

Filed on behalf of: Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

v.

GENZYME CORPORATION,
Patent Owner.

Case No. IPR2023-00608
U.S. Patent No. 9,051,542

DECLARATION OF MANSOOR M. AMIJI, R.PH., PH.D.

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I. INTRODUCTION

1. I have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP on behalf of Petitioners Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation as an independent expert consultant in the above-referenced *inter partes* review (“IPR”) proceeding to provide information and opinions on the teachings of the prior art and the state of the art, as relevant to the issued claims of U.S. Patent No. 9,051,542 (“the ’542 patent”) (Ex. 1001). It is my understanding that the ’542 patent identifies Genzyme Corporation as its assignee, and I have been informed by counsel that this is consistent with their understanding of recorded assignments for this patent. I further understand that Genzyme is a wholly owned subsidiary of Aventis, Inc.

II. QUALIFICATIONS

2. I am an expert in the field of pharmaceutical sciences and drug formulation development and characterization. Specifically, I specialize in drug formulation development and targeted delivery of therapeutics and have extensive experience with formulations and prevention of aggregation of colloidal systems, including protein and peptide drug products, which generally apply the same scientific principles as virus formulations. I have been an expert in this field since prior to June 2004. I have relied upon my training, knowledge, and experience in the relevant art to form my opinions.

3. In 1988, I graduated with honors from Northeastern University and received a Bachelor of Science degree in Pharmacy and became a Registered Pharmacist in Massachusetts. In 1992, I received a Ph.D. in Pharmaceutical Science/Pharmaceutics from the School of Pharmacy and Pharmaceutical Sciences at Purdue University, under the supervision of Professor Kinam Park. My dissertation focused on biomaterials and water-soluble polymers. During my graduate studies at Purdue University, I took several pharmaceutics courses and had hands-on training in pharmaceutical formulations.

4. After receiving my Ph.D. in 1992, I worked as a Senior Research Scientist for Columbia Research Laboratories (CRL) in Madison, Wisconsin. At CRL, I worked on polymeric delivery systems for various types of therapeutic agents.

5. I am currently a University Distinguished Professor and Professor of Pharmaceutical Sciences in the School of Pharmacy, Bouve College of Health Sciences at Northeastern University in Boston, Massachusetts. I am also jointly appointed as a Professor of Chemical Engineering in the College of Engineering at Northeastern University. I am also currently an Affiliate Faculty Member in the Department of Biomedical Engineering at Northeastern University. I have taught and carried out research in pharmaceutical sciences at Northeastern University since 1993, and from 2010 to 2016, I served as the Chairman of the Department of

Pharmaceutical Sciences. In 2000, I was a Visiting Research Scholar in the Department of Chemical Engineering at the Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts, in the laboratory of Professor Robert Langer.

6. As a tenured faculty member at Northeastern University, I have over 27 years of experience in teaching drug formulations to both graduate and undergraduate students. In theory and laboratory courses that I have taught and continue to teach, I extensively cover the manufacturing, composition, and pharmacokinetics of pharmaceutical formulations and delivery systems. I also serve as a consultant to several pharmaceutical, biotechnology, and medical device companies regarding product development and drug delivery.

7. I have published extensively over the course of my career and am ranked as a Thompson-Reuters Highly Cited (top 1%) author in Pharmacology and Toxicology. I have coauthored over 70 book chapters and more than 400 peer reviewed scientific articles. The topics of these materials include the design and development of pharmaceutical dosage forms, pharmacokinetics, drug metabolism, dose delivery and controlled release systems, and the use/formulation of related excipients and methods. I have been involved in and consulted on multiple projects over the years, both in industry and academia, about the aforementioned topics. To that end, I have taught courses in pharmaceutics; drug design, evaluation, and development; dosage forms; and pharmacokinetics.

8. I have served as a grant reviewer for the National Institutes of Health, the Department of Defense, the United States Department of Agriculture, and the American Chemical Society. I am a member of several professional and industrial societies, including the American Association of Pharmaceutical Sciences (AAPS) and the Controlled Release Society (CRS), and have participated as a reviewer for more than 50 scientific journals. I have also received a number of professional awards and honors, including the Nano Science and Technology Institute (NSTI) Fellowship Award for Outstanding Contributions towards the Advancement in Nanotechnology, Microtechnology, and Biotechnology in 2006; a Fellowship and Meritorious Manuscript Award from the AAPS in 2007; the Tsuneji Nagai Award from the CRS in 2012; the Northeastern University School of Pharmacy Distinguished Alumni Award in 2016; and Purdue University College of Pharmacy Distinguished Alumni Award in 2019. Over the course of my career, I have advised numerous post-doctoral associates, doctoral students, master's students, visiting scientists, and research fellows.

9. I am also the inventor or co-inventor on a number of issued patents and pending patent applications, including nine issued within the United States.

10. I lecture extensively on various topics on the leading edge of modern pharmaceutical sciences, and I regularly attend numerous worldwide pharmaceutical conferences. I have submitted over 400 papers to conferences and journals in the

field of pharmaceutical sciences. I have been an invited speaker at national and international scientific conferences.

11. I am a founder of and scientific advisor to many pharmaceutical companies, including Nemucore Medical Innovations and Targagenix, Inc., which have licensed my patents on lipid-based drug delivery systems and are in the process of developing a commercial product.

12. In addition to serving as a long-term member of the AAPS Association and on the Scientific Advisory Board of the CRS, I have also served as a permanent member of the National Institutes of Health's grant review panel and many other public funding agencies in the U.S. and across the world. I am an Editor of the journal Drug Delivery and Translational Research and Associate Editor of several peer-reviewed journals and on the editorial board of about a half dozen other scientific journals.

13. Additional details concerning my background, training, and experience are contained in my current *Curriculum Vitae*, attached hereto as Exhibit A.

14. Based on my education, training, and experience, including my research expertise in pharmaceutical product development and drug formulation development of over 30 years, including in the June 2004 time frame, I believe that I am qualified to provide useful technical information regarding the subject matter of this case.

III. MATERIALS CONSIDERED

15. Exhibit B provides a list of the materials I considered in preparing this declaration. I also relied on my experience, education, and knowledge of the art.

IV. SUMMARY OF OPINIONS

16. In my opinion, challenged claims 1, 2, 5, and 6 of the '542 patent all recite well-known virus vector formulations comprising classic excipients routinely used in prior art viral compositions, with features (e.g., virus titer, ionic strength, pH, and reduced aggregation) already achieved by prior art virus formulations or would have been reasonably expected to be achieved through routine optimization. In my opinion, all of the challenged claims would have been obvious.

17. Although the '542 patent alleges that its formulations having an elevated ionic strength while maintaining acceptable osmolarity provided a new solution for aggregation-related problems in adeno-associated virus (AAV) formulations, excipients that provide elevated ionic strength while maintaining acceptable osmolarity had been used in virus formulations, and various methods for eliminating aggregation had been readily available as of June 2004.

18. First, claims 1, 2, 5, and 6 would have been obvious over Evans, Huang, and Mingozi in view of the prior common knowledge of those skilled in the art, as reflected in Wright, Clark, Gatlin, Croyle, and Liu. Evans, which relates to stable and high titer virus formulations, teaches and claims multiple limitations of claim 1,

including the recited virus titer level, pH, multivalent ions, and ionic strength. Evans further demonstrates improved stability of its adenovirus formulations and teaches that AAV can also be used in its compositions. Because those skilled in the art had achieved stable AAV formulations with virus concentrations measured in the vg/ml range recited in the challenged claim 1, using AAV in Evans's compositions at such concentrations would have been obvious and expected to have no "significant aggregation." Claim 1 is thus obvious.

19. Dependent claim 2, which additionally recites using a well-known and commercially available non-ionic surfactant, poloxamer 188 (Pluronic® F68), also would have been obvious over the reported routine use of that component in earlier virus formulations. Dependent claims 5 and 6, which further recite an average particle radius range or AAV vector particle recovery rate after passing through a 0.22 µm filter, would have been understood by a person of ordinary skill in the art ("POSA") to merely describe a natural feature of the AAV formulations of claim 1 that prevent aggregation and thus would have been obvious like claim 1. Even if claims 5 and 6 require further reduced aggregation, these claims would still have been obvious in view of known prior methods of reducing virus aggregation that had achieved no observable aggregation and improved viral formulation stability.

20. Second, claims 1, 2, 5, and 6 also would have been obvious over Frei, Huang, and Mingozi in view of the prior common knowledge of those skilled in the art, as reflected in Wright, Clark, Gatlin, Croyle, and Liu.

21. Frei provides high titer virus formulations with improved stability and demonstrates a stable high-titer adenovirus formulation (“D-1” formulation) that meets most limitations of the challenged claim 1 except the rAAV and ionic strength limitations. It would have been obvious to use rAAV in Frei’s D-1 formulation in view of Frei’s teachings and prior knowledge of AAV’s superior stability and therapeutic benefits. Achieving the recited AAV concentration in vg/ml units would also have been obvious in view of prior successes reported by Huang and Mingozi. Frei’s recommendation of using up to 10 mg/ml sodium chloride (NaCl) to improve virus particle stability would have further motivated a POSA to increase the NaCl concentration in the D-1 formulation with a reasonable expectation that the resulting viral formulation having an ionic strength over 200 mM would be stable and have no aggregation.

22. Frei also expressly teaches using Pluronic® F68 in its formulations, and thus, renders claim 2 obvious. Even if claims 5 and 6 require further reduced aggregation, those recited features would also have been routine optimization and obvious in view of Frei’s success and prior successes of eliminating aggregation from high titer virus formulations.

23. Third, the challenged claims 1, 2, 5, and 6 would also have been obvious over Liu in view of Huang, Mingozi, and the prior knowledge of those skilled in the art, as reflected in Wright, Clark, Croyle, and Gatlin.

24. Liu focuses on virus production and purification and storage of purified high yield virus with improved stability. Liu describes an adenovirus composition in Example 17 that meets all limitations of claim 1 except the recited rAAV vector particles at the recited concentration in vg/ml. It would have been obvious to use rAAV in Liu's Example 17 composition in view of Liu's express teachings and prior knowledge of AAV's superior stability and therapeutic benefits. Increasing the virus concentration in Liu's compositions to the recited vg/ml level would have been obvious in view of Huang and Mingozi's teachings of stable AAV compositions having 10^{13} to 10^{14} vg/ml virus particles and prior knowledge reflected in Wright, Clark, Croyle, and Gatlin. Because Liu reported no sign of aggregation of its stable compositions in Example 17 and because AAV was known to be more stable than adenovirus, using rAAV at an increased concentration in Liu's Example 17 compositions at the recited concentration would have been expected to have no "significant aggregation" as recited in claim 1. To the extent the obvious variants of Liu's composition do not prevent aggregation, minimizing any residual aggregation would have required only routine optimization and would have been obvious.

25. Claim 2 would also have been obvious over Liu in view of its teaching of using Pluronic F68 in preferred embodiments. Even if claims 5 and 6 require further reduced aggregation, those recited features would also have been achieved through routine optimization and were obvious in view of Liu's success and prior successes of other references in eliminating aggregation from high titer virus formulations.

26. Fourth, the challenged claims 1, 2, 5, and 6 also would have been obvious over Lochrie in view of Huang, Mingozi, Johnson, and Liu and the prior knowledge of those skilled in the art, as reflected in Wright, Clark, Croyle, and Gatlin.

