UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. and
NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioner,

v.

GENZYME CORPORATION,
Patent Owner.

Case No. IPR2023-01044
Patent No. 10,429,288

PETITION FOR *INTER PARTES REVIEW*

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I. INTRODUCTION

U.S. Patent No. 10,429,288 ("the '288 patent") relates to analyzing recombinant adeno-associated virus ("rAAV") preparations using analytical ultracentrifugation ("AUC"). Critical to allowance of the '288 patent was Patent Owner's argument that the claimed invention was "surprising" and that "one of skill in the art would not have predicted with a reasonable expectation of success that methods described for a virus such as adenovirus could be applied to [rAAV] particles." Ex.1002, 541. That is, Patent Owner represented that AUC had not been used to analyze rAAV particles in a printed publication. As illustrated herein, the prior art objectively indicates that representation was incorrect.

A published patent application referred to herein as "Le Bec" describes using AUC to characterize rAAV preparations. Le Bec was not before the Examiner, and it shows conclusively that application of AUC to rAAV was not "surprising." Indeed, Le Bec used the same instrumentation, software, and analysis that researchers had relied on for decades to characterize heterogeneous compositions of biomacromolecules—a fact acknowledged in the patent and evidenced by the prior art. Moreover, Le Bec ascribes no particular fanfare to its use of AUC on rAAV preparations; instead Le Bec treats it as what it is—the routine use of a decades old technique to characterize rAAV preparations. The claims challenged in this petition recite various parameters that could be employed when performing AUC on rAAV

particles, but those parameters were well known and a matter of routine optimization. Because the method was already known, there is nothing inventive about the claimed subject matter of the ’288 patent.

Accordingly, Petitioner respectfully submits the challenged claims are unpatentable. That position is supported by the art of record, the POSA’s knowledge, Patent Owner’s admissions, and the declaration of Dr. Steven Berkowitz (Ex.1020), an expert in AUC, including its application to viral preparations.

II. REQUIREMENTS FOR *INTER PARTES* REVIEW UNDER 37 C.F.R. §42.104

A. Grounds for Standing

Petitioners certify that (1) the ’288 patent is available for *inter partes* review (“IPR”) based on its January 20, 2015, priority date (Ex.1001, (60)), and (2) Petitioners are not barred or estopped from requesting review on the grounds identified.

B. Identification of Challenges

Pursuant to 37 C.F.R. §§42.104(b) and 42.22(a)(1), Petitioners request review and cancellation of the claims of the ’288 patent on the following grounds:

Ground	Claim(s)	Basis	References
1	1, 17-18, 20-21, 23, 32-33	§102	Anticipated by *Le Bec*
2	1, 17-18, 20-21, 23, 32-33	§103	Obvious over *Le Bec* alone
3	1-3, 17-26, 31-33	§103	Obvious over *Le Bec* in view of *Berkowitz* and *Cole*
4	27-30	§103	Obvious over *Le Bec* in view of *Berkowitz* and *Schuck* and/or *Zhao*
5	34	§103	Obvious over *Colosi* in view of *Le Bec*

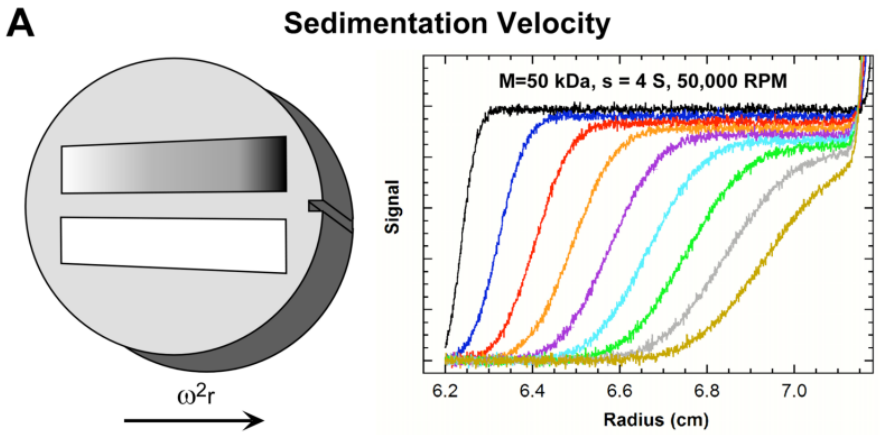
III. BACKGROUND

A. Analytical Ultracentrifugation

Theodore Svedberg invented the analytical ultracentrifuge in 1925, and the fundamental principles of analytical ultracentrifugation have changed little since. Ex.1020, ¶¶9-13; Ex.1005, 144 ("For over 75 years, analytical ultracentrifugation [] has proven to be a powerful method for characterizing solutions of macromolecules."); Ex.1001, 19:22-28; Ex.1006, 163.

The claims of the '288 patent involve a type of AUC called sedimentation velocity analytical ultracentrifugation ("SV-AUC"). SV-AUC involves spinning a sample fast enough to force suspended particles or dissolved molecular species to migrate in solution while collecting data on their differing sedimentation velocities (i.e. rates of movement). Ex.1020, ¶¶13-21. A boundary of particles moving at the same rate forms in response to the gravitational force, and a series of scans are taken

to measure the rate of movement (sedimentation velocity) of the boundaries over time. Ex.1020, ¶¶25-26; Ex.1006, 165.



Ex.1005, 162, Fig. 1 (depicting sedimentation velocity cells—a reference cell and a sample cell—and the data obtained from scans over time). Sedimentation velocity is a function of particle characteristics such as mass, shape, and density and is reported as a sedimentation coefficient (i.e., the rate at which a particle moves in response to acceleration) measured in Svedberg units, S (10^{-13} s). Ex.1005, 146; Ex.1020, ¶¶16-21. SV-AUC can separate, identify, and quantify different species of particles in a heterogenous mixture by their sedimentation coefficients. Ex.1020, ¶16.

The Lamm equation (which was derived in 1929 by Ole Lamm) describes the sedimentation and diffusion of a solute under ultracentrifugation and is used to determine sedimentation coefficients. Ex.1020, ¶139; Ex.1005, 146; Ex.1011, 228. In 2000, Peter Schuck created a publicly available, freely downloadable computer program called SEDFIT, which uses a distribution of Lamm equation solutions to

directly model the sedimentation boundary, and greatly increased the information derivable from AUC experiments. Ex.1020, ¶171; *see also* Ex.1004, 17. SEDFIT can graphically display sedimentation coefficient distribution profiles and determine relative concentrations of species in a sample. Ex.1020, ¶¶27-40. The SEDFIT software was widely used and frequently cited in publications. Ex.1020, ¶27; Ex.1005, 166-167, 173; Ex.1006, 167. It was also discussed in the '288 patent. Ex.1001, 21:4-19.

B. Using SV-AUC to Characterize Wide Varieties of Particle Sizes, Including Viral Preparations

After the release of SEDFIT and other, similar analysis tools, those in the field saw the benefits of AUC for a wide variety of applications. Ex.1020, ¶26. In the early 2000s, companies were developing gene therapy products comprising viral-based vectors, which required rigorous assessments of purity, potency, and safety during and after their manufacture. *See*, *e.g.*, Ex.1004, 16 ("To achieve drug licensure, drug product quality (with regard to homogeneity and purity) and consistency of manufacturing are of key importance."); Ex.1008, 1 ("[I]t has been speculated that nonfunctional empty virions in clinical vector lots may reduce efficiency of therapeutic gene transduction in the liver by competing with the fully packaged therapeutic vector particles for receptor uptake."). SV-AUC was seen as a powerful and useful tool for assessing the homogeneity of viral vectors used in gene

therapy applications. Ex.1004, 17; *see also* Ex.1005, 145 ("The range of molecular weights suitable for AUC exceeds that of any other solution technique from a few hundred Daltons (e.g., peptides, dyes, oligosaccharides) to several hundred-million Daltons (e.g., viruses, organelles).").

Moreover, SV-AUC had been identified as a useful tool for characterizing rAAV particles before the priority date for the '288 patent. Ex.1020, ¶¶41-44, 64-71; Ex.1019, 4-5; Ex.1006. An article from 1999 compared sedimentation coefficients of AAV virus-like particles determined by analytical ultracentrifugation. Ex.1009, 373-374, Fig. 3. A 2012 Assessment Report from the European Medicines Agency identified analytical ultracentrifugation as a method used to "determine mass, density and distribution profiles" for Glybera, a gene therapy product comprising a replication deficient AAV vector. Ex.1010, 15. Distribution profiles are a hallmark of SV-AUC (*see, e.g.*, Ex.1003, 6:3-10), indicating characterization of rAAV particles in pharmaceutical compositions via SV-AUC was known. An article from 2014 commented that "analytic ultracentrifugation technology is a powerful tool for quantitative characterization of structural heterogeneity of rAAV preparations, allowing precise and selective observation of viral capsid sedimentation in real time…. For future studies, it may be necessary to use analytic ultracentrifugation for further characterization of compositions of clinical rAAV lots." Ex.1008, 6.

Indeed, the use of SV-AUC with rAAV particles was not just theoretical, it had already been performed and reported in a published patent application ("Le Bec"). *See* Ex.1003. Before the '288 patent application was filed, the field was aware of SV-AUC, its usefulness in characterizing biomolecules of different types, and its actual application to the development of gene therapy technologies by identifying and quantifying rAAV particles.

IV. THE '288 PATENT

The '288 patent purports to have developed "methods to characterize preparations of recombinant viral particles using analytical ultracentrifugation." Ex.1001, Abstract. The patent acknowledges that AUC is a tool that "may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes" (Ex.1001, 19:25-28) and that it had been around for decades (Ex.1001, 19:22-24 ("AUC analysis has been well characterized over many decades and is highly versatile.")). The '288 patent claims methods that involve characterizing rAAV preparations under boundary sedimentation velocity conditions. Ex.1001, 54:20-58:47. However, the concept of using SV-AUC to analyze preparations of rAAV particles was already well-known before the '288 patent's priority date.

A. The Challenged Claims

The ’288 patent claims various methods that involve using SV-AUC to characterize a preparation of rAAV particles. Claims 1-3 and 17-34 are challenged herein.[1] Claim 1 is representative and recites:

> 1. A method of characterizing a preparation of recombinant adeno-associated viral (AAV) particles comprising the steps of
>
> a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,
>
> b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and
>
> c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle.

Ex.1001, 54:20-33.

The steps of “subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions” and “plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation

[1] The remainder of the claims of the ’288 patent are challenged in the concurrently filed petition, IPR2023-01045.

coefficient in Svedberg units (S)" are common to all challenged claims. The claims that depend from claim 1 specify the parameters used for the AUC method. Independent claim 2 (and claim 3 which depends therefrom) involve comparison to a standard curve. And claims 33 and 34 use the method of claim 1 to evaluate the production of rAAV particles (claim 33) or to prepare rAAV particles with reduced empty capsids (claim 34). These additional steps are not novel.

B. Patent Owner's Admissions in the Specification

The '288 patent's disclosure includes many admissions concerning the elements of the challenged claims known in the art, the motivation to develop the claimed methods, and the expectation of success in doing so. For example, the '288 patent acknowledges that "[t]he generation of recombinant viral vectors for the clinic *requires* an analytical method that monitors drug product quality with regard to homogeneity, purity and consistency of manufacturing" (Ex.1001, 1:38-41 (emphasis added)). This mirrors the need long-recognized by others for viral compositions used in gene therapy as well as pharmaceutical compositions generally and provides strong motivation to develop the claimed methods. *See*, *e.g.*, Ex.1004, 16-17, Ex.1005, 149, Ex.1008, 6.

