UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

Genzyme Corporation,

Patent Owner

U.S. Patent No. 11,698,377

"Methods for Detecting AAV"

IPR2025-00167

PETITION FOR *INTER PARTES* REVIEW

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EX1006	Shytuhina *et al*., "Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine," *Journal of Chromatography A* 1364 (2014): 192-197 ("Shytuhina")
EX1007	Alqahtani, "Analysis of purified wild type and mutant adenovirus particles by SILAC based quantitative proteomics," *Journal of General Virology* 95.11 (2014): 2504-2511 ("Alqahtani")
EX1008	Anacleto and Boyd, "Calibration of ion spray mass spectra using cluster ions," *Organic Mass Spectrometry* 27.6 (1992): 660-666 ("Anacleto")
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EX1013	Burova and Ioffe, "Chromatographic purification of recombinant adenoviral and adeno-associated viral vectors: methods and implications," *Gene Therapy* 12.1 (2005): S5-S17 ("Burova")
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EX1021	Davis *et al*., "Rational Design and Engineering of a Modified Adeno-Associated Virus (AAV1)-Based Vector System for Enhanced Retrograde Gene Delivery," *Neurosurgery* 76.2 (2015): 216-225 ("Davis")
EX1022	Dong *et al*., "Proteomics analysis of co-purifying cellular proteins associated with rAAV vectors," *PLoS One* 9.2 (2014): e86453 ("Dong")
EX1023	Glish and Vachet, "The Basics of Mass Spectrometry in the Twenty-First Century," *Nature Reviews Drug Discovery* 2.2 (2003): 140-150 ("Glish")
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EX1031	Rayaprolu *et al*. "Comparative analysis of adeno-associated virus capsid stability and dynamics," *Journal of Virology* 87.24 (2013): 13150-13160 ("Rayaprolu")
EX1032	Rhoads *et al*., "Neutron-Encoded Mass Signatures for Quantitative Top-Down Proteomics," *Analytical Chemistry* 86.5 (2014): 2314-2319 ("Rhoads")
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EX1036	Siuzdak, "Probing Viruses with Mass Spectrometry," *Journal of Mass Spectrometry* 33.3 (1998): 203-211 ("Siuzdak")
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LIST OF CHALLENGED CLAIMS

Claim	Element
1 [pre]	A method to determine the serotype of an adeno-associated virus (AAV) particle comprising
1[a]	a) denaturing the AAV particle,
1[b]	b) directly subjecting the denatured AAV particle to liquid chromatography/mass spectroscopy (LC/MS) intact protein analysis, and
1[c]	c) determining the masses of VP1, VP2 and VP3 of the AAV particle;
1[d]	wherein the specific combination of masses of VP1 VP2 and VP3 are indicative of the AAV serotype,
1[e]	and wherein the method is performed in the absence of a gel separation step.
2[pre]	A method to determine the serotype of a viral particle comprising
2[a]	a) denaturing the viral particle,
2[b]	b) directly subjecting the denatured viral particle to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, and
2[c]	c) determining the masses of one or more capsid proteins of the viral particle;
2[d]	wherein the specific combination of masses of the one or more capsid proteins are indicative of the virus serotype,
2[e]	and wherein the method is performed in the absence of a gel separation step.

Claim	Element
3	The method of claim 1, wherein the calculated masses of VP1, VP2 and VP3 are compared to the theoretical masses of VPl, VP2 and VP3 of one or more AAV serotypes.
4	The method of claim 1, wherein the AAV particle is denatured with acetic acid, guanidine hydrochloride, and/or an organic solvent.
5	The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
6	The method of claim 1, wherein the liquid chromatography is reverse phase chromatography.
7	The method of claim 6, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography.
8	The method of claim 1, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
9	The method of claim 1, wherein the mass spectrometry comprises assisted calibration.
10	The method of claim 9, wherein sodium iodide is used as a calibrant.
11	The method of claim 1, wherein the AAV particle is a recombinant AAV (rAAV) particle.
12	The method of claim 1, wherein the AAV particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, anAAV4 capsid, an AAV5 capsid, anAAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an

Claim	Element
	AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587 A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, anAAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV/human bocavirus virus 1), an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid.
13	The method of claim 2, wherein the calculated masses of the one or more capsid proteins are compared to the theoretical masses of the one or more capsid proteins of one or more virus serotypes.
14	The method of claim 2, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography.
15	The method of claim 2, wherein the liquid chromatography is reverse phase chromatography.
16	The method of claim 15, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography.
17	The method of claim 2, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC).
18	The method of claim 2, wherein the mass spectrometry comprises assisted calibration.
19	The method of claim 18, wherein sodium iodide is used as a calibrant.
20	The method of claim 2, wherein the viral particle comprises a viral vector encoding a heterologous transgene.

Sarepta Therapeutics, Inc. ("Sarepta" or "Petitioner") respectfully requests *inter partes* review of claims 1-20 (the "challenged claims") of U.S. Patent No. 11,698,377 ("the '377 patent") (EX1001). The '377 patent is assigned to Genzyme Corporation.

I. INTRODUCTION

Adeno-associated virus (AAV) has been studied for decades as a useful tool to deliver therapeutic genes to patients to treat diseases such as Duchenne Muscular Dystrophy, cystic fibrosis, Parkinson's disease, hemophilia B, and Canavan disease. For both preclinical and clinical process development, researchers have sought to develop efficient and accurate analytical methods to monitor the purity and stability of AAV compositions.

For years before the earliest priority date for the '377 patent, researchers were using methods such as reversed-phase high pressure liquid chromatography (RP-HPLC), ultra-performance liquid chromatography (UPLC), and liquid chromatography-mass spectrometry (LC-MS) of both enzymatically digested and intact proteins, to monitor the purity and stability of preparations of viral particles and other proteins. It was also well known in the art that widely available software could be used to calibrate a mass spectrometer, and also to deconvolute and interpret mass spectrometry (MS) data. Similarly, the choice of calibrant for a spectrometer would have required nothing more than routine experimentation.

The challenged claims are directed to methods for the analysis of preparations of AAV particles, and viral particles more generally. In particular, the challenged claims are directed to methods using LC-MS of intact proteins to analyze and characterize viral particles, including their serotype. The challenged claims recite straightforward and well known aspects of LC-MS methods, such as choosing a C4 or C8 column, using RP-HPLC and UPLC, and calibrating the mass spectrometer using software and particular calibrants. All of these aspects of the claimed methods were known in the prior art for years before the earliest possible priority date for the '377 patent.

Challenged claims 1-20 are obvious over the combination of two prior art references, Satkunanathan and Shytuhina. Challenged claims 8 and 17 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Ansong. Challenged claims 9, 10, 18, and 19 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Byeon.

Satkunanathan and Shytuhina. Satkunanathan studied three different AAV serotypes (AAV2, AAV5, and AAV8), focusing in particular on analyzing the protein composition present in purified AAV vectors of each serotype. Satkunanathan used LC-MS/MS, involving enzymatic digestion of proteins before MS analysis, to identify the proteins present in preparations of these purified AAV

vectors. Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy. Among the unique proteins identified by LC-MS/MS in each purified preparation, Satkunanathan identified AAV2, AAV5, and AAV8 capsid proteins.

A POSA would have understood, however, that, given the structure of the AAV capsid proteins, vp1, vp2, and vp3, it would have been difficult, perhaps impossible, using Satkunanathan's method, to determine whether a fragment towards the C terminus of a given capsid protein originated from vp1, vp2, or vp3. A POSA would further have understood that enzymatic digestion is a laborious, time-consuming process, which can introduce artificial modifications, such as cyclization of N-terminal glutamine, and deamidation.

A POSA at the time would have been motivated to improve methods for characterizing AAV proteins for process development. A POSA would have understood that more precise, accurate characterization to improve process development would involve the capability to distinguish AAV serotypes, identify PTMs, and monitor degradation products.

Shytuhina is directed towards development of a vaccine against Chikungunya virus, a mosquito-borne virus that causes acute illness including fever, rash, and severe arthralgia. Shytuhina discloses the development and application of an RP-HPLC-MS method for analysis of intact viral structural proteins for process

development for a vaccine. In particular, Shytuhina disclosed using their HPLC-MS method to identify PTMs of intact viral structural proteins. Shytuhina discusses monitoring PTMs as a key element of process development.

The Chikungunya virus-like particle (VLP) that Shutuhina studied has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein.

