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. 12,298,313

"Methods for Detecting AAV"

IPR2025-0166

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")
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EX1040	*Genzyme Corp. v. Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC*, Second Amended Complaint, C.A. No. 24-cv-00882-RGA (D. Del.)

LIST OF CHALLENGED CLAIMS

Claim	Element
1 [pre]	A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles, the method comprising
1[a]	a) denaturing the AAV particles;
1[b]	b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis;
1[c]	c) determining the masses of the one or more VPs; and
1[d]	d) determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses,
1[e]	wherein the VPs comprise VP1, VP2 and VP3 capsid proteins, and wherein the method is performed in the absence of a gel separation step.
2	The method of claim 1, wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination.
3	The method of claim 2, wherein the post-translational modification is N-terminal acetylation.
4	The method of claim 1, wherein the AAV particles are denatured using detergent, heat, high salt or buffer with low or high pHs.
5	The method of claim 1, wherein the liquid chromatography is reverse phase chromatography.

Claim	Element
6	The method of claim 5, wherein the reverse phase chromatography is C8 reverse phase chromatography.
7	The method of claim 1, further comprising determining the sequence of one or more VPs that has undergone post-translational modifications.
8	The method of claim 7, wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination.
9	The method of claim 8, wherein the post-translational modification is N-terminal acetylation.
10	The method of claim 7, wherein the sequences of VP1, VP2 and VP3 are determined.
11[pre]	A method of determining the heterogeneity of viral particles in a preparation of adeno-associated virus (AAV) particles comprising VP1, VP2 and VP3 capsid proteins, the method comprising
11[a]	a) denaturing the AAV particles;
11[b]	b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, thereby separating the peaks of the VP1, VP2 and VP3 capsid proteins;
11[c]	c) deconvoluting the peaks of the VP1, VP2 and VP3 capsid proteins; and
11[d]	d) determining the masses of one or more of the VP1, VP2 and VP3 capsid proteins and additional capsid proteins within one or more of the deconvoluted peaks,

Claim	Element
11[e]	wherein the method is performed in the absence of a gel separation step.
12	The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are variant capsids.
13	The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are capsid amino acid substitutions.
14	The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are truncated capsids.
15	The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are modified capsids.
16	The method of claim 15, wherein the modifications of the modified capsids are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination.
17	The method of claim 16, wherein the modification is N-terminal acetylation.
18	The method of claim 11, wherein the liquid chromatography is reverse phase chromatography.
19	The method of claim 18, wherein the reverse phase chromatography is C8 reverse phase chromatography.
20[pre]	A method of preparing a pharmaceutical composition of adeno-associated virus (AAV) particles, the method comprising:
20[a]	monitoring AAV particles for consistency and/or identity;

Claim	Element
20[b]	wherein the AAV particles comprise viral proteins (VPs) comprising VP1, VP2 and VP3 capsid proteins of an AAV particle capsid,
20[c]	wherein the AAV particle is monitored for consistency and/or identity by:
20[d]	a) extracting an aliquot of an AAV particle preparation;
20[e]	b) denaturing the AAV particles;
20[f]	c) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis;
20[g]	d) determining the masses of one or more VPs of the AAV particles; and
20[h]	e) comparing the determined masses of the one or more VPs to theoretical masses of corresponding VPs, wherein the theoretical masses of corresponding VPs are those VPs of known AAV serotypes and/or those that have not undergone undesired post-translational modifications; and
20[i]	f) determining if there is any deviation of the determined masses of the one or more VPs from the theoretical masses of the corresponding VPs;
20[j]	wherein the determination of any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs thereby monitors the AAV particles for consistency and/or identity;
20[k]	wherein the monitoring for consistency and/or identity is performed in the absence of a gel separation step; and

Claim	Element
20[l]	wherein if less than an undesirable amount of deviation is determined during the monitoring for consistency and/or identity, the AAV particles are combined with one or more pharmaceutically acceptable excipients to form the pharmaceutical composition.
21	The method of claim 20, wherein the monitoring of the AAV particles for consistency and/or identity includes determining the serotype of the AAV particles based on the comparison of the determined masses of the VPs to the theoretical masses of the corresponding VPs.
22	The method of claim 20, wherein a determination of any actual deviation in masses reflects heterogeneity in the AAV particle preparation.
23	The method of claim 22, wherein the heterogeneity in the AAV particle preparation is due to mixed AAV capsid serotypes, variant AAV capsid proteins, AAV capsid protein amino acid substitutions, truncated AAV capsid proteins or modified AAV capsid proteins.
24	The method of claim 21, wherein the undesired post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination.
25	The method of claim 21, wherein the AAV particles are denatured using detergent, heat, high salt or buffer with low or high pHs.
26	The method of claim 21, wherein the liquid chromatography is reverse phase chromatography.
27	The method of claim 26, wherein the reverse phase chromatography is C8 reverse phase chromatography.

Sarepta Therapeutics, Inc. ("Sarepta" or "Petitioner") respectfully requests *inter partes* review of claims 1-27 (the "challenged claims") of U.S. Patent No. 12,298,313 ("the '313 patent") (EX1001). The '313 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 '313 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, including liquid chromatography-tandem MS (LC-MS/MS), 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.

The challenged claims are directed to methods for the analysis of preparations of AAV particles. In particular, the challenged claims are directed to methods using LC-MS of intact proteins to analyze and characterize AAV particles, including their serotype and the presence of post-translational modifications (PTMs). The challenged claims recite straightforward and well known aspects of LC-MS methods, such as choosing a C8 column, and using RP-HPLC. All of these aspects of the claimed methods were known in the prior art for years before the earliest possible priority date for the ’313 patent.

Challenged claims 1-27 are obvious over the combination of two prior art references, Satkunanathan and Shytuhina. Challenged claims 6, 19, and 27 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Yuan. Challenged claims 7-10 are also obvious over the additional combination of three prior art references, Satkunanathan, Shytuhina, and Zabrouskov.

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 Shytuhina 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 1-27 are therefore obvious over the combination of Satkunanathan and Shytuhina.

Satkunanathan, Shytuhina, and Yuan. A POSA would have understood the need for routine optimization of column chromatography conditions, such as the column, column length, solvent, and solvent gradient for example, in carrying out liquid chromatography for LC-MS methods, such as those disclosed in Shytuhina. Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. Yuan tested both C4 and C8 columns, finding that either column could be used with equal efficiency to purify the L1 capsid protein. A POSA would therefore have likely tested both C4 and C8 columns, in applying Shytuhina's intact LC-MS method to the characterization of deamidation PTMs in viral proteins.

Challenged claims 6, 19, and 27 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Yuan.

Satkunanathan, Shytuhina, and Zabrouskov. A POSA at the time would have understood that the MS/MS technique (also known as "tandem" MS) used in Satkunanathan could be combined with the intact LC-MS method of Shytuhina, such that intact proteins analyzed using LC-MS could be further analyzed using a second MS step that would provide protein sequence information. For example,

Zabrouskov used intact MS/MS to characterize a specific PTM on ribonuclease A (RNase A). Zabrouskov obtained sequence information allowing characterization of the particular amino acids that were subject to deamidation. A POSA would have understood the benefits of carrying out intact LC-MS/MS, *e.g.*, more precise characterization of PTMs through obtaining sequence information, and would have been motivated to combine Satkunanathan and Shytuhina with Zabrouskov.

Challenged claims 7-10 are therefore also obvious over the combination of Satkunanathan, Shytuhina, and Zabrouskov.

Petitioner respectfully submits that the challenged claims (1-27) 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 ’313 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 ’313 patent.

C. Related Patent Office Proceedings

This is the first petition challenging a claim of the ’313 patent.

Petitioner is concurrently filing petitions requesting *inter partes* review of the claims of two related patents, U.S. Patent No. 11,698,377 (“the ’377 patent”) and U.S. Patent No. 12,123,880 (“the ’880 patent”). The ’313 patent issued from U.S. Application No. 19/013,863, which is a continuation of application No. 18/801,293, which is a division of application No. 18/321,542, which issued as U.S. Patent No. 12,123,880, which is a division of application No. 16/325,653, filed as application No. PCT/US2017/046814, which issued as U.S. Patent No. 11,698,377.