The '288 patent also describes AUC as a well-known method within the ability of a skilled artisan to optimize. For example, the patent notes that "AUC analysis has been well characterized over many decades and is highly versatile" and

that it "may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes." Ex.1001, 19:22-28. The '288 patent also admits that a person skilled in the art would know how to optimize AUC for use with rAAV particles. *See, e.g.*, Ex.1001, 26:4-6 ("It is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles."); *id.*, 28:62-64 ("Suitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art."). The '288 patent claims merely recite an application of AUC analysis to an rAAV preparation, which Le Bec had already achieved.

C. Prosecution of the '288 Patent

As originally filed, the claims in the application leading to the '288 patent were directed to the use of SV-AUC to characterize preparations of recombinant viral particles in general. The Examiner issued a non-final rejection of all pending claims, as anticipated or obvious over references teaching SV-AUC methods and principles, including Cole. Ex.1002, 516-529 (citing Ex.1005).

To overcome the Examiner's rejections, Patent Owner amended the claims "to recite that the viral particles are recombinant adeno-associated viral (AAV) particles." Ex.1002, 539. Patent Owner argued that the cited references were silent regarding rAAV particles. *Id.*, 539-540. Patent Owner also argued—without evidentiary support—that "one of skill in the art would not have predicted with a

reasonable expectation of success that methods described for a virus such as adenovirus could be applied to recombinant AAV particles" and that the application was based on "the inventors' surprising finding that AUC could be applied to preparations of recombinant AAV particles to identify variant recombinant viral genomes or empty viral capsid particles impurities with incredible sensitivity, precision, and accuracy." *Id.*, 541.

The Examiner accepted Patent Owner's arguments when allowing the claims, stating that:

> Analytical ultracentrifugation (AUC) was well-described in the art as a means to separate biological molecules, including viruses. While it would be obvious to apply AUC to separation of AAV particles and routine to optimize said parameters, AUC was not noted as having been applied to AAV separation routinely….Applicants have surprisingly found parameters for AUC that allow for high levels of separation of AAV particles, including accurate separation of AAV subtypes and empty particles.

Ex.1002, 561. The Examiner's allowance was in error because the Examiner (1) was not aware of relevant art asserted herein (e.g., Le Bec), and (2) was led astray by Patent Owner's argument that the ability to apply SV-AUC to rAAV particles was surprising or unexpected. *See* Ex.1020, ¶¶52-56.

V. ASSERTED ART

A. Le Bec

Le Bec is a PCT Publication of International Application Number PCT/EP2014/052978, entitled "Methods for the Production of Double-Stranded AAV Viral Particles." Ex.1003 ("Le Bec"), (21). Le Bec was published on August 21, 2014 and qualifies as prior art under AIA §102(a)(1). *Id.* Le Bec was published in French, and a certified translation has been submitted with this petition. Ex.1003. The Examiner did not consider Le Bec during the prosecution of the '288 patent. Ex.1002. In the EPO, observations have been filed by third parties raising lack of novelty and inventiveness of claims nearly identical to those in the '288 patent. Ex.1012. Le Bec is analogous art because it is from the same field of endeavor as the claimed invention and reasonably pertinent to the problem faced by the inventor. Ex.1020, ¶66.

Le Bec discloses methods to produce double stranded/"self-complementary" AAV particles ("scAAV"), a type of rAAV particles. Le Bec describes the production and purification methods employed to make its scAAV compositions using two different methods, one involving insect derived sf9 cells, and another employing human embryonic kidney (HEK293) cells. Ex.1003, 1:33-3:2.

Le Bec reports characterization data for its AAV preparations, including "[a]nalysis of empty and full AAV viral particles" using "analytical

ultracentrifugation." Ex.1003, 15:17-18. Le Bec explains that "[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation" and that "[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100 µl or 400 µl of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT." Ex.1003, 15:23-28. Further, Le Bec notes that the densities of empty and full AAV viral particles had been reported in the literature, and that "[k]nowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation." *Id.*, 18:1-6.

Figures 2 and 3 of Le Bec depict C(s) v. S plots from the AUC analyses:

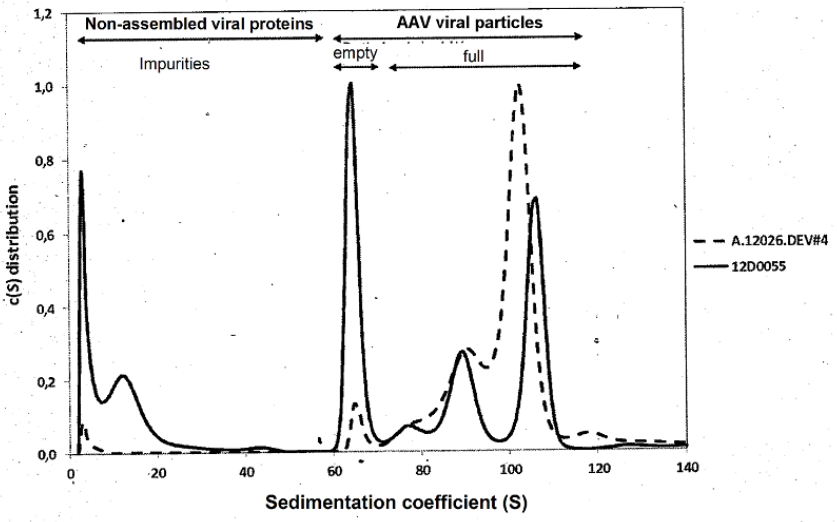


Figure 2

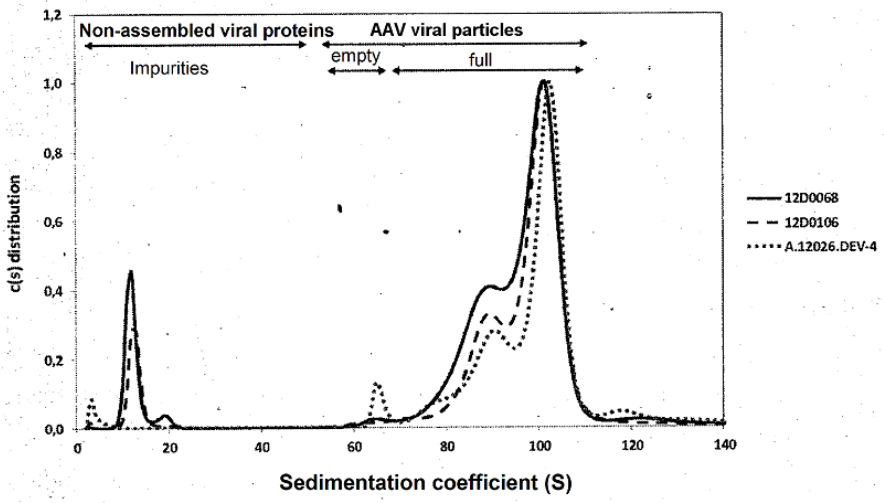


Figure 3

Figure 2 compares the AAV particles produced in sf9 cells with those prepared in HEK293 cells. Ex.1003, Fig. 2. Le Bec explains that the distribution profile reflects the different populations of viral proteins: (1) < 60S represents non-assembled viral proteins or contaminants, (2) the peak at around 65S represents empty AAV particles, (3) a peak at 90S corresponds to particles with the viral

genome in single-stranded form, and (4) a peak at 105S corresponds to particles with the viral genome in double-stranded form. Ex.1003, 18:10-32.

The results in Figure 2 show that the viral preparation made with HEK293 cells has less than 25% full particles (only 10% of which contain the double stranded genome). Ex.1003, 19:1-5. Empty rAAV particles are a substantial portion of the composition, as shown by the large peak at 65S. Ex.1003, Fig. 2. In contrast, viral particles made with sf9 cells contained very few empty particles and were enriched in particles containing the double stranded genome. Ex.1003, 19:7-15.

Thus, AUC's applicability to characterizing heterogeneous compositions of rAAV particles was known and described in a printed publication (Le Bec) before the priority date of the '288 patent. Nothing in Le Bec suggests that the use of AUC to characterize rAAV particle compositions was considered unexpected or required more than routine optimization or experimentation. Ex.1003.

B. Berkowitz[2]

Berkowitz is a scientific article entitled "Adenovirus homogeneity by analytical ultracentrifugation." It was published in 2007 and qualifies as prior art under AIA §102(a)(1). Ex.1004. Berkowitz is cited in the '288 patent specification,

[2]Berkowitz was authored by Petitioner's expert, Dr. Steven Berkowitz.

but it was not relied on by the Examiner during prosecution to support a rejection. Ex.1002, 516-529.

Berkowitz is analogous art from the same field of endeavor as the claimed invention and reasonably pertinent to the problem faced by the inventor. Ex.1020, ¶159. Berkowitz teaches the use of analytical ultracentrifugation to characterize the homogeneity of viral preparations. *Id.*, ¶¶158-163, 180.

Berkowitz explains that for all drug biologic products requiring marketing approval, "drug product quality (with regard to homogeneity and purity) and consistency of manufacturing are of key importance." *Id.* Berkowitz notes that an adenoviral preparation might include empty capsids, incomplete or aberrant particles, sub particles, and/or aggregates. Ex.1004, 16-17.

Berkowitz uses AUC to assess the homogeneity of viral preparations, noting that it had been used historically. Ex.1004, 17. Berkowitz explains that technological improvements make SV-AUC a promising tool for analyzing biological samples. *Id.* Berkowitz further teaches that "simple peak area integration, taking into account radial dilution effects…allows the percentage of [empty capsid] material present in a virus preparation to be readily calculated." Ex.1004, 21. Berkowitz provides examples of these calculations for empty capsids and aggregates in Table 2. Ex.1004, 29 (Table 2).

C. Cole

Cole is a chapter in METHODS IN CELL BIOLOGY entitled "Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium." It was published in 2008 and qualifies as prior art under AIA §102(a)(1). Ex.1005. Cole was cited by the Examiner during prosecution of the '288 patent. Ex.1002, 520-523, 525-526.

Cole is analogous art from the same field of endeavor as the claimed invention and reasonably pertinent to the problem faced by the inventor. Ex.1020, ¶165. Cole provides a general overview of various analytical ultracentrifugation techniques—including SV-AUC—and explains the parameters to be adjusted depending on the experimental system and samples being tested.

Cole reviews the developments in using AUC-based techniques to quantitatively analyze macromolecules in solution. Cole notes that AUC is considered "a versatile and powerful method," with "broad applications for the study of biomacromolecules in a wide range of solvents and over a wide range of solvent concentrations." Ex.1005, 144.

Cole differentiates between sedimentation velocity ("SV-AUC") and sedimentation equilibrium ("SE-AUC"). Cole explains SV-AUC's use of fast rotor speeds to measure the speed at which particles transit the sector cell in response to the gravitational field, from which one can derive information about particle size and

shape. Ex.1005, 145-149, 161-168. Cole notes that SE-AUC uses lower speeds and provides information on molar masses, stoichiometries, association constants, and solution nonideality in the sample. Ex.1005, 145,147, 168-173; *see also* 162, Fig 1.