Shytuhina identified E1 and E2 PTMs, including N-glycosylation and acylation, by matching the observed mass to the theoretical molecular weight. Shytuhina states that they likely also observed deamidation of E2. Shytuhina notes that Chikungunya particles produced in different cell lines exhibit different PTMs. Shytuhina states that it is therefore important to monitor PTMs to ensure product lot-to-lot consistency throughout the vaccine development cycle.

A POSA would have understood that the intact LC-MS method of Shytuhina would have allowed efficient, reliable, accurate analysis of individual AAV capsid proteins, including distinguishing vp1, vp2, and vp3 from one another and from those of other serotypes, and assessing PTMs and other variations and truncations. A POSA would also have understood at the relevant time that the different capsid protein sequences would be reflected in different masses identified through LC-MS

and that therefore the determined masses of the capsid proteins would be indicative of AAV serotypes such as those Satkunanathan studied. A POSA would therefore have been motivated to combine Satkunanathan and Shytuhina.

Challenged claims 1-20 are therefore obvious over the combination of Satkunanathan and Shytuhina.

Satkunanathan, Shytuhina, and Ansong. For the reasons discussed above, a POSA would have been motivated to combine Satkunanathan and Shytuhina. Ansong discloses the use of intact LC-MS with a UPLC column to study the proteome of *Salmonella typhimurium*. Using intact LC-MS with UPLC, Ansong identified 563 unique proteins including 1665 proteoforms generated by PTMs. They found 12 proteins with an N-terminal acetylation modification, among other PTMs. A POSA would have been aware that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to analyze proteins for in-process control. A POSA would therefore have likely tested Ansong's UPLC approach, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins.

Challenged claims 8 and 17 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Ansong.

Satkunanathan, Shytuhina, and Byeon. For the reasons discussed above, a

POSA would have been motivated to combine Satkunanathan and Shytuhina. Byeon discloses the use of LC-MS analysis to identify the molecular structure of a variant peak detected by RP-HPLC of rHu-EPO (Human Recombinant Erythropoietin). Byeon discloses that the mass spectrometer was calibrated with NaI before acquisition. Byeon also discloses that the mass spectra obtained in experiments were processed with a Maximum Entropy program (MaxEnt) to deconvolute multiply charged ESI data.

A POSA would have understood that LC-MS systems require calibration. A POSA would have been motivated to use Byeon's method of assisted calibration to calibrate the mass spectrometer because such assisted calibration was an efficient and straightforward method of calibration. In particular, a POSA would have been motivated to use Byeon's method to avoid complications resulting from adding a calibrant to the sample being analyzed, which would have required ensuring compatibility between the sample and the internal calibrant. In addition, a POSA would have understood NaI to be a desirable and commonly available calibrant, including for assisted calibration using software.

Challenged claims 9, 10, 18, and 19 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Byeon.

Petitioner respectfully submits that the challenged claims (1-20) are unpatentable. This conclusion is supported by the prior art of record and the

declaration of Dr. Joshua J. Coon (EX1004), an expert in the area of biological mass spectrometry (EX1005). EX1003, ¶¶2-48.

II. MANDATORY NOTICES

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

Petitioner identifies Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC as real parties-in-interest.

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

Petitioner identifies the following related matters. The '377 patent is being asserted in currently-pending litigation: *Genzyme Corp. v. Sarepta Therapeutics, Inc.*, C.A. No. 24-cv-00882-RGA (D. Del.). EX1040.

Petitioner is unaware of any other lawsuits involving the '377 patent.

C. Related Patent Office Proceedings

This is the first petition challenging a claim of the '377 patent.

Petitioner is concurrently filing petitions requesting *inter partes* review of the claims of two related patents, U.S. Patent No. 12,123,880 ("the '880 patent") and U.S. Patent No. 12,298,313 ("the '313 patent"). The '377 patent issued from U.S. Patent Application No. 16/325,653, filed as application No. PCT/US2017/046814 on August 14, 2017.

D. Lead and Back-up Counsel and Service Information

Petitioner provides the following counsel and service information. Pursuant

to 37 C.F.R. §42.10(b), a Power of Attorney accompanies this Petition.

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III. REQUIREMENTS FOR IPR

A. Payment of Fees

The undersigned authorizes the Office to charge the fee required for this Petition for *inter partes* review to Deposit Account No. 50-5708.

B. Grounds for Standing

Petitioner certifies that the ’377 patent is available for IPR and Petitioner is not barred or estopped from requesting IPR on the grounds identified herein. Petitioner further certifies that the prohibitions of 35 U.S.C. §§315 (a)-(b) are inapplicable.

C. Statement of Relief Requested

Petitioner respectfully requests review and cancellation of claims 1-20 of the '377 patent. The challenged claims should be found unpatentable on the following grounds:

Prior Art References
Satkunanathan (EX1005); published in 2014; prior art under AIA §102(a)(1).
Shytuhina (EX1006), published in 2014; prior art under AIA §102(a)(1).
Ansong (EX1009), published in 2013; prior art under AIA §102(a)(1).
Byeon (EX1014), published in 2015; prior art under AIA §102(a)(1).

Ground	**Claims**	**Description**
1	1-20	Obvious in view of Satkunanathan and Shytuhina
2	8, 17	Obvious in view of Satkunanathan, Shytuhina, and Ansong
3	9, 10, 18, 19	Obvious in view of Satkunanathan, Shytuhina, and Byeon

Ground	**Claims**	**Description**
1	1-20	Obvious in view of Satkunanathan and Shytuhina
2	8, 17	Obvious in view of Satkunanathan, Shytuhina, and Ansong
3	9, 10, 18, 19	Obvious in view of Satkunanathan, Shytuhina, and Byeon

Satkunanathan, Shytuhina, Ansong, and Byeon were not cited to or considered by the Patent Office during prosecution. EX1001 ("References Cited"); EX1002.

IV. BACKGROUND OF THE TECHNOLOGY

Well over a decade before the earliest possible priority date for the '377 patent, researchers were using liquid chromatography, RP-HPLC in particular, to detect and quantify viral capsid proteins for purification process development. EX1037, Abstract; EX1003, ¶¶91-97, 188; *see also*, EX1003, ¶¶62-63. MS had also been used to explore the properties of viruses, including PTMs, for more than a decade before the earliest priority date for the '377 patent. EX1036, Abstract, 205-206; EX1003, ¶¶189; *see also id.*, 98-204. A 1998 review, "Probing Viruses with Mass Spectrometry," stated, "[m]ass measuring viral proteins is now routine and since viruses are typically well characterized, in that the capsid proteins and DNA (or RNA) sequences are known, identifying a virus based on the mass of the protein and enzymatic digestion fragments is relatively straightforward." EX1036, Abstract, 205; EX1033, 11; EX1003, ¶189.

At least as early as 2002, LC-MS had been used to analyze viral composition. EX1016; EX1003, ¶¶193; *see also id.*, ¶¶128-165, ¶¶188-204. Chelius disclosed the use of LC-MS/MS to analyze the composition of adenovirus particles. EX1016, Abstract; EX1003, ¶193. LC-MS/MS was also used before the priority date for process development for rAAV, including identifying the capsid proteins of different

serotypes and identifying PTMs. EX1022, Abstract, 2; EX1005, Abstract, Supplementary Table S1; EX1003, ¶¶194-96. Satkunanathan used LC-MS/MS to explore cellular components associated with three different serotypes of rAAV, specifically, AAV2, AAV5, and AAV8. EX1005, Abstract; EX1003, ¶195. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶¶202-03.

Years before the earliest possible priority date, researchers had also used LC-MS to analyze intact monoclonal antibodies, after reduction and separation using RP-HPLC. EX1012, 1416; EX1003, ¶176; *see also id.*, ¶¶166-87. Bondarenko stated that "it is very attractive to separate the intact protein isoforms by liquid chromatography and then perform on-line mass and top-down [intact] analyses to determine the sites of modifications and their abundances in one short assay." EX1012, 1416; EX1003, ¶¶173, 178.

Also before the priority date, researchers were using LC-MS to analyze intact viral structural proteins for process development. EX1006, Abstract; EX1003, ¶¶166-187, 204. Shytuhina applied LC-MS to analyze intact, undigested viral structural proteins to evaluate PTMs. EX1006, Abstract, 193-94; EX1003, ¶¶204, 266-278.

V. THE '377 PATENT

The ’377 patent is titled “Methods for Detecting AAV.” EX1001. The patent names Xiaoying Jin, Catherine O’Riordan, Lin Liu, and Kate Zhang as inventors. EX1001; EX1003, ¶205. The ’377 patent issued on July 11, 2023. EX1001; EX1003, ¶205.