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 ’313 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 1-27 of the ’313 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).
Yuan (EX1037); published in 1998; prior art under AIA §102(a)(1).
Zabrouskov (EX1038); published in 2005; prior art under AIA §102(a)(1).

Ground	Claims	Description
1	1-27	Obvious in view of Satkunanathan and Shytuhina
2	6, 19, 27	Obvious in view of Satkunanathan, Shytuhina, and Yuan
3	7-10	Obvious in view of Satkunanathan, Shytuhina, and Zabrouskov

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

IV. BACKGROUND

A. Overview of the Technology

Well over a decade before the earliest possible priority date for the '313 patent, researchers were using liquid chromatography, RP-HPLC in particular, to detect and quantify viral capsid proteins for purification process development. EX1037, Abstract; EX1003, ¶188; *see also id.*, ¶¶95-97. 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 '313 patent. EX1036, Abstract, 205-206; EX1003, ¶189; *see also id.*, ¶¶98-127. 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; *see also id.*, 205; EX1033 (Richards), 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, ¶¶194-196. 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.

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. 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, ¶204, *see also id*., ¶¶166-187. Shytuhina applied LC-MS to analyze undigested, intact viral structural proteins to evaluate PTMs. EX1006, Abstract, 193-94; EX1003, ¶204. Researchers before the priority date were also using intact LC-MS/MS to characterize PTMs further through obtaining protein sequence information, as Zabrouskov did. EX1038, Abstract, 989, Figure 2; EX1003, ¶¶179-183.

B. THE '313 PATENT

The '313 patent is titled "Methods for Detecting AAV." EX1001; EX1003, ¶205. The patent names Xiaoying Jin, Catherine O'Riordan, Lin Liu, and Kate Zhang as inventors. EX1001; EX1003, ¶205. The '313 patent issued on May 13, 2025. EX1001; EX1003, ¶205.

The ’313 patent is assigned to Genzyme Corporation. EX1001; EX1003, ¶206.

1. The Claims

The challenged claims are reproduced in the list above. EX1003, ¶208. Claims 1, 11, and 20 are independent claims. EX1001, 83:2-18, 43-58, 84:15-50; EX1003, ¶209. Claims 2, 4, 5, and 7 depend from claim 1, claim 3 depends from dependent claim 2, claim 6 depends from dependent claim 5, claims 8 and 10 depend from dependent claim 7, and claim 9 depends from dependent claim 8. EX1001, 83:19-42; EX1003, ¶209. Claims 12-15 and 18 depend from claim 11, claim 16 depends from dependent claim 15, claim 17 depends from dependent claim 16, and claim 19 depends from dependent claim 18. EX1001, 83:59-84:14; EX1003, ¶209. Claims 21 and 22 depend from claim 20, claim 23 depends from dependent claim 22, claims 24-26 depend from dependent claim 21, and claim 27 depends from dependent claim 26. EX1001, 84:51-85:7; EX1003, ¶209.

The challenged claims are directed to the use of LC-MS intact protein analysis to characterize preparations of AAV. EX1003, ¶207. The claims require denaturing the viral particles and then subjecting them to intact LC-MS. *Id.* Dependent claims specify particular denaturing methods, and particular types of chromatography. Certain claims also involve characterization of viral proteins having various mutations and modifications.

2. The Specification

The specification of the ’313 patent discusses using LC-MS as an analytical tool to evaluate viral preparations. EX1001, 2:10-24; EX1003, ¶210. The specification discusses using LC-MS to discern characteristics of the viral preparation including the identity of the capsid serotype. EX1001, 2:14-17; 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, 2:17-20; EX1003, ¶210. The specification describes “Examples” of LC-MS and LC-MS/MS for characterization of rAAV viral capsid proteins. EX1001, 51:24-56:45; EX1003, ¶211. The specification discusses methods of denaturing rAAV samples before LC-MS analysis. EX1001, 54:24-34; EX1003, ¶212.

The specification discusses LC-MS intact protein analysis. EX1001, 53:34-51; 54:35-55:7; EX1003, ¶213. The specification also discusses LC-MS/MS peptide mapping. EX1001, 53:63-54:20; 55:8-50; 62:57-63:9; EX1003, ¶214. The specification does not disclose LC-MS/MS analysis of intact proteins; EX1003, ¶214.

3. The Prosecution History

The applicants filed a preliminary amendment, and a terminal disclaimer. EX1002, 147-54, 229-31; EX1003, ¶215. The applicants also filed an Amendment

after Allowance, which was entered by the Examiner. EX1002, 272-79, 283-85; EX1003, ¶215.

4. Priority Date

The ’313 patent claims priority to U.S. Provisional Application No. 62/375,314, filed August 15, 2016 (“the ’314 provisional”). EX1003, ¶216. The ’313 patent issued from U.S. Patent Application 19/013,863, filed January 8, 2025, and is a continuation of U.S. Patent Application No. 18/801,293, filed August 12, 2024, which is a divisional of U.S. Patent Application No. 18/321,542 (U.S. Pat. No. 12,123,880), filed May 22, 2023, which is a divisional of U.S. Patent Application No. 16/325,653 (U.S. Pat. No. 11,698,377), which adopts the international filing date of August 14, 2017, which is a National Phase application under 35 U.S.C. § 371 of International Application No. PCT/US2017/046814, filed August 14, 2017, which claims the priority benefit of the ’314 provisional. EX1003, ¶217.

The earliest possible priority date for the ’313 patent claims is therefore the filing date of the ’314 provisional, August 15, 2016. EX1003, ¶218.

V. LEVEL OF ORDINARY SKILL IN THE ART

A POSA in the technical field of the ’313 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, ¶¶210-222. 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.*, ¶222.

VI. OVERVIEW OF THE PRIOR ART

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 '313 patent. EX1005; EX1003, ¶228. Therefore, Satkunanathan is AIA §102(a)(1) prior art. EX1005; EX1003, ¶228.

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, ¶229. 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, ¶230. Satkunanathan states that their work is directed towards improving the production of AAV vectors for gene therapy.

EX1005, Abstract, 930; EX1003, ¶232. 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, ¶232.

Satkunanathan digested purified and concentrated vector samples with trypsin before LC-MS/MS. EX1005, 930-31; EX1003, ¶¶233-238. 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, ¶239. 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, 932; EX1003, ¶240. Three MS runs were performed for each batch of samples. EX1005, 932; EX1003, ¶241.

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, ¶236. Notably, Dong had identified oxidation of methionine as a PTM found in AAV2 capsid proteins. EX1022, Abstract, 2; EX1003, ¶236.

Out of eight proteins found in at least two serotypes, five were shared by AAV2 and AAV5, indicating a relative similarity between AAV2 and AAV5 vectors. EX1005, 932, Supplementary Table S1; EX1003, ¶243. Twenty-six were

unique to individual serotypes of vectors. EX1005, 932, Supplementary Table S1; EX1003, ¶243. 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 (Satkunanathan), Supplementary Table S1 (excerpt) (yellow highlights added); EX1003, ¶¶244-245.

Satkunanathan also found a serotype-specific role for an AAV-associated cellular protein, YB1. EX1005, Abstract, 938; EX1003, ¶¶246-252, 370-371. 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, ¶¶246-252, 370-371.

Satkunanathan therefore teaches the importance of identifying and characterizing different AAV serotypes accurately for rAAV vector purification and production. EX1003 ¶249. 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, ¶249.

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, ¶250.

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 '313 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1006; EX1003, ¶256.

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, ¶257. In particular, Shytuhina disclosed using their HPLC-MS method to identify PTMs on intact viral structural proteins. EX1006, Abstract; EX1003, ¶257.

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, ¶¶258-262. 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, ¶263.

