extent of osteomyelofibrosis and multifocal ossification after the treatment using G-CSF/CY was definitely higher compared to other methods. Furthermore, massive granulopoiesis and a substantial increase in spleen weight could be observed in this test group, emphasizing the increased stem cell mobilization.

The reduced CFU-C capacity of bone marrow cells after G-CSF/CY administration must be regarded as a result of an increased mobilization of progenitor cells into the blood. This is supported by the multiple end point analysis.

TABLE 1

Dosage and assignment of animals to test group		
Test groups	Weekly dosage during weeks 1 to 4	Number of animals per test group
G-CSF/CY group		
G-CSF administration sc. followed by	250 μg/kg on 3 successive days +	(n = 8)

TABLE 1-continued

Dosage and assignment of animals to test group

Test groups	Weekly dosage during weeks 1 to 4	Number of animals per test group
CY administration ip. (n = 16)	50 mg/kg immediately after G-CSF administration on the 3rd day	
CY/G-CSF group		
CY administration ip. after 24 hrs followed by G-CSF administration (n = 10)	50 mg/kg + 250 μg/kg on 3 successive days	(n = 5)
CY group		
ip. (n = 10)	50 mg/kg	(n = 5)
Control group		
(n = 10)	0.9% NaCl solution ip. and sc.	(n = 5)

After 6 weeks (2 weeks with no treatment) another examination was conducted in satellite groups.

TABLE 2

Bone marrow cell numbers (BMC) of mice after 4 weeks of treatment and after a 2 weeks period with no treatment († = animal died untimely)

Treatment group	BMC ($\times 10^6$/femur) 4 weeks treatment	BMC ($\times 10^6$/femur) After 2 weeks without treatment
Control	13.2	14.7
	†	15.9
	8.6	20.6
	21.3	18.1
	18	16.6
Median value	15.28	17.18
CY alone	13.6	12.7
	7.8	8.3
	13	6.1
	7.6	10.3
	2.9	9.9
Median value	8.98	9.46
G-CSF + CY	5.6	4.7
	7.4	9.5
	0.75	3.4
	2.1	0.48
	1.6	1.32
	7.8	2.2
	0.65	0.55
	1.2	0.72
Median value	3.39	2.86
CY + G-CSF	3.5	9.4
	3.5	7.4
	7.3	3.5
	7.9	9.1
	0.25	12.5
Median value	4.49	8.38

TABLE 3

Histopathological findings in bone marrow (femur)

	CY	G-CSF/CY	CY/G-CSF
After 4 weeks treatment			
Cellular decline	++	(+)	+/++
Fat cells	+/++	(+)	+

TABLE 3-continued

Histopathological findings in bone marrow (femur)

	CY	G-CSF/CY	CY/G-CSF
Hyperemia	—	—	+
Increased single cell necrosis	++	++	+
Stimulated granulopoiesis	±0	+++	++
Osteomyelofibrosis or multifocal ossification	1.5, +++	8/8, ++/+++	
After 2 weeks with no treatment			
Fat cells	(+)	(+)	(+)
Stimulated granulopoiesis	(+)	+/++	+/++
Osteomyelofibrosis or multifocal ossification	3/5, (+)/+	8/8, +++	3.5, +/++

(+) = minimal
+ = faint
++ = moderate
+++ = marked

TABLE 4

Histopathological findings in the spleen

	CY alone	G-CSF/CY	CY/G-CSF
State after 4 weeks treatment			
Cellular decline Follicle cells (lymphocytes)	+	—	—
Cellular decline Reticulum cells	+	—	—
Stimulated granulopoiesis	—	+++[1]	+/++[2]
Loss of follicle structure		+++	+/++
Increased single cell necrosis		++	(+)
After 2 weeks with no treatment			
Stimulated hematopoiesis	—	+	+

[1]All maturity stages
[2]Mainly myeloblastic cells

TABLE 5

Spleen weight († = animal died untimely)

Treatment group	Spleen weight (g) after 4 weeks treatment	Spleen weight (g) after 2 weeks with no treatment
Control	0.141	0.135
	†	0.090
	0.148	0.127
	0.410	0.133
	0.161	0.150
Median value	0.215	0.127
CY alone	0.107	0.183
	0.100	0.121
	0.174	0.235
	0.082	0.199
	0.189	0.133
Median value	0.130	0.174
G-CSF + CY	0.523	0.194
	0.448	0.248
	0.497	0.181
	0.477	0.262
	0.523	0.219

TABLE 5-continued

Treatment group	Spleen weight (†) Spleen weight (g) after 4 weeks treatment	Spleen weight (g) after 2 weeks with no treatment
	0.492	0.261
	0.483	0.153
	0.486	0.273
Median value	0.491	0.224
CY + G-CSF	0.263	0.177
	0.218	0.228
	0.225	0.208
	0.324	0.218
	0.254	0.232
Median value	0.257	0.213

TABLE 6

Leukocyte number (WBC) († = animal died untimely)