The ’377 patent is assigned to Genzyme Corporation. EX1001; EX1003, ¶206.

A. The Claims

The challenged claims are reproduced in the list above. *See also* EX1003, ¶¶207-08. Claims 1 and 2 are independent claims. EX1001, 109:19-44; EX1003, ¶209. Claims 3-6, 8, 9, 11, and 12 depend from claim 1, claim 7 depends from dependent claim 6, claim 10 depends from dependent claim 9, claims 13-15, 17, 18, and 20 depend from claim 2, claim 16 depends from dependent claim 15, and claim 19 depends from dependent claim 18. EX1001, 109:44-110:58; EX1003, ¶209.

The challenged claims are directed to the use of LC-MS intact protein analysis to characterize preparations of AAV and other viral particles. EX1003, ¶207. The claims require denaturing the viral particles and then subjecting them to intact LC-MS. *Id.* Dependent claims specify particular denaturing methods, particular types of chromatography, and particular methods of calibration and particular calibrants for the mass spectrometer.

B. The Specification

The specification of the ’377 patent discusses using LC-MS as an analytical tool to evaluate viral preparations. EX1001, 2:3-17; EX1003, ¶¶210-214. The specification discusses using LC-MS to discern characteristics of the viral preparation including the identity of the capsid serotype. EX1001, 2:7-13; EX1003, ¶210. The specification states that the method can be used as an AAV serotype identity test or to monitor viral capsid protein heterogeneity in rAAV gene therapy development. EX1001, 2:10-13; EX1003, ¶210. The specification describes “Examples” of LC-MS and LC-MS/MS for characterization of rAAV viral capsid proteins. EX1001, 51:20-56:47; EX1003, ¶211. The specification discusses methods of denaturing rAAV samples before LC-MS analysis. EX1001, 54:20-30; EX1003, ¶212.

The specification discusses LC-MS intact protein analysis. EX1001, 53:30-47, 54:31-55:4; EX1003, ¶213. The specification also discusses LC-MS/MS peptide mapping. EX1001, 53:61-54:19, 55:5-48, 62:43-62; EX1003, ¶214. The specification does not disclose LC-MS/MS analysis of intact proteins. EX1003, ¶214.

C. The Prosecution History

The applicants filed preliminary amendments amending the claims. EX1002, 520-30, 1515-24; EX1003, ¶215.

On July 9, 2021, the Examiner issued a Non-final rejection, rejecting all of the pending claims over several pieces of prior art. EX1002, 1794-1801; EX1003, ¶216. The Examiner rejected various claims as anticipated by Van Vliet *et al*., "Adeno-associated virus capsid serotype identification: Analytical methods development and application," *Journal of Virological Methods*, 159 (2009) 167-77 ("Vliet"). EX1002, 1794-98; EX1003, ¶216.

The Examiner rejected various claims as obvious over the combination of Vliet with Bark *et al*., "High-Temperature Protein Mass Mapping Using a Thermophilic Protease," *J. Am. Chem. Soc*. 2001, *123*, 1774-75 ("Bark"). EX1002, 1798-1800; EX1003, ¶217.

In response to this rejection, on November 9, 2021, the applicants amended the claims. EX1002, 1820-35; EX1003, ¶218. The claim amendments included adding the limitation "wherein the method is performed in the absence of a gel separation step." EX1002, 1821-23; EX1003, ¶218.

On November 17, 2021, the Examiner issued a final rejection. EX1002, 2110-2122; EX1003, ¶219. Here, the Examiner rejected various claims over the combination of Vliet and Bark. EX1002, 2110-2122; EX1003, ¶219.

In response, the applicants again amended the claims, adding the limitations "directly subjecting the denatured AAV particle to liquid chromatography/mass

spectrometry (LC/MS) intact protein analysis." EX1002, 2146 (emphasis original); EX1003, ¶220.

On April 28, 2022, the Examiner issued another non-final rejection, again rejecting the claims over Vliet and Bark. EX1002, 2179-89; EX1003, ¶221.

In response, the applicants canceled various claims in response to the rejection. EX1002, 2204-11; EX1003, ¶222.

On November 8, 2022, the Examiner issued a final rejection, again over the combination of Vliet and Bark. EX1002, 2221-26; EX1003, ¶223.

On February 3, 2023, the applicants responded, canceling a claim. EX1002, 2246-52; EX1003, ¶224.

D. Priority Date

The '377 patent claims priority to U.S. Provisional Application No. 62/375,314, filed on August 15, 2016 ("'314 provisional"). The '377 patent issued from U.S. Patent Application No. 16/325,653, filed as application No. PCT/US2017/046814 on August 14, 2017. The earliest possible priority date for the '377 patent claims is therefore the filing date of the '314 provisional, August 15, 2016. EX1001; EX1003, ¶¶225-27.

VI. LEVEL OF ORDINARY SKILL IN THE ART

A POSA in the technical field of the '377 patent would have had at least a Ph.D. in biochemistry, chemistry, pharmaceutical sciences, or a related field, and

between one and four years of post-doctoral experience in the field of chemistry or pharmaceutical sciences, including analytical techniques such as chromatography and mass spectrometry. EX1003, ¶¶228-31. Alternatively, a POSA would have had at least a Master's or Bachelor's Degree in biochemistry, chemistry, pharmaceutical sciences, or a related field, with a corresponding number of additional years of experience in the field of chemistry or pharmaceutical sciences. *Id.*

VII. OVERVIEW OF THE PRIOR ART

Before the earliest possible priority date for the '377 patent, researchers had been using LC-MS, including intact LC-MS, to analyze viral capsid proteins and other associated viral proteins, for process development. *See, e.g.*, EX1006; EX1005; EX1003, ¶¶232-36.

Researchers were using intact LC-MS to study post-translational modifications of viral proteins for process development. EX1006; EX1003, ¶¶232-36.

Researchers in the AAV field specifically were also using LC-MS techniques to identify and analyze AAV capsid proteins and their associated cellular co-purifying proteins, for research and for process development. EX1005; EX1022; EX1003, ¶¶232-36.

AAV researchers were well aware of the importance of studying and comparing different AAV serotypes for process development and for understanding

the different functions and capabilities of the different serotypes, which influence their suitability for use as therapeutic gene delivery vectors in different kinds of gene therapies. EX1005; ¶¶232-36.

236. LC-MS techniques, moreover, were well known to be a powerful tool for analyzing differences among various AAV serotypes. EX1005; EX1003, ¶¶232-36.

A. Satkunanathan

Satkunanathan, titled "Establishment of a Novel Cell Line for the Enhanced Production of Recombinant Adeno-Associated Virus Vectors for Gene Therapy," was published in November 2014, more than one year before the earliest possible filing date for the '377 patent. EX1005. Therefore, Satkunanathan is AIA §102(a)(1) prior art. EX1005; EX1003, ¶237.

Satkunanathan studied three different AAV serotypes (AAV2, AAV5, and AAV8), focusing in particular on analyzing the protein composition present in purified AAV vectors of each serotype. EX1005, Abstract; EX1003, ¶238. Satkunanathan used LC-MS/MS, involving enzymatic digestion of proteins before MS analysis, to identify the proteins present in preparations of these purified AAV vectors. EX1005, Abstract, 930-31; EX1003, ¶¶239-40. Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy. EX1005, Abstract, 930; EX1003, ¶241. Satkunanathan discusses the

problem of pre-existing immune responses in patients, requiring administration of higher titers and, presumably, the design of vectors based on different AAV serotypes. EX1005, Abstract; EX1003, ¶241, *see also* ¶¶72-85.

Satkunanathan digested purified and concentrated vector samples with trypsin before LC-MS/MS. EX1005, 930-31; EX1003, ¶¶242-48. Equal amounts of total proteins from three different types of purified AAV vector samples, that is, AAV2-GFP, AAV5-GFP, and AAV8-GFP were subjected to LC-MS/MS analysis. EX1005, 932; EX1003, ¶¶249-65. To minimize data variation, three batches of samples were prepared for each type of vector, with each batch pooled from 40 tissue culture plates (150 mm diameter). EX1005; EX1003, ¶250. Three MS runs were performed for each batch of samples. EX1005; EX1003, ¶250.

For data analysis, including mass spectra processing and database searching, up to two missed tryptic cleavages were considered, and methionine oxidation was set as a dynamic modification. EX1005, 931; EX1003, ¶245. Notably, Dong had identified oxidation of methionine as a PTM found in the AAV2 capsid proteins. EX1022, Abstract, 2; EX1003, ¶246.