Cole teaches that a common application of SV-AUC is to determine the distribution of sedimentation coefficients for different macromolecules, *e.g.*, by their C(s) values, explaining that "often a simple relationship between s [sedimentation coefficient] and M [effective particle mass] may be used to identify particular peaks as belonging to certain oligomers (e.g., dimer, trimer, etc.) or certain fragments of the monomer." Ex.1005, 149. In particular, "[s]edimentation coefficient distributions are used widely in the pharmaceutical industry to assess the stability of protein formulations and to characterize preparations of inherently heterogeneous samples (e.g., vaccines based on bacterial cell wall preparations)." *Id.*

Cole also describes the instrumentation routinely used in SV-AUC analysis. Analytical ultracentrifuges are distinguished from other high-speed centrifuges by "the specialized rotors, sample holders and optical systems that permit the observation of samples during sedimentation." Ex.1005, 150. Samples are loaded into cells that permit the passage of light allowing for optical assessments during centrifugation. These "fundamental measurements" are known as "scans" and "are acquired at intervals ranging from minutes (for velocity sedimentation) to hours (for equilibrium sedimentation)." Ex.1005, 151. Cole instructs how to determine and

optimize parameters for an SV-AUC experiment and how to perform the analysis steps necessary to interpret the data. Ex.1005, 161-168.

D. Schuck

Schuck is a scientific article entitled "Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling." It was published in 2000 and qualifies as prior art under AIA §102(a)(1). Ex.1007. Schuck was cited by the Examiner during prosecution of the '288 patent. Ex.1002, 525-527.

Schuck is analogous art from the same field of endeavor as the claimed invention and reasonably pertinent to the problem faced by the inventor. Ex.1020, ¶169. Specifically, Schuck describes methods for analyzing AUC data using SEDFIT. Shuck describes SEDFIT as "a method for direct boundary modeling for the size-distribution analysis in sedimentation velocity analytical ultracentrifugation." Ex.1007, 1615. Schuck also explains what scientists must consider when analyzing SV-AUC data, including with respect to regularization of the data.

E. Zhao

Zhao is a scientific review entitled "Current Methods in Sedimentation Velocity and Sedimentation Equilibrium Analytical Ultracentrifugation." It was published in 2013 and qualifies as prior art under AIA §102(a)(1). Ex.1016. Zhao

was not considered by the Examiner during the prosecution of the ’288 patent. Ex.1002, 516-529.

Zhao is analogous art from the same field of endeavor as the claimed invention and reasonably pertinent to the problem faced by the inventor. Ex.1020, ¶174. Specifically, Zhao reviews basic principles of AUC analysis and highlights the “significant progress” made in interpreting AUC data. Ex.1016, Abstract. Zhao teaches about SV-AUC data analysis and methods to effect regularization of SV-AUC data.

F. Colosi

Colosi is U.S. Pat. Pub. Application. No. 2009/0017542, entitled “High-efficiency wild-type-free AAV helper functions.” Colosi was published in 2009 and qualifies as prior art under AIA §102(a)(1). Ex.1015. Colosi was cited by the Examiner during prosecution of the ’288 patent. Ex.1002, 527-529. Colosi teaches “methods and compositions for producing high titer, wild-type-free preparations of recombinant AAV (‘rAAV’) virions.” Ex.1015, Abstract.

VI. LEVEL OF ORDINARY SKILL IN THE ART

A POSA working in the field of the ’288 patent on January 19, 2015, would have possessed at least a B.S. in biology, chemistry, chemical engineering, biochemistry, biophysics, pharmaceutical science, or a related discipline, with two or more years of industry, laboratory, and/or clinical experience in analyzing or

characterizing biomolecules, including viruses or viral vectors. Such a person may be familiar with, or consult with someone familiar with, the development, formulation, and/or administration of viral vectors for gene therapy and quality standards required to market such products. Ex.1020, ¶58.

VII. CLAIM CONSTRUCTION

The Board construes claims per *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005). 37 C.F.R. §42.100(b). Claims should only be construed to the extent necessary to resolve a controversy. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). For this proceeding, no terms require express construction, because the prior art's disclosures are commensurate with the '288 patent disclosures and Patent Owner's admissions during prosecution. The prior art reads on the claims under any construction consistent with *Phillips*. For purposes of this proceeding, the petition analyzes the claim terms under their "plain and ordinary meaning."[3]

[3] Petitioners reserve the right to argue that claim construction is necessary in another forum. For example, Patent Owner's infringement and validity positions in the co-pending litigation may raise controversies that require resolution through claim constructions not implicated here given the similarities between the prior art and the '288 patent.

VIII. GROUND 1: CLAIMS 1, 17-18, 20-21, 23, AND 32-33 ARE ANTICIPATED BY LE BEC

"To anticipate, a prior art reference must place the [invention] in the possession of the public." *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc*., 471 F.3d 1369, 1375 (Fed. Cir. 2006). "[A] reference can anticipate a claim even if it 'd[oes] not expressly spell out' all the limitations arranged or combined as in the claim, if a person of skill in the art, reading the reference, would 'at once envisage' the claimed arrangement or combination." *Kennametal, Inc. v. Ingersoll Cutting Tool Co.*, 780 F.3d 1376, 1381 (Fed. Cir. 2015) (quoting *In re Petering*, 49 CCPA 993, 301 F.2d 676, 681 (1962)); *see also Novo Nordisk Pharms., Inc. v. Bio-Tech. Gen. Corp.*, 424 F.3d 1347, 1355 (Fed. Cir. 2005) (anticipation does not require actual performance of suggestions in a disclosure).

A. Claim 1 is Anticipated by Le Bec

Claim 1 recites a method of characterizing an rAAV preparation by subjecting it to boundary sedimentation conditions, plotting the results, and analyzing the relative amounts of different species therein. Claim 1 is anticipated by Le Bec.

1. "A method of characterizing a preparation of recombinant adeno-associated viral (AAV) particles comprising the steps of"

To the extent the preamble is construed to be limiting,[4] it is taught by Le Bec. Specifically, Le Bec teaches a method for analytically separating, detecting, and quantifying the presence of empty capsids, full capsids, and aggregates in an rAAV preparation. Ex.1003, 15:17-23, 16:26-29, 18:1-6; Ex.1020, ¶72.

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals"

Le Bec teaches subjecting an rAAV preparation to ultracentrifugation under boundary sedimentation velocity conditions. The '288 patent explains the terms "'sedimentation velocity conditions' or 'boundary sedimentation velocity conditions' may refer to any experimental conditions under which a sample solution is subjected to sedimentation velocity analysis." Ex.1001, 17:17-20. Le Bec describes subjecting

[4] "[A] preamble is not limiting 'where a patentee defines a structurally complete invention in the claim body and uses the preamble only to state a purpose or intended use for the invention'." *Catalina Mktg. Int'l, Inc. v. Coolsavings.com, Inc.*, 289 F.3d 801, 808 (Fed. Cir. 2002) (quoting *Rowe v. Dror*, 112 F.3d 473, 478 (Fed. Cir. 1997)).

an rAAV preparation to SV-AUC conditions to determine the sedimentation coefficients of various rAAV particles. Ex.1003, 15:17-23. SV-AUC determines sedimentation coefficients by looking at boundary sedimentation velocity data. Ex.1020, ¶¶73-78; Ex.1005, 146-147. Le Bec's determination of the "sedimentation coefficient[s] of the various AAV viral particles," and plotting those coefficients, confirms that Le Bec was measuring the velocities at which different sedimentation boundaries were moving and, thereby, performing SV-AUC. Ex.1020, ¶¶77-78; *see also* Ex.1005, 146-147.

Furthermore, the AUC parameters described in Le Bec result in boundary sedimentation conditions for rAAV-sized particles. Ex.1020, ¶¶75-76; Ex.1001, 17:17-20. Le Bec details the parameters of the ultracentrifugation procedure: vectors "are concentrated and formulated in PBS buffer" and "[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100µl or 400µl of undiluted pure vectors, sedimentation was followed by the absorbance at the wavelength of 276 nm, the sedimentation coefficient was obtained using the software SEDFIT." Ex.1003, 14:15-16; 15:23-28. Le Bec's rotor speed of 16,000 rpm falls within the range identified in the '288 patent for use with AAV particles (between 10,000 rpm and 20,000 rpm). Ex.1001, 29-30 (Table 1). Similarly, Le Bec's disclosure of measuring absorbance at 276 nm is consistent with the '288 patent's explanation that

"[i]n some embodiments, the absorbance is at about 230nm, 260nm or 280nm." *See* Ex.1001, 4:36-37; *see also* Ex.1001, 29-30 (Table 1).

Le Bec further reports acquiring data in "real-time," which means that the sedimentation of rAAV particles was monitored at time intervals. Ex.1020, ¶79. The distribution profiles reported in Le Bec in Figures 2 and 3 and the calculation of sedimentation coefficients of the various populations using the SEDFIT software requires that sedimentation was monitored at time intervals. Ex.1020, ¶79. Thus, a POSA would understand and at once envisage that Le Bec applied boundary sedimentation velocity conditions to rAAV particles. *Kennametal*, 780 F.3d at 1381.

3. "b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)"

Le Bec plots a "differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S)." Ex.1020, ¶¶80-81. Specifically, Le Bec describes Figures 2 and 3 as "a graph showing the analytical ultracentrifugation distribution profile." Ex.1003, 6:4-10. Each graph plots the "C(s) distribution" on one axis and the "sedimentation coefficient (S)" on the other:

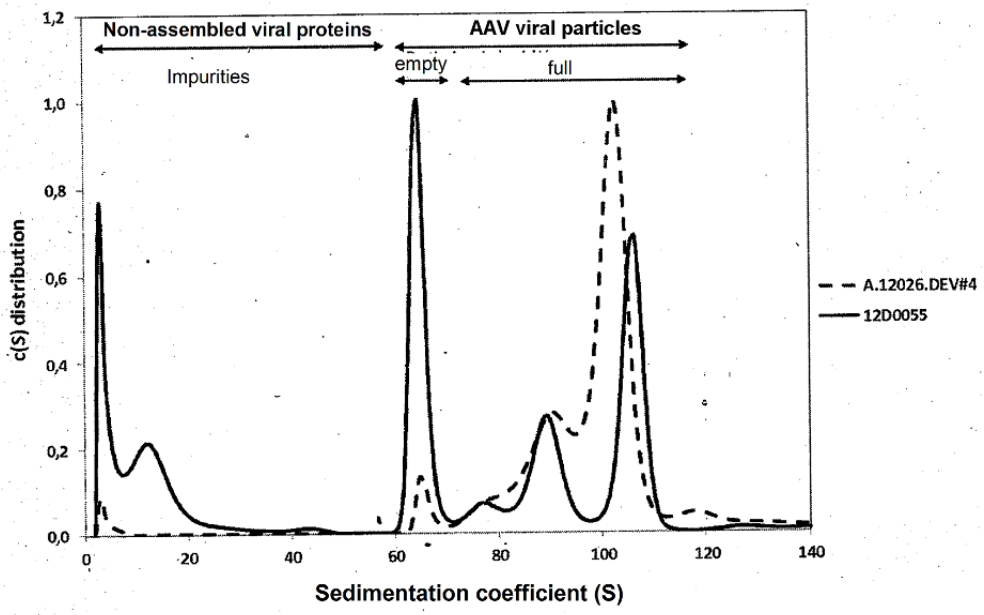


Figure 2

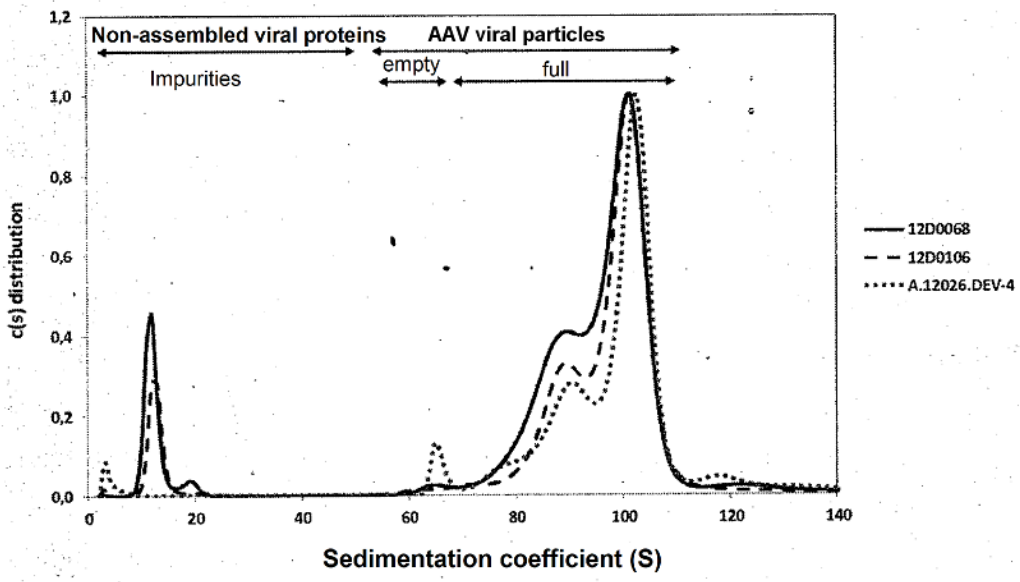


Figure 3

Ex.1003, Figs. 2 and 3; Ex.1020, ¶¶80-81.

4. "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle"

Le Bec teaches use of AUC to determine the "quantification of empty and full viral particles" and reports the results of that quantification in the form of a percentage. For example, Le Bec explains that "[t]he distribution profile…confirms that the HEK293 system essentially produced very few full AAV particles (< 25%). Ex.1003, 19:1-5; *see also* 19:12-15 (sf9 system sample essentially composed of full AAV particles (> 80%)). Le Bec teaches that the "distribution profile of the species allows the identification of two categories of populations" and differentiates peaks as corresponding to empty or full particles. Ex.1003, 18:11-27; *see also* Figs. 2 and 3. A POSA would understand the disclosed percentages to be relative concentrations of the corresponding rAAV particle species in the sample. Ex.1020, ¶¶82-84; *see also* Ex.1003, 8:10-13.

Furthermore, a POSA would understand that obtaining the percentages/relative concentrations disclosed in Le Bec using a distribution profile involves integrating the area under the curve for the peaks of interest. Ex.1020, ¶84; *Novo Nordisk*, 424 F.3d at 1355. Le Bec disclosed that the SEDFIT software was used to determine sedimentation coefficients, and the C(s) v. S distribution profile graphs depicted in Figures 2 and 3 of Le Bec are the type generated by SEDFIT.

Ex.1020, ¶¶27-40, 83. In Version 14.4d of SEDFIT, which was publicly available and freely downloadable before the '288 patent's priority date and at the time of Le Bec, users could determine relative concentrations by integrating areas under the curve. Ex.1020, ¶¶27-40. A POSA was aware of this functionality of the SEDFIT software and how to implement it. Ex.1020, ¶27. Claim 1 is anticipated.

B. Claim 17 is Anticipated by Le Bec

Claim 17 depends from claim 1, and further requires that "the sedimentation of recombinant AAV particles is monitored by absorbance or interference." Le Bec discloses that its rAAV particle sedimentation is monitored by absorbance: "sedimentation was followed by absorbance at the wavelength of 276 nm." Ex.1003, 15:25-26; Ex.1020, ¶135. Claim 17 is anticipated.

C. Claim 18 is Anticipated by Le Bec

Claim 18 depends from claim 1, and further requires that "the preparation is an aqueous solution." Le Bec discloses that "[t]he AAV viral particles are concentrated and formulated in a PBS buffer." Ex.1003, 14:15-14:16. A POSA would know PBS is an aqueous buffer. Ex.1020, ¶137. Claim 18 is anticipated.

D. Claim 20 is Anticipated by Le Bec

Claim 20 depends from claim 1, and further requires that "the C(s) values are determined by an algorithm that comprises Lamm equation solutions." Le Bec discloses that "the sedimentation coefficient of the different populations was

obtained using the software SEDFIT" (Ex.1003, 15:27-28), which was known to implement an algorithm that provides Lamm equation solutions to determine C(s) values (Ex.1007, 1610; Ex.1016, 5; Ex.1020, ¶139). Claim 20 is anticipated.

E. Claim 21 is Anticipated by Le Bec

Claim 21 depends from claim 20, and further requires that "the algorithm is the SEDFIT algorithm." Le Bec expressly discloses using SEDFIT. Ex.1003, 15:27-28; Ex.1020, ¶141. Claim 21 is anticipated.

F. Claim 23 is Anticipated by Le Bec

Claim 23 depends from claim 1 and further requires that "the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell." Le Bec indicates that "analytical ultracentrifugation" was performed, and sedimentation coefficients determined. Ex.1003, 15:17-23, 18:1-6. Sedimentation velocity experiments require the use a special sector-shaped velocity cell. *See, e.g.*, Ex.1005, 144, 161-162; Ex.1011, 220; Ex.1013, 11-14; Ex.1020, ¶143. A POSA would at once envisage that Le Bec used an ultracentrifuge velocity cell to generate the data therein. *Kennametal*, 780 F.3d at 1381; *Novo Nordisk*, 424 F.3d at 1347. Claim 23 is anticipated.

G. Claim 32 is Anticipated by Le Bec

Claim 32 depends from claim 1 and further requires that "the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm and/or

at about 4°C. to about 20° C." Importantly, the rotor speed and temperature ranges are claimed as alternatives. Le Bec discloses that "centrifugation of the samples was carried out at a speed of 16,000 rpm," which is within the claimed range. Ex.1003, 15:23-25; Ex.1020, ¶145. Claim 32 is anticipated.

H. Claim 33 is Anticipated by Le Bec

Claim 33 recites a method of evaluating a process to produce rAAV particle preparations by analyzing the relative amounts of different species therein following application of the method of claim 1.

1. "A method of evaluating a process for the production of recombinant AAV particles comprising"

To the extent the preamble is construed to be limiting (fn.5, *supra*), it is taught by Le Bec. Le Bec explains that its invention relates "to a method for producing an scAAV vector." Ex.1003, 4:12-13. In a section discussing "[a]nalyses and characterization," Le Bec teaches the use of "analytical ultracentrifugation" to analyze empty and full AAV particles. Ex.1003, 14:20-15:28. Le Bec teaches that SV-AUC can be used to evaluate and compare rAAV production processes. Ex.1003, 20:2-7; Ex.1020, ¶147.

2. "the method of claim 1,"

Claim 1 is anticipated by Le Bec. *See* §VIII.A.

3. "wherein an increase in the relative amount of recombinant AAV particles comprising intact AAV genomes compared to the relative amount of empty capsid particles and/or recombinant AAV capsid particles with variant recombinant AAV genomes compared to a reference preparation of recombinant AAV particles indicates an improvement in the production of recombinant AAV particles."

The final limitation of claim 33 merely describes how to interpret the information generated by the claimed SV-AUC method and should not be afforded any patentable weight. Under the "printed matter" doctrine, "[c]laim limitations directed to the content of information and lacking a requisite functional relationship are not entitled to patentable weight…." *Praxair Distribution, Inc. v. Mallinckrodt Hosp. Prod. IP Ltd.*, 890 F.3d 1024, 1032 (Fed. Cir. 2018); *see also id*. at 1031 ("Claim limitations directed to printed matter are not entitled to patentable weight unless the printed matter is functionally related to the substrate on which the printed matter is applied.").

In considering whether claimed information is functionally related, one must consider "whether the printed matter merely informs people of the claimed information, or whether it instead interacts with the other elements of the claim to create a new functionality in a claimed device or to cause a specific action in a claimed process." *C R Bard Inc. v. AngioDynamics, Inc.*, 979 F.3d 1372, 1381 (Fed. Cir. 2020). In *Praxair*, claims that merely required providing a physician with

information and/or required the physician to evaluate the information—a "think about it" step—were not given patentable weight, unlike claims that required the physician to take a specific action based on the information provided. *Praxair*, 890 F.3d at 1033-1035.

Here, the final limitation of claim 33 seeks to claim the content of information provided by the SV-AUC analysis without a functional relationship, and thus it is entitled to no patentable weight. The limitation merely recites that an increase of intact genome AAV particles "indicates"—i.e., informs the experimenter of—an improvement in the process tested. And there is no functional relationship to the remainder of the claim because the experimenter is not required to do anything with the information or take any action based on the knowledge.

Additionally, this limitation should not be accorded any patentable weight, because it merely recites the relationship between the tested composition and associated result of the method. *See, e.g.*, *Minton v. Nat'l Ass'n of Sec. Dealers, Inc.*, 336 F.3d 1373, 1381 (Fed. Cir. 2003) ("A whereby [or wherein] clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited."); *see also Texas Instruments Inc. v. U.S. Int'l Trade Comm'n*, 988 F.2d 1165, 1169 (Fed. Cir. 1993).

To the extent such data analysis limitations are given any patentable weight, they were nevertheless disclosed in Le Bec. For example, Le Bec discloses that a

known problem in the production of double stranded rAAV vectors is "the percentage of empty AAV viral particles, i.e. particles lacking viral DNA," because those particles are both inactive and contribute to immunogenicity. Ex.1003, 3:16-21. Le Bec recognizes the increased number of intact rAAV particles compared to the number of empty rAAV particles in the sf9-based system—as measured by SV-AUC—is an improvement over the HEK293-based preparation. Ex.1003, 3:26-29, 18:27-32; Ex.1020, ¶149. Claim 33 is anticipated.

IX. GROUND 2: CLAIMS 1, 17-18, 20-21, 23, and 32-33 ARE OBVIOUS IN VIEW OF LE BEC ALONE

Claims 1, 17-18, 20-21, 23, and 32-33 are anticipated in view of Le Bec, but to the extent not anticipated, these claims are obvious over Le Bec alone. A single prior art reference can invalidate a patent claim for obviousness if it would have been obvious to modify that reference to arrive at the patented invention. *Koninklijke Philips N.V. v. Google LLC*, 948 F.3d 1330, 1338 (Fed. Cir. 2020).