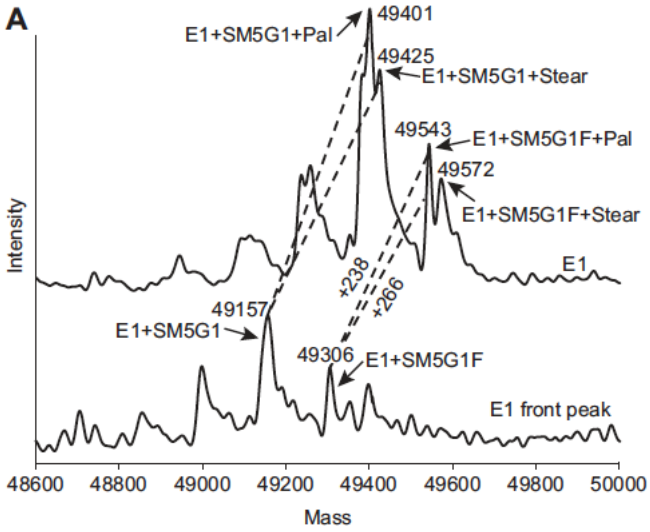
Shytuhina discloses that HPLC was an attractive analytical tool, in light of its high sensitivity and reproducibility. EX1006, 192; EX1003, ¶264. Shytuhina notes that HPLC had been applied for the identification and quantitation of viral proteins and VLPs from a variety of other viruses, including serotypes of adenovirus (types 3 and 5), influenza, lentivirus, Sendai virus, poliovirus, human papillomavirus VLP, and Hepatitis B VLP. EX1006, 192-93; EX1003, ¶¶264-265.

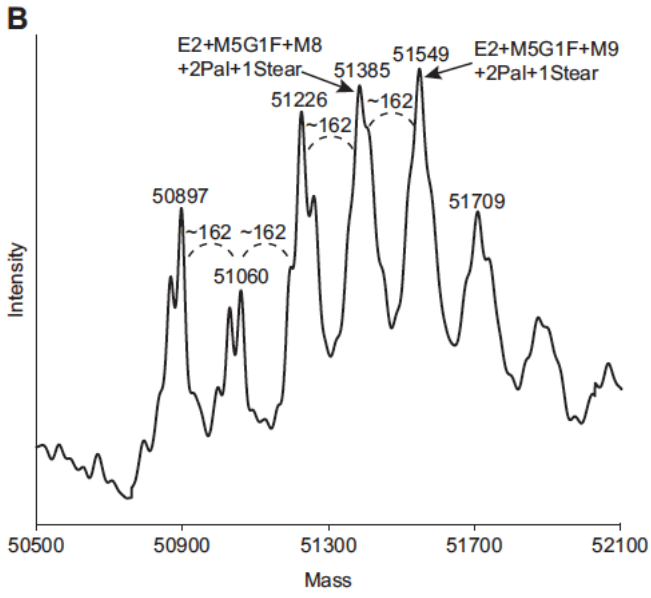
The Chikungunya VLP has three structural proteins – E1 (envelope protein 1), E2 (envelope protein 2), and a capsid protein. EX1006, 193; EX1003, ¶266. The capsid and envelope are organized as follows: an outer surface composed of 240 copies of glycoproteins E1 and E2 embedded in a lipid bilayer surrounding a nucleocapsid made of 240 copies of capsid protein. EX1006, 193; EX1003, ¶266.

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, ¶267. This assay would evaluate and quantitate the mass and purity of the vaccine product. EX1006, 193; EX1003, ¶267. This method would be a tool to assess both

protein degradation and PTMs for formulation and process development. EX1006, 193; EX1003, ¶267.

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, ¶268. Shytuhina discusses monitoring PTMs as a key element of process development. EX1006, 196-97; EX1003, ¶268. PTMs were identified by comparing observed masses to theoretical masses of expected N-glycoforms with and without acylation. EX1006, 194; EX1003, ¶282. 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, ¶¶269-295, ¶421. Figure. 2, reproduced below, shows the deconvoluted spectra for E1 (Fig. 2A) and E2 (Fig. 2B).





Id., 194-95, Fig. 2; EX1003, ¶295.

As shown in the figures above, multiple masses were observed with each representing different glycosylation and acylation modifications. EX1006, 194-95, Fig. 2; EX1003, ¶296. 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, ¶297.

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, Figure. 2; EX1003, ¶298. They detected a small amount of glycosylated but deacylated E1. EX1006, 194-95, Figure 2A, bottom trace; EX1003, ¶299. 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, ¶299.

Fig. 2B shows that E2 contained two N-glycosylations and three acylations. EX1006, 194-95, Figure 2; EX1003, ¶300.

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, ¶301. 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, ¶¶301-302.

Shytuhina explains that Chikungunya VLPs produced in different cell lines exhibit different PTMs. EX1006, 196-97 EX1003, ¶303. 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, ¶303.

C. Yuan

Yuan, titled, "Reversed-phase high-performance liquid chromatography of virus-like particles," was published in 1998, more than a year before the earliest possible priority date for the '313 patent (August 15, 2016), and is therefore AIA §102(a)(1) prior art. EX1037; EX1003, ¶304.

Yuan discloses an RP-HPLC method for analysis of virus-like particles (VLPs) of human papillomavirus (HPV) for process development. EX1037, Abstract; EX1003, ¶¶305-307. In particular, Yuan's method is directed towards identifying the L1 capsid protein that comprises 90-95% of HPV capsids. EX1037, 21; EX1003, ¶306. Yuan explains that the HPV capsid is typically composed of 72 pentameric capsomers of L1 arranged on a skewed icosahedral lattice. EX1037, 21; EX1003, ¶306. A second structural protein, L2, represents the remaining 5-10% of the capsid. EX1037, 21; EX1003, ¶306. Yuan states that their method is quantitative and can be used to facilitate HPV purification process development. EX1037, 21; EX1003, ¶¶307-312.

Yuan discusses the importance of the first step of dissociating the VLPs before placing them on the HPLC column. EX1037, 22; EX1003, ¶¶313-327. Yuan explains that the VLPs they used in the analysis were approximately 55 nm in diameter and contained a single capsid protein, L1, with a molecular mass of about 55,000. EX1037, 22; EX1003, ¶313. Yuan explains that to quantitate the L1 capsid protein, it was necessary to dissociate the VLPs prior to RP-HPLC analysis. EX1037, 22; EX1003, ¶313.

Yuan tested both C4 and C8 columns, noting that "either the C4 or C8 column can be used with equal efficiency of separation providing calibration is carried out with purified reference standard material." EX1037, 23; EX1003, ¶319.

D. Zabrouskov

Zabrouskov, "Stepwise Deamidation of Ribonuclease A at Five Sites Determined by Top Down Mass Spectrometry," was published in 2006, more than a year before the earliest possible priority date for the '313 patent (August 15, 2016), and is therefore AIA 102(a)(1) prior art. EX1038; EX1003, ¶328.

Zabrouskov used intact MS/MS to identify multiple deamidation sites, at asparagine and glutamine, on Ribonuclease A (RNase A). EX1038, Abstract; EX1003, ¶329. Zabrouskov also carried out site-specific quantitation of deamidation at the various sites in RNase A. EX1038, Abstract; EX1003, ¶331.

Zabrouskov notes, nonetheless, that it was known in the art that deamidation poses a special challenge, in that the covalent $–NH_2 \rightarrow –OH$ modification produces only a 0.984 Da mass increase, closely matching the 1.002 Da spacing of the molecular ion isotope peaks. EX1038, 987; EX1003, ¶333. As a result, it is difficult to distinguish deamidated forms from ^{13}C isotopic forms. EX1003, ¶333.

Despite this known difficulty, Zabrouskov successfully used intact MS/MS to distinguish five stepwise deamidation sites in RNase A. EX1038, 987; EX1003, ¶¶334-361.

According to Zabrouskov, rates of deamidation of asparagine and glutamine residues depend on protein primary sequence, three-dimensional structure, and

solution parameters – increased pH and temperature and enhanced denaturation accelerate deamidation. EX1038, 987; EX1003, ¶335.

Deamidation of Asn is generally favored over that of Gln, in part through operation of a cyclic imide reaction mechanism that also favors the Asn67-Gly68 sequence found in RNase A, while other neighboring residues also show an influence statistically. EX1038, 987; EX1003, ¶336. However, some Asn and Gln residues are extremely resistant to *in vivo* deamidation. EX1038, 987; EX1003, ¶336.