27. Lochrie describes methods to achieve purified and stable stocks of rAAV virions. Lochrie's rAAV formulation in Example 2 contains AAV at a concentration almost reaching claim 1's recited vg/ml level, with a total ionic strength almost reaching claim 1's recited level, and at a pH almost reaching claim 1's recited pH range. Increasing the rAAV concentration in Lochrie's Example 2 composition to the recited level of exceeding 1×10^{13} vg/ml also would have been obvious in view of Huang and Mingozi's teachings of stable AAV compositions having 10^{13} to 10^{14} vg/ml and prior knowledge reflected in Wright, Clark, Croyle, and Gatlin. Johnson's achievement of stable purified rAAV formulations with no aggregation at pH 7.5 would have rendered the recited pH limitation obvious. It also

would have been obvious to increase the ionic strength of obvious variants of Lochrie's Example 2 composition to above 200 mM in view of Liu's teachings and success. In view of prior successes of eliminating aggregation from high titer virus formulations, a POSA would not expect the resulting obvious variants of Lochrie Example 2 formulation to have any "significant aggregation." To the extent the obvious variants of Lochrie's composition do not prevent aggregation, minimizing any residual aggregation would have required only routine optimization and would have been obvious.

28. Claim 2 would have been obvious in view of the previously reported routine uses of Pluronic® F68 in AAV compositions. Claims 5 and 6, which essentially require the rAAV vector particles to be in monomeric and non-aggregated form, would also have been obvious over Lochrie, Huang, Mingozzi, Johnson, and Liu in view of the prior knowledge and successes of eliminating aggregation from virus formulations.

29. In sum, in my opinion, the challenged claims of the '542 patent merely repackaged well-known elements of prior art viral formulations without making any surprising contribution to the field. All of the challenged claims would have been obvious and unpatentable.

V. BACKGROUND AND STATE OF THE ART

A. Adeno-Associated Viral Vectors Were Known as “Convenient,” “Promising,” and “Attractive” Gene Therapy Delivery Systems

30. Adeno-associated virus (AAV) is a replication-deficient member of the parvovirus family, and contains a protein shell surrounding and protecting a small, single-stranded DNA genome of approximately 4.7 kilobases (kb). Ex.1006, 10497. There are six serotypes of AAV described in the art, among which the serotype AAV-2 had been most extensively studied by June 2004. *Id.* As early as 1982, researchers had isolated a molecular clone for AAV-2, which resulted in the development of the first AAV-based vector for use in gene therapy. Ex.1007, 174. AAV vectors are generated by deleting the viral rep and cap genes of the wild-type virus, and replacing them with control elements, making AAV vectors free from all viral genes. Ex.1006, 10497. Recombinant AAV (rAAV) does not contain viral coding sequences and, thus, can serve as a protein-based nanoparticles engineered to safely traffic and deliver its DNA cargo into the nucleus of a cell. *Id.*; Ex.1007, 174.

31. Years before June 2004, AAV had been widely known as “a promising vector for human gene transfer” and had “received considerable attention in the field of gene therapy, because of [its] ability to mediate long-term gene transfer in the absence of significant toxicity.” Ex.1007, 174. AAV’s other well-known advantageous characteristics for use as a gene transfer vector include its ability to

“infect both dividing and non-dividing cells and establish a latent state with high frequency.” *Id.*; Ex.1010, 2:27-28 (“AAV can infect a wide range of host cells, including non-dividing cells.”). AAV vectors were also known to be less immunogenic than other viral vectors, such as vectors based on adenoviruses, “a factor which may contribute to enhanced duration of therapeutic gene expression in vivo.” Ex.1007, 174. Other well-known desired attributes of AAV vectors include “high affinity for the target tissue, suitable promoters to maximize gene expression, proper regulatory elements to synchronize transcription in response to biochemical stimuli and the ability to accommodate the desired transgene of interest.” Ex.1013, 1281.

32. AAV vectors had been shown in a number of investigative studies to transfer genes to nondividing target cells and mediate long-term gene transduction in a variety of tissues, including lung, liver, muscle, central nervous system, and eye. Ex.1006, 10497; Ex. 1007, 174; Ex.1009, [0002]; Ex.1012, S-9 (similarly acknowledging “the ability of [AAV vectors] to mediate long-term, robust in vivo gene expression in numerous nondividing cell types (i.e., hepatocytes, neurons, and skeletal myocytes”); Ex.1005, S286 (“AAV vectors lead to efficient gene transfer in the liver”). By June 2004, AAV vectors became one of the most actively investigated gene therapy vehicles. Ex. 1006, 10497; Ex.1012, Abstract.

33. As of June 2004, AAV vectors capable of expressing therapeutic gene products had been successfully formulated and “shown significant promise for human gene therapy.” Ex.1007, 174 (repeatedly emphasizing that AAV-based vectors were “particularly promising for human gene transfer”). It was also well known that “because AAV and adenovirus are both non-enveloped viruses developed as gene transfer vectors, studies on the latter can provide guidance for AAV vector formulation development.” *Id.* Indeed, some researchers had initiated side-by-side formulation studies with both adenovirus and AAV, due to AAV’s “increasing[] popular[ity] for clinical applications.” Ex.1013, 1281-82.

34. AAV was known to be “stable at a wide range of physical and chemical conditions, which lends itself to production, storage, and transportation requirements.” EX. 1010, 3:1-2. It was also reported to be “significantly more stable than the adenovirus.” Ex. 1013, 1283. Thus, those skilled in the art would have understood that stable formulations of adenovirus should be applicable to the inherently more stable AAV vectors. Ex. 1003, 3:10-14 (teaching that the formulations disclosed therein “which show enhanced storage stability include but are not limited to adenovirus, adeno-associated virus . . .”) ; Ex.1004, 8:18-28 (teaching that “[a] wide range of viruses can be used in the compositions of the present invention” with improved stability, including “adeno-associated virus”); Ex.1009, [0008] (teaching that for its viral vector particle compositions, “[s]uitable

viral vector particles include, for example, . . . adeno-associated viral vector particles (AAV vector particles)").

B. High Titer AAV Compositions Had been Desired and Achieved by Known Production and Purification Techniques

35. Whereas the production of viral vectors from transfected cells can be measured by particles/cell or particle units (PU)/cell, the titer or yield of viral vector formulations can also be measured in vector genomes (vg)/ml, genome copies (gc)/ml, virus particles (vp)/ml, or capsid particles (cp)/ml. *E.g.*, Ex. 1012, S-9; Ex.1009, [00126]; Ex.1005, S286; Ex.1003, claim 2; Ex.1007, 175.

36. Among these titer or yield measurements, vector genomes (vg)/ml and genome copies (gc)/ml have been used by researchers interchangeably, since both represent the measured copies of functional vectors containing the to-be-transferred gene. By comparison, capsid particles or virus particles per ml takes into account all capsid proteins, including capsids containing the full viral vector and empty capsids that are viral particles that are incomplete, damaged, or lacking genetic material. Ex.1009, [00281].

37. It was also known that "[t]hese empty capsids, whose size and surface characteristics are similar to that of genome-containing vector particles," may contribute to the undesired aggregation. Ex.1007, 175; *see also* '542 patent (Ex. 1001), 1:60-64 (noting "excess empty capsids are co-purified [by column chromatography techniques] and contribute to particle concentration," thereby

lowering “[t]he effective vector concentration limit”). Thus, while vector genomes (vg)/ml or genome copies (gc)/ml more accurately reflect the true copies of therapeutically useful viral vectors, both categories of measurements (vg or gc/ml and vp or cp/ml) are equivalent for assessing concentration-induced virus aggregation.

38. Empty capsids are undesired because they “do[] not efficiently function to transfer the gene of interest into the host cell.” Ex.1010, 11:27-28. Thus, before June 2004, those skilled in the art had routinely controlled the empty capsid percentages to low levels. For example, Liu teaches that “[m]ost preferably, less than about 10% of the adenoviral vector particles in the composition are empty capsids. Ideally, less than about 5% of the adenoviral vector particles in the composition are empty capsids. Optimally, practically 0% of the adenoviral vector particles in the composition are empty capsids (e.g., no empty capsids are detectable).” Ex.1009, [00281]. Lochrie also teaches “efficient and commercially available methods” of removing empty capsids from rAAV composition stocks “with minimal loss to packaged capsids contained therein.” Ex.1010, 5:2-7. Lochrie further teaches that after rAAV compositions are purified by its methods, “even more preferably about 5 % to about 1 % or less of the resulting stock comprises empty capsids.” *Id.*, 7:1-3. Other researchers also routinely separated full (genome-containing) AAV vector particles from the lighter empty capsids by density-based purification methods, such

as cesium chloride or iodixanol gradient ultracentrifugation. Ex.1007, 175; Ex.1006, 10497.

39. As of June 2004, it was well known that high productivity of AAV vectors and high AAV vector yield in virus formulations would be required for clinical uses. For instance, Mingozi acknowledges that an average dose of 10^{12} vg/kg of body weight were required to achieve expression of a therapeutic transgene in dogs, which corresponds to a dose of approximately 3.2×10^{13} vg for a 60 kg human.¹ Ex.1006, 10498; Ex.1017 (citing Ex.1016). Mingozi further shows successful expression of the tested therapeutic transgene in mice using rAAV formulations at doses of 10^{11} or 2.5×10^{11} vg/vector, which correspond to doses of

¹ Because the average weight of humans is 60 kg, an average 10^{12} vg dose in dogs can be converted based on an approximate surface area ratio of dog versus human of 0.54 to approximately 10^{12} vg/kg $\times$ 0.54 $\times$ 60 kg $\approx$ 3.2×10^{13} vg.

approximately 1.9×10^{13} vg and 4.8×10^{13} vg, respectively, for a 60 kg human.² Ex.1006, 10498; Ex.1017 (citing Ex.1016). Thus, there had been a strong desire in the art to achieve a high titer of virus in AAV vector formulations to maximize the vector doses and gene transfer efficiency.

40. It was well known by June 2004 that viral vector formulations are typically administered parenterally, i.e., non-orally, to patients for gene therapy. Ex.1003, 20:16-18 (“The preferred methods of delivery of the recombinant viruses described herein are intramuscular injection and needle-free injection. An especially preferred method is intramuscular delivery”), 7:1-8 (emphasizing that the stable virus formulations are compatible with “parenteral administration”); *see also* Ex.1006, 10497 (describing injection into the splenic capsule “for efficient delivery to the portal circulation” or into the tail vein of mice); Ex.1007, 176-178 (mentioning “vascular delivery,” “intraocular injection,” and “intramuscular injection”).

² My conversion is based on the known average lab mouse weight of approximately 25 g. Thus, a 10^{11} vg dose in mice can be converted to 4×10^{12} vg/kg in mice. A corresponding human dose can be calculated based on an approximate surface area ratio of mouse v. human of 0.08 as 4×10^{12} vg/kg $\times$ 0.08 $\times$ 60 kg $\approx$ 1.9×10^{13} vg. A 2.5×10^{11} vg dose in mice can be converted to 10^{13} vg/kg in mice. A corresponding human dose can be calculated as 10^{13} vg/kg $\times$ 0.08 $\times$ 60 kg $\approx$ 4.8×10^{13} vg.

41. Before June 2004, it was known that “[t]o achieve high levels of gene transfer and ensure the safety of AAV vector administration, it is desirable to **deliver high doses of vector in small volumes.**” Ex.1005, S286 (emphasis added). Indeed, it was common sense to a formulator that injection volume is one of the most important considerations in the development of a commercial product and is selected based on the proposed injection route. Ex.1008, 405. For example, Gatlin teaches that although a product administered by an intravenous route can have a volume of approximately 10 ml, other parenteral routes require much smaller volumes (e.g., 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery). *Id.*; see also *id.* at 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections); *id.* at 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL” for intramuscular injection). Thus, those skilled in the art would have understood that it is desirable to prepare AAV virus formulations containing highly concentrated viral vectors in small volumes for effective and safe clinical use.

42. Multiple routine viral vector production and purification techniques had been developed before June 2004 and had achieved high vector productivity and high yields in virus formulations that were particularly useful in clinical applications. Ex.1009, [0003] (citing prior art methods for culturing adenovirus packaging cells, lysing such cells, and purifying adenoviral vector compositions). For instance,

advances in vector production technology had resulted in the routine isolation of $>10^4$ rAAV viral particles per cell “that should permit widespread use of this technology for clinical applications.” Ex.1012, Abstract, S-9. With advances in transient transfection production techniques and the development of stable cell lines for production of large-scale preparations, yields of $> 10^{14}$ rAAV particles per large-scale preparation had been achieved long before June 2004. *Id.*, S-10-S-12.