A. Applying SV-AUC to rAAV Particles was Obvious

Le Bec discloses "methods for producing double-stranded AAV viral particles…for therapeutic applications such as gene therapy." Ex.1003, 1:5-9. Le Bec commented on the then-current limitations in producing scAAV vectors, including the high percentage of empty AAV particles "with an inactive product contributing to the immunogenicity of the vector." *Id.*, 3:16-22. Others similarly

acknowledged the problem with having excess empty capsids in a gene therapy product comprising AAV particles. Ex.1008, 1. Thus, POSAs were motivated to assess virus preparations for such undesirable contaminants. *See Spectrum Pharms., Inc. v. Sandoz Inc.*, 802 F.3d 1326, 1334 (Fed. Cir. 2015) ("A physician would not likely want to administer a contaminant or a less pure material to a patient if one could use a pure material. Thus, there is always in such cases a motivation to aim for obtaining a pure, resolved material."). Indeed, the '288 patent acknowledged as much. *See* §IV.B.

The art taught that SV-AUC was a powerful tool for assessing the homogeneity of viral vectors used in gene therapy applications. Ex.1004, 17; Ex.1008, 6; Ex.1019, 5 ("Critical information about aggregation, empty capsids, virus subparticles, and other lower molecular weight species was gained from AUC experiments…. The aggregates, empty capsids, and low molecular weight degradation products can also be quantified."). The '288 patent itself notes that "AUC analysis has been well characterized over many decades and is highly versatile" and that it "may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes." Ex.1001, 19:22-28. The many uses of SV-AUC were well known and explained in the prior art, including for assessing the homogeneity of viral preparations. Ex.1004. In view of (1) the importance of drug product quality (with regard to homogeneity

and purity) to achieve licensure (Ex.1004, 16; Ex.1008, 1, 6), and (2) the recognized benefits of using boundary sedimentation velocity analysis for analyzing such aspects of biological samples (Ex.1003, 17:30-18:27; Ex.1005 145, 149, 161-168), a POSA would have been motivated to choose SV-AUC when seeking to characterize a preparation of rAAV particles to assess homogeneity. Ex.1020, ¶180; Ex.1019, 4-5; *Spectrum*, 802 F.3d at 1334.

A POSA would have reasonably expected success in applying SV-AUC to rAAV particles because the sedimentation coefficients of empty and full rAAV particles were known and reported in the literature, and because Le Bec taught that rAAV particles (full, empty, and aggregate) could be effectively separated, characterized, and quantified by analytical ultracentrifugation. Ex.1003, 15:19-23; Ex.1020, ¶181. Patent Owner has not described or claimed any changes to SV-AUC. *See Southwire Co. v. Cerro Wire LLC*, 870 F.3d 1306, 1311 (Fed. Cir. 2017) (affirming obviousness finding where the patented steps did not differ in any material way from the process disclosed in the prior art). *In re Woodruff*, 919 F.2d 1575, 1578 (Fed. Cir. 1990) ("It is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable."). Instead, Patent Owner incorrectly claims to be the first to apply SV-AUC to rAAV particles, ignoring Le Bec's teachings.

B. Using Integration to Determine Relative Concentrations was Obvious

To the extent Patent Owner argues that Le Bec does not describe integrating the area under each peak to determine the relative concentration of each particle, doing so would have been obvious.

As explained above, a POSA would have been motivated to use SV-AUC to assess homogeneity of rAAV preparations and accurately quantify the rAAV species in a consistent and reliable way for clinical lots of drug product. *See* Ex.1008, 1; Ex.1020, ¶180. Integrating the area under the peaks generated in an SV-AUC experiment is a standard technique used to determine the relative concentrations of components in the analyzed sample. Ex.1020, ¶182. Indeed, Le Bec itself provides motivation to integrate peak areas of C(s) v. S plots: to assess levels of impurities such as empty particles and aggregates as well as particles containing single- and double-stranded genomes, and to compare the efficiency of different rAAV manufacturing platforms. Ex.1003, 3:16-22; 15:27-28; 17:29:18:6.

Moreover, the SEDFIT software used for analyzing SV-AUC data included the ability to calculate concentration information based on integration of peaks selected by the user. Ex.1020, ¶¶27-40. A POSA would have been motivated to use the integration function in the SEDFIT software to determine the relative concentrations of rAAV species in Le Bec's samples with a reasonable expectation

of success in view of Le Bec's own use of SEDFIT to report relative concentrations. Ex.1003, 15:18-28; Ex.1020, ¶182. Using a process disclosed in the prior art for achieving the same purpose is obvious. *See KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 401 (2007); *Southwire,* 870 F.3d at 1311; *In re Woodruff*, 919 F.2d at 1578.

X. GROUND 3: CLAIMS 1-3, 17-26, AND 31-33 ARE OBVIOUS OVER LE BEC IN VIEW OF BERKOWITZ AND/OR COLE

A. Claims 1, 17-18, 20-21, 23, and 32-33 are Obvious Over Le Bec in View of Berkowitz and Cole

Le Bec anticipates and renders obvious each of claims 1, 17-25, and 33, but to the extent any limitation is found missing, it would have been obvious in view of Berkowitz and Cole.

1. Claim 1

Claim 1 is anticipated and obvious over Le Bec alone (§§VIII.A and IX), and further would have been obvious over Le Bec in view of Berkowitz.

Berkowitz instructs as to the "overall capability of boundary sedimentation velocity analysis," refers to it as "conventional," and describes it as "a uniquely useful characterization tool that can assess adenovirus product quality and manufacturing consistency." Ex.1004, 16-18. Subjecting Le Bec's preparation of rAAV particles to SV-AUC, would have been obvious to a POSA based on the teachings of Berkowitz regarding the uses of SV-AUC to characterize viral preparations for gene therapy applications, including to identify and quantify empty

capsids, intact monomers, aggregates, and incompletely formed species. Ex.1004, 17; Ex.1020, ¶¶199-200; *KSR*, 550 U.S. at 401. A POSA would have been motivated to apply Berkowitz's teachings to Le Bec's preparations in view of the (1) importance of drug product quality (with regard to homogeneity and purity) to achieve licensure, and (2) recognized benefits of using boundary sedimentation velocity analysis for analyzing such aspects of biological samples. Ex.1003, 17:30-18:27; Ex.1004, 16; Ex.1005 145, 149, 161-168; Ex.1008, 1, 6; Ex.1020, ¶201; *Spectrum*, 802 F.3d at 1334. And the POSA would have reasonably expected success because the SV-AUC method was well known and within the skill of an ordinary skilled artisan to optimize, and the art recognized no difficulties in applying SV-AUC to rAAV. Ex.1001, 19:22-28; Ex.1020, ¶202; *KSR*, 550 U.S. at 401.

Furthermore, although determining relative concentrations of viral particles by integrating areas under the curves was anticipated by and/or obvious in view of Le Bec (§§VIII.A.4 and IX), it would have further been obvious in view of Berkowitz. Berkowitz expressly teaches that "quantification" of each particle would be accomplished by integration: "simple peak area integration…allows the percentage of EC [empty capsid] material present in a virus preparation to be readily calculated." Ex.1004, 20. A POSA would have been motivated to apply Berkowitz's teachings to Le Bec's SV-AUC data to accurately quantify the rAAV species in a consistent and reliable way for clinical lots of drug product. *See* Ex.1008, 1; Ex.1020,

¶¶180, 199-201; *KSR*, 550 U.S. at 401; *Unwired Planet, LLC v. Google Inc.*, 841 F.3d 995, 1003 (Fed. Cir. 2016) ("For the technique's use to be obvious, the skilled artisan need only be able to recognize, based on her background knowledge, its potential to improve the device and be able to apply the technique.").

2. Claim 17

Claim 17 is anticipated by Le Bec (Section VIII.B), and further would have been obvious over Le Bec in view of Cole. Le Bec teaches monitoring by absorbance. Ex.1003, 15:23-28. Cole teaches that "[t]hree optical systems are available for the analytical ultracentrifuge (absorbance, interference, and fluorescence) that permit precise and selective observation of sedimentation in real time." Ex.1005, 144; *see also id.*, 152-155 (describing the use of absorbance—"the most frequently used detector"—and interference). A POSA would have been motivated to choose a method of detection that would be effective in detecting sedimentation of rAAV particles. Ex.1020, ¶227. Here, there is a finite number of solutions (just three) that were obvious to try. Of the three options, fluorescence would likely be incompatible with a pharmaceutical drug product. *Id.* Absorbance or interference would have been implemented with a reasonable expectation of success and would yield predictable results. *Id.*; *KSR*, 550 U.S. at 401; *see also* Ex.1013, 18.

3. Claim 18

Claim 18 is anticipated by Le Bec (§VIII.C), and further would have been obvious over Le Bec in view of Cole. A POSA would have been motivated to choose a buffer that is compatible with SV-AUC. Le Bec describes using PBS (phosphate buffered saline) which is an aqueous solution. Cole teaches that "[m]ost of the commonly used buffer components are compatible with AUC experiments." Ex.1005, 160. In view of the teachings of Le Bec and Cole, use of an aqueous buffer is obvious to try and would yield predictable results. *KSR*, 550 U.S. at 401; Ex.1020, ¶228.

4. Claims 20 and 21

Claims 20 and 21 are anticipated by Le Bec (§VIII.E-F), and further would have been obvious over Le Bec in view of Berkowitz and Cole. The Lamm equation was known since 1929 as the equation that that describes sedimentation of a component or molecular species under ultracentrifugation. Ex.1011, 228; Ex.1020, ¶231. Modern computer programs and models (such as SEDFIT) use solutions to the Lamm equation to generate a sedimentation coefficient distribution. Ex.1005, 166-167; Ex.1011, 231-233. Le Bec indicates that sedimentation coefficients were determined using the SEDFIT software. Ex.1003, 15:26-28. Berkowitz indicates that "[d]ata analysis was conducted using…Peter Shuck's software program SEDFIT." Ex.1004, 18. And Cole discussed use of the Lamm equation (Ex.1005, 146-147),

including how the SEDFIT software "simulates the sedimentation boundaries for each point using a numerical solution of the Lamm equation" (Ex.1005, 166-167). A POSA would have been motivated to determine C(s) values in order to determine purity and homogeneity of the sample and would have reasonably expected success using an algorithm that comprises Lamm equation solutions—like SEDFIT—based on the teachings of Le Bec, Cole, and Berkowitz. *KSR*, 550 U.S. at 401; Ex.1020, ¶¶180, 231.

5. Claim 23

Claim 23 is anticipated by Le Bec (§s VIII.H), and further would have been obvious over Le Bec in view of Cole. Le Bec demonstrates that SV-AUC was performed and sedimentation coefficients determined. Ex.1003, 15:17-23, 18:1-6. Cole teaches that sedimentation velocity "experiments are carried out in two-channel cells with sector-shaped compartments (Fig. 1) in order to prevent convection, which would occur if the cell walls were not parallel to radial lines." Ex.1005, 161-162, Fig. 1. The '288 patent also confirms that "[w]hen using the ProteomeLab™ XL-1, sample is loaded into the sample sector of a two-sector velocity cell, a vehicle control (e.g., PBS without recombinant viral) is loaded into the corresponding reference sector." Ex.1001, 22:30-33. To prevent convection and ensure accurate data, to the extent not inherent in Le Bec, a POSA would have been motivated to use a sector shaped velocity cell when running a sedimentation velocity experiment, and would

have done so with a reasonable expectation of success. *KSR*, 550 U.S. at 401; Ex.1020, ¶233.

6. Claim 32

Claim 32 is anticipated by Le Bec (§VIII.G), and further would have been obvious over Le Bec in view of Cole. Le Bec discloses that "centrifugation of the samples was carried out at a speed of 16,000 rpm" (Ex.1003, 15:23-25), which is within the claimed range and is *prima facie* obvious. *In re Wertheim*, 541 F.2d at 267; *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003).