Zabrouskov states that in "bottom-up" MS proteomics, which uses enzymatic digestion of proteins before MS analysis, initial digestion of the protein gives peptides with mass spectra that often provide a fast, reliable identification of a protein, but are much less useful in characterizing PTMs. EX1038, 987; EX1003, ¶¶337-361, 377.

Zabrouskov calculated percentages of deamidated products in the various reactions. EX1038, 990; EX1003, ¶¶338-362. For example, Zabrouskov reported that, for the monodeamidated fraction, Asn67 was the first residue to deamidate and the deamidation of this reside was nearly complete in 1 hour (0:1:2 deamidations at a 5:43:52 ratio), with <10% monodeamidation at other sites. EX1038, 990; EX1003, ¶362.

VII. 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 "heterogeneity," the express definition in the specification has been applied to the analysis. Finally, for the term "variants/variant," the petition analyzes the challenged claims according to the construction that Genzyme has proposed in the litigation for this term – to mean "AAV mutant capsid protein[s]." EX1003, ¶365.

VIII. DETAILED EXPLANATION OF GROUNDS

A. Ground 1: Claims 1-27 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. 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. EX1003, ¶¶366-367. 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.*, ¶368.

Satkunanathan studied proteins that co-purify along with each of three different AAV serotypes, AAV2, AAV5, and AAV8. EX1003, ¶¶369, 372. As part

of this analysis, Satkunanathan also identified capsid proteins for each of the three serotypes studied. EX1003, ¶373. In addition, Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. *Id.* 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.*, ¶377; *see also id.*, ¶¶64-85.

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). EX1003, ¶¶374-375; *see also id.*, ¶¶86-94. 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, ¶375.

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 and other variations and truncations. EX1003, ¶378. Shytuhina discloses the use of intact LC-MS of viral structural proteins to identify and monitor PTMs to improve process development. *Id.*, ¶376.

1. Claim 1: "A method of detecting post-translational modifications of one or more viral proteins (VPs) in a preparation of adeno-associated virus (AAV) particles, the method 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, ¶380. In addition, Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification. EX1005, 931; EX1003, ¶380. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the sequence of some capsid proteins containing this PTM. EX1003, ¶380. A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶380.

Shytuhina uses intact LC-MS to identify various post-translational modifications, glycosylation and acylation, of viral structural proteins. EX1006, 194; EX1003, ¶381. Shytuhina observed several different glycosylation and

acylation modifications on each of the two viral structural proteins analyzed by intact LC-MS. EX1003, 194-195, Fig. 2; EX1003, ¶381. Shytuhina therefore used intact LC-MS to detect post-translational modifications of viral proteins in a preparation of viral particles. EX1003, ¶381.

A POSA would have understood that applying Shytuhina's intact LC-MS method to Satkunanathan's study of variations among different AAV serotypes would have provided a method of detecting post-translational modifications of the AAV capsid proteins in a preparation of AAV particle. EX1003, ¶382.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶379-383.

(a) "a) denaturing the AAV particles;"

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, ¶385.

For the first step, Shytuhina teaches incubating the samples with 5% Zwittergent 3-12 detergent. EX1006, 193-94; EX1003, ¶386. 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, ¶386. A POSA at the time would have understood that "solubilizing" glycoproteins involves denaturing them. EX1003, ¶386.

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, ¶387. 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, ¶387.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶384-388.

(b) "b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis;"

See claim 1(a). Shytuhina discloses LC-MS intact protein analysis of denatured viral structural proteins. EX1006, 193-94; EX1003, ¶390.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶389-392.

(c) "c) determining the masses of the one or more VPs; and"

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, ¶394.

Shytuhina determined the masses of two viral structural proteins containing various different PTMs using intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶395.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶393-396.

(d) "d) determining any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs that have not undergone post-translational modifications to detect a deviation in the compared masses,"

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, ¶398.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶397-399.

(e) "wherein the VPs comprise VP1, VP2 and VP3 capsid proteins, 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, ¶401.

Shytuhina identified and distinguished two structural viral proteins, E1 and E2, using intact LC-MS in the absence of a gel separation step. EX1006, 193-95, Fig. 2; EX1003, ¶402. As discussed above, 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, ¶402. No gel separation step was used at any point as part of this process. EX1003, ¶402.

The masses of the AAV capsid proteins (87, 73, and 62 kDa) are similar to the masses of the Chikungunya structural proteins (approximately 55 and 33 kDa) studied by Shytuhina. EX1003, ¶403. As a result, a POSA would have understood that it would have been straightforward to apply Shytuhina's intact LC-MS method to identify AAV capsid proteins. *Id.*

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶400-404.

2. Claim 2: "The method of claim 1, wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation,

glycosylation, truncation and ubiquitination."

See claim 1. Satkunanathan uses LC-MS/MS with methionine oxidation set as a dynamic modification. EX1005, 931; EX1003, ¶406. A POSA at the time would have understood that methionine oxidation was a post-translational modification of AAV capsid proteins. EX1022, 2; EX1003, ¶406.

Satkunanathan identified capsid proteins for each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶407. A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation in vivo. EX1011, 7920; EX1003, ¶407.

Shytuhina uses intact LC-MS to identify various post-translational modifications, including glycosylation, of viral structural proteins. EX1006, 194; EX1003, ¶¶408, 413.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶405-409.

3. Claim 3: "The method of claim 2, wherein the post-translational modification is N-terminal acetylation."

See claim 2. A POSA would have expected that, given Shytuhina's identification of post-translational modifications including various different glycosylations and acylations, Shytuhina's intact LC-MS method would identify the

known post-translational modification of N-terminal acetylation of AAV capsid proteins. EX1003, ¶414.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶410-415.

4. Claim 4: "The method of claim 1, wherein the AAV particles are denatured using detergent, heat, high salt or buffer with low or high pHs."

See claim 1. Shytuhina teaches denaturing the VLP samples with 5% Zwittergent 3-12 detergent. EX1006, 193-94; EX1003, ¶417.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. EX1003, ¶¶ 416-419.

5. Claim 5: "The method of claim 1, wherein the liquid chromatography is reverse phase chromatography."

See claim 1. For intact LC-MS, Shytuhina used an RP-HPLC separation coupled to the mass spectrometer. EX1006, 193; EX1003, ¶421.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. EX1003, ¶¶420-422.

6. Claim 6: "The method of claim 5, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claim 5. Shytuhina discloses that the specific RP-HPLC column they used prior to LC-MS was a C4 column. EX1006, 193; EX1003, ¶¶424, 427. 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, ¶¶424, 427.

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, ¶428.

Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. EX1037, Abstract; EX1003, ¶429. 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; EX1003, ¶429.

Satkunanathan used a reversed phase C18 column for LC-MS/MS. EX1005, 931; EX1003, ¶430. 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, ¶430.

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. EX1003, ¶431. 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. *Id.*

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶423-432.

7. Claim 7: "The method of claim 1, further comprising determining the sequence of one or more VPs that has undergone post-translational modifications."

See claim 1. A POSA would have understood that Satkunanathan's LC-MS/MS method determined the sequences of the fragments in the mixtures of protein fragments analyzed. EX1003, ¶434. For example, using LC-MS/MS, Satkunanathan identified AAV capsid proteins from three different serotypes by matching the sequence of the proteins as determined by MS/MS against a database, specifically protein FASTA databases. EX1005, 931, Supplementary Table S1; EX1003, ¶434.

Given that Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification, and that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the sequence of some capsid proteins containing this PTM. EX1005, 931; EX1022, Abstract, 2; EX1003, ¶435. A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶435.

As demonstrated in references such as Zabrouskov, for example, a POSA at the time would have understood how to combine Shytuhina's intact LC-MS method

with Satkunanathan's LC-MS/MS method, to determine the sequences of the AAV capsid proteins, vp1, vp2, vp3, and further characterize their post-translational modifications. EX1038, Abstract; EX1003, ¶436.