Out of eight proteins found to be by two serotypes, five were shared by AAV2 and AAV5, indicating a relative similarity between AAV2 and AAV5 vectors. EX1005, 932, Supplementary Table S1; EX1003, ¶253. Twenty-six were unique to individual serotypes of vectors. EX1005, 932, Supplementary Table S1; EX1003,

¶253 As shown in the excerpt from Supplementary Table S1 below, among the unique proteins for each serotype, Satkunanathan identified AAV2, AAV5, and AAV8 capsid proteins:

SUPPLEMENTARY TABLE S1. (CONTINUED)

Protein ID	*AAV2*	*AAV5*	*AAV8*
Actin, gamma 1 OS=Homo sapiens GN=ACTG1 PE=3 SV=1 - [F5H0N0_HUMAN]		+	
Annexin A2 (Fragment) OS=Homo sapiens GN=ANXA2 PE=4 SV=1 - [H0YKZ7_HUMAN]		+	
ATP synthase subunit alpha OS=Homo sapiens PE=2 SV=1 - [B4DY56_HUMAN]		+	
ATP synthase-coupling factor 6, mitochondrial OS=Homo sapiens GN=ATP5J PE=1 SV=1 - [ATP5J_HUMAN]		+	
Capsid protein VP1 OS=Adeno-associated virus 2 (isolate Srivastava/1982) PE=1 SV=2 - [CAPSD_AAV2S]	+		
Capsid protein OS=Adeno-associated virus - 5 GN=cap PE=1 SV=1 - [Q9YIJ1_9VIRU]		+	
Capsid protein OS=Adeno-associated virus - 8 PE=1 SV=1 - [Q8JQF8_9VIRU]			+

EX1005, Supplementary Table S1 (excerpt) (yellow highlights added); EX1003, ¶¶254-55.

Satkunanathan also found a serotype-specific role for an AAV-associated cellular protein, YB1. EX1005, Abstract, 938; EX1003, ¶256. Satkunanathan found that knockdown of YB1 improved AAV2 and AAV8 production by 45- and 9-fold, respectively, but had no significant effect on AAV5 production. EX1005, 938; EX1003, ¶256.

Satkunanathan therefore teaches the importance of identifying and characterizing different AAV serotypes accurately for rAAV vector purification and production. EX1003, ¶259. Satkunanathan's discussion of the problem of pre-existing immunity among patients to various AAV serotypes further underscores the

need to ensure the serotypic purity of any preparation of rAAV for possible clinical use. EX1005, 929; EX1003, ¶259.

Satkunanathan also teaches that as of 2014, it was routine to identify capsid proteins of different AAV serotypes using LC-MS/MS. EX1005, Supplementary Table S1; EX1003, ¶260.

B. Shytuhina

Shytuhina, titled, "Development and application of a reversed-phase high-performance liquid chromatographic method for quantitation and characterization of a Chikungunya virus-like particle vaccine," was published in 2014, more than a year before the earliest possible priority date for the '377 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1006; EX1003, ¶266.

Shytuhina discloses the development and application of an RP-HPLC-MS method for analysis of intact viral structural proteins for process development for a vaccine. EX1006, Abstract; EX1003, ¶¶267-313. In particular, Shytuhina disclosed using their HPLC-MS method to identify PTMs on intact viral structural proteins. EX1006, Abstract; EX1003, ¶267.

Shytuhina is directed towards development of a vaccine against Chikungunya virus, a mosquito-borne virus that causes acute illness including fever, rash, and severe arthralgia. EX1006, Abstract, 192; EX1003, ¶268. Shytuhina states that to support process and formulation development effectively, it is highly desirable to

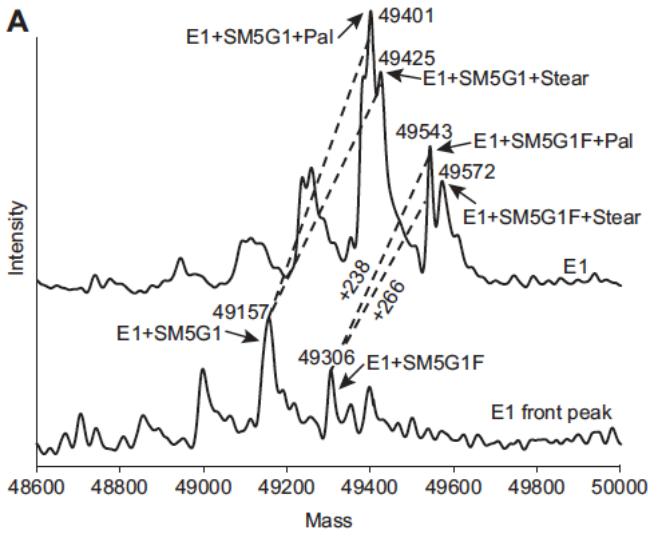
have a sensitive and robust method available that can be automated to measure both vaccine purity and antigen specific vaccine mass. EX1006, 192; EX1003, ¶273.

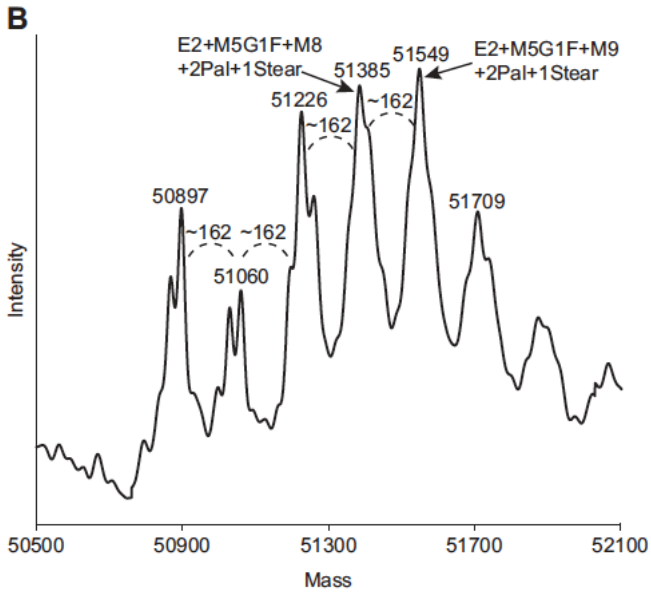
Shytuhina discloses that HPLC was an attractive analytical tool, in light of its high sensitivity and reproducibility. EX1006, 192; EX1003, ¶274. Shytuhina notes that HPLC had been applied for the identification and quantitation of viral proteins and VLPs from a variety of other viruses, including serotypes of adenovirus (types 3 and 5), influenza, lentivirus, Sendai virus, poliovirus, human papillomavirus VLP, and Hepatitis B VLP. EX1006, 192-93; EX1003, ¶274.

The Chikungunya VLP has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. EX1006, 193; EX1003, ¶276. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein. EX1006, 193; EX1003, ¶276.

Shytuhina states that their goal was to develop a RP-HPLC assay that would separate E1, E2, and capsid proteins of Chikungunya VLPs. EX1006, 193; EX1003, ¶277. This assay would evaluate and quantitate the mass and purity of the vaccine product. EX1006, 193; EX1003, ¶277. This method would be a tool to assess both protein degradation and PTMs for formulation and process development. EX1006, 193; EX1003, ¶277.

Shytuhina used LC-MS intact protein analysis to validate their RP-HPLC method, and to identify specific PTMs on the E1 and E2 proteins. EX1006, 193-96; EX1003, ¶278. Shytuhina discusses monitoring PTMs as a key element of process development. EX1006, 196-97; EX1003, ¶278. PTMs were identified by comparing observed masses to theoretical masses of expected N-glycoforms with and without acylation. EX1006, 194; EX1003, ¶292. Shytuhina first carried out RP-HPLC, and then carried out RP-HPLC-MS, where the chromatography was online with the mass spectrometer. EX1006, 193-94; EX1003, ¶¶293-310. Figure. 2, reproduced below, shows the deconvoluted spectra for E1 (Fig. 2A) and E2 (Fig. 2B).





EX1006, 194-95, Fig. 2; EX1003, ¶305.

As shown in the figures above, multiple masses were observed with each representing different glycosylation and acylation modifications. EX1006, 194-95, Fig. 2; EX1003, ¶306. Based on known glycoprotein acylation and expected N-linked glycans, Shytuhina identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight. EX1006, 194-95, Fig. 2; EX1003, ¶307.