Although it is an alternative limitation, the temperature range would have been obvious over Le Bec in view of Cole. A POSA would have been motivated to choose a temperature range that would allow the AUC experiment to function without impacting the sample. Cole teaches that ultracentrifugation should be performed between 4ºC-35ºC and indicates that "most experiments are conducted at 20ºC." Ex.1005, 163. It is within the purview of a POSA to optimize parameters such as centrifugation speed and temperature (Ex.1001, 26:4-6), and they would have motivated to pick parameters within the claimed ranges with a reasonable expectation of success based on the teachings of Le Bec and Cole. *KSR*, 550 U.S. at 401; Ex.1020, ¶238.

7. Claim 33

Claim 33 is anticipated and obvious in view of Le Bec (§§VIII.I and IX), and further would have been obvious over Le Bec in view of Berkowitz.

Le Bec teaches that empty rAAV particles are undesirable (Ex.1003, 2:15-17) and that empty and full rAAV particles can be distinguished via SV-AUC (Ex.1033, 18:1-6). Berkowitz teaches the importance of purity (i.e. homogeneity) in pharmaceutical preparations (Ex.1004, 16), and that sedimentation distribution data can be used to compare/evaluate different purification processes (*id.*, 20-21). Given the desire for full rAAV particles in gene therapy products, a POSA would have been motivated to monitor the homogeneity rAAV particles using SV-AUC during purification and would have reasonably expected success doing so. Ex.1020, ¶240; Ex.1004, 16; Ex.1008, 6; *Spectrum*, 802 F.3d at 1334; *KSR*, 550 U.S. at 401.

B. Claim 2 is Obvious Over Le Bec in View of Berkowitz and Cole

Claim 2 recites a method of assessing "genome integrity" in a preparation of rAAV particles by subjecting it to boundary sedimentation conditions, plotting the data, and analyzing the relative amounts of different species therein with the use of a standard curve generated from rAAV particles of known genome sizes. As discussed above, Le Bec anticipates the claim limitations directed to performing boundary SV-AUC on rAAV preparations (§VIII.A.2) and plotting the data as C(s) v. S graphs (§VIII.A.3). Prior to Le Bec, the expected sedimentation coefficients for

empty and full rAAV particles were known. Ex.1003, 17:30-33. To the extent a POSA wanted to estimate genome size of other viral particles based on sedimentation coefficients, a POSA would have been motivated to use a standard curve and would have had a reasonable expectation of success in doing so.

1. "A method to assess vector genome integrity of recombinant AAV particles in a preparation of recombinant AAV particles comprising"

To the extent the preamble is construed to be limiting (fn.5, *supra*), it is taught by Le Bec. Le Bec teaches a method that can detect the presence of full and empty rAAV particles (and aggregates), with particular focus on the purity and quantification of full viral particles. Ex.1003, 15:17-23, 16:25-29, 18:1-6; Ex.1020, ¶¶204-205. Le Bec compared two production systems and deemed the one producing a greater percentage of full AAV particles superior (i.e., having superior genome integrity). Ex.1003, 19:1-15.

2. "a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant AAV particles is monitored at time intervals,"

This limitation is anticipated in Le Bec as described above for claim 1. *See* §VIII.A.2.

3. "b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S)"

This limitation is anticipated in Le Bec as described above for claim 1. *See* §VIII.A.3.

4. "c) identifying species of recombinant AAV particles in the preparation by presence of peaks on the plot corresponding to an S value"

Le Bec identifies rAAV particle species based on peaks appearing at certain S-values on its C(s) v. S plots, with empty particles identified by the peak at 65S, and full particles identified at the peaks at 90S and 105S (single-stranded and double-stranded, respectively). Ex.1003, 18:8-32; *see also* Figures 2 and 3; Ex.1020, ¶208.

5. "wherein the genome size of a particular species of recombinant AAV particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant AAV particles comprising encapsidated AAV genomes of known nucleotide sizes"

A POSA would have been motivated to generate a standard curve and would have reasonably expected success using such a curve, in view of the teachings of Le Bec, Cole, and Berkowitz, and the general knowledge in the field.

The use of reference standards or standard curves was routinely applied to sedimentation coefficients and AUC experiments. Ex.1020, ¶¶209-210. For example, in a sedimentation analysis using a sucrose density gradient centrifugation experiment, reference proteins—including empty and full AAV particles—were run

on a parallel sucrose gradient. Ex.1014, 1455, 1457 (Fig. 3) (noting a sedimentation coefficient of 60S for empty AAV particles and a sedimentation coefficient of 110S for full AAV particles).

Cole's 2008 article explains the math behind the forces at issue:

$$s \equiv \frac{v}{\omega^2 r} = \frac{M_b}{f}$$

where s is the sedimentation coefficient, M_b is the effective or buoyant mass of the particle, and f is the frictional coefficient. Ex.1005, 146. In a hypothetical system where the rAAV particles are uniform and have an identical frictional coefficient, the sedimentation coefficient (s) will be proportional to the effective particle mass (M_b). Ex.1013, 9-10; Ex.1020, ¶¶211-214. With a few reference points, a POSA would understand how to mathematically estimate the mass corresponding to a given sedimentation coefficient. *Id.*

Le Bec identifies the sedimentation coefficients of full and empty rAAV particles, reporting a higher sedimentation coefficient for full viral particles than for empty viral particles. Ex.1003, 15:17-28; 17:20-19:15. The data (with empty capsids traveling at 65S and full capsids moving at between 80S to 110S (Ex.1003, 18:21-25)), mirrors what the inventors in Le Bec expected (i.e. 60S for empty particles and 110 S for full particles (Ex.1003, 17:30-35)), with no unexpected deviations. Full

capsids—having greater mass—have higher sedimentation coefficients than empty capsids.

This is consistent with what a POSA would expect because full particles have more mass than empty particles. Ex.1005, 146; Ex.1020, ¶214. A POSA, understanding the complications arising from nonfunctional rAAV particles and the desire for high-quality drug product (Ex.1004, 16; Ex.1008, 1, 6) would see the benefit of using a standard curve to identify the genome size of a particular species of recombinant AAV particles, and would be able to predictably utilize a standard curve to assess purity (Ex.1020, ¶214). Claim 2 is obvious. *KSR*, 550 U.S. at 401.

C. Claim 3 is Obvious Over Le Bec in View of Berkowitz, Cole and the Knowledge of a POSA

Claim 3 depends from claim 2 and further requires "c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant AAV particle." This limitation is anticipated and/or obvious in view of Le Bec as described above for claim 1. *See* §§VIII.A.4 and IX.

D. Claim 19 is Obvious Over Le Bec in View of Berkowitz, Cole, and the Knowledge of a POSA

Claim 19 depends from claim 18 (which in turn depends from claim 1) and comprises "wherein the monitoring further comprises comparison to a reference

sample, wherein the reference sample comprises the aqueous solution without recombinant AAV particles."

Le Bec does not expressly disclose the use of a reference sample, but reference samples are routinely used to control for the optical attributes of the solution itself, as taught by the prior art. Ex.1020, ¶¶23-24, 230-231. Berkowitz teaches that "even for normal boundary sedimentation velocity experiments steps still need to be taken to exactly match the buffer matrix composition between the reference and the sample solutions to eliminate artifacts caused by the sedimentation of buffer components." Ex.1004, 21. Cole teaches a general method of SV-AUC where a sample is loaded in the cell along with a reference buffer without the species of interest: "For the actual run, each synthetic boundary cell is loaded with 430 µl in the reference sector and 420 µl of sample solution in the sample sector." Ex.1005, 163. To ensure accurate data and to eliminate the possibility of buffer artifacts interfering with sedimentation detection, a POSA would have been motivated to use a reference sample comprised of an aqueous solution having no AAV particles and would have had a reasonable expectation of success in doing so. Ex.1020, ¶231. Claim 19 claim is obvious.

E. Claims 22, 24, and 25 are Obvious Over Le Bec in View of Berkowitz, Cole, and the Knowledge of a POSA

Claims 22, 24, and 25 depend from claim 1 and recite that "sedimentation is monitored until the recombinant AAV particles with the lowest density sediments to the bottom of a sector of an ultracentrifuge" (claim 22); "sedimentation is monitored until recombinant AAV particles sediment to the bottom of an ultracentrifuge velocity cell" (claim 24); and "sedimentation is monitored until the recombinant AAV particles with the lowest density sediments and clears an optical window (claim 25)."

Figures 2 and 3 of Le Bec depict sedimentation coefficient data for particles having sedimentation coefficients approaching zero. Ex.1003, Figs 2 and 3. Figure 3 of Le Bec shows a peak between 10S and 20S, and very low readings for sedimentation coefficients less than 10S. *Id.* The fact that Le Bec obtained data for species with such low sedimentation coefficients would indicate to a POSA that the samples were allowed to run for a long period of time, such that it is likely the rAAV particles with the lowest density had cleared an optical window and reached the bottom of a sector of an ultracentrifuge. Ex.1020, ¶233.

In the interest of ensuring accurate data regarding the purity and homogeneity of the rAAV pharmaceutical drug product (Ex.1004, 16; Ex.1008, 6), a POSA would be motivated to acquire data during the sedimentation until all species of interest had

completely sedimented. Ex.1020, ¶233. Cole teaches that, in determining rotor speeds for SV-AUC experiments, "[i]t should take a boundary at least 2 h to sediment the full length of the cell (1.5 cm maximum), to ensure sufficient scans will be acquired." Ex.1005, 164. Similarly, Berkowitz reports that for its adenoviral SV-AUC experiments, "scans covering the full range of boundary movement were analyzed." Ex.1004, 17. It is within the purview of a POSA to optimize parameters such as run time (Ex.1001, 26:4-6), and in view of the above teachings, a POSA would have reasonably expected success monitoring sedimentation of rAAV particles in an SV-AUC experiment until the recombinant AAV particles with the lowest density sediments to the bottom and/or and clears an optical window. Ex.1020, ¶233; *KSR*, 550 U.S. at 401.

F. Claim 26 is Obvious Over Le Bec in View of Cole

Claim 26 depends from claim 22 and further recites that "at least 30 scans are used to monitor sedimentation of recombinant AAV particles." Le Bec displays data from multiple scans. Cole teaches that when monitoring via optical absorbance (as Le Bec did), "it is necessary to consider the longer scan times and adjust the rotor speed so that at least 30-40 scans are recorded during the movement of the boundary across the cell." Ex.1005, 164. Based on Cole, a POSA would be motivated to collect a sufficient number of scans to ensure robust data. Ex.1020, ¶235. It is within the purview of a POSA to optimize SV-AUC parameters (Ex.1001, 26:4-6, 28:62-64).

Thus, a POSA would have reasonably expected success in modifying the SV-AUC parameters as needed to collect at least 30 scans, especially because Cole suggests that collecting 30-40 scans is necessary. *KSR*, 550 U.S. at 401 ("If a person of ordinary skill in the art can implement a predictable variation, and would see the benefit of doing so, §103 likely bars its patentability.").