Zabrouskov used MS/MS of intact proteins ("top-down MS/MS") to analyze deamidation of RNase A. EX1038, Abstract; EX1003, ¶437. Zabrouskov identified multiple deamidation sites, at asparagine and glutamine, on RNase A. EX1038, Abstract; EX1003, ¶437.

As part of the MS/MS analysis, Zabrouskov determined the sequences of the fragments generated from the intact RNase A proteins during MS/MS analysis. EX1037, Figures 2-4, 989-90; EX1003, ¶338-361, ¶438.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶ 433-439.

8. Claim 8: "The method of claim 7, wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation, and ubiquitination."

See claim 7. EX1003, ¶¶441-450. A POSA would further have understood Zabrouskov's teaching that deamidation was a particularly challenging post-translational modification to characterize, given "the special challenge" that "its covalent $–NH_2 \rightarrow –OH$ modification causes an only 0.984 Da mass increase, closely

matching the 1.002 Da spacing of the molecular ion isotope peaks." EX1038, 987; EX1003, ¶451.

A POSA would therefore have understood that the known post-translational modifications of AAV capsid proteins, N-terminal truncation and acetylation, would have been more easily characterized by intact LC-MS/MS than the deamidation modifications characterized by Zabrouskov. EX1003, ¶452.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. EX1003, ¶¶440-453.

9. Claim 9: "The method of claim 8, wherein the post-translational modification is N-terminal acetylation."

See claim 8. EX1003, ¶¶454-466.

10. Claim 10: "The method of claim 7, wherein the sequences of VP1, VP2 and VP3 are determined."

See claim 7. EX1003, ¶¶467-473.

11. Claim 11: "A method of determining the heterogeneity of viral particles in a preparation of adeno-associated virus (AAV) particles comprising VP1, VP2 and VP3 capsid proteins, the method comprising"

"Heterogeneity" is defined in the '313 patent as follows:

> "Heterogeneity" when used in reference to an AAV capsid refers to an AAV capsid characterized by one or more capsid polypeptides observed to deviate from a reference mass of a VP1, VP2, and/or VP3 polypeptide, or fragment thereof. A reference mass may include, without limitation, a theoretical, predicted, or expected mass of a VP1, VP2, and/or VP3 polypeptide, e.g., of a known AAV serotype. For example, an AAV capsid may be said to display heterogeneity if it

> demonstrates one or more of the following properties (without limitation): a mixed serotype, a variant capsid, a capsid amino acid substitution, a truncated capsid, or a modified capsid.

EX1001, 19:54-65; EX1003, ¶475.

Satkunanathan identified capsid proteins for each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶476.

Satkunanathan uses LC-MS/MS with methionine oxidation set as a dynamic modification. EX1005, 931; EX1003, ¶477. A POSA at the time would have understood that methionine oxidation was a PTM of AAV capsid proteins. EX1022, 2; EX1003, ¶477. A POSA would therefore have understood that Satkunanathan likely identified certain AAV capsid proteins carrying this post-translational modification. EX1003, ¶477.

A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶478. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶202.

A POSA at the time would further have understood that PTMs vary from protein molecule to protein molecule. EX1006, 194-95, Fig. 2; EX1003, ¶479. Shytuhina discloses that, using intact LC-MS, they identified multiple different

glycosylation and acylation modifications for each of the viral structural proteins they studied. EX1006, 194-95, Fig. 2; EX1003, ¶479.

A POSA would therefore have understood that Shytuhina's intact LC-MS method, which identified heterogeneous populations of viral structural proteins, could have been applied to identify heterogeneous populations of the capsid proteins Satkunanathan studied, some of which likely carried PTMs, including N-terminal acetylation and truncation. EX1003, ¶480.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶474-481.

(a) "a) denaturing the AAV particles;"

See claim 1(a). EX1003, ¶¶482-486.

(b) "b) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis, thereby separating the peaks of the VP1, VP2 and VP3 capsid proteins;"

See claim 1(b). EX1003, ¶¶487-495.

(c) "c) deconvoluting the peaks of the VP1, VP2 and VP3 capsid proteins; and"

Shytuhina characterized post-translational modifications for each of the two viral structural proteins, E1 and E2, using intact LC-MS. EX1006, 194-95, Fig. 2; EX1003, ¶497. Using intact LC-MS, for example, Shytuhina separated and characterized two different PTMs for the E1 structural protein. EX1006, 194-95,

Fig. 2A; EX1003, ¶497. Shytuhina also separated and characterized a small amount of glycosylated but deacylated E1 protein by intact LC-MS. EX1006, 194-95, Fig. 1, Fig. 2A; EX1003, ¶497.

Shytuhina used software to deconvolute the MS results. *Id.*, 193-94; EX1003, ¶498.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶496-499.

(d) "d) determining the masses of one or more of the VP1, VP2 and VP3 capsid proteins and additional capsid proteins within one or more of the deconvoluted peaks,"

See claim 1(c). EX1003, ¶¶500-506.

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

See element (e) of claim 1. EX1003, ¶¶507-510.

12. Claim 12: "The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are variant capsids."

See claim 11. Satkunanathan identified capsid proteins for each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶512. Satkunanathan uses LC-MS/MS with methionine oxidation set as a dynamic modification. EX1005, p931; EX1003, ¶512. A POSA at the time would have understood that methionine oxidation was a PTM of AAV capsid proteins.

EX1022, 2; EX1003, ¶512. Given that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the sequence of some capsid proteins containing this PTM. EX1005, 931; EX1022, Abstract, 2; EX1003, ¶512.

A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶513. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶202.

Shytuhina discloses LC-MS intact protein analysis of viral structural proteins. EX1006, 193-94; EX1003, ¶514. Using intact LC-MS, Shytuhina separated and characterized different post-translationally modified forms of viral structural proteins E1 and E2 from one another and from unmodified forms of the proteins. EX1006, 194-95, Fig. 2; EX1003, ¶514.

Using intact LC-MS, for example, Shytuhina separated and characterized two different PTMs for the E1 structural protein, in addition to identifying E1 unmodified protein. EX1006, 194-95, Fig. 2A; EX1003, ¶515. Specifically, a POSA would have understood that Shytuhina's intact LC-MS method involved, as discussed above, deconvoluting the peaks obtained using software and identifying

within the deconvoluted peaks modified forms of E1 and E2. EX1006, 194-95, Fig. 2; EX1003, ¶515.

A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high resolution to separate and characterize at least some capsid protein variants of the AAV serotypes studied in Satkunanathan, from one another. EX1003, ¶516.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶511-517.

13. Claim 13: "The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are capsid amino acid substitutions."

See claim 11. EX1003, ¶¶519-521. A POSA would have understood that a variety of different AAV serotypes, and versions of those serotypes with amino acid modifications to the capsid, including single amino acid substitutions and larger insertions, deletions, and truncations, had been discovered and engineered. *Id.*, ¶522.

A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high resolution to separate capsid proteins with amino acid substitutions from capsid

proteins without such substitutions and to characterize those amino acid substitutions. EX1003, ¶523.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶¶518-524.

14. Claim 14: "The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are truncated capsids."

See claim 11. EX1003, ¶¶526-530. A POSA at the time would have further understood that AAV capsid proteins are post-translationally modified by N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶530. Becerra states that after truncation of the N-terminal methionine, the following alanine becomes acetylated *in vivo*. EX1011, 7920; EX1003, ¶530.

A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high resolution to separate and characterize varying forms of at least some capsid proteins, such as those studied in Satkunanathan, from one another, including AAVs with truncated capsid proteins. EX1003, ¶531.

A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high

resolution to separate and characterize at least some capsid protein variants of the AAV serotypes studied in Satkunanathan, from one another. EX1003, ¶517.

A POSA would have been motivated to use Shytuhina's intact LC-MS method to monitor AAV composition for process development, including identifying truncated capsids. EX1003, ¶532.

Satkunanathan, in combination with Shytuhina, therefore meets this limitation. *Id.*, ¶525-533.

15. Claim 15: "The method of claim 11, wherein the additional capsid proteins within one or more of the deconvoluted peaks are modified capsids."

See claims 11, 14. EX1003, ¶¶534-542.