Shytuhina detected one N-glycosylation and one acylation – either palmitoylation (Pal) or stearoylation (Stear) – for the majority of E1 (Figure. 2A, top trace). EX1006, 194-95, Fig. 2; EX1003, ¶308. They detected a small amount of glycosylated but deacylated E1. EX1006, 194-95, Figure 2A, bottom trace; EX1003, ¶309. Shytuhina explains that this result was not surprising, given that protein

acylation is a reversible process, and enzymatic depalmitoylation of viral glycoprotein had been demonstrated. EX1006, 194-95, Figure 2; EX1003, ¶309.

Fig. 2B shows that E2 contained two N-glycosylations and three acylations. EX1006, 194-95, Figure 2; EX1003, ¶310.

Shytuhina states that they likely observed deamidation of one of the viral structural proteins they studied, the E2 protein of chikungunya virus. EX1006, 196; EX1003, ¶311. Specifically, in carrying out RP-HPLC, they observed an E2 degradant peak that eluted slightly earlier than the regular E2, suggesting that the degradant was more hydrophilic. EX1006, 194, 196, Figure 1A; EX1003, ¶311.

Shytuhina explains that Chikungunya VLPs produced in different cell lines exhibit different PTMs. EX1006, 196-97; EX1003, ¶313. Shytuhina states that it is therefore important to monitor PTMs to ensure product lot-to-lot consistency throughout the vaccine development cycle. EX1006., 197; EX1003, ¶313.

C. Ansong

Ansong, titled, "Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella Typhimurium* in response to infection-like conditions," was published on June 18, 2013, more than a year before the earliest possible priority date for the '377 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1009; EX1003, ¶314.

Ansong used intact LC-MS with a UPLC column to study the proteome of *Salmonella typhimurium*. EX1009, Abstract, 10153-54; EX1003, ¶¶314-23. Ansong states that a number of factors contributed to the improved proteome coverage using their platform compared with prior studies. EX1009, Abstract, 10154; EX1003, ¶319. Ansong states that these factors included the use of a UPLC system with long columns (80 cm) and long gradients (250 min), and particular software tool for intact protein identification based on spectral alignment. EX1009, Abstract, 10154; EX1003, ¶¶320-21.

Using intact LC-MS with UPLC, Ansong identified 563 unique proteins including 1665 proteoforms generated by PTMs. EX1009, Abstract; EX1003, ¶322. They found 12 proteins with an N-terminal acetylation modification, among other PTMs. EX1009, Abstract, 10154-55; EX1003, ¶323.

D. Byeon

Byeon, titled, "Structural Identification of a Non-Glycosylated Variant at Ser126 for O-Glycosylation Site from EPO BRP, Human Recombinant Erythropoietin by LC/MS Analysis," was published online on May 27, 2015, more than a year before the earliest possible priority date for the '377 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1014; EX1003, ¶324.

Byeon used LC-MS analysis to identify the molecular structure of a variant peak detected by RP-HPLC of rHu-EPO (Human Recombinant Erythropoietin).

EX1014, Abstract; EX1003, ¶¶325-33. Byeon used UPLC-MS for the analysis, and calibrated the mass spectrometer, which was calibrated with NaI before acquisition. EX1014, 497; EX1003, ¶¶325-27. Byeon also discloses that the mass spectra obtained in experiments were processed with a Maximum Entropy program (MaxEnt) to deconvolute multiply charged ESI data. EX1014, 497; EX1003, ¶328. Byeon concluded that their method could be used to monitor rHu-Epo-related degradation on the carbohydrate structure and therefore could be applied as a purity test method. EX1014, 504; EX1003, ¶333.

VIII. CLAIM CONSTRUCTION

In this petition, the challenged claims have generally been analyzed according to their plain and ordinary meaning. For certain defined terms, such as "heterologous," the express definition in the specification has been applied to the analysis. EX1003, ¶¶337; *see also id.*, ¶¶ 334-36.

IX. DETAILED EXPLANATION OF GROUNDS

A. Ground 1: Claims 1-20 Are Obvious Over Satkunanathan and Shytuhina

A POSA would have been motivated to combine Satkunanathan and Shytuhina because both are directed towards efficient and precise methods for analysis of viral particle composition for the purpose of process development. EX1003, ¶¶338-533. Both use liquid chromatography-mass spectrometry techniques to characterize purified viral preparations, identifying properties such as

differences among different viral serotypes, or post-translational modifications. *Id.*, ¶339. Satkunanathan's method, however, involves enzymatically digesting proteins before subjecting them to LC-MS, while Shytuhina's method involves carrying out MS on intact proteins that have not been enzymatically digested. *Id.*, ¶¶339-40.

Satkunanathan studied proteins that co-purify along with each of three different AAV serotypes, AAV2, AAV5, and AAV8. *Id.*, ¶¶340-41. As part of this analysis, Satukunanathan also identified capsid proteins for each of the three serotypes studied. *Id.*, ¶¶342-44. In addition, Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. *Id.*, ¶345. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the identification of some capsid proteins containing this PTM. *Id.*

A POSA would have understood that, given the structure of the AAV genome, LC-MS analysis involving enzymatic digestion before MS analysis would not have enabled reliable and accurate separation and characterization of the AAV viral capsid proteins (vp1, vp2, and vp3) from one another, and from the capsid proteins of different serotypes. *Id.*, ¶¶64-71, 349.

A POSA at the time would have been motivated to improve methods for characterizing AAV proteins for process development, as Satkunanathan teaches, including identifying and characterizing different serotypes, identifying and

characterizing PTMs, and identifying and characterizing individual AAV capsid proteins (vp1, vp2, and vp3). *Id.*, ¶¶347, 350. A POSA would also have been aware that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶347.

In particular, a POSA would have understood that the intact LC-MS method of Shytuhina would have allowed efficient, reliable, accurate analysis of individual AAV capsid proteins, including distinguishing vp1, vp2, and vp3 from one another and from those of other serotypes, and assessing PTMs. EX1003, ¶350. Shytuhina discloses the use of intact LC-MS of viral structural proteins to identify and monitor PTMs to improve process development. *Id.*, ¶353.

1. Claim 1: "A method to determine the serotype of an adeno-associated virus (AAV) particle comprising"

Using LC-MS/MS, Satkunanathan identified capsid proteins from each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶¶351-52.

Shytuhina discloses LC-MS intact protein analysis of viral structural proteins. EX1006, 193-94; EX1003, ¶353. Using intact LC-MS, Shytuhina separated different post-translationally modified forms of viral structural proteins E1 and E2 from one another. EX1006, 194-95, Fig. 2; EX1003, ¶¶353-54.

A POSA would have understood that the AAV capsid protein masses differ from one another by substantially more than the masses of the post-translationally modified viral structural proteins in Shytuhina differ from one another or from the unmodified proteins. A POSA would therefore have also understood that the intact LC-MS method of Shytuhina, applied to the capsid proteins identified by LC-MS/MS in Satkunanathan, would have identified the three different capsid proteins. EX1003, ¶355.

A POSA would further have understood that a variety of different serotypes of AAV had been discovered, with varying degrees of homology to one another. *See, e.g.*, EX1017, Abstract, 14783-87, Fig. 2, Fig. 3, Fig. 4. A POSA would have been motivated to use Shytuhina's intact LC-MS method to monitor AAV composition for process development, including identifying different AAV serotypes. EX1003, ¶¶356-57.

Satkunanathan discloses that the capsid proteins of different AAV serotypes have different sequences, can uniquely identify a particular AAV serotype, and determine key properties of the particular serotype. EX1005, 930, 938. A POSA would further have understood at the relevant time that the different capsid protein sequences would be reflected in different masses identified through LC-MS and that therefore the determined masses of the capsid proteins would be indicative of the AAV serotype. EX1003, ¶358.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶351-59.

(a) "a) denaturing the AAV particle,"

Shytuhina teaches carrying out two denaturing steps – treatment with Zwittergent and RP-HPLC – before subjecting the denatured viral particles to LC-MS intact protein analysis. EX1006, 193-94; EX1003, ¶¶360-61.

For the first step, Shytuhina teaches incubating the samples with 5% Zwittergent 3-12 detergent. EX1006, 193-94; EX1003, ¶362. Shytuhina states that pre-treatment with Zwittergent improved recovery, and that "Zwittergent 3-12 detergent is believed to solubilize and stabilize the glycoproteins [viral structural proteins] and prevent non-specific binding during chromatography." EX1006, 194; EX1003, ¶362. A POSA at the time would have understood that "solubilizing" glycoproteins involves denaturing them. EX1003, ¶362.