43. As of 2004, researchers had also routinely achieved high titers/yields of AAV in virus formulations using known production and purification methods. For instance, Liu teaches achieving high-yield production of viral vector particles of about 1×10^{15} particle units/cell, their purification, concentration, and preparation in buffers to form storage compositions. *E.g.*, Ex.1009, [0068], [00187], [00203], [00371]. Clark reports that improved and refined chromatography-based purification methods had achieved increased AAV “vector purity, biological potency, and process throughput.” Ex.1012, S12. By using such purification techniques, “[r]ecoveries were on average $>70\%$ with purity in excess of 95% .” *Id.* at S12-13. Potter likewise described an improved protocol for large-scale production of rAAV in a high ionic strength (500 mM NaCl) buffer “consisting of three sequential chromatography purification steps resulting in highly purified (99.9% pure) and infectious (particle-to-infectivity ratios less than 10) vector preparations.” Ex.1011, 429; *see also id.* at 417-419. Additionally, Mingozzi used repeated cesium chloride

(CsCl) gradient centrifugation to purify AAV-2 and AAV-5 vectors and achieved yields of “ $>10^{13}$ vg/ml.” Ex.1006, 10497.

44. Acknowledging the routine nature of these production and purification techniques, Wright taught that “AAV vectors are typically prepared at final purified concentrations in the range of 10^{11} to 10^{13} vg/ml.” Ex. 1007, 176. Wright also reported achieving stable virus formulations containing purified AAV vectors at concentrations “ $\geq 10^{14}$ capsid particles (cp)/ml.” *Id.* at 175.

45. Likewise, Evans taught that a “preferred aspect” of its invention is a virus formulation that shows enhanced stability characteristics “with a virus concentration in the range from about 1×10^7 vp/mL (virus particles/milliliter) to about 1×10^{13} vp/mL.” Ex.1003, 8:5-11. The claims of Evans further recite virus formations “with a virus concentration in the range from about 1×10^7 vp/mL to about 1×10^{13} vp/mL.” *See, e.g., id.* at claims 2, 3, 7.

46. Thus, as of June 2004, researchers had been continuously advancing AAV production and purification technologies and had achieved stable AAV vector formulations containing high titer purified AAV vectors, including titers of more than 10^{13} vg/ml.

C. Aggregation at High Virus Concentration Was a Known and Resolved Formulation Issue

47. In virus formulations, virus particles may interact with each other to form aggregates, which had been identified as “a significant issue for vector

formulation and stability” well before June 2004. Ex.1007, 175. Because aggregation involves inter-particle interactions, the nature of the viral genome packaged in the particles, the family classification of the virus, and its disease-causing properties (or lack thereof) do not impact the propensity of the particles to aggregate. Thus, empty capsids, which only differ from genome-containing virus particles in terms of what is packaged inside the particle, would have been expected to have the same propensity to aggregate as genome-containing particles.

48. The '542 patent admits that aggregation of AAV particles and increased aggregation at higher AAV vector concentrations had been a known formulation problem before June 2004. Ex.1001, 1:41-53 (“The solubility of purified AAV2 virus particles is limited, and aggregation of AAV2 particles has been described as a problem”). What the '542 patent ignores, however, was the increasing understanding among researchers of the factors contributing to aggregation prior to 2004 and the prior successful approaches that had already achieved a reduction of aggregation and improvement of virus formulation stability.

49. For instance, Huang acknowledged that “at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions” and that “[t]he size of aggregates appears to be concentration dependent.” Ex.1005, S286. Huang then developed new formulations to tackle that

problem and reported that some formulations “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” *Id.*

50. Wright similarly noted the aggregation problem with AAV vectors. Ex.1007, 175. Wright taught that viral aggregation is problematic because some target tissues “contain[] intercellular gaps of 0.1 to 1 μm that could allow the passage of non-aggregated AAV particles (diameter of 0.026 μm), but may significantly inhibit the passage of aggregated vectors.” *Id.* Wright further noted that “the variability in particle size associated with aggregation may also adversely affect *in vivo* gene transfer.” *Id.* While acknowledging that “[t]he mechanism of vector aggregation is not well understood,” Wright taught that “purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration.” *Id.* Wright further observed that freeze-thaw cycling exacerbates vector aggregation, and can lead to aggregation even at vector concentrations below 10^{14} cp/ml. *Id.* Wright taught that purification methods that do not separate full genome-containing vector particles from empty capsids could cause aggregation at lower vector concentrations because “[t]hese empty capsids, whose size and surface characteristics are similar to that of genome-containing vector particles, contribute to particle aggregation.” *Id.*

51. Wright further probed the mechanisms and contributing factors of AAV aggregation in an effort to seek solutions. Specifically, Wright explained that initial

aggregation “could be reversed by adjusting buffer pH” and that “non-ionic surfactants do not prevent or reverse aggregation induced at high vector concentrations or elevated temperatures, suggesting that hydrophobic interactions alone do not mediate the strong interparticle interactions involved in this phenomenon.” *Id.* at 176.

52. In addition, Wright acknowledged that “loss of rAAV following a 0.2- μ m filtration step correlates with the extent of vector aggregation.” *Id.* This is because AAV vector particles are about 20-30 nm in diameter. *Id.* (stating non-aggregated AAV particles have a diameter of 0.026 μ m). Thus, under appropriate filtration conditions and filter specifications, monomeric AAV can readily pass through the pores of a 0.2- μ m filter, which has a cutoff size approximately 10-fold larger than a single AAV vector particle. However, interparticle aggregates of 10 or more vector particles can no longer pass through a 0.2- μ m filter and would be lost during the filtration step. Accordingly, AAV vector compositions with large particle aggregates show substantially reduced recovery after filtration through a 0.2- μ m filter. Conversely, virus formulations with small particle aggregates lose only low percentages of AAV vectors following a 0.2- μ m filtration step.

53. Johnson further tested the impact of pH on aggregation of purified rAAV vector particles, reporting that at pH 7.2 and all lower tested pHs, the AAV particles “were aggregated and were not evenly distributed in the field but were

found in clumps,” and “[m]any aggregates were found containing thousands of particles.” Ex.1019, 585, 589. When the pH is increased to pH 7.5, “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” *Id* at 589.

54. Liu also acknowledged the problem of virus particle aggregation, teaching that “[t]he determination of the relative aggregation can be used to determine if the viral vector particle composition is suitable and/or whether the contents of the composition should be modified, e.g., by ***addition of surfactants which reduce aggregation.***” Ex.1009, [00263] (emphasis added).

55. Importantly, prior purification and formulation methods had achieved high titer AAV vector formulations with substantially reduced aggregation (i.e., small particle aggregation) and long-term stability. For instance, Wright described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are ***stable in a non-aggregated, monomeric state*** when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added). Huang likewise discussed the successful reduction of AAV vector aggregation in high-titer formulations having $5\text{-}10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. And Mingoizzi reported no issues relating to particle aggregation in its high-titer AAV preparations having concentrations of greater than 10^{13} vg/ml described therein. Ex.1006, 10497.

56. As another example, Evans focused on developing virus vector formulations for use in gene therapy having improved stability, and disclosed virus formulations that “show improved stability when stored in about the 2-8 °C range while also being compatible with parenteral administration.” Ex.1003, Abstract, 1:16-19. Evans describes recombinant virus formulations that show “enhanced stability characteristics” “with a virus concentration in the range from about 1×10^7 vp/mL (virus particles/milliliter) to about 1×10^{13} vp/mL.” *E.g., id.* at 8:5-11. Evans further demonstrates improved stability after short-term and long-term storage of its exemplary adenoviral formulations. *E.g., id.*, Examples 3 and 13. Because these formulations showed substantially improved stability when stored, a POSA would have recognized that the Evan’s formulations necessarily prevented aggregation.

57. Liu also demonstrates in working example 17 the ability of a high ionic strength composition to effectively maintain a stable population of adenoviral vector particles while being stored for 7 days during the viral vector particle production and/or purification process. Ex.1009, [00365]. In Example 17, Liu describes producing adenovirus vectors, purifying them through two rounds of TTF, and then storing the particles in a storage buffer comprising 25 mM Tris, 300 mM NaCl, 5 mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5. *Id.* Liu reports that when the resulting composition was maintained at about 4 °C for 7 days in the storage buffer, visual inspection showed “no signs of settling or precipitation over

the 7 day period.” *Id.* at [00367], [00369]. Liu further reports that “results of the HPLC PU assay performed on the samples of the sterile bag composition showed no significant decrease in particle number over the 7 day test period.” *Id.*, Table 15. Thus, Liu also achieved stable storage compositions containing purified adenoviral particles without significant loss of virus particle titer and without observed aggregation.

58. As of June 2004, the UV absorbance ratio for the wavelengths A320/A260, which is a measure of the turbidity of a dispersion, was known to “indicate the aggregation of the virus particles” in prepared adenovirus formulations. Ex.1004, 12:12-17. It was also known that “[p]urified, free [adeno]virus particles display a [A320/A260] ratio of about 0.22-0.30:1.” Ex.1015, 6:59-63.

59. Frei, which teaches developing high titer virus formulations with improved stability, had demonstrated that in four different adenovirus formulations, i.e., D-1, D-2, D-3, and S-1, having virus concentrations of $1.3\text{-}1.66 \times 10^{13}$ particles/ml, the light scattering ratios were 0.22 to 0.26, which were all within the range of monomeric virus and indicate no observed aggregation in its formulations. Ex.1004, 12:12-17; Ex.1015, 6:59-63.

60. In addition, as of June 2004, it had been routine to indicate the level of aggregation in a virus preparation by measuring the recovery rate after passing the virus formulations through a 0.2 or 0.22 μm filter. For instance, Wright reported

observing that “loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation.” Ex.1007, 175.

D. Known Formulation Techniques Had Achieved Stable Virus Compositions with Multivalent Ions, Elevated Ionic Strength, and pH of 7.5 to 8.0

1. It Had Been Routine to Use Monovalent and Multivalent Ions in Virus Formulations to Increase Ionic Strength, Maintain Osmolarity, and Achieve Improved Stability

61. The ionic strength of a solution is a measure of the concentration of ions in that solution, which can be calculated by the following well-known formula:

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2$$

62. In this formula, I is the ionic strength, c_i is the concentration of each ion (cation or anion) in molar units (mol/L), and z_i is the charge of each ion. Ex.1020, 216; *see also*, Ex.1001, 12:54-59 (describing the same formulation but replacing I with μ). In other words, ionic strength is calculated as half of the sum of the molar concentration of each ion multiplied by its valence squared.

63. For example, if a composition has 250 mM NaCl and 10 mM MgCl₂, the total ionic strength is ½ the sum of the concentration of Na⁺ in NaCl (250 mM) times the square of the charge of Na⁺ ((+1)²), the concentration of Cl⁻ in NaCl (250 mM) times the square of the charge of Cl⁻ ((-1)²), the concentration of Mg²⁺ in MgCl₂ (10 mM) times the square of the charge of Mg²⁺ ((+2)²), and the concentration of Cl⁻ in MgCl₂ (20 mM) times the square of the charge of Cl⁻((-1)²). Thus, the ionic

strength of this composition is $\frac{1}{2} \times (250 \text{ mM} \times (+1)^2 + 250 \text{ mM} \times (-1)^2 + 10 \text{ mM} \times (+2)^2 + 20 \text{ mM} \times (-1)^2) = 280 \text{ mM}$. Based on this well-accepted formula, it had been common knowledge among formulators long before June 2004 that multivalent ions make a significant contribution to the ionic strength of a formulation. Ex.1020, 216 (“The greater effectiveness of ions of higher charge on a specific property, compared with the effectiveness of the same number of singly charged ions, generally coincides with the ionic strengths calculated in Eq 8[the equation above]”).