16. Claim 16: "The method of claim 15, wherein the modifications of the modified capsids are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination."

See claim 15. EX1003, ¶¶543-551.

17. Claim 17: "The method of claim 16, wherein the modification is N-terminal acetylation."

See claim 16. EX1003, ¶¶552-560.

18. Claim 18: "The method of claim 11, wherein the liquid chromatography is reverse phase chromatography."

See claims 5, 11. EX1003, ¶¶561-564.

19. Claim 19: "The method of claim 18, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claims 6, 18. EX1003, ¶¶565-572.

20. Claim 20: "A method of preparing a pharmaceutical composition of adeno-associated virus (AAV) particles, the method comprising:

Satkunanathan is expressly directed towards enhanced production of AAV vectors for gene therapy. EX1005, Abstract; EX1003, ¶574.

Satkunanathan states: "The significance of our findings should be considered in the context of future development of production methods for AAV-based gene therapy products." EX1005, 930; EX1003, ¶575.

A POSA at the time would therefore have understood Satkunanathan to be directed towards methods of preparing a pharmaceutical composition of AAV particles. EX1003, ¶576.

Shytuhina is similarly expressly directed towards an analytical method for monitoring viral particles for process development of pharmaceuticals, including vaccines. EX1006, Abstract, 197 ("We demonstrated that this RP-HPLC method could support process development by monitoring product purity, and support formulation development by monitoring the product protein degradation"); EX1003, ¶¶577-578.

Because Shytuhina discloses a method to support both process and formulation development, a POSA would therefore have understood Shytuhina to disclose a method for preparing a pharmaceutical composition. EX1003, ¶579.

As discussed above, a POSA at the time would have applied Shytuhina's method to process and formulation development of AAV vectors for gene therapy. EX1003, ¶580.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶573-581.

(a) "monitoring AAV particles for consistency and/or identity"

See claim 20 preamble. EX1003, ¶¶574-588. A POSA would have further understood that applying Shytuhina's method for monitoring product degradation monitors the viral particles for consistency and identity. EX1006, 197; EX1003, ¶589.

As discussed above, a POSA at the time would have applied Shytuhina's method to process and formulation development of AAV vectors for gene therapy. EX1003, ¶590.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶582-591.

(b) "wherein the AAV particles comprise viral proteins (VPs) comprising VP1, VP2 and VP3 capsid proteins of an AAV particle capsid,"

Shytuhina identified and distinguished two structural viral proteins, E1 and E2, using intact LC-MS. EX1006, 193-95, Fig. 2; EX1003, ¶593. As discussed

above, 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, ¶593.

Satkunanathan identified capsid proteins using LC-MS/MS for each of three different AAV serotypes, AAV2, AAV5, and AAV8; EX1003, ¶594. Moreover, the masses of the AAV capsid proteins (87, 73, and 62 kDa) are similar to the masses of the Chikungunya structural proteins (approximately 55 and 33 kDa) studied by Shytuhina. EX1003, ¶594. As a result, a POSA would have understood that it would have been straightforward to apply Shytuhina's intact LC-MS method to identify AAV capsid proteins. *Id.*

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶592-595.

(c) "wherein the AAV particle is monitored for consistency and/or identity by:"

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 were known to be subject to post-translational modification. EX1003, ¶596.

Satkunanathan is expressly directed towards enhanced production of AAV

vectors for gene therapy. EX1005, Abstract; EX1003, ¶597. Satkunanathan states: "The significance of our findings should be considered in the context of future development of production methods for AAV-based gene therapy products." EX1005, 930; EX1003, ¶598.

Shytuhina discloses using their RP-HPLC method to monitor viral particles for process and formulation development. EX1006 (Shytuhina), 197; EX1003, ¶¶600-604. A POSA would have understood that monitoring product degradation and PTMs to ensure product lot-to-lot consistency constitutes monitoring the viral particles for consistency and identity. EX1003, ¶¶603-604. As discussed above, a POSA at the time would have applied Shytuhina's method to process and formulation development of AAV vectors for gene therapy. *Id.*, ¶605.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶596-606.

(d) "a) extracting an aliquot of an AAV particle preparation;"

Satkunanathan discloses that the samples of viral particles subjected to LC-MS/MS analysis were first "extracted." EX1005, 931; EX1003, ¶608. A POSA would have understood that the samples had been obtained by removing an aliquot or aliquots from Satkunanathan's purified preparation. EX1005, 930-31; EX1003, ¶608.

Shytuhina discloses that they prepared VLP samples from mammalian and insect cells. EX1006, 193; EX1003, ¶609.

Shytuhina further discloses that after these purifications, "[s]amples containing CHIKV VLPs" were analyzed on the initial RP-HPLC column, before being subjected to LC-MS. EX1005, 193; EX1003, ¶610.

A POSA would have understood that obtaining these "samples" for analysis would have involved removing or extracting an aliquot from the VLP preparations. EX1003, ¶611.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶607-612.

(e) "b) denaturing the AAV particles;"

See claim 1(a). EX1003, ¶¶613-617.

(f) "c) subjecting the denatured AAV particles to liquid chromatography/mass spectrometry (LC/MS) intact protein analysis;

See claim 1(b). EX1003, ¶¶618-621.

(g) "d) determining the masses of one or more VPs of the AAV particles; and "

See claim 1(c). EX1003, ¶¶622-625.

(h) "e) comparing the determined masses of the one or more VPs to theoretical masses of corresponding VPs, wherein the theoretical masses of corresponding VPs are those VPs of known AAV serotypes and/or those that have not undergone undesired post-translational

modifications; and"

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, ¶627.

Using intact LC-MS, Shytuhina determined the masses of, and separated and characterized different post-translationally modified forms of viral structural proteins E1 and E2. EX1006, 194-95, Fig. 2; EX1003, ¶628.

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." EX1003, 194; EX1003, ¶629.

Satkunanathan, in combination with Shytuhina, thus meets this limitation. *Id.*, ¶¶626-630.

(i) "f) determining if there is any deviation of the determined masses of the one or more VPs from the theoretical masses of the corresponding VPs;"

See claim 1(d). EX1003, ¶¶631-635.

(j) "wherein the determination of any deviation of the determined masses of the one or more VPs from the theoretical masses of corresponding VPs thereby monitors the AAV particles for consistency and/or identity;"

See claim 20 preamble. EX1003, ¶¶637-642. A POSA would have further understood that applying Shytuhina's method, as discussed above, for monitoring product degradation, monitors the viral particles for consistency and identity. EX1006, 197; EX1003, ¶643.

Moreover, Shytuhina states expressly that it is important to monitor PTMs "to ensure product lot-to-lot consistency." EX1006, 197; EX1003, ¶644.

Shytuhina expressly discloses, as discussed above, that they compared the masses they determined through intact LC-MS with known theoretical masses for the E1 and E2 viral proteins, and, through that comparison, identified PTMs. EX1006, 194; EX1003, ¶645.

As discussed above, a POSA at the time would have applied Shytuhina's method to process and formulation development of AAV vectors for gene therapy. EX1003, ¶646.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶636-647.

(k) "wherein the monitoring for consistency and/or identity is performed in the absence of a gel separation step; and"

See element (e) of claim 1. EX1003, ¶¶648-660.

(l) "wherein if less than an undesirable amount of deviation is determined during the monitoring for consistency and/or identity, the AAV particles are

combined with one or more pharmaceutically acceptable excipients to form the pharmaceutical composition."

See claim 20 preamble. EX1003, ¶¶662-667. Moreover, Shytuhina states expressly that it is important to monitor PTMs "to ensure product lot-to-lot consistency." EX1005, 197; EX1003, ¶668.

A POSA would have understood that each lot of viral particles monitored in this fashion would be evaluated to determine whether the purity was sufficient to use the viral particle preparation as part of the pharmaceutical preparation. EX1003, ¶669.

A POSA would further have understood that this process of monitoring PTMs to ensure product lot-to-lot consistency would involve evaluating the amount of deviation from the theoretical masses of the viral proteins to determine whether the amount of deviation was less than a particular threshold deemed undesirable. EX1003, ¶670.