For the second step, Shytuhina carried out an RP-HPLC separation, before subjecting the denatured samples to intact LC-MS, which then involved another RP-HPLC column coupled to the mass spectrometer. EX1006, 193; EX1003, ¶363. Shytuhina states that for the initial RP-HPLC separation (before LC-MS), they used TFA in the mobile phase on the column, and that "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." EX1006, 194; EX1003, ¶363.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶360-64.

(b) "b) directly subjecting the denatured AAV particle to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, and"

See claim 1(a). After the initial RP-HPLC, the denatured viral particles are then separated and analyzed by intact LC-MS using a Waters Acquity LC system coupled to a Synapt G2 mass spectrometer. The denatured viral particles are therefore directly subjected to LC-MS intact protein analysis. EX1003, ¶367.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶365-68.

(c) "c) determining the masses of VP1, VP2 and VP3 of the AAV particle;"

Using LC-MS/MS, Satkunanathan determined the masses of capsid proteins for each of three different AAV serotypes, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶370.

Shytuhina determined the masses of two viral structural proteins containing various different PTMs using intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶371.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. EX1003, ¶¶369-73.

(d) "wherein the specific combination of masses of VP1, VP2 and VP3 are indicative of the AAV serotype,"

Using LC-MS/MS, Satkunanathan determined the masses of capsid proteins for each of three different AAV serotypes, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶375.

Shytuhina determined the masses of two viral structural proteins using intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶376.

A POSA at the relevant time would have understood that the AAV capsid protein masses differ from one another by substantially more than the masses of the post-translationally modified viral structural proteins in Shytuhina differ from one another or from the unmodified proteins. EX1003, ¶377. A POSA would therefore have also understood that the intact LC-MS method of Shytuhina, applied to the capsid proteins identified by LC-MS/MS in Satkunanathan, would have identified the three different capsid proteins. *Id.*

Satkunanathan discloses that the capsid proteins of different AAV serotypes have different sequences, can uniquely identify a particular AAV serotype, and determine key properties of the particular serotype. EX1005, 930, 938; EX1003, ¶378. A POSA would further have understood at the relevant time that the different capsid protein sequences would be reflected in different masses identified through

LC-MS and that therefore the determined masses of the capsid proteins could be indicative of the AAV serotype. EX1003, ¶378.

A POSA would therefore have similarly understood that the specific combination of the masses of VP1, VP2, and VP3 could be indicative of the particular AAV serotype. *Id.*, ¶379.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶374-80.

(e) "and wherein the method is performed in the absence of a gel separation step."

Satkunanathan identified capsid proteins for each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶381.

Shytuhina used RP-HPLC to separate the viral proteins, a capsid protein and two other structural proteins, before subjecting the structural proteins to intact LC-MS. EX1006, 193-95, Fig. 2; EX1003, ¶382. No gel separation step was used at any point as part of this process. EX1003, ¶382.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶381-83.

2. Claim 2: "A method to determine the serotype of a viral particle comprising"

See claim preamble. EX1003, ¶¶384-93.

(a) "a) denaturing the viral particle,"

See claim 1(a). EX1003, ¶¶394-98.

(b) "b) directly subjecting the denatured viral particle to liquid chromatography/mass spectrometry (LC-MS) intact protein analysis, and"

See claim 1(b). EX1003, ¶¶399-402.

(c) "c) determining the masses of one or more capsid proteins of the viral particle;"

See claim 1(c). EX1003, ¶¶402-07.

(d) "wherein the specific combination of masses of the one or more capsid proteins are indicative of the virus serotype,"

See claim 1(d). EX1003, ¶¶408-15.

(e) "and wherein the method is performed in the absence of a gel separation step."

See claim 1(e). EX1003, ¶¶416-18.

3. Claim 3: "The method of claim 1, wherein the calculated masses of VP1, VP2 and VP3 are compared to the theoretical masses of VPl, VP2 and VP3 of one or more AAV serotypes."

See Claim 1. Shytuhina compared the calculated masses of the E1 and E2 viral structural proteins to their theoretical masses as part of their analysis. EX1006, 194; EX1003, ¶423. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶423.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. EX1003, ¶¶419-24.

4. Claim 4: "The method of claim 1, wherein the AAV particle is denatured with acetic acid, guanidine hydrochloride, and/or an organic solvent."

See claim 1. Shytuhina teaches carrying out two denaturing steps – treatment with Zwittergent and RP-HPLC – before subjecting the denatured viral particles to LC-MS intact protein analysis. EX1006, 193-94; EX1003, ¶426.

For the second denaturing step prior to LC-MS (the initial RP-HPLC), they used TFA in the mobile phase on the column, and that "TFA appeared to help disassemble the VLPs and facilitate the binding of proteins to the stationary phase." EX1006, 194; EX1003, ¶428.

Specifically, for this initial RP-HPLC, mobile phase B contained 30% acetonitrile (ACN), 70% 2-propanol, and 0.1% trifluoroacetic acid (TFA). A POSA would have understood that both acetonitrile and 2-propanol are organic solvents. EX1029, 11199-200, Table I, Table II (testing various different organic solvents including acetonitrile and 2-propanol on a C8 column, and comparing their ability to separate large denatured peptides with and without TFA); EX1003, ¶429.

A POSA would therefore have understood that the combination of the organic solvents acetonitrile and 2-propanol with TFA in the mobile phase disassembled, *i.e.*, denatured, the VLPs. EX1003, ¶430.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶425-431.

5. Claim 5: "The method of claim 1, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography."

See claim 1. For intact LC-MS, Shytuhina used an RP-HPLC separation coupled to the mass spectrometer. EX1006, 193; EX1003, ¶¶432-33.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. EX1003, ¶¶432-34.

6. Claim 6: "The method of claim 1, wherein the liquid chromatography is reverse phase chromatography."

See claims 1, 5. EX1003, ¶¶435-37.

7. Claim 7: "The method of claim 6, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography."

See claim 6. Shytuhina discloses that the specific RP-HPLC column they used prior to LC-MS was a C4 column. EX1006, 193; EX1003, ¶442. Although Shytuhina does not expressly disclose the column used for the RP-HPLC coupled to the mass spectrometer (the LC-MS intact protein analysis), a POSA would have understood it was likely that they used the same C4 column that had successfully separated E1 and E2 in the prior RP-HPLC step. EX1003, ¶442.

In any event, a POSA at the time would have understood that it was generally necessary to test a few different columns to optimize the desired separation. EX1037, 22-23; EX1003, ¶443.

Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. EX1037, Abstract; EX1003, ¶444. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. EX1037, 23.

Satkunanathan used a reversed phase C18 column for LC-MS/MS. EX1005, 931. Mahoney used a C8 column for RP-HPLC of large peptides. EX1029, 11199-200, Table I, Table II (testing various different organic solvents including acetonitrile and 2-propanol on a C8 column, and comparing their ability to separate large denatured peptides with and without TFA); EX1003, ¶445.

A POSA would have understood that these columns, C4, C8, and C18, were commonly used columns for separation of viral structural proteins, including AAV capsid proteins. A POSA would therefore have likely tested all of them, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins. EX1003, ¶446.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶437-47.

8. Claim 8: "The method of claim 1, wherein the liquid

chromatography is ultra-performance liquid chromatography (UPLC).”

See claim 1. Shytuhina’s method involves separation of the viral proteins using RP-HPLC for the liquid chromatography coupled to the mass spectrometer. EX1006, 193; EX1003, ¶449.

A POSA would have been aware that researchers were using top-down (intact) LC-MS with UPLC to study mixtures of proteins and to identify PTMs. EX1009; EX1003, ¶450. A POSA would have been aware of the advantages UPLC could offer, set out in Ansong, such as the efficient separation and increased sampling of proteins in the mixture and identification of PTMs, using long columns and long gradients. EX1009, 10154; EX1003, ¶450.

A POSA would have further understood that UPLC is a chromatographic technique that, for example, can use sub-2 μm particles, mobile phases at high linear velocities, and instrumentation that operates at high pressure. EX1020, 1019; EX1003, ¶451.

A POSA would have been aware that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to quantify proteins for in-process control. EX1020, 1019; EX1003, ¶452.

A POSA would therefore have likely tested UPLC, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins. EX1003, ¶453.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶448-54.