64. Ionic strength calculations for compositions containing phosphate-based buffers require an additional step to account for the pH-dependent dissociation of the dihydrogen phosphate (H_2PO_4^-) anion. For example, sodium phosphate buffer contains disodium phosphate (Na_2HPO_4) and monosodium phosphate (NaH_2PO_4), and it was well known that the dihydrogen phosphate (H_2PO_4^-) anion, after dissociating from NaH_2PO_4 , undergoes additional dissociation to H^+ and monohydrogen phosphate anion (HPO_4^{2-}). Ex.1020, 218-19, 226. For compositions containing a sodium phosphate buffer, the concentrations of the sodium ion, dihydrogen phosphate anion (H_2PO_4^-), and monohydrogen phosphate anion (HPO_4^{2-}) at a given pH can be calculated by using the Henderson-Hasselbalch equation shown below:

$$\text{pH} = \text{pK}_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

Id. at 226. The Henderson-Hasselbalch equation accounts for the relationship between the pH of acids (in aqueous solutions) and their pKa (acid dissociation constant). *Id.* In the equation, $[A^-]$ is the concentration of a conjugate base, and $[HA]$ is the concentration of an acid. At a known pH, with the knowledge of the sodium phosphate buffer's concentration in a composition and the dissociation rate of $H_2PO_4^-$ to H^+ and HPO_4^{2-} , a POSA can calculate the concentration of HPO_4^{2-} and $H_2PO_4^-$ in the composition, which are $[A^-]$ and $[HA]$ in the equation, respectively.

65. Osmolarity is a measure of the number of particles present in solution and is equal to the sum of the concentrations of the solutes which have the capacity to exert an osmotic force across the membrane. *Id.* at 613. Tonicity is a measure of the effect of a solution's osmolarity on a cell or other body fluid. *Id.* An isotonic solution has the same osmotic pressure as the cell or body fluid. *Id.* It was well known as of June 2004 that it is preferable to maintain the osmolarity of a pharmaceutical composition to be as close to isotonic as possible, especially for parenteral administration, to reduce injection pain. Ex.1008, 410-411; Ex.1021, 1525 ("Solutions to be administered subcutaneously require strict attention to tonicity adjustment, otherwise irritation of the plentiful supply of nerve endings in this anatomical area would give rise to pronounced pain"). The same principle applies to virus compositions for therapeutic uses. For instance, Evans taught that its virus formulations with enhanced stability should also have a suitable range of "total

osmolarity which both promotes long term stability at temperature of 2-8 °C, or higher, while also making the formulation useful for parenteral, and especially intramuscular, injection.” Ex.1003, 10:18-21.

66. Before June 2004, it had been preferable to use elevated ionic strength solutions to achieve improved purity and to store the purified viral vectors in virus formulations with improved stability. *E.g.*, Ex.1009, Example 17 (demonstrating successful storage of adenovirus particles at an ionic strength >300 mM); *see also e.g.*, Ex.1003, 9:16-22, 11:13-21 (teaching viral formulations with enhanced viral stability contain a salt “at an ionic strength which is physiologically acceptable to the host,” such as NaCl “added at concentration within a range of upwards of 250 mM NaCl”); Ex.1011, 417-419 (disclosing an “improved protocol” for production of preclinical grade rAAV that involved eluting and storing the stocks in a high ionic strength (500 mM NaCl) buffer); Ex.1004, 5:34-6:10 (recommending using up to 10 mg/ml NaCl to stabilize virus formulation and reduce aggregation).

67. Decades before 2004, formulators had been routinely adjusting pH, buffer species, and solvents to optimize stability of drug formulations. Ex.1020, 239 (“Consideration of the mechanism of the reaction and the way in which the reaction rate is influenced by pH, buffer species and solvent permits selection of the optimum conditions for drug stability.”). Before 2004, it had also been well known that a combination of monovalent salts and multivalent salts can effectively increase the

ionic strength of adenovirus and AAV compositions while maintaining the osmolarity within a suitable range to improve stability of the virus formulation. Ex.1003, 11:22-30; Ex.1009, [00181]; Ex.1008, 411. For example, Evans taught that adding salts to virus formulations can “attain the desired ionic strength or osmolarity” and that NaCl in a range of about 25 mM to about 250 mM can be used to maintain the total osmolarity of its stable virus formulation in the suitable range. Ex.1003, 9:19-20, 11:22-30. Evans further taught that “[a]n additional component which further stabilizes the added viral component comprise[s] the addition of at least one salt of a divalent cation, including but not necessarily limited to MgCl₂, CaCl₂ and MnCl₂.” *Id.* at 9:6-8.

68. Liu further demonstrates that using high ionic strength solutions to maintain adenovirus vectors during the production and purification processes achieved stable storage compositions. Ex.1009, [00365]-[00370]. Specifically, Liu demonstrates that an adenovirus vector composition comprising a buffer of 25 mM Tris, 300 mM NaCl, 5 mM MgCl₂, 0.0025% polysorbate 80, and 5% trehalose at pH 7.5 was stable when stored over 7 days, with “no signs of settling or precipitation,” *i.e.*, aggregation, over the 7-day period and “no significant decrease in particle number.” *Id.* at [00366], [00369], Table 15.

69. Evans similarly describes adenovirus vector or AAV vector formulations with improved stability, which comprise (1) Tris to buffer the pH “from

about pH 7.5 to about pH 8.5;” (2) “NaCl which is added at concentration within a range of upwards of 250 mM NaCl;” and (3) the divalent cation MgCl_2 “in a range from about 0.1 mM to about 10 mM.” Ex.1003, 11:13-19. Evans tested the stability of candidate formulations using adenoviral preparations and found that they exhibited good stability after short-term and longer-term storage. *E.g., id.*, Examples 3, 13. In claim 5, Evans further recites purified viral vector formulations with a virus concentration in the range from about 1×10^7 vp/ml to about 1×10^{13} vp/ml and a total osmolarity in a range from about 200 mOs/L to about 800 mOs/L and comprising a Tris buffer, at a pH from about 7.5 to about 8.5, sodium chloride from about 25 mM to about 250 mM, and a divalent cation selected from the group consisting of MgCl_2 and CaCl_2 in an amount from about 0.1 mM to about 5 mM. *Id.* at 36:25-27. Evans teaches that the viral vector formulations described therein “are amenable to prolonged storage at 2 °C to 8 °C and higher for periods approaching two years,” “show enhanced storage stability,” and are suitable for use with “adenovirus, adeno-associated virus”. *Id.* at 3:10-14, 7:16-18.

70. Frei similarly teaches the compositions of its invention preferably contain pharmaceutically acceptable “divalent metal salt stabilizers, such as magnesium salts,” and pharmaceutically acceptable monovalent salt stabilizers such as sodium chloride. Ex.1004, 5:31-6:6. Frei explains that, in addition to stabilizing the composition, sodium chloride may “result[] in a more pharmaceutically elegant

presentation that may have reduced antigenicity potential due to protein aggregates.” *Id.* at 6:7-10. Frei teaches that the sodium chloride salt in the virus compositions is preferably in an amount of up to 10.0 mg/ml. *Id.* at 5:34-36, 6:5-6.

71. As discussed above, a POSA would have understood that particle aggregation is the most common physical instability observed when developing virus pharmaceutical compositions, and that by passing the stability tests described in Evans, Frei, and Liu, their formulations necessarily prevented aggregation. Thus, as of 2004, researchers had understood the benefits of elevated ionic strength in adenovirus and AAV vector formulations and achieved elevated ionic strength virus formulations by utilizing monovalent and multivalent ions to achieve improved stability by preventing particle aggregation.

2. It Had Been Routine to Optimize pH to Improve Stability of Viral Formulations

72. It was well known that a buffer system with appropriate pH “help[s] maintain an environment in which the drug is stable throughout the commercial shelf life of the product” and that “[o]ptimization of pH is a ***simple but very useful*** solution for” solving stability problems in pharmaceutical formulations. Ex.1018, Chapter 1, 12 (emphasis added); Ex.1008, 415; Ex.1020, 335 (“Buffers are used to maintain the pH of a medicinal at an optimal value”), 239 (“Consideration of the mechanism of the reaction and the way in which the reaction rate is influenced by pH, buffer species and solvent permits selection of the optimum conditions for drug

stability.”). Before June 2004, formulators had routinely designed parenteral formulations to have a narrow pH range, i.e., the acidity or alkalinity would be contained within a small window. Ex.1020, 230 (“The evidence for enhanced stability of systems when these are maintained within a narrow range of pH ... is abundant”). This is because “[t]he more a pH deviates from physiological conditions and the higher the buffer capacity, the more likely the product will contribute to tissue damage or injection pain.” Ex.1008, 415.

73. Decades before 2004, those skilled in the art had observed the impact of pH on virus particle aggregation level and reported the superiority of pH 7.5 over lower tested pHs in minimizing undesired AAV particle aggregation in purified AAV compositions. For instance, Johnson reports that in purified AAV compositions, at pH 7.2 and all lower tested pHs, the AAV particles “were aggregated and were not evenly distributed in the field but were found in clumps,” and “[m]any aggregates were found containing thousands of particles.” Ex.1019, 589. But when the pH is increased to pH 7.5, “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” *Id.*

74. As of 2004, those skilled in the art had routinely maintained viral vector formulations at pH of 7.5-8.0. For instance, Frei teaches that “[m]aintenance of pH is important for viral preparations because at pH below 7.0 and above 8.5 the live virus particles are vulnerable to losing viability due to physical and biological

instability.” Ex.1004, 2:6-8. Frei then taught generating multiple stable adenovirus formulations with pH between 7.5 to 8. *Id.* at 22:25-23:20. Liu taught purifying and storing adenovirus formulations in elevated ionic strength buffers “at pH 7.5” and achieving stability for 7 days. Ex.1009, [00366], [00369], Table 15.

75. Evans similarly taught that the pH range of its stable virus formulations is preferably from about 7.5 to about 8.5, and further claimed purified virus vector formulations with a virus concentration in the range from about 1×10^7 vp/ml to about 1×10^{13} vp/ml and a total osmolarity in a range from about 200 mOs/L to about 800 mOs/L and preferably comprising a Tris buffer, “at a pH from about 7.5 to about 8.5,” sodium chloride from about 25 mM to about 250 mM, and a divalent cation selected from the group consisting of MgCl_2 and CaCl_2 in an amount from about 0.1 mM to about 5 mM. Ex.1003, 36:25-27. Evans further exemplified multiple adenovirus compositions, all but two of which had pH values of 7.5, 8.0, or between pH 7.5 and 8.0. *Id.* at 21:27-24:16. Evans further reported that while its pH 7.5 compositions achieved optimum long-term stability when stored at 15 °C, pH 7.0-7.5 was optimum for long-term storage at 25 °C, and pH 8.0-8.5 was optimum for long-term storage at 2-8 °C. *Id.* at 27:26-29.

76. Conventional and routine buffers that had been used for decades to maintain the pH of pharmaceutical compositions include citric acid buffers,

acitrisaminomethane (“Tris”) buffer, and phosphate buffers. Ex.1008, 408, Table 17.3.

77. Thus, long before June 2004, a pH range of 7.5-8.0 was well-known and routinely used in pharmaceutical formulations with clinical applications, including AAV vector formulations. Given that formulators regularly optimized pH for pharmaceutical compositions and deemed such adjustments fairly “simple,” it would have been routine to optimize the pH of AAV formulations within a narrow range close to physiological pH by using well known buffers to achieve improved stability. Ex.1008, 411, 415; Ex.1018, Chapter 1, 12; Ex. 1020, 335.