A POSA would further have understood that if the amount of deviation from the theoretical viral protein masses was below this threshold, then the preparation of viral particles would continue to move through the process to be combined with pharmaceutically acceptable excipients to form the pharmaceutical composition. EX1003, ¶671.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶661-672.

21. Claim 21: "The method of claim 20, wherein the monitoring of the AAV particles for consistency and/or identity includes determining the serotype of the AAV particles based on the comparison of the determined masses of the VPs to the theoretical masses of the corresponding VPs."

See claim 20. Using LC-MS/MS, Satkunanathan identified capsid proteins from each of the three serotypes tested, AAV2, AAV5, and AAV8. EX1005, Supplementary Table S1; EX1003, ¶674. Satkunanathan, moreover, taught the importance of differences among the serotypes for development of therapies based on AAV vectors. EX1005, 930 ("We reason that by analyzing host cellular proteins coproduced and copurified with AAV vectors, we may identify differences in protein composition among the three AAV serotypes AAV2, AAV5, and AAV8; better understand the role of cellular proteins in AAV assembly; and ultimately improve AAV vector production"); EX1003, ¶674.

Shytuhina discloses LC-MS intact protein analysis of viral structural proteins. EX1006, 193-94; EX1003, ¶675. Using intact LC-MS, Shytuhina separated and characterized different post-translationally modified forms of viral structural proteins E1 and E2. EX1005, 194-95, Fig. 2; EX1003, ¶675.

Using intact LC-MS, for example, Shytuhina separated and characterized two different PTMs for the E1 structural protein. EX1005, 194-95, Fig. 2A; EX1003, ¶676.

A POSA would further have understood, particularly in light of Shytuhina's identification and separation of glycosylated but deacylated E1 from glycosylated and acylated E1, that Shytuhina's intact LC-MS method had sufficiently high resolution to characterize AAV capsid proteins from at least some different serotypes, such as those studied in Satkunanathan (AAV2, AAV5, and AAV8). EX1003, ¶677.

A POSA would further have understood that numerous AAV serotypes had been discovered, with varying degrees of homology to known serotypes and to one another. *See, e.g.*, EX1017, Abstract, 14783-87, Fig. 2, Fig. 3, Fig. 4; EX1003, ¶678. A POSA would have been motivated to use Shytuhina's intact LC-MS method to monitor AAV composition for process development, including characterizing different AAV serotypes. EX1003, ¶678.

Satkunanathan, in combination with Shytuhina, therefore meets this additional limitation. *Id.*, ¶¶673-679.

22. Claim 22: "The method of claim 20, wherein a determination of any actual deviation in masses reflects heterogeneity in the AAV particle preparation."

See claim 20. A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation in vivo. EX1011, 7920; EX1003, ¶681.

A POSA at the time would have further understood that PTMs vary from protein molecule to protein molecule. EX1006, 194-95, Fig. 2; EX1003, ¶682. Shytuhina discloses that, using intact LC-MS, they characterized different glycosylation and acylation modifications for each of the viral structural proteins they studied. EX1006, 194-95, Fig. 2; EX1003, ¶682.

A POSA would therefore have understood that Shytuhina's intact LC-MS method, which identified heterogeneous populations of viral structural proteins, could be applied to identify heterogeneous populations of the capsid proteins Satkunanathan studied, some of which likely carried PTMs. EX1003, ¶683. A POSA would further have understood that different PTMs in the capsid proteins of each different AAV serotype would have led to heterogeneity within that population of particles. EX1003, ¶683.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶680-684.

23. Claim 23: "The method of claim 22, wherein the heterogeneity in the AAV particle preparation is due to mixed AAV capsid serotypes, variant AAV capsid proteins, AAV capsid protein amino acid substitutions, truncated AAV capsid proteins or modified capsid proteins."

See claim 22. A POSA would have understood that it had been known since the 1980s that AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶686. Before the priority date, researchers had also used MS techniques to study mutant viral proteins in proteomics studies. EX1007, Abstract, 2509; EX1003, ¶202.

A POSA at the time would have further understood that PTMs vary from protein molecule to protein molecule. EX1006, 194-95, Fig. 2; EX1003, ¶687. Shytuhina discloses that, using intact LC-MS, they characterized different glycosylation and acylation modifications for each of the viral structural proteins they studied. EX1006, 194-95, Fig. 2; EX1003, ¶687.

A POSA would therefore have understood that Shytuhina's intact LC-MS method, which identified heterogeneous populations of viral structural proteins, could be applied to identify heterogeneous populations of the capsid proteins Satkunanathan studied, some of which likely carried PTMs. EX1003, ¶688. A POSA would further have understood that different PTMs in the capsid proteins of each different AAV serotype would have led to heterogeneity within that population of particles. EX1003, ¶688.

Therefore, the combination of Satkunanathan and Shytuhina meets this limitation. *Id.*, ¶¶685-689.

24. Claim 24: "The method of claim 21, wherein the undesired

post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation and ubiquitination."

See claims 2, 21. EX1003, ¶¶690-694.

25. Claim 25: "The method of claim 21, wherein the AAV particles are denatured using detergent, heat, high salt or buffer with low or high pHs."

See claims 4, 21. EX1003, ¶¶695-698.

26. Claim 26: "The method of claim 21, wherein the liquid chromatography is reverse phase chromatography."

See claims 5, 21. EX1003, ¶¶699-702.

27. Claim 27: "The method of claim 26, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claims 6, 26. EX1003, ¶¶703-710.

28. 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. EX1003, ¶711. The techniques required to make the claimed combination, namely, RP-HPLC, intact LC-MS, intact LC-MS/MS, and application of software to deconvolute and interpret MS data, were well known to people of ordinary 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 identifying several different post-translational modifications. *Id.*, ¶712.

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.*, ¶713.

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.*, ¶714.

Moreover, a POSA would have a reasonable chance of success in separating AAV capsid proteins and characterizing them, including any PTMs, by intact LC-MS. *Id.*, ¶715.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶716.

Moreover, researchers in the field for years before the relevant date had been successfully separating reduced monoclonal antibodies and identifying PTMs using intact LC-MS. *Id.*, ¶717. 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.*

Researchers in the field for years before the relevant date had also been using intact LC-MS/MS to characterize PTMs such as deamidation, obtaining sequencing information as part of the analysis. *Id.*, ¶718.

29. 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 the Asserted Claims. *Id.*, ¶719. Petitioner is not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '313 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.* Similarly,

[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 '313 patent. There is no nexus between the commercial success of Elevidys® and the analytical method recited in the challenged claims.

Petitioner is not aware of any licenses directed specifically to the ’313 patent or the subject matter recited in challenged claims 1-27. *Id.*

Finally, Petitioner is not aware of any unexpected results having a nexus to the claimed subject matter. *Id.*, ¶720. The ’313 patent does not disclose unexpected properties of the claimed formulation. *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 characterizing 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 1-27 of the ’313 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. *Id.*, ¶721. Thus, secondary considerations do not alter the conclusion that claims 1-27 of the ’313 patent are obvious over the combination of Satkunanathan and Shytuhina. *Id.*

B. Ground 2: Claims 6, 19, and 27 Are Obvious Over Satkunanathan, Shytuhina, and Yuan

Dependent claims 6, 19, and 27 are also obvious over the combination of Satkunanathan, Shytuhina, and Yuan. EX1003, ¶¶722-23. Petitioner incorporates its discussion of Ground 1 in its entirety herein. *Id.*, ¶723.

1. Claim 6: "The method of claim 5, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claim 5 of Ground 1. 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 were known to be subject to post-translational modification. EX1003, ¶724.

Shytuhina carried out an initial RP-HPLC step, which separated and denatured the proteins, and then carried out a second RP-HPLC step, coupled to the mass spectrometer – this second step subjected the denatured viral structural proteins to LC-MS intact protein analysis. *Id.*, ¶725.

Shytuhina discloses that the specific RP-HPLC column they used for the first RP-HPLC step was a C4 column. EX1006, 193; EX1003, ¶726. Although Shytuhina does not expressly disclose the column used for the second RP-HPLC, coupled to the mass spectrometer, 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, ¶726.