9. Claim 9: "The method of claim 1, wherein the mass spectrometry comprises assisted calibration."

See claim 1. A POSA at the time would have understood that LC-MS systems require calibration, and that calibration could be carried out with an external or internal calibrant. EX1014, 497; EX1008; EX1003, ¶456.

A POSA would further have understood that the term "assisted calibration" in the patent refers to "using software to correlate a peak and/or position of a known standard (e.g., a calibrant) to a specific mass to charge (m/z) ratio." EX1001, 21:47-56; EX1003, ¶457.

A POSA at the relevant time would have been aware that calibration could be carried out using commonly available software, *i.e.*, via assisted calibration. EX1014, 497; EX1003, ¶458.

The combination of Satkunanathan and Shytuhina therefore discloses the limitation. EX1003, ¶¶455-59.

10. Claim 10: "The method of claim 9, wherein sodium iodide is used as a calibrant."

See claim 9. A POSA would have understood NaI to be a desirable and commonly available calibrant, including for assisted calibration using software. EX1008, 665; EX1014, 497; EX1003, ¶464.

The combination of Satkunanathan and Shytuhina therefore discloses the limitation. EX1003, ¶¶460-65.

11. Claim 11: "The method of claim 1, wherein the AAV particle is a recombinant AAV (rAAV) particle."

See claim 1. A POSA at the time would have understood that a recombinant AAV particle (rAAV) is an AAV particle that has been produced through some type of genetic engineering. EX1005, Abstract; EX1003, ¶467.

A POSA would further have understood that the specification of the '377 patent does not expressly define "recombinant AAV particle," but states: "A rAAV vector can be packaged into an AAV virus capsid to generate a 'recombinant adeno-associated viral particle (rAAV particle).'" EX1001, 16:62-64; EX1003, ¶468. In turn, the specification states that "A 'recombinant AAV vector (rAAV vector)' refers to a polynucleotide vector comprising one or more heterologous sequences (i.e., nucleic acid sequence not of AAV origin) that are flanked by at least one, e.g., two, AAV inverted terminal repeat sequences (ITRs)." EX1001, 16:42-46; EX1003, ¶468.

A POSA at the time would have understood that rAAV gene therapy vectors were specifically designed to deliver a heterologous transgene to a variety of different target cells. EX1025, Abstract, 289-97; EX1003, ¶469.

Satkunanathan discloses rAAV particles containing a heterologous GFP transgene. EX1005, 930; EX1003, ¶470.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. EX1003, ¶¶466-71.

12. Claim 12: "The method of claim 1, wherein the AAV particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, anAAV4 capsid, an AAV5 capsid, anAAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV LK03 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV DJ8 capsid, an AAV2 N587 A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, anAAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV/human bocavirus virus 1), an AAV2HBKO capsid, an AAVPHP.B capsid or an AAVPHP.eB capsid."

See claim 1. Satkunanathan studied rAAV particles comprising three different capsid serotypes: AAV2, AAV5, and AAV8. EX1005, Abstract; EX1003, ¶473.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶472-74.

13. Claim 13: "The method of claim 2, wherein the calculated masses of the one or more capsid proteins are compared to

the theoretical masses of the one or more capsid proteins of one or more virus serotypes."

See claim 2. Shytuhina states that "[b]ased on known glycoprotein acylation and expected N-linked glycans, we identified glycoprotein modifications in several major peaks by matching the observed mass to the theoretical molecular weight." EX1006, 194; EX1003, ¶479.

Therefore, the combination of Satkunanathan and Shytuhina meets the additional limitation of dependent claim 13. EX1003, ¶¶475-80.

14. Claim 14: "The method of claim 2, wherein the liquid chromatography is reverse phase liquid chromatography, size exclusion chromatography, hydrophilic interaction liquid chromatography, or cation exchange chromatography."

See claims 2, 5; EX1003, ¶¶481-83.

15. Claim 15: "The method of claim 2, wherein the liquid chromatography is reverse phase chromatography."

See claims 2, 6; EX1003, ¶¶484-86.

16. Claim 16: "The method of claim 15, wherein the reverse phase chromatography is a C4 or C8 reverse chromatography."

See claims 7, 15; EX1003, ¶¶487-96.

17. Claim 17: "The method of claim 2, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC)."

See claims 2, 8; EX1003, ¶¶497-503.

18. Claim 18: "The method of claim 2, wherein the mass spectrometry comprises assisted calibration."

See claims 2, 9; EX1003, ¶¶504-08.

19. Claim 19: "The method of claim 18, wherein sodium iodide is used as a calibrant.

See claims 10, 18; EX1003, ¶¶509-14.

20. Claim 20: "The method of claim 2, wherein the viral particle comprises a viral vector encoding a heterologous transgene."

See claim 2. A POSA would have understood that the specification of the '377 patent states: "'Heterologous' means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated." EX1001, 17:33-35; EX1003, ¶516.

A POSA would further have understood that the specification of the '377 patent states: "The term 'transgene' refers to a nucleic acid that is introduced into a cell and is capable of being transcribed into RNA and optionally, translated and/or expressed under appropriate conditions." EX1001, 17:42-45; EX1003, ¶517.

A POSA at the time would have understood that a recombinant AAV particle (rAAV) is an AAV particle that has been produced through some type of genetic engineering. EX1005, Abstract; EX1003, ¶518.

A POSA at the time would have understood that rAAV gene therapy vectors were specifically designed to deliver a heterologous transgene to a variety of different target cells. EX1025, Abstract, 289-97; EX1003, ¶519.

Satkunanathan discloses rAAV particles containing a heterologous GFP transgene. EX1005, 930; EX1003, ¶520.

Therefore, the combination of Satkunanathan and Shytuhina meets the additional limitation of dependent claim 20. EX1003, ¶¶515-21.

21. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combinations

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina to arrive at the claimed combinations recited in claims 1-20. EX1003, ¶522. The techniques required to make the claimed combinations, namely, RP-HPLC, UPLC, intact LC-MS, and application of software to calibrate a spectrometer, and also to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. *Id*. Similarly, the choice of calibrant for the spectrometer would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully characterizing several different post-translational modifications. *Id.*, ¶523.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶524.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*, ¶525.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶526.

A POSA would have been able with only routine experimentation to select a calibrant and carry out assisted calibration of a mass spectrometer, as Byeon disclosed. *Id.*, ¶527.

Moreover, a POSA would have had a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS. *Id.*, ¶528.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶529.

Moreover, researchers in the field for years before the relevant date had been successfully separating and characterizing reduced monoclonal antibodies and identifying PTMs, using intact LC-MS. *Id.*, ¶530. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies. *Id.*

22. Secondary Considerations Do Not Change the Conclusion of Obviousness

For evidence of "secondary considerations" to be informative of obviousness, there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in challenged claims 1-20 of the '377 patent. EX1003, ¶531. Petitioner is not aware of any secondary considerations of non-obviousness with the required nexus to these claims of the '377 patent. *Id.* For example, Petitioner is not aware of any commercial success attributable to an analytical method of monitoring an AAV preparation using the well known technique of intact LC-MS.[1] *Id.*

[1] If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys® – there is no nexus to the challenged claims of the '377 patent. There is no nexus between the commercial success of Elevidys® and the analytical method recited in the challenged claims.

Similarly, Petitioner is not aware of any licenses directed specifically to the '377 patent or the subject matter recited in challenged claims 1-20. *Id.*

Finally, Petitioner is not aware of any unexpected results having a nexus to the claimed subject matter. *Id.*, ¶532. The '377 patent does not disclose unexpected properties of the claimed methods. *Id.* Intact LC-MS had been used successfully for years before the relevant date as an analytical technique to monitor proteins, including monoclonal antibodies, and viral structural proteins, for process development. *Id.* The use of LC-MS/MS to identify the capsid proteins of different AAV serotypes had been disclosed in Satkunanathan, and the use of intact LC-MS to monitor viral structural proteins, including identifying their PTMs, for process development had been disclosed in Shytuhina. *Id.*

To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-20 of the '377 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. *Id.*, ¶533. Thus, secondary considerations do not alter the conclusion that claims 1-20 of the '377 patent are obvious over the combination of Satkunanathan and Shytuhina. *Id.*

B. Ground 2: Claims 8 and 17 Are Obvious Over Satkunanathan, Shytuhina, and Ansong

Dependent claims 8 and 17 are also obvious over the combination of Satkunanathan, Shytuhina, and Ansong. EX1003, ¶¶534-68. Petitioner incorporates its discussion of Ground 1 in its entirety herein.

1. Claim 8: "The method of claim 1, wherein the liquid chromatography is ultra-performance liquid chromatography (UPLC)."