VI. THE ’542 PATENT

A. The Disclosure of the ’542 Patent

78. The ’542 patent, entitled “Compositions and Methods to Prevent AAV Vector Aggregation,” relates to compositions and methods for preparation of concentrated stock solutions of AAV virions. Ex.1001, (54), Abstract. The ’542 patent states that “[t]he present invention is based in part on the observation that solution ionic strength is an important parameter in AAV vector aggregation, implicating the involvement of ionic interactions between virus particles in the aggregation process.” *Id.* at 4:53-57. The ’542 patent further states that “[t]he observation that elevated ionic strength increases AAV2 vector solubility regardless of the identity of the charged excipient supports the hypothesis that ionic strength of

solution per se, rather than interactions involving a specific ionic species, is the relevant physico-chemical parameter. A threshold ionic strength of at least 200 mM is required to prevent aggregation at vector particle concentrations.” *Id.* at 4:57-64.

79. But the ’542 patent also admits that it “is ***well established*** for protein therapeutics” that “an important aspect of vector stability is solubility during preparation and storage,” and that “a few studies have been reported describing systematic efforts to optimize AAV vector formulation and stability.” *Id.* at 2:49-51 (emphasis added). The patent further admits that high salt concentrations were known to increase AAV 2 vector solubility. *Id.* at 4:67-5:4 (noting “highly concentrated AAV2 vectors recovered from gradients generally remain soluble in concentrated CsCl.”). These admissions are consistent with the common knowledge in 2004 that AAV vectors require elevated concentrations of salt to prevent the formation of insoluble aggregates. As I explained above, it had been known that the ionic strength of virus vector formulations, and AAV solutions in particular, is an important parameter for solubility and stability, and the prior art had already used high ionic strength solutions in virus formulations to achieve improved stability.

B. The Claims of the ’542 Patent

80. Claim 1, the only independent claim of the ’542 patent, recites:

A composition for the storage of purified, recombinant
adeno-associated virus (AAV) vector particles,
comprising:

purified, recombinant AAV vector particles at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml;

a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and

excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate; wherein the ionic strength of the composition is greater than 200 mM, and wherein the purified AAV vector particles are stored in the composition without significant aggregation.

'542 patent (Ex. 1001), 14: 5-26. As I explain below, claim 1 recites nothing more than a known buffering system for storing a known product at known concentrations and under known conditions. Thus, claim 1 would have been obvious.

81. Dependent claim 2 recites that the composition further comprises Pluronic® F68. *Id.* at 14:27-28. Dependent claim 5 recites that the purified, recombinant AAV vector particles have an average particle radius of less than about 20 nm as measured by dynamic light scattering. *Id.* at 14:34-37. Dependent claim 6 recites that recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition through a 0.22 μ m filter. *Id.* at 14:38-41. As I explain below, these dependent claims recite either routinely used formulation

excipients or well-known virus stability features, and thus, also would have been obvious just like claim 1.

VII. LEVEL OF ORDINARY SKILL IN THE ART

82. In my opinion, a POSA working in the field of the '542 patent on June 1, 2004, would have possessed at least a BS in biology, chemistry, chemical engineering, biochemistry, pharmaceutical science, or a related discipline, with at least four years of industry, laboratory, and/or clinical experience in formulating or developing dispersions for therapeutic biologics, such as proteins or vectors for gene delivery. Such person may be familiar with, or consult with someone familiar with, the development and/or administration of viral vectors for gene therapy.

83. For this declaration, I have applied this definition of a POSA, and my opinions are offered from the perspective of a POSA as that hypothetical person would have understood matters on June 1, 2004, which I understand is the relevant date for the analysis.

VIII. CLAIM CONSTRUCTION

84. I have been informed by counsel that claims should only be construed to the extent necessary to resolve any controversy. Here, in my opinion, no terms need construction.

IX. OBVIOUSNESS ANALYSIS

A. Legal Standard

85. I have been informed by counsel and I understand that an obviousness analysis asks if the differences between the patented subject matter and the prior art are such that the subject matter as a whole would have been obvious, at the time the invention was made, to a POSA to which the subject matter pertains.

86. I have been informed by counsel and I understand that obviousness is a factual inquiry, and that the factors guiding the analysis include: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; (4) and any so-called objective secondary factors of nonobviousness (for example, commercial success, long-felt but unmet need, unexpected results, and copying).

87. I have been informed by counsel and I understand that obviousness may be shown by a combination of prior art references. I understand that it is important to consider whether there was a reason (e.g., a teaching, suggestion, or motivation) to combine elements found in the prior art, although an explicit teaching, suggestion, or motivation to combine prior art references is not required. Rather, the following factors may be considered in evaluating the existence of a motivation to combine: (1) the inter-related teachings of multiple references; (2) the effects of demands

known to those of ordinary skill in the field or present in the marketplace; and (3) the background knowledge possessed by a POSA.

88. I have been informed by counsel and I understand that, to evaluate the obviousness of the '542 patent claims over a given combination of prior art references, I should analyze whether the prior art references disclose every limitation of the challenged claims either explicitly or inherently, as those references are read by the POSA at the time of the invention. Then I am to determine whether that combination makes the claimed invention, as a whole, obvious to the POSA by a preponderance of the evidence, at the time of the invention. I understand that the preponderance of the evidence standard is satisfied as long as the proposition is more likely to be true than not true.

89. I have been informed by counsel and I understand that a patent claiming a combination of elements from the prior art is obvious if the alleged improvement is no more than the predictable use of prior art elements according to their established functions. When there is a design need or market pressure to solve a problem and the prior art suggests a finite number of identified, predictable solutions, a skilled artisan has good reason to pursue the known options within their technical grasp. If this leads to the anticipated success, it is likely that the product is not one of innovation but rather one of ordinary skill and common sense.

90. I have been informed by counsel and I understand that under an obviousness analysis, all that is required is a reasonable expectation of success in combining the prior art references to arrive at the claimed embodiments. A guarantee or absolute certainty of success based on the prior art is not required.

B. Summary of Prior Art References

1. PCT Publication of International Application No. PCT/US01/07194, “ADENOVIRUS FORMULATIONS” (“Evans”)

91. Evans is a PCT Publication of International Application Number PCT/US01/07194 entitled “Adenovirus Formulations,” which was published in 2001. EX.1003 (21). I was informed that Evans is prior art to the ’542 patent.

92. Evans discloses viral formulations for use in gene therapy. *Id.*, Abstract, 1:15-16. Evans teaches that there was a need “for the development of a recombinant virus liquid formulation that is stable for approximately 1-2 years at 2-8 °C and compatible with parenteral administration.” *Id.* at 1:30-34, 2:26-28. In satisfying that need, Evans discloses that “[t]he present invention relates to **stabilized virus formulations** and related pharmaceutical products for use in gene therapy and/or vaccine applications.” *Id.* at 3:2-3 (emphasis added).

93. Evans discloses that its formulations comprise a buffer, a salt, a divalent cation, and a non-ionic detergent. *Id.* at 1:19-21, 3:7-8. Evans then further provides examples of components, concentration ranges, and pH ranges of its virus

formulations. *See id.* at 8:22-11:4. Specifically, Evans discloses that the pH range is “preferably . . . from about 7.5 to about 8.5” and that “[t]he preferred divalent cations are MgCl_2 and CaCl_2 at a concentration ranging from about 0.1 mM to about 5 mM.” *Id.* at 8:27-28, 9:8-9. Evans further discloses that a preferred aspect of the invention is a recombinant viral vector formulation that “shows enhanced stability characteristics with a virus concentration in the formulation ranges from about 1×10^7 virus particles/ml (vp/ml) to about 1×10^{13} vp/ml.” *Id.* at 8:5-11.

94. Evans teaches that adding monovalent and multivalent salts to virus vector formulations can “attain the desired ionic strength or osmolarity” and that NaCl in a range of about 25 mM to about 250 mM can be used to maintain the total osmolarity of its stable virus formulation in a suitable range. *Id.* at 5:16-22, 11:25-27. Evans further teaches that “[a]n additional component which further stabilizes the added viral component comprise the addition of at least one salt of a divalent cation, including but not necessarily limited to MgCl_2 , CaCl_2 and MnCl_2 .” *Id.* at 9:6-8.

95. Evans tested the stability of candidate adenovirus formulations and found that they exhibited good stability after both short-term and longer-term storage. *E.g., id.*, Examples 3 and 13. Evans then further specifically claimed virus formulations comprising purified virus with a concentration in the range from about 1×10^7 vp/ml to about 1×10^{13} vp/ml, a buffer acceptable for human parenteral use at

a pH from about 7.5 to about 8.5, NaCl from about 25 mM to about 250 mM, a divalent cation selected from MgCl₂ and CaCl₂ in an amount from about 0.1 mM to about 5 mM, and a non-ionic detergent. *Id.*, claim 5.

96. Evans teaches that “[t]he adenoviral-based formulations of the present invention are amenable to prolonged storage at 2 °C to 8 °C and higher for periods approaching two years,” “show enhanced storage stability” and are applicable to adenovirus, adeno-associated virus, retroviruses, herpes virus, vaccinia virus, rotavirus, pox viruses.” *Id.* at 3:10-14, 7:16-18.

2. PCT Publication of International Application No. PCT/US99/01873, “COMPOSITIONS COMPRISING VIRUSES AND METHODS FOR CONCENTRATING VIRUS PREPARATIONS” (“Frei”)

97. Frei is a PCT Publication of International Application No. PCT/US99/01873 entitled “Compositions Comprising Viruses and Methods For Concentrating Virus Preparations,” which was published in 1999. Ex.1004, (21). I was informed that Frei is prior art to the ’542 patent.

98. Frei teaches achieving virus compositions with enhanced stability and that “[t]he formulation of the present invention has the additional advantage of having the ability to **stabilize high concentrations of virus.**” *E.g., id.* at 4:29-30, 7:7-8 (emphasis added). Frei discloses that its compositions are buffered to “maintain pH in a range from about 7 to about 8.5.” *Id.*, Abstract. Frei explains that it is important to maintain pH to such a range in viral preparations “because at pH below

7.0 and above 8.5 the live virus particles are vulnerable to losing viability due to physical and chemical instability.” *Id.* at 2:6-8, 6:17-19. Frei then describes narrower preferred pH ranges. *Id.* at 6:26-30.

99. Frei further teaches that the composition of its invention preferably contains pharmaceutically acceptable “divalent metal salt stabilizers, such as magnesium salts,” and pharmaceutically acceptable monovalent salt stabilizers such as sodium chloride. *Id.* at 5:31-6:6. Frei explains that, in addition to stabilizing the composition, sodium chloride may “result[] in a more pharmaceutically elegant presentation that may have reduced antigenicity potential due to protein aggregates.” *Id.* at 6:7-10. Frei teaches that the concentration of the divalent salt, e.g., magnesium salt, in the virus compositions is preferably “from about 0.1 to 1 mg/mL” and that the sodium chloride salt in the virus compositions is preferably “in an amount of 0.1 to 10.0 mg/ml,” which can be converted into a molar range of approximately 17.1 to 171 mM based on the molecular weight of NaCl (58.44 g/mol).³ *Id.* at 5:34-36, 6:5-6. Frei teaches that a wide range of viruses can be used in the compositions of the invention, including adenoviruses and adeno-associated virus. *Id.* at 8:18-24.

³ The molar concentration of 0.1 mg/ml NaCl = $0.1/58.44 \approx 17.1$ mM; the molar concentration of 100 mg/ml NaCl = $100/58.44 \approx 171$ mM.

100. Frei also teaches generating multiple exemplary stable adenovirus formulations. Specifically, Frei discloses an adenovirus final formulation as Example D-1, which comprises “20 mM NaPi, 100 mM NaCl, 2 mM MgCl₂, 2% sucrose, 10% glycerol,” with a pH 8 at 2-10 °C. *Id.* at 22:24-27. The concentration of the virus in this formulation is 1.6×10^{13} particles/ml. *Id.* at 22:31. Frei further discloses that the A320/A260 ratio of this formulation is 0.22.” *Id.* at 22:30. A POSA would have understood that this ratio indicates that the formulation contains monomeric virus particles and no observable aggregation. Ex.1015, 6:59-63 (“Free virus particles display a light scattering ratio” at A320/A260 nm “of about 0.22-0.30:1.”).