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, ¶727.

Yuan discloses a RP-HPLC method to detect a capsid protein of HPV, the L1 protein. EX1037, Abstract; EX1003, ¶728. 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; EX1003, ¶728.

Satkunanathan used a reversed phase C18 column for LC-MS/MS. EX1005, 931; EX1003, ¶729.

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. EX1003, ¶730. 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, in combination with Yuan's method of testing columns such as C4 and C8, to optimize the LC-MS separation and protein identification. *Id.*

Satkunanathan, in combination with Shytuhina and Yuan, therefore meets the additional limitation of dependent claim 6. EX1003, ¶¶724-731.

2. Claim 19: "The method of claim 18, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claim 18 of Ground 1, claim 6. EX1003, ¶¶732-740.

3. Claim 27: "The method of claim 26, wherein the reverse phase chromatography is C8 reverse phase chromatography."

See claims 26 of Ground 1, claim 6. EX1003, ¶¶741-749.

4. 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 and Shytuhina with Yuan. EX1003, ¶750. The techniques required to make the claimed combination, namely, RP-HPLC, intact LC-MS, and application of software 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.*

In particular, optimizing RP-HPLC for a particular separation would have required only routine experimentation. *Id.*, ¶751. As Yuan demonstrates, researchers at the time routinely tested columns such as C4 and C8 columns, including for separation of viral structural proteins, to determine which column separated most effectively and efficiently. *Id.*

Yuan successfully used both C4 and C8 columns to detect the L1 capsid protein of HPV. Yuan expressly found that either the C4 or C8 column could be used with equal efficiency of separation. *Id.*, ¶752.

Shytuhina successfully separated glycoproteins by liquid chromatography, specifically RP-HPLC, and analyzed them by intact LC-MS, successfully identifying several different post-translational modifications. *Id.*, ¶753.

Intact LC-MS analysis of AAV capsid proteins would have been even more straightforward for a POSA than working with the glycoproteins Shytuhina analyzed. *Id.*, ¶754. Glycoproteins are more variable and therefore more difficult to analyze than AAV capsid proteins by intact LC-MS. *Id.*

A POSA therefore, 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.*, ¶755.

A POSA would have had a reasonable chance of success that a C8 column would have successfully separated the AAV capsid proteins. *Id.*, ¶756. The HPV L1 capsid protein is approximately the same size as the AAV capsid proteins (55 kDa), further demonstrating that a POSA would have had a reasonable chance of success in separating the AAV capsid proteins on a C8 column. *Id.*

Moreover, a POSA would have a reasonable chance of success in separating AAV capsid proteins and identifying them, including any PTMs, by intact LC-MS. *Id.*, ¶757.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶758.

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.*, ¶759. 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.*

5. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner incorporates herein in its entirety the discussion of secondary considerations from Ground 1. *See also* EX1003, ¶¶760-763.

C. Ground 3: Claims 7-10 Are Obvious Over Satkunanathan, Shytuhina, and Zabrouskov

Dependent claims 7, 8, 9, and 10 are also obvious over the combination of Satkunanathan, Shytuhina, and Zabrouskov. EX1003, ¶764. Petitioner herein incorporates Ground 1 in its entirety. *Id.*

1. Claim 7: "The method of claim 1, further comprising

determining the sequence of one or more VPs that has undergone post-translational modifications."

See claim 1 of Ground 1. 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. EX1003, ¶765.

A POSA would further have understood that Satkunanathan's LC-MS/MS method determined the sequences of the fragments in the mixtures of protein fragments analyzed. *Id*., ¶766. For example, using LC-MS/MS, Satkunanathan identified AAV capsid proteins from three different serotypes by matching the sequence of the proteins as determined by MS/MS against a database, specifically protein FASTA databases. EX1005, 931, Supplementary Table S1; EX1003, ¶766.

Given that Satkunanathan's settings included the PTM methionine oxidation as a dynamic modification, and that Dong had determined that AAV capsid proteins undergo this PTM, Satkunanathan's analysis likely included the sequence of some capsid proteins containing this PTM. EX1005, 931; EX1022, Abstract, 2; EX1003, ¶767. As a POSA would have understood, it had been known since the 1980s that

AAV capsid proteins undergo N-terminal truncation and acetylation *in vivo*. EX1011, 7920; EX1003, ¶767.

Relying on Zabrouskov, a POSA would have understood to combine Shytuhina's intact LC-MS method with Satkunanathan's LC-MS/MS method, to determine the sequences of the AAV capsid proteins, vp1, vp2, vp3, and further characterize their post-translational modifications. EX1038, Abstract; EX1003, ¶768.

Zabrouskov used MS/MS of intact proteins ("top-down MS/MS") to analyze deamidation of RNase A. EX1038, Abstract; EX1003, ¶769. Zabrouskov identified multiple deamidation sites, at asparagine and glutamine, on RNase A. EX1038, Abstract; EX1003, ¶769.

As part of the MS/MS analysis, Zabrouskov determined the sequences of the fragments generated from the intact RNase A proteins during MS/MS analysis. EX1038, Figures 2-4, pp. 989-90; EX1003, ¶770.

The combination of Satkunanathan and Shytuhina with Zabrouskov discloses this limitation. EX1003, ¶¶765-771.

2. Claim 8: "The method of claim 7, wherein the post-translational modifications are selected from the group consisting of acetylation, deacetylation, deamidation, glycosylation, truncation, and ubiquitination."

See claim 7. EX1003, ¶¶772-782. A POSA would further have understood Zabrouskov's teaching that deamidation was a particularly challenging post-translational modification to characterize, given "the special challenge" that "its covalent $–NH_2 \rightarrow –OH$ modification causes an only 0.984 Da mass increase, closely matching the 1.002 Da spacing of the molecular ion isotope peaks." EX1038, 987; EX1003, ¶783.

A POSA would therefore have understood that the known post-translational modifications of AAV capsid proteins, N-terminal truncation and acetylation, would have been more easily characterized by intact LC-MS/MS than the deamidation modifications characterized by Zabrouskov. EX1003, ¶784.

Satkunanathan, in combination with Shytuhina and Zabrouskov, therefore meets this limitation. EX1003, ¶¶772-785.

3. Claim 9: "The method of claim 8, wherein the post-translational modification is N-terminal acetylation."

See claim 8. EX1003, ¶¶786-798.

4. Claim 10: "The method of claim 7, wherein the sequences of VP1, VP2 and VP3 are determined."

See claim 7. EX1003, ¶¶799-805.

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 Zabrouskov. EX1003, ¶806. The techniques

required to make the claimed combination, namely, RP-HPLC, intact LC-MS, intact LC-MS/MS, and application of software to deconvolute and interpret MS data, were well known to people of ordinary 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 identifying several different post-translational modifications. *Id.*, ¶807.

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.*, ¶808.

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.*, ¶809.

Moreover, a POSA would have a reasonable chance of success in separating AAV capsid proteins and characterizing them, including any PTMs, by intact LC-MS. *Id.*, ¶810.

Satkunanathan successfully identified and distinguished three different AAV serotypes, and Shytuhina successfully separated and characterized viral structural glycoproteins with multiple different PTMs. *Id.*, ¶811.

Moreover, researchers in the field for years before the relevant date had been successfully separating reduced monoclonal antibodies and identifying PTMs using intact LC-MS. *Id.*, ¶812. 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.*

A POSA would also have had a reasonable expectation of success in further combining Satkunanathan with Shytuhina and Zabrouskov to carry out intact LC-MS/MS to provide further characterization of post-translational modifications of AAV capsid proteins by obtaining the sequences of the modified capsid proteins through tandem MS. *Id.*, ¶813. As demonstrated by Zabrouskov, researchers in the field for years before the earliest possible priority date for the ’313 patent had been using intact LC-MS/MS to characterize post-translational modifications of proteins through obtaining the masses and sequences of the modified proteins. *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. *See also* EX1003, ¶¶814-817.

IX. CONCLUSION

Sarepta respectfully requests institution of IPR for claims 1-27 of the ’313 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 13,064. 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.

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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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