See claim 1 of Ground 1. The combination of Satkunanathan, Shytuhina, and Ansong discloses the additional limitation of dependent claim 8. EX1003, ¶535. A POSA would have been motivated to apply Shytuhina's efficient and precise intact LC-MS method to improve on Satkunanathan's use of LC-MS/MS to optimize process development for AAV vectors, particularly in light of differences among the different AAV serotypes and the fact that the AAV capsid proteins (vp1, vp2, vp3) were known to be subject to post-translational modification. *Id.*

A POSA would further have been motivated to combine Ansong's use of UPLC with the methods of Satkunanathan and Shytuhina. Ansong carried out intact LC-MS using UPLC to analyze the proteome of *Salmonella typhimurium* and identify multiple different PTMs. EX1009, Abstract; EX1003, ¶536.

Shytuhina's method involves separation of the viral proteins using RP-HPLC for the liquid chromatography coupled to the mass spectrometer. EX1006, 193; EX1003, ¶537.

A POSA would have been aware that researchers were using top-down (intact) LC-MS with UPLC to study mixtures of proteins and to identify PTMs. EX1009; EX1003, ¶538. A POSA would have been aware of the advantages UPLC could offer, set out in Ansong, such as the efficient separation and increased sampling of proteins in the mixture and identification of PTMs, using long columns and long gradients. EX1009, 10154; EX1003, ¶538.

A POSA would have further understood that UPLC is a chromatographic technique that, for example, can use sub-2 μm particles, mobile phases at high linear velocities, and instrumentation that operates at high pressure. EX1020, 1019; EX1003, ¶539.

A POSA would have been aware that UPLC could offer certain benefits as compared with HPLC, including increased resolution, sensitivity and speed of the chromatographic analysis, which are desirable for a chromatographic method to quantify proteins for in-process control. EX1020, 1019; EX1003, ¶540.

A POSA would therefore have likely tested Ansong's UPLC approach, in applying Shytuhina's intact LC-MS method to the separation and identification of AAV capsid proteins. EX1003, ¶541.

Satkunanathan, in combination with Shytuhina and Ansong, therefore meets this limitation. *Id.*, ¶¶535-42.

2. Claim 17: "The method of claim 2, wherein the liquid

chromatography is ultra-performance liquid chromatography (UPLC).

See claim 8, claim 2 of Ground 1. EX1003, ¶¶543-50.

3. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina and Ansong. EX1003, ¶551. The techniques required to make the claimed combinations, namely, RP-HPLC, UPLC, intact LC-MS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully characterizing several different post-translational modifications. *Id.*, ¶552.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶553.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*, ¶554.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶555.

A POSA would have been able with only routine experimentation to select a calibrant and carry out assisted calibration of a mass spectrometer, as Byeon disclosed. *Id.*, ¶556.

Moreover, a POSA would have had a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS. *Id.*, ¶557.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶558.

Moreover, researchers in the field for years before the relevant date had been successfully separating and characterizing reduced monoclonal antibodies and identifying PTMs, using intact LC-MS. *Id.*, ¶559. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies. *Id.*, ¶559.

4. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner incorporates herein in its entirety the discussion of secondary considerations from Ground 1. EX1003, ¶560.

C. Ground 3: Claims 9, 10, 18, 19 Are Obvious Over Satkunanathan, Shytuhina, and Byeon

Dependent claims 9, 10, 18, and 19 are also obvious over the combination of Satkunanathan, Shytuhina, and Byeon. EX1003, ¶¶561-97. Petitioner herein incorporates Grounds 1 and 2 in their entirety.

1. Claim 9: "The method of claim 1, wherein the mass spectrometry comprises assisted calibration."

See claim 1 of Ground 1. Satkunanathan and Shytuhina, in combination with Byeon, disclose the additional limitation of dependent claim 9. EX1003, ¶562. As discussed above, a POSA would have been motivated to apply Shytuhina's efficient and precise intact LC-MS method to improve on Satkunanathan's use of LC-MS/MS to optimize process development for AAV vectors, particularly in light of differences among the different AAV serotypes and the fact that the AAV capsid proteins (vp1, vp2, vp3) were known to be subject to post-translational modification. *Id.*

A POSA at the time would have understood that LC-MS systems require calibration. EX1014, 497; EX1008; EX1003, ¶563.

A POSA would further have understood that the term "assisted calibration" in the patent refers to "using software to correlate a peak and/or position of a known

standard (e.g., a calibrant) to a specific mass to charge (m/z) ratio." EX1001, 21:47-56; EX1003, ¶564.

As I discussed above, a POSA at the relevant time would have been aware that calibration could be carried out using commonly available software, *i.e.*, via assisted calibration. EX1014, 497; EX1003, ¶565.

A POSA would have been motivated to use Byeon's method of assisted calibration to calibrate the mass spectrometer because such assisted calibration was an efficient and straightforward method of calibration. EX1003, ¶566. In particular, a POSA would have been motivated to use Byeon's method of external, assisted calibration to avoid complications resulting from adding a calibrant to the sample being analyzed, which would have required ensuring compatibility between the sample and the internal calibrant. *Id.*

The combination of Satkunanathan and Shytuhina with Byeon discloses this limitation. *Id.*, ¶¶562-67.

2. Claim 10: "The method of claim 9, wherein sodium iodide is used as a calibrant."

See claim 9. A POSA would have understood NaI to be a desirable and commonly available calibrant, including for assisted external calibration using software. EX1008, 665; EX1014, 497; EX1003, ¶568-74.

The combination of Satkunanathan and Shytuhina with Byeon discloses this limitation. EX1003, ¶568-74.

3. Claim 18: "The method of claim 2, wherein the mass spectrometry comprises assisted calibration."

See claim 9, claim 2 of Ground 1; EX1003, ¶575-80.

4. Claim 19: "The method of claim 18, wherein sodium iodide is used as a calibrant."

See claim 10; EX1003, ¶581-87.

5. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

A POSA would have had a reasonable expectation of success in combining Satkunanathan with Shytuhina and Byeon. EX1003, ¶588. The techniques required to make the claimed combinations, namely, RP-HPLC, UPLC, intact LC-MS, and application of software to calibrate a spectrometer, and also to deconvolute and interpret MS data, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. *Id.* Similarly, the choice of calibrant for the spectrometer would have required nothing more than routine experimentation. *Id.*

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully characterizing several different post-translational modifications. *Id.*, ¶589.

Using nothing more than routine experimentation, a POSA would have been able to select and optimize a UPLC column for separation followed by analysis using MS, as Ansong showed. *Id.*, ¶590.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*, ¶591.

A POSA furthermore, using nothing more than routine experimentation, would have been able to select the best column, C4 or C8, for example, to optimize the separation by RP-HPLC, and then the analysis by MS. *Id.*, ¶592.

A POSA would have been able with only routine experimentation to select a calibrant and carry out assisted calibration of a mass spectrometer, as Byeon disclosed. *Id.*, ¶593.

Moreover, a POSA would have had a reasonable chance of success in separating AAV capsid proteins and identifying them, including PTMs, by intact LC-MS. *Id.*, ¶594.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶595.

Moreover, researchers in the field for years before the relevant date had been successfully separating and characterizing reduced monoclonal antibodies and identifying PTMs, using intact LC-MS. *Id.*, ¶596. Applying intact LC-MS to AAV capsid proteins at the relevant date would have been considerably more routine and straightforward than applying the technique to monoclonal antibodies. *Id.*

6. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner incorporates herein in its entirety the discussion of secondary considerations from Ground 1. EX1003, ¶597.

X. CONCLUSION

Sarepta respectfully requests institution of IPR for claims 1-20 of the ’377 patent based on the grounds specified in this Petition.

Respectfully submitted,

December 2, 2025

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WORD COUNT CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), Petitioner hereby certifies, in accordance with and reliance on the word count provided by the word-processing system used to prepare this Petition, that the number of words in this paper is 10,282. Pursuant to 37 C.F.R. §42.24(d), this word count excludes the table of contents, table of authorities, mandatory notices under §42.8, certificate of service, certificate of word count, appendix of exhibits, and any claim listing.

Respectfully submitted,

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §42.6 (e) and 37 C.F.R. §42.105, I hereby certify that on December 2, 2025, I caused the foregoing Petition for *Inter Partes* Review, Power of Attorney, and Exhibits 1001–1040 to be served on Patent Owner by depositing them for shipment with Federal Express to the correspondence address of record listed on the Patent Center:

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