3. PCT Publication of International Application No. PCT/US02/35049, “VIRAL VECTOR PRODUCTION METHODS AND COMPOSITIONS” (“Liu”)

101. Liu is a PCT Publication of International Application Number PCT/US02/35049, entitled “VIRAL VECTOR PRODUCTION METHODS AND COMPOSITIONS,” and was published in 2002. Ex.1009, (21). I was informed that Liu is prior art to the '542 patent.

102. Liu describes methods of purifying virus vector particles to achieve formulations “comprising a significant number and concentration of viral vector particles (e.g., at least about 1×10^{12} PU),” wherein the composition has low levels of impurities. *Id.* at [0006]. Liu teaches that an advantage of its methods is “the

reduction in the number of empty viral vector particles (viral vector particles that are incomplete, damaged, or lacking genetic material) or ‘empty capsids.’” *Id.* at [00281]. Liu teaches that preferably, less than about 10%, preferably less than about 5%, and optimally, practically 0% of the viral vector particles in the composition are empty capsids. *Id.*

103. Liu focuses on techniques for effective viral vector production and purification and achieved highly pure adenovirus formulations. *Id.*, Examples 1-19. Liu teaches achieving high-yield production of viral vector particles of about 1×10^{15} particle units/cell, their purification, concentration, and preparation in buffers to form storage compositions. *E.g., id.* at [0068], [00187], [00203], [00371].

104. Liu achieved highly pure virus formulations through utilizing salt concentrations that “may improve separation of the viral vector particle composition” during purification. *Id.* at [00223]. For instance, Liu teaches loading purification columns with a solution (e.g., buffer) comprising at least about 400 mM of a monovalent salt (e.g., NaCl) and that the buffer preferably comprises at least about 500-1200 mM, and, more preferably, about 1000 mM, of a monovalent salt (e.g., NaCl). *Id.* Liu also loaded and/or eluted the viral vector particles with a composition comprising at least about 250-600 mM of a divalent salt (e.g., MgCl_2). *Id.* Liu further claims using various purification methods to achieve viral vector

particle compositions that maintain “an ionic strength of about 0.5-1.2 M for a sufficient period of time.” *Id.*, claim 123.

105. Liu teaches “increasing the ionic strength of the composition to at least about 0.5 M to form an increased ionic strength composition and subjecting the increased ionic strength composition to tangential flow filtration to obtain a filtered composition, wherein the filtered composition comprises less non-viral-encapsidated DNA, non-viral vector particle component protein, or both, than a substantially identical composition.” *Id.* at [00183]. Liu further teaches that its experiments demonstrated that “high salt filtration, organic solvent filtration, and combination high salt/organic solvent filtration results in a decrease of non-viral encapsidated polynucleotide concentration in an adenoviral vector particle composition comprising at least about 6×10^{10} adenoviral vector particles without any appreciable loss in total adenoviral vector particles.” *Id.* at [00360], [00364].

106. Liu demonstrates in working Example 17 the ability of storage compositions to “effectively maintain a stable population of adenoviral vector particles during the viral vector particle production and/or purification process.” *Id.* at [00365]. In Example 17, Liu describes lysing adenoviral vector particle-infected cells by microfluidization and clarifying the cell lysate by microfiltration through a triple-filter clarification filter. *Id.* at [00366]. The clarified lysate was then subjected to diafiltration by tangential flow filtration with a benzon nuclease buffer and

subjected to Benzonase® digestion at 35 °C for 4 hours. *Id.* The viral vector composition was further subjected to tangential flow filtration “to obtain an adenoviral vector particle composition comprising a population of adenoviral vector particles in a temporary storage buffer (25 mM Tris, 300 mM NaCl, 5 mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5).” *Id.* The resulting composition was maintained at about 4°C for 7 days in the storage buffer. *Id.* at [00367]. Liu reports that visual inspection showed “no signs of settling or precipitation over the 7 day period.” *Id.* at [00369]. Liu further measured the virus particle numbers by UV adsorption at 260 nm and reverse phase HPLC and reports that “results of the HPLC [infectious particle] assay performed on the samples of the sterile bag composition showed no significant decrease in particle number over the 7 day test period.” *Id.*; *see also id.* at Table 15.

107. Thus, Liu teaches the benefits of using high ionic strength buffers to purify, elute, and store viral vector compositions, and further demonstrates the stability of purified adenoviral particles in a storage composition comprising high ionic strength buffers. *Id.* at [00371].

4. PCT Publication of International Application No. PCT/US02/37944, “METHODS FOR PRODUCING STOCKS OF RECOMBINANT AAV VIRIONS” (“Lochrie”)

108. Lochrie is a PCT Publication of International Application Number PCT/US02/37944, entitled “METHODS FOR PRODUCING STOCKS OF

RECOMBINANT AAV VIRIONS,” and was filed in 2002 and published on June 5, 2003. Ex.1009, (21). I was informed that Lochrie is prior art to the ’542 patent.

109. Lochrie “relates to methods for purifying adeno-associated virus (AAV) virions,” particularly “efficient and commercially viable methods for producing stocks of rAAV virions with reduced amounts of empty capsids.” *Id.* at 1:10-13, 5:2-5. Lochrie teaches that its purification methods “produce[] a stock of rAAV virions substantially free of empty AAV capsids, such as a stock wherein at least 75 % to about 99 % or more of the AAV virions present in the stock are packaged AAV capsids.” *Id.* at 7:1-3. Lochrie acknowledges rAAV virions can be purified from the host cell “using a variety of conventional purification methods, such as column chromatography, CsCl gradients, and the like.” *Id.* at 24:5-7. Lochrie then treated the purified rAAV stock to further remove empty capsids. *Id.* at 24:24-25. Example 2 of Lochrie describes production of rAAV virions, their purification via a POROS 20HE™ column, and formulation of the purified AAV stock “in 20 mM NaH₂PO₄, 150 mM NaCl, 5% sorbitol, and 0.1 % Tween-80, at pH 7.4 at a concentration of 4×10^{12} vector genomes/milliliter (vg/mL).” *Id.*, Example 2, 29:7-9.

5. Huang et al, “Aggregation of AAV Vectors, its Impact on Liver directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency” 1 MOL. THER. S286 (2000) (“Huang”)

110. Huang is an abstract published in the Supplemental issue of Molecular Therapy, a peer-reviewed scientific journal, in 2000. Ex. 1005. I was informed that Huang is prior art to the '542 patent.

111. Huang focused on preventing aggregation in pharmaceutical formulations containing a high concentration of AAV virus vector particles. *Id.* at S286. Huang teaches that “[t]o achieve high levels of gene transfer and ensure the safety of AAV vector administration, it is desirable to deliver high doses of vector in small volumes.” *Id.* Huang noted the formation of AAV aggregates at high concentrations and the concentration-dependency of the aggregate size. *Id.* Huang further reported its observation that compared to a formulation with a virus concentration of $1\text{-}5 \times 10^{12}$ genome copies/ml, the gene transfer efficiency of a formulation containing the same vector administered at the same dose but with an increased virus concentration of $5\text{-}10 \times 10^{13}$ genome copies per ml was 10-100-fold lower. *Id.*

112. Huang thus performed “a series of formulation studies” “to prevent and dissolve AAV aggregates.” *Id.* Huang reported that some of its formulations

achieved “a 30-50% reduction in the size of aggregates at high vector concentrations.” *Id.*

6. Mingozi et al., “Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector” 76 J. Virology 10497-10502 (2002) (“Mingozi”)

113. Mingozi is an article published in the Journal of Virology, a peer-reviewed scientific journal, in 2002. Ex.1006, 10497. I was informed that Mingozi is prior art to the '542 patent.

114. Mingozi, entitled “Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector,” teaches preparing AAV vectors at high concentrations reported in units of genomes/ml (vg/ml) and achieving improved gene transfer efficiency with such preparations. *Id.* at Abstract, 10501. Mingozi teaches that AAV vectors “have been shown to efficiently transfer genes into nondividing target cells,” and that “[a]n excellent safety profile combined with reduced potential for activation of inflammatory or cellular immune responses has made this vector system attractive for clinical application and treatment of genetic disorders.” *Id.* at 10497.

115. Mingozi further teaches that it was shown that infusion of doses of an AAV-Factor IX vector at 10^{12} vector genomes [vg]/kg of body weight can result in sustained Factor IX expression in dogs, which corresponds to a dose of approximately 1.9×10^{13} vg for a 60 kg human. *Id.* Mingozi then produced and

purified AAV-2 and AAV-5 vectors at a concentration of $>10^{13}$ vg/ml by repeated cesium chloride (CsCl) gradient centrifugation, and reported the efficiency of hepatic gene transfer in mice using the purified vectors. *Id.* These successful formulations contained rAAV at doses of 10^{11} or 2.5×10^{11} vg/vector, which correspond to doses of approximately 1.9×10^{13} vg and 4.8×10^{13} vg, respectively, for a 60 kg human. *Id.* at 10498, 10501. Based on these results, Mingozzi concluded that “[t]hese data further encourage development of the AAV vector system as a delivery vehicle for hepatic gene transfer.” *Id.* at 10501.

7. Wright et al., “Recombinant adeno-associated virus: Formulation challenges and strategies for a gene therapy vector” 6 CURR. OPIN. DRUG DISCOVERY 174-177 (2003) (‘Wright’)

116. Wright is a review article published in Current Opinion in Drug Discovery, a peer-reviewed scientific journal, in March 2003. Ex.1007, 174. I note that the named inventors of the ’542 patent are listed as co-authors of Wright. I was informed that Wright is prior art.

117. Wright teaches that AAV “is a promising vector for human gene transfer” and has “received considerable attention in the field of gene therapy, because of [its] ability to mediate long-term gene transfer in the absence of significant toxicity.” *Id.* at 174. Wright summarized the superior characteristics of AAV vectors, which contribute to its popularity in the field of gene therapy, and discusses the development of AAV formulations, emphasizing that “because AAV

and adenovirus are both non-enveloped viruses developed as gene transfer vectors, studies on the latter can provide guidance for AAV vector formulation development.” *Id.* at 174.

118. Wright acknowledges that aggregation of AAV particles is “a significant and not fully resolved issue for adenovirus vectors aggregation” and that “purification conditions that may affect aggregation include buffer ionic strength and pH, shear and vector concentration.” *Id.* at 175. Thus, before the ’542 patent, Wright had already acknowledged that challenges to AAV formulation included particle aggregation and summarized the progresses and available tools in resolving those known issues. *Id.* at 174-75.

119. Wright teaches that “AAV vectors are typically prepared at final purified concentrations in the range of 10^{11} to 10^{13} vg/ml.” *Id.* at 176. Wright cited Huang for previously identifying that rAAV underwent concentration-dependent aggregation and confirmed Huang’s observation, reporting that “aggregation of vector frequently occur[s] in a time- and concentration-dependent manner in vector preparations at concentrations $\geq 10^{14}$ capsid particles (cp)/ml.” *Id.* Wright also teaches that $\geq 10^{14}$ capsid particles (cp)/ml corresponds to $\geq 10^{13}$ vg/ml. *Id.* at 176. Wright notes that “[t]he tendency of AAV vectors to aggregate at high concentrations is a factor to consider during the development of vector purification methods.” *Id.* Wright notes that “empty capsids, whose size and surface

characteristics are similar to that of genome containing vector particles, contribute to particle aggregation, and their presence may result in aggregation at lower vector genome concentrations than would be observed in their absence. *Id.* at 175. Wright assumed “that full vector particles and empty capsids aggregate by a similar mechanism” and concluded that “a preparation of AAV vectors containing a 10-fold excess of empty capsids should have a similar risk of aggregation at concentrations of $\geq 10^{13}$ vg/ml.” *Id.* at 175-76.