G. Claim 31 is Obvious Over Le Bec in View of Cole and the Knowledge of a POSA

Claim 31 depends from claim 1, and further requires "wherein the sedimentation of recombinant AAV particles is monitored about every 10-60 seconds." Le Bec performed SV-AUC in "real-time" (Ex.1003, 15:23), but did not specify how frequently scans were taken. Cole teaches scan times of 60-300 seconds for an absorbance-based approach. Ex.1005, 152; Table II. The lower end of Cole's range overlaps with the claimed range. Where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 267 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 1577 (Fed. Cir. 1990) (Where prior art taught concentrations of "about 1-5%" and the claim was "more than 5%," the ranges overlapped because "about 1-5%" allowed for concentrations slightly above 5%). It is within the purview of a POSA to optimize SV-AUC parameters like scan rate (Ex.1001, 26:4-6, 28:62-64), and a POSA would have been motivated to take more frequent scans (at the bottom of the Cole range),

to collect more robust data. Ex.1020, ¶237. And a POSA would have reasonably expected success because Cole taught collecting scans every 60 seconds was possible on the ultracentrifuge. *KSR*, 550 U.S. at 401.

XI. GROUND 4: CLAIMS 27-30 ARE OBVIOUS OVER LE BEC IN VIEW OF BERKOWITZ AND SCHUCK AND/OR ZHAO

Limitations relating to certain mathematical operations in SV-AUC data analysis in claims 27-30 of the '288 patent are obvious in view of the knowledge of a POSA, particularly in view of the disclosures of Berkowitz, Schuck, and/or Zhao.

A. Claim 27 is Obvious Over Le Bec in View of Berkowitz and Zhao

Claim 27 depends from claim 20, and further requires that "a regularization is applied to a fitting level with a confidence level of F statistic of at least about 0.68."

A POSA would understand that a regularization parameter needs to be chosen to perform the SEDFIT data analysis described in Le Bec. Ex.1020, ¶¶27-40, 242. Regularization is a data analysis technique used to prevent overfitting of models to training data. *Id.*; Ex.1005, 166 ("The resulting c(s) function is often quite ''spiky,'' and a regularization procedure is performed to produce a smoother distribution function."); Ex.1016, 10 (regularization is necessary to deal with "misleading details of the peak structure" and minimize "over-interpretation."). A POSA would be motivated to choose a regularization confidence level that smooths the distribution without introducing artifacts into the data. Ex.1020, ¶¶242-243.

Berkowitz describes using the SEDFIT software to characterize SV-AUC data from recombinant adenoviral preparations where "[a] regularization confidence level of 0.68 was employed." Ex.1004, 18. Further, Berkowitz demonstrated that this data analysis procedure resulted in an SV-AUC assay for adenoviral preparations that successfully discriminated between full, empty, and aggregate particles. *See* Ex.1004, 27-30. Others have recognized that using a value of 0.68 "strikes an excellent balance between the resolution of the distribution and the accuracy of its content." Ex.1016, 30. And Schuck teaches that "when the parameter for the maximum entropy regularization is adjusted to a probability of p = 0.68, significantly smoother curves are obtained, which are much more robust against small errors in the model." Ex.1007, 1611. In view of these teachings and with the ability to optimize SV-AUC parameters and data analysis (Ex.1001, 26:4-6, 28:62-64), a POSA would have been motivated to choose a confidence level of F statistic of at least about 0.68 and would have had a reasonable expectation of success doing so. Ex.1020, ¶243; *KSR*, 550 U.S. at 401.

B. Claims 28-29 are Obvious Over Le Bec in View of Berkowitz, Schuck, and Zhao

Claims 28 and 29 depend from claim 27 and further require that the regularization is "a second derivative regularization" (claim 28) or "Max entropy

regularization" (claim 29). These dependent claims are obvious over Le Bec in view of the combination of Berkowitz, and Schuck and/or Zhao.

A explained above, regularization is used to produce a smoother distribution and a POSA applying SV-AUC to rAAV particles would be motivated to choose a regularization method for more robust data. Ex.1020, ¶¶244-245. Schuck teaches that during the development of the SEDFIT software, "[a]s regularization methods, maximum entropy regularization and Tikhonov–Phillips regularization with a second derivative operator were studied." Ex.1007, 1617. Schuck further provides an example of the successful use of maximum entropy regularization: "Shown are results for $\alpha = 0$ (distributions with spikes, at 10-fold reduced scale) and with maximum entropy regularization and adjusted to a probability of $p = 0.68$ (smooth curves)." Ex.1007, 1611. (Fig. 1 inset). Moreover, Zhao confirms that both methods are available in SEDFIT and that "for typical SV data…both Tikhonov and maximum entropy regularization will result in very similar distributions." Ex.1016, 11. In view of these teachings and with the ability to optimize SV-AUC parameters and data analysis (Ex.1001, 26:4-6, 28:62-64), a POSA would have been motivated to choose either "second derivative regularization" or "Max entropy regularization" to analyze the rAAV SV-AUC data, and would have reasonably expected success in doing so because both regularization methods are incorporated into the SEDFIT

software described in Le Bec. Ex.1020, ¶¶245-246; *KSR*, 550 U.S. at 401. Claims 28 and 29 are obvious.

C. Claim 30 is Obvious Over Le Bec in View of Berkowitz, Cole and the Knowledge of a POSA

Claim 30 depends from claim 20, and further requires that each of the following C(s) parameters be held constant.

1. "resolution of about 200 S to about 5000 S,"

In SEDFIT, the resolution parameter is the number of values taken between the minimum and maximum of the distribution range. Ex.1020, ¶248. The higher the resolution value chosen, the more computing time required to process the data. *Id.* Although Le Bec does not expressly disclose the precise resolution it used to produce its C(s) v. S plots depicted in Figures 2 and 3, choosing the resolution parameter is routine and within a POSA's skill to optimize. Ex.1001, 26:4-6 ("It is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles."); *id.*, 28:62-64 ("Suitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art."). Given the range of the C(s) v. S plots in Le Bec, a POSA would have been motivated to use a resolution of at least 200 to properly visualize the different rAAV species. Ex.1020, ¶248. A POSA would not have wanted to use a resolution greater than 5000 due to the amount of computational time that would

have been required. There is nothing inventive about the recited resolution range. *KSR*, 550 U.S. at 401.

2. "S min is about 1 S to about 100 S,"

Although Le Bec does not expressly disclose the precise S min value used in its SEDFIT analysis of its SV-AUC data, the C(s) v. S plots in Figures 2 and 3 have an axis with data recorded at a minimum S value of about 1, thus a POSA would understand Le Bec to teach an S min value of about 1S to about 100S for AAV particles. Ex.1003, Figs. 2 and 3; Ex.1020, ¶249. This limitation is inherently anticipated. Where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 267 (CCPA 1976). And choosing the minimum S-value parameter is routine and within a POSA's skill to optimize. Ex.1001, 26:4-6; Ex.1020, ¶251. Given the known sedimentation value of empty rAAV particles at 60S (Ex.1003, 17:30-18:1), using an S min value between 1S and 100S would be obvious. Ex.1020, ¶249.

3. "S max is about 100 S to about 5000 S,"

Although Le Bec does not expressly disclose the precise S max value it used in its SEDFIT analysis of its SV-AUC data, the C(s) v. S plots in Figures 2 and 3 have an axis with data recorded in excess of 100S, up to at least 140S. Ex.1003, Figs. 2 and 3. Plotting this data would require that the S max value used in Le Bec was greater than 100S. Ex.1020, ¶250. And where the species of interest have

sedimentation coefficients in the range of 60S to 110S (as taught by Le Bec), particles having a sedimentation coefficient exceeding 5000 S would not likely be present or of interest. This limitation is inherently anticipated. Where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 267 (CCPA 1976). And choosing the maximum S-value parameter is routine and within a POSA's skill to optimize. Ex.1001, 26:4-6.

4. "frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software"

The frictional ratio is a number that represents the deviation of the frictional coefficient of the particle from the frictional coefficient of a perfect sphere. Particles having the same frictional coefficient as a perfect sphere have a frictional ratio of 1.0. Ex.1020, ¶252. The SEDFIT software allows the user to define the frictional ratio, or to allow the software to perform more complex analyses "that do not assume a single value." Ex.1005, 166. Le Bec necessarily applied a frictional ratio that resulted in the data disclosed in Figures 2 and 3.

A POSA using the SEDFIT software would be required to decide how to set the frictional ratio to perform the data analysis. A POSA would be motivated to set the frictional ratio at 1.0 if the frictional ratio were unknown and the particle was assumed to be a perfect sphere. Ex.1020, ¶¶252-253. Analyses using a fixed

frictional ratio were known in the art. *See* Ex.1016, 1611. A POSA also would have been motivated to allow the SEDFIT software determine the frictional ratio (i.e. leave it to float to a value determined by the software), since that approach often returns more accurate analysis. Ex.1020, ¶253. Choosing the frictional ratio is routine and within a POSA's skill to optimize. Ex.1001, 26:4-6. A POSA would have been able to implement either approach with a reasonable expectation of success because the selection of frictional ratio is one of the features of the SEDFIT software. Ex.1020, ¶253. There is nothing inventive about the recited resolution range. *KSR*, 550 U.S. at 401. Claim 30 is obvious.

XII. GROUND 5: CLAIM 34 IS OBVIOUS OVER COLOSI IN VIEW OF LE BEC

A. Claim 34 is Obvious Over Colosi in View Of Le Bec

Claim 34 recites a method of preparing rAAV particles using host cells and nucleic acids, isolating the rAAV particles, and analyzing them according to the methods of claim 1. During prosecution, the Examiner relied on Colosi when rejecting this claim. Ex.1002, 492-494, 527-529. Patent Owner did not distinguish Colosi or refute the Examiner's position that it taught "a method of producing recombinant AAV (rAAV) virions" relevant to the claim elements discussed below. Instead, Patent Owner overcame the rejection by arguing that the prior art did not contemplate using AUC with AAV particles. Ex.1002, 541-542. This is incorrect in

view of Le Bec. A POSA would have been motivated to characterize Colosi's rAAV particles with Le Bec's SV-AUC method. Claim 34 is obvious.

1. "A method for preparing recombinant AAV particles with reduced empty capsids and/or recombinant AAV particles comprising variant genomes, the method comprising…"

To the extent the preamble is construed to be limiting (fn.5, *supra*), Colosi teaches a method for producing "high titer, wild-type-free preparations" of rAAV particles. Ex.1015, Abstract. It would have been obvious to analyze those rAAV particles with the method of claim 1 in view of Le Bec, which itself expressly discloses "a method for producing double-stranded rAAV particles with an improvement in the ratio of particles containing viral DNA to empty particles with an increased percentage of particles containing a double-stranded genome." Ex.1003, 3:25-29.

2. "a) culturing host cells under conditions suitable for recombinant AAV production, wherein the cells comprise"

Colosi expressly discloses "culturing the host cell to produce rAAV virions" as one of the steps of the disclosed method. Ex.1015, [0021], claims 5 and 13. Le Bec also discloses culturing host cells under conditions suitable for rAAV production. For example, Le Bec describes culturing host HEK293 cells "transfected with plasmids coding for all the elements necessary for the production of an rAAV."