Treatment group	Animal number	WBC (× $10^6/\mu l$) Time (weeks) 0	1	2	3	4	6
Control	1	5.6	5.2	9.4	3.3	6.6	—
	2	10	6.3	6.9	†	†	†
	3	7	4.8	7	3.4	6.8	—
	4	7.6	7.6	7.7	3.9	7.7	
	5	9.1	6	11	3.5	6.3	—
	6	8.4	5.1	9.5	6.2	7.5	4.5
	7	2.5	2.3	5.3	5	5.7	3
	8	9.5	7.3	12.2	8.5	13.7	7.8
	9	6.3	4.3	8.8	4.2	10.1	4.5
	10	6	5.9	8.5	5.9	8	6.4
Median value		7.20	5.48	8.63	4.88	8.04	5.24
CY alone	1	4.8	2.9	5	7.6	4	—
	2	9	7.1	5.3	2.7	2.8	—
	3	8.6	5.9	7.3	5.2	7	—
	4	6.3	4.8	6.3	6.1	4.8	—
	5	5.3	4.5	4.9	8.6	2.9	—
	6	6.4	5	5.1	6.7	4.1	3.6
	7	6.8	4.7	4.9	5	3.6	3.2
	8	5.6	5.7	4.8	4.1	3.7	4.6
	9	5.8	4.7	5.9	4.1	7.1	5.9
	10	9.4	6	7.1	4	2.8	5.9
Median value		6.80	5.13	5.66	5.41	4.28	4.64
G-CSF + CY	1	6.1	11.7	27.7	33.4	39.4	—
	2	6.8	12.4	46.5	43.7	30.5	—
	3	5.6	11.2	30.3	58.78	40.6	—
	4	4.3	11.7	35.3	51.4	40.7	—
	5	6.2	22.4	62.2	55.4	66.7	—
	6	6.2	12.6	49.2	84.2	85.4	—
	7	7.1	16.8	84.8	105.4	102.3	—
	8	8	18.6	82	117.6	70.1	—
	9	3	5.4	42.7	15.8	33.3	2.2
	10	4.6	18.2	62.8	69	33.2	3.9
	11	5.8	10.9	29.1	40	33.5	2.7
	12	5	12.7	43.8	51.9	28.1	6.4
	13	5.8	14.6	28.8	33.4	20	4.6
	14	6.2	10.2	50	36.8	45.4	3.6

TABLE 6-continued

Leukocyte number (WBC) († = animal died untimely)

Treatment group	Animal number	WBC (× $10^6/\mu l$) Time (weeks) 0	1	2	3	4	6
	15	8.6	20.2	30	36.2	25.3	5.2
	16	5.9	12.8	26.8	45.5	54.8	4.9
Median value		6.00	14.04	48.08	55.16	44.60	4.19
CY + G-CSF	1	6.7	6.1	6.9	9.5	12.68	—
	2	9.1	7.1	11.08	11.6	3.78	—
	3	9.1	11.1	4.5	9.6	5.9	—
	4	6.9	5.2	10.4	16.6	5	—
	5	6.3	3.9	7	#	11.6	—
	6	9.6	8.3	5	6.9	4.1	4.6
	7	6.6	6.3	8.6	7.1	7.5	4.2
	8	6.1	4.4	5.4	3.8	3.18	2.4
	9	10.3	7.6	4.8	4.2	6.2	3.7
	10	8.2	6.5	5.4	6.2	4.3	3.2
Median value		7.89	6.65	6.91	8.39	6.42	3.62

What is claimed is:

1. A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment, comprising administering to the patient a hematopoietic stem cell mobilizing-effective amount of G-CSF; and thereafter administering to the patient a disease treating-effective amount of at least one chemotherapeutic agent.

2. The method of claim **1**, wherein the disease is a tumor disease.

3. The method of claim **1**, wherein the G-CSF is recombinant G-CSF.

4. The method of claim **1**, wherein the at least one chemotherapeutic agent opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells.

5. The method of claim **1**, wherein the at least one chemotherapeutic agent is cyclophosphamide.

6. The method of claim **1**, wherein the G-CSF is administered once per day over 2–3 consecutive days, and the chemotherapeutic agent is administered immediately after the final administration of G-CSF, or on a fourth consecutive day.

7. A pharmaceutical kit, comprising

a first component comprising G-CSF;

a second component comprising at least one chemotherapeutic agent; and

a third component comprising instructions for the administration of the G-CSF prior to the onset of administration of the at least one chemotherapeutic agent.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that on this 13th of April, 2018, I caused the BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED (CONFIDENTIAL AND NON-CONFIDENTIAL) to be filed with the Clerk of the Court using the NextGen System. I also caused a true and correct copy of the BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED (CONFIDENTIAL AND NON-CONFIDENTIAL) to be electronically served, pursuant to agreement of the parties, on Defendants-Appellees Sandoz Inc., Sandoz International GmbH, Sandoz GmbH, and Lek Pharmaceuticals, d.d.'s counsel of record as follows:

MORRISON & FOERSTER LLP
Deanne Maynard (DMaynard@mofo.com)
Brian Robert Matsui (BMatsui@mofo.com)
Erik Jeffrey Olson (EJOlson@mofo.com)
Eric C. Pai (EPai@mofo.com)
Bryan Leitch (BLeitch@mofo.com)

/s/ Nicholas Groombridge
Nicholas Groombridge

CERTIFICATE OF COMPLIANCE

This brief complies with the type-volume limitation of Federal Circuit Rule 32(a). The brief contains 13,778 words, excluding parts of the brief exempted by Fed. R. App. P. 32(f) and Federal Circuit Rule 32(b). The word count includes the words counted by the Microsoft Word 2016 function and the words included in the images within the brief. This brief also complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6). The brief has been prepared in a proportionally spaced typeface using Microsoft Word 2016 in 14-point font of Times New Roman.

Dated: April 13, 2018

/s/ Nicholas Groombridge
Nicholas Groombridge
Attorney for Appellants

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

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/s/ Nicholas Groombridge
(Signature of Attorney)

Nicholas Groombridge
(Name of Attorney)

Appellants
(State whether representing appellant, appellee, etc.)

4/13/2018
(Date)