120. In addition, Wright acknowledges that “loss of rAAV following a 0.2- μ m filtration step correlates with the extent of vector aggregation” and that some target tissues “contain[] intercellular gaps of 0.1 to 1 μ m that could allow the passage of non-aggregated AAV particles (diameter of 0.026 μ m), but may significantly inhibit the passage of aggregated vectors.” *Id.* Wright further noted that “the variability in particle size associated with aggregation may also adversely affect in vivo gene transfer.” *Id.*

121. Despite the noted challenges, Wright reports that “highly purified vector preparations at concentrations of 5×10^{13} cp/ml [capsid particles/ml] are stable in a non-aggregated, monomeric state when stored at 2 to 8°C” without freeze-thaw cycles. *Id.* Wright further explains that while “non-ionic surfactants do not prevent or reverse aggregation induced at high vector concentrations or elevated temperatures,” initial aggregation “could be reversed by adjusting buffer pH,” *Id.*

8. Johnson et al., “Effect of Environmental pH on Adenovirus-Associated Virus” in Proceedings of The Society For Experimental Biology And Medicine 150, 585-590 (1975)

122. Johnson is an article published in a peer-reviewed scientific journal, Proceedings of The Society For Experimental Biology And Medicine, in 1975. I was informed that Johnson is prior art to the '542 patent.

123. Johnson explores the effect of pH on AAV particle infection activity and aggregation. Ex.1019, 585. Specifically, Johnson infected cultured cells with AAV at different pH, detected the infection activity of the AAV through immunofluorescence assays, purified the AAV on isopycnic CsCl gradients, and tested the level of AAV particle aggregation in the purified AAV composition at different pH by electron microscopy. *Id.* at 585-86.

124. Johnson teaches that the pH of a purified AAV composition affects AAV particle aggregation. *Id.* at 589. Johnson reports that at pH 7.2 and all lower tested pHs, the AAV particles “were aggregated and were not evenly distributed in the field but were found in clumps,” and “[m]any aggregates were found containing thousands of particles.” *Id.* But when the pH is increased to pH 7.5, “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” *Id.*

125. Based on these results, Johnson concludes that “[t]he greatest effect of pH appeared to be its influence on the aggregation of the viral particles” and that

“increasingly large aggregates of particles [were shown] as the pH was lowered” from 7.5. *Id.* at 589-90.

9. Clark, K. R., “Recent advances in recombinant adeno-associated virus vector production” 61 KIDNEY INT. S9-S15 (2002) (‘Clark’)

126. Clark is a review article published in a Supplemental issue of *Kidney International*, a peer-reviewed scientific journal, in 2002. Ex.1012, S-9. I was informed that Clark is prior art to the ’542 patent.

127. Clark discusses advances in technologies in AAV particle production that achieved “significant increase in production capacity.” *Id.*, Abstract. Clark notes that “[i]nterest in recombinant adeno-associated virus vectors (rAAV) has steadily increased because of the ability of this vector to mediate long-term, robust in vivo gene expression in numerous nondividing cell types (i.e., hepatocytes, neurons, and skeletalmyocytes).” *Id.* at S-9. Clark teaches that “[e]fficient in vivo gene transfer via rAAV transduction requires reasonably high multiplicities of infection,” and that “a clinical dose in humans will require 10^{12} to 10^{14} rAAV particles, depending on the level of therapeutic protein expression needed for treatment efficacy.” *Id.*

128. Clark then discusses “the recent advances in vector production technology that have resulted in the routine isolation of $>10^4$ recombinant viral particles per cell.” *Id.* Specifically, Clark teaches that while earlier transfection methods could achieve yields of between 10^2 to 10^3 viral particles, improved

methods capable of achieving increased AAV capsid expression levels improved the transient transfection vector yields by approximately 10-fold. *Id.* at S-10. Clark further teaches that with additional advances in stable cell line production, increased AAV vector yields of $> 10^{14}$ rAAV particles can be achieved by large-scale preparation. *Id.* at S-12.

129. Clark teaches that another significant advance in AAV vector technology is several refined chromatography-based purification methods. *Id.* Clarks reports that “[i]mportantly, vector purity, biological potency, and process throughput are all increased using these chromatographic methods.” *Id.* By using these new purification techniques, “[r]ecoveries were on average $>70\%$ with purity in excess of 95% .” *Id.* at S-12-S-13.

10. Gatlin et al. “Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parenteral Products”, in INJECTABLE DRUG DEVELOPMENT: TECHNIQUES TO REDUCE PAIN AND IRRITATION (Gupta, P.K & Brazeau, G.A. eds., 1999)

130. Gatlin is a chapter on “Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parenteral Products” from a textbook titled “Injectable Drug Development” and published in 1999. Ex.1008, 401-402. I was informed that Gatlin is prior art to the ’542 patent.

131. Gatlin provides formulation strategies for developing parenteral products. *Id.* at 401. Gatlin emphasizes “the two formulation parameters, pH and

tonicity, that are usually associated with tissue damage and injection pain,” and “provides a parenteral product development outline” that includes guidance on ways in which pH and tonicity “may be modified to minimize tissue damage and pain caused by a parenteral product.” *Id.* at 401-402. Gatlin teaches that a buffer system with appropriate pH “help[s] maintain an environment in which the drug is stable throughout the commercial shelf life of the product.” *Id.* at 415. Gatlin further teaches that commonly used pH buffers include Trisaminomethane (Tris) and phosphate buffers. *Id.* at 408, Table 17.3.

132. Gatlin further teaches that injection volume is one of the most important considerations in the development of a commercial parenteral product and is selected based on the proposed injection route. *Id.* at 405. For example, Gatlin states that a product administered by an intravenous route can have a volume of approximately 10 ml, but other parenteral routes require much smaller volumes (e.g., 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery). *Id.*; *see also id.* at 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections); *id.* at 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL” for intramuscular injection).

11. Croyle et al., “Development of formulations that enhance physical stability of viral vectors for gene therapy” 8 GENE THER. 1281-1290 (2001) (“Croyle”)

133. Croyle is an article published in Gene Therapy, a peer-reviewed scientific journal, in 2001. Ex.1013, 1281. I was informed that Croyle is prior art to the '542 patent.

134. Croyle discusses the development of adenovirus and AAV viral vector formulations by selecting appropriate buffer systems, cryoprotectants, and storage conditions. *Id.*, Abstract. Croyle acknowledges that “excipients employed in vector formulations must be suitable for use in vivo.” *Id.* To enhance the physical stability of viral vectors for gene therapy, Croyle selected excipients “from a panel of pharmaceutically acceptable compounds known to promote the physical stability of protein and peptide-based therapeutics currently on the market.” *Id.* at 1281-82.

135. Croyle explains that “[w]e chose the adenovirus as our model vector, because it can easily be produced in quantities sufficient for real time assessment of viral stability. Studies were also initiated with adeno-associated virus, as this vector system is becoming increasingly popular for clinical applications.” *Id.* Through its testing, Croyle concluded that “[a]deno-associated virus (AAV) was not as sensitive to pH changes upon freezing as the adenovirus at the concentrations studied.” *Id.* Croyle also notes that AAV “is significantly more stable than the adenovirus.” *Id.* at 1281, 1283.

12. Potter et. al, “Streamlined Large-Scale Production of Recombinant Adenovirus-Associated Virus (rAAV) Vectors” in Methods in Enzymology, Vol. 346, Gene Therapy Methods (M Ian Phillips, ed. 2002)

136. Potter is a chapter on “Streamlined Large-Scale Production of Recombinant Adenovirus-Associated Virus (rAAV) Vectors” from a textbook titled “Methods In Enzymology” Vol. 346, “Gene Therapy Methods,” and published in 2002. Ex.1011, 413. I was informed that Potter is prior art to the ’542 patent.

137. Potter describes “an improved protocol adapted for large-scale production of a preclinical grade rAAV” that “consists of three sequential chromatography purification steps resulting in a highly purified (99.9% pure) and infectious (particle-to-infectivity ratios less than 10) vector preparations.” *Id.* at 429. Potter acknowledges that “[r]ecent progresses in development of rAAV production protocols” “has allowed testing of purified vectors in high titers in a variety of animal models” and that “[m]any of these trials have resulted in successful therapies derived from long-term expression of transgenes delivered by rAAV.” *Id.* at 413. Potter then describes “a preindustrial scale-up protocol carried out in a cell factory format (about 10^9 cells per factory), allowing a modest facility to increase vector production at least 10- to 100- fold.” *Id.*

138. Potter reports having previously “developed a new rAAV purification protocol resulting in higher yield and improved infectivity of viral particles” and then discusses “further improvements in the production protocol” that incorporated

“new chromatography purification steps” that involve eluting and storing the purified rAAV vectors in a high ionic strength buffer. *Id.* at 419 (eluting the purified rAAV vectors in a buffer containing 0.5 M NaCl), 422 (describing the eluted product as a “virus stock,” which implies storage). Potter further describes a new centrifugation method “that allows separation of full and empty particles.” *Id.* at 413, 429. Potter teaches that “[r]emoval of empty particles” “improves the overall quality of a viral preparation.” *Id.* at 429.

139. Potter reported yields of $1.2\text{-}4.2 \times 10^{12}$ total infectious particles, and “characterize[s] the purified rAAV in terms of purity, infectivity, and packaged particle composition.” *Id.* at 413-14, 419. Potter concluded that “[t]he described protocol was successfully implemented for the production the National Reference Standard rAAV vector.” *Id.* at 429.

C. Claims 1, 5, and 6 Would Have Been Obvious Over Evans in View of Huang and Mingozi

140. In my opinion, claims 1, 5, and 6 of the '542 patent cover virus formulations that contain known and routinely used virus concentrations, multivalent salts, ionic strength, and pH ranges, and would have been obvious to a POSA over Evans in view of Huang and Mingozi as of June 2004, based on the general knowledge in the art as evidenced by Wright, Clark, Gatlin, Croyle, and Liu.

1. Claim 1 Would Have Been Obvious Over Evans in View of Huang and Mingozi

141. Evans teaches preparing stable compositions, including rAAV compositions, comprising (1) purified recombinant virus particles at a concentration reaching about 1×10^{13} vp/ml; (2) a pH buffer that maintains the pH of the composition between 7.5 and 8.5; (3) a multivalent ion (MgCl_2); and (4) an ionic strength of greater than 200 mM, and which a POSA would have understood as having “no significant aggregation” as recited in claim 1. In view of teachings in Huang and Mingozi, as well as prior knowledge as evidenced by Wright, Clark, Gatlin, Croyle, and Liu, it would have been obvious to remove any empty capsids from Evans’s AAV compositions while maintaining Evans’s virus concentrations to achieve $> 1 \times 10^{13}$ vg/ml as recited in challenged claim 1 while also preventing virus particle aggregation in the composition.

a. “A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particles”

142. Evans teaches a “need remains for the development of a recombinant virus liquid formulation that is stable for approximately 1-2 years at 2-8 °C and compatible with parenteral administration,” and reports that “[t]he present invention addresses and meets these needs by disclosing improved recombinant virus liquid formulations which show enhanced stability for longer periods of time at temperatures in the range of 2-8 °C.” *E.g.*, Ex.1003, 2:26-32; *see also id.*, 3:10-12

(noting that the viral-vector based formulations disclosed therein “are amenable to prolonged storage at 2 °C to 8 °C and higher for periods approaching two years.”). Evans provides “stabilized virus formulations and related pharmaceutical products for use in gene therapy and/or vaccine applications.” *Id.* at 3:2-3. Evans further teaches using its formulations to stabilize viral vector particles that were “purified by column chromatography.” *Id.* at 7:29-30, 21:12-13. Evans further teaches and that “[t]he **recombinant viruses** of the present invention which show enhanced storage stability include but are not limited to adenovirus, **adeno-associated virus...**” *Id.*, 3:12-14 (emphases added); *see also id.*, 7:16-18.