Ex.1003, 6:23-25; *see also id.*, 12:31-13:15 (disclosing specific culture conditions). Ex.1003, 19, Table 1.

a. "i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR,"

Colosi teaches that "[t]he AAV genome generally comprises an internal non-repeating genome flanked on each end by inverted terminal repeats (ITRs)." Ex.1015, [0008]. Colosi further explains that by "'AAV ITRs' it is meant the art-recognized regions found at each end of the AAV genome which function together in cis as origins of DNA replication and as packaging signals for the viral-genome." *Id.*, [0034]. Le Bec discloses that its HEK293 host cells are transfected with "the plasmid encoding the viral genome of the AAV vector (scSMN transgene)." Ex.1003, 12:29-30. Elsewhere, Le Bec describes the use of "a sequence coding for a transgene of interest comprised between AAV ITRs" and the titration of the resulting rAAV vector via qPCR using primer pairs "corresponding to an amplicon located in the ITR region of the transgene plasmid." Ex.1003, 4:17-18; 14:27-29.

b. "ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a mutated p5 promoter wherein rep expression from the p5 promoter is reduced compared to a wild-type p5 promoter"

Colosi defines the phrase "lacks an intact p5 promoter region" as "a nucleotide sequence that either lacks a p5 promoter region or that contains a non-functional p5

promoter region" and includes nucleotide sequences where the p5 promoter region "has been rendered non-functional by one or more mutations." Ex.1015, [0059]. Further, claim 1 of Colosi recites a nucleic acid molecule comprising "an AAV rep coding region; an AAV cap coding region; and a nucleotide sequence comprising a modified AAV p5 promoter region, such that the modified AAV p5 promoter region no longer functions in transcription initiation." Ex.1015, claim 1. The Examiner relied on Colosi in finding this limitation taught by the prior art.

c. "iii) nucleic acid encoding AAV helper virus functions;"

The compositions disclosed in Colosi are described as including "novel nucleic acids encoding AAV helper functions and AAV helper function vectors." Ex.1015, Abstract. The field of invention is described as relating to "AAV helper function constructs that provide for high-efficiency rAAV production but do not generate wild-type AAV." *Id.*, [0002]. And claim 1 of Colosi recites "[a] nucleic acid molecule which encodes one or more AAV helper functions." *Id.*, claim 1.

3. "b) lysing the host cells to release recombinant AAV particles;"

After the host cells are cultured, they must be lysed to free the rAAV particles from the cells. The Examiner relied on Colosi in finding this limitation taught by the prior art, which Patent Owner never disputed. Ex.1002, 492-494, 527-529. Further, that the host cells would need to be lysed was taught in Le Bec, which explains that

following transection, the HEK293 host cells "are then mechanically lysed." Ex.1003, 13:24-25. Le Bec goes on to perform several assays to characterize the rAAV particles generated, including SV-AUC. A POSA would have been motivated to characterize the rAAV particles (as discussed below), and would have been motivated to lyse the cells first to perform such analyses with a reasonable expectation of success in view of Le Bec.

4. "c) isolating the recombinant AAV particles produced by the host cell;"

After the host cells are lysed and the rAAV particles released, the rAAV particles must be isolated for testing. The Examiner relied on Colosi in finding this limitation taught by the prior art, which Patent Owner never disputed. Ex.1002, 492-494, 527-529. Further, Le Bec describes that following host cell lysis, clarification, digestion, and column chromatography steps are performed and "[t]he AAV viral particles are concentrated and formulated in a PBS buffer by tangential filtration (TFF) using a cut-off threshold of 100 KDa to be at a final concentration of approximately > 10^{11} vg/ml and filtered through a 0.2μm filter." Ex.1003, 14:15-19. These steps would isolate the rAAV particles.

5. "d) analyzing the recombinant AAV particles for the presence of empty capsids and/or recombinant AAV particles with variant genomes by analytical ultracentrifugation by the method of claim 1."

Claim 1 is anticipated by and/or obvious in view of Le Bec. *See* §§VIII.A and IX. A POSA would have been motivated to use the characterization method of Le Bec to assess the purity and homogeneity of the rAAV particles being made by the method in Colosi because it would characterize the viral preparations and allow for an assessment of the purity and homogeneity of the rAAV particles. Ex.1020, ¶257. Further, a POSA would have reasonably expected success in applying SV-AUC to the rAAV particles of Colosi because Le Bec teaches that SV-AUC can separate, identify, and quantify rAAV populations in a heterogenous sample. *Id.*

XIII. SECONDARY CONSIDERATIONS

Petitioner is unaware of any objective evidence of nonobviousness that would outweigh the compelling conclusion of obviousness set forth above and reserves the right to address any such evidence submitted in this proceeding.

XIV. DISCRETIONARY DENIAL IS NOT WARRANTED

Institution should not be denied under 35 U.S.C. §325(d) because the arguments and evidence presented here were not previously and/or properly considered by the Office. *Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6 (PTAB Feb. 13, 2020); *Becton, Dickinson*

& Co. v. B. Braun Melsungen AG, IPR2017-01586, Paper 8 (PTAB Dec. 15, 2017) (precedential).

A. The Prior Art and Arguments Presented to the Office Were Not the Same or Substantially the Same

During prosecution the Examiner did not consider or cite Le Bec, a critical reference teaching the use of SV-AUC with rAAV particles that anticipates many of the challenged claims. Indeed, the Examiner appeared to lack any knowledge of Le Bec, commenting in the notice of allowance that "AUC was not noted as having been applied to AAV separation routinely," (Ex.1002, 561), despite the teachings of Le Bec to the contrary. The Zhao reference was also not before the Examiner. Ex.1002, 516-529. And while Berkowitz was cited by Patent Owner in the background, it was not relied on or discussed by the Examiner in any of the office actions. Ex.1002, 516-529. Three of the references relied on this petition—Cole, Schuck, and Colosi—were considered by the Examiner and relied on to reject pending claims during prosecution (Ex.1002, 520-523; 525-527), but the challenges presented here are materially different because Le Bec is the primary reference, and each of Cole, Schuck, and Colosi are secondary references.

Moreover, the art asserted in Grounds 1-5 and Petitioners' associated arguments are not cumulative with those substantively considered during prosecution because the crux of Patent Owner's prosecution argument was that

although SV-AUC was well known, it would not have been obvious to apply it to rAAV particles. Ex.1002, 541. Specifically, Patent Owner argued that the art relied on by the Examiner was "completely silent regarding recombinant AAV particles" and that "one of skill in the art would not have predicted with a reasonable expectation of success that methods described for a virus such as adenovirus could be applied to recombinant AAV particles." Ex.1002, 541. Patent Owner also argued that "[o]ne of skill in the art would not have assumed that the use of AUC would have allowed for the characterization of variant recombinant viral genomes or empty viral capsid particles in a preparation of rAAV particles, much less with the sensitivity, precision, and accuracy discussed below." *Id*. The Examiner credited these arguments when allowing these claims. Ex.1002, 561-562. Because Le Bec shows these arguments are incorrect, Grounds 1-5 present a substantially different argument than considered by the Examiner.

Furthermore, because the Examiner did not apply the primary reference Le Bec as prior art—and indeed was apparently unaware of Le Bec—there was no consideration given to the combinations of references asserted in Grounds 1-5 or Petitioners' rationales for motivation to combine and reasonable expectation of success based on the asserted art. *St. Jude Medical, LLC v. Snyders Heart Valve LLC*, Case No. IPR2018-00105, Paper 15 at 12 (PTAB May 3, 2018) (instituting where "evidence of record does not demonstrate that the Examiner considered the

references in the combinations relied upon by Petitioner or addressed arguments similar to those Petitioner now presents"). Thus, *Becton Dickenson* Factors (a), (b), and (d) support institution.

B. The Office Erred in a Manner Material to the Patentability of the Challenged Claims

The Board need not reach Part Two of the *Advanced Bionics* framework. But if it does, the *Becton Dickenson* Factors also favor institution.

As explained above, the Examiner did not substantively evaluate Petitioners' primary asserted art or any of the combinations presented. Thus, factor (c) favors institution. *Microsoft Corporation v. SurfCast, Inc.*, IPR2022-00590, Paper 9 at 15 (PTAB Oct. 7, 2022) (finding factor (c) favors institution because the cited art "was not extensively evaluated during examination and was not the basis for a rejection"); *Amazon.com, Inc. v. M2M Sols. LLC*, IPR2019-01205, Paper 14 at 16 (PTAB Jan. 27, 2020) ("a reference that 'was neither applied against the claims nor discussed by the Examiner' does not weigh in favor of exercising the Board's discretion under §325(d) to deny a petition").

Factor (e) also supports institution in view the Examiner's mistakes. The Examiner was led astray by Patent Owner's unsupported attorney argument that a person or ordinary skilled would not have expected that AUC—a well-known method recognized for its ability to analyze a wide range of molecules (from peptides

and oligosaccharides to viruses and organelles)—could be employed to characterize recombinant AAV particles. Indeed, Le Bec not only teaches exactly the method Patent Owner argues would have been unexpected, the inventors in Le Bec did not even comment on the novelty of using SV-AUC with AAV particles. The Examiner erred in allowing the claims based on Patent Owner's factually incorrect and unsupported arguments.

In addition to presenting art and arguments that were not considered by the Examiner, Petitioners also provide Dr. Steven Berkowitz's declaration, which further explains a POSA's understanding of the art as of January 19, 2015. Dr. Berkowitz confirms that application of SV-AUC to rAAV particles was not surprising or unexpected. Ex.1020, ¶¶52-56. Thus, *Becton Dickenson* Factor (f) likewise favors institution. *Celltrion, Inc. v. Genentech, Inc.*, No. IPR2017-01140, Paper 31 at 13-14 (PTAB Jan. 25, 2018) (instituting when, "taking the expert declaration…into account, Petitioner's testimonial evidence presents the prior art in a new light.").

Accordingly, institution should not be denied under 35 U.S.C. §325(d).

XV. MANDATORY NOTICES UNDER 37 C.F.R. §42.8

Pursuant to 37 C.F.R. §42.8, Petitioner states as follows:

A. Real Parties-in-Interest (37 C.F.R. §42.8(b)(1))

Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation are the real parties-in-interest.

B. Related Matters (37 C.F.R. §42.8(b)(2))

The '288 patent has been asserted against Petitioners in an action for infringement: *Genzyme Corporation and Aventis Inc. v. Novartis Gene Therapies, Inc., and Novartis Pharmaceuticals Corporation*, Case No. 1:23-cv-00554-RGA (D. Del.).

C. Lead and Backup Counsel and Service Information (37 C.F.R. §§42.8(b)(3) and (b)(4))

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XVI. CERTIFICATION UNDER 37 C.F.R §42.24(D)

Pursuant to 37 C.F.R. §42.24(a)(1)(i), the foregoing PETITION FOR *INTER PARTES* REVIEW contains 13,960 words, excluding parts of this Petition exempted under §42.24(a), as measured by the word-processing system used to prepare this paper.

Respectfully submitted,

Date: June 30, 2023

By: /John D. Livingstone/
John D. Livingstone, Reg. No. 59,613
Counsel for Petitioner

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105(a), the undersigned certifies that on June 30, 2023, a copy of the foregoing **Petition for *Inter Partes* Review** and **the associated powers of attorney** were served by FedEx Priority Overnight on the correspondence address of record indicated in the Patent Office's Patent Center website for U.S. Patent No. 10,429,288:

Lisa P. Rasmussen
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A courtesy copy of the foregoing was also served by FedEx Priority Overnight upon the following counsel of record for the Patent Owner in litigation pending before the United States District Court for the District of Delaware Case No. 1:23-cv-00554:

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