143. Thus, Evans expressly teaches a composition for the storage of purified, virus vector particles comprising purified, recombinant virus vector particles and further teaches that the virus vector particles in its stable composition can be rAAV vector particles.

144. Evans also reports stability testing results from storing multiple exemplary purified adenovirus formulations. *See, e.g., id.*, Examples 3, 4, 7-11, 13, 14. Evans further specifically claims a virus formulation comprising: a purified virus from about 1×10^7 particles/ml to about 1×10^{13} particles/ml; a buffer acceptable for human parenteral use at a pH from about 7.5 to about 8.5; sodium chloride from about 25 mM to about 250 mM; a divalent cation selected from MgCl_2 and CaCl_2 in

an amount from about 0.1 mM to about 5 mM; and a non-ionic detergent. *Id.* at 36:3-27 (claims 1, 3-5).

145. Although Evans's claims 1 and 3-5 do not specify the recited virus vector particles as rAAV vector particles, as explained above, a POSA would have understood these disclosures cover a composition comprising rAAV vector particles in view of Evans's express teachings that "[t]he recombinant viruses of the present invention which show enhanced storage stability include" "adeno-associated virus." *Id.* at 3:12-14.

146. A POSA also would have picked rAAV vector particles to use in Evans's claim 5 formulations in view of prior knowledge of the advantages and attractiveness of rAAV. For instance, Mingozzi reported preparing compositions of purified AAV-2 and AAV-5 having concentrations ">10¹³vg/ml" and successfully utilizing those compositions for gene transfer in mice. Ex.1006, 10497-98.

147. Like Mingozzi, Wright also reflects a POSA's prior knowledge of preparing AAV compositions for gene therapy, and further motivates the use of AAV in Evans's claim 5 formulation. Ex.1007, 174-75; Ex.1012, S-9. Specifically, Wright discusses the development of compositions for AAV and teaches that "because AAV and adenovirus are both non-enveloped viruses developed as gene transfer vectors, studies on the latter can provide guidance for AAV vector formulation development." *Id.* Thus, the prior knowledge reflected in Wright, just

like Mingozi, also would have motivated a POSA to use purified, recombinant AAV vector particles in Evans's claim 5 formulation.

148. A POSA also would have had a reasonable expectation of success in using purified, recombinant AAV vector particles in Evans's claim 5 formulation in view of Evans's express teachings and the common knowledge that AAV is similar to and more stable than adenovirus from a formulation perspective. For example, Evans demonstrates successful development of multiple adenovirus compositions that achieved optimum long-term stability when stored at 2-8 °C, 15 °C, and 25 °C. Ex.1003, 21:27-24:16, 27:26-29. Evans further teaches that "[t]he recombinant viruses of the present invention which show enhanced storage stability include but are not limited to adenovirus and "adeno-associated virus." *Id.* at 3:12-14. And Croyle observed that AAV is significantly more stable than adenovirus. Ex.1013, 1283.

149. In addition, as I explained in section V.C, the nature of the viral genome, the family classification of the virus, and its disease-causing properties (or lack thereof) do not impact the propensity of the particles to aggregate. Thus, in view of Evans's teachings that its stable recombinant virus formulations with improved stability include adenovirus as well as AAV and the prior knowledge of superior stability of AAV over adenovirus, those skilled in the art would have reasonably

expected Evans's claim 5 composition to provide similar, if not better stability for storing AAV particles.

150. Thus, in my opinion, Evans renders obvious "[a] composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particles," as recited in challenged claim 1.

b. "at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml"

151. Evans's claim 5 formulation contains virus particles at a "concentration in the range from about 1×10^7 virus particles per ml (vp/ml) to about 1×10^{13} vp/ml." Ex.1003, claim 3 (from which claim 5 depends). I understand "about" to mean more or less than the stated value. Thus, a POSA would have understood that "about 1×10^{13} vp/ml" includes (1) virus particle concentrations of 1×10^{13} vp/ml, (2) virus particle concentrations that are slightly below 1×10^{13} vp/ml, and (3) virus particle concentrations that are *slightly above 1×10^{13} vp/ml*.

152. As I explained earlier in section V.B, the difference between vector particle/ml and vector genome/ml is that the former also includes any empty capsids that may be present in the composition. Thus, if Evans's claim 5 composition contained no empty capsids, then its concentration of "[a little more than] 1×10^{13} vp/ml" exceeds 1×10^{13} vg/ml, as required by the challenged claim 1.

153. If Evans's claim 5 composition contained some empty capsids, a POSA would have been motivated to remove those knowingly undesired empty capsids from Evans's formulation while still maintaining Evans's high titer to achieve a concentration of vector-containing AAV particles exceeding 1×10^{13} vg/ml.

154. As I explained in section V.B, it was common knowledge that empty AAV capsids reduces the efficiency of AAV gene therapies (by virtue of having no genome). Ex.1007, 175; *see also* Ex.1010, 11:27-28 (removing empty capsids because they "do[] not efficiently function to transfer the gene of interest into the host cell."). These well-known drawbacks of empty capsids would have provided a strong motivation to remove empty capsids from the purified AAV particles used in Evans's claim 5 virus formulation.

155. A POSA would have been motivated to maintain Evans's high concentrations of AAV vectors after removing empty capsids (if any) from Evans's formulations in view of prior knowledge of the desired high AAV doses for gene therapy. For instance, Huang teaches that "[t]o achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes." Ex.1005, S286. Mingozzi also reported that administering AAV vector average doses of 10^{12} vg/kg to dogs resulted in sustained transgene expression. Ex.1006, 10497. As I explained in section V.B, average doses of 10^{12} vg/kg correspond to average doses of 1.9×10^{13} vector genomes for a 60 kg human.

156. In addition, AAV-based gene therapies are typically administered parenterally, which require the high doses of rAAV vector particles to be concentrated in a small volume of only a few milliliters. Ex.1007, 175, 176, 177; Ex.1006, 10497; Ex.1008, 417-18. For instance, Gatlin teaches that a drug product administered parenterally generally requires small volumes. Ex.1008, 405 (teaching using volumes as small as 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery); *see also id.*, 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections), 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL” for intramuscular injection). Thus, a POSA would have been motivated to develop formulations having vector genome concentrations exceeding 1×10^{13} vg/ml to prepare therapeutically useful amounts of AAV particles via parenteral administration.

157. A POSA also would have had a reasonable expectation of success in achieving such concentrations in view of the teachings of Evans and Huang. For instance, Evans teaches that its formulations provide improved stability for virus vector formulations at “varying degrees of virus concentration” for pharmaceutical administration, indicating that Evans did not consider virus concentration to be a limiting factor that would impact the stability of the formulations described therein. Ex.1003, 7:31-33. In addition, because empty AAV capsids and genome-containing vector particles were known to have similar size and surface characteristics (and thus

have similar contributions to aggregation), removing empty AAV capsids while maintaining a concentration exceeding 1×10^{13} vg/ml would be expected to have no impact on the stability of Evan's claim 5 formulation before any empty capsids were removed. Ex.1007, 175.

158. By June 2004, methods of producing AAV, removing empty capsids, and concentrating AAV to achieve high yields of AAV vectors were well-known. For instance, Clark reported that stable cell lines can yield $> 1 \times 10^{14}$ AAV particles per large-scale preparation. Ex.1012, S-12. Wright taught the use of cesium chloride and iodixanol gradient ultracentrifugation for separating "genome-containing[] vector particles from the lighter empty capsids." Ex.1007, 175. Liu taught an advantage of its purification methods is "the reduction in the number of empty viral vector particles (viral vector particles that are incomplete, damaged, or lacking genetic material) or 'empty capsids.'" Ex.1009, [00281]. Liu taught that by utilizing such methods, less than about 10%, less than about 5%, or practically 0% of the viral vector particles in the composition are empty capsids. *Id.* A POSA would have understood that such methods could be used to successfully remove empty capsids while maintaining Evans's claim 5 purified virus concentration. And if the virus concentration of Evans's composition dropped after removing empty capsids, a POSA would have known how to concentrate the remaining genome-containing particles. For instance, Huang further taught concentrating viral vector preparations

“to different concentrations.” Ex.1005, S286. Liu also teaches using conventional ultrafiltration steps to concentrate virus. Ex.1009, [0005].

159. Huang’s and Mingozzi’s successes in achieving AAV formulations having concentrations above 1×10^{13} vg/ml would have further provided a reasonable expectation of success. Huang described AAV compositions having concentrations of $5\text{--}10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. As explained above, those skilled in the art would understand that genome copies/ml and virus genome/ml are virus concentration units used interchangeably to reflect the number of genome-containing particles. Huang, thus, teaches achieving AAV concentrations of $5\text{--}10 \times 10^{13}$ vg/ml. Although Huang acknowledges that gene transfer efficiency was lower with compositions having these AAV concentrations, Huang reports developing multiple AAV formulations, which effectively reduced aggregation by up to 50%. *Id.* In view of these teachings, a POSA would have reasonably expected to achieve a high concentration of AAV particles, such as $5\text{--}10 \times 10^{13}$ vg/ml, in Evans’s claim 5 virus formulations.

160. Prior successes of achieving high concentration AAV formulations had also been reported by others in the art. For instance, Mingozzi reported preparing compositions containing purified AAV-2 and AAV-5 at concentrations of “ $>10^{13}$ vg/ml as determined by quantitative slot blot analysis,” and then using those compositions to achieve successful gene transfer in mice. Ex.1006, 10497-98. These

teachings would have further reinforced a POSA's reasonable expectation of success in achieving high concentration AAV formulations based on Evans's claim 5 composition.

161. Thus, in my opinion, the claim limitation "at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml" would have been obvious over Evans in view of Huang and Mingozi.

c. "a pH buffer, wherein the pH of the composition is between 7.5 and 8.0"

162. Evans's claim 5 formulation contains a buffer "acceptable for human parenteral use, preferably a Tris buffer, at a pH from about 7.5 to about 8.5." Ex.1003, claim 3 (from which claim 5 depends). As I explained in section V.D.2, Tris buffer had been known as one of the most common buffers to maintain pH of pharmaceutical compositions. Ex.1008, 408, Table 17.3. Thus, Evans's claim 5 formulation contains "a pH buffer" recited in challenged claim 1.

163. Moreover, the pH range of Evans's claim 5 formulation largely overlaps with the recited pH range of "between 7.5 and 8.0" in the challenged claim 1, with the same lower limit of pH 7.5. It would have been obvious to select a pH of 7.5 to 8.0 in view of Evans's own teachings and prior knowledge of the suitability of pH 7.5-8.0 for stable viral formulations for therapeutic uses.

164. It had been common knowledge years before 2004 that for a parenterally administered drug composition, "[t]he more a pH deviates from

physiological conditions and the higher the buffer capacity, the more likely the product will contribute to tissue damage or injection pain.” Ex.1008, 415. Because human physiological pH is closer to 7.5 than 8.5, a POSA would have been motivated to select a pH within the lower end of the pH range of “about 7.5 to about 8.5” in Evans’s disclosed claim 5 virus formulation.

165. Moreover, long before 2004, the impact of pH on rAAV viral formulation aggregation level and stability had been investigated, and Johnson had reported that at pH 7.5, there is no aggregation of purified rAAV in the viral composition. Ex.1019, 589. Those skilled in the art with this prior knowledge would further be motivated to pick pH 7.5 in the recited range for Evans’s claim 5 formulation to control aggregation and improve stability.

166. Evans’s teachings also provide a reasonable expectation of success in selecting a pH between 7.5 to 8.0 for Evans’s claim 5 formulation. Specifically, Evans prepared adenovirus formulations with pH values of 7.5, 8.0, or between pH 7.5 and 8.0, and tested the formulations for short- and longer-term stability. Ex.1003, Example 4. Evans reported that the pH of optimum stability for its adenovirus formulations at 15°C, 25°C, and 2-8°C was 7.5, 7.0-7.5, and 8.0-8.5, respectively. *Id.* at 21:27-24:16, 27:25-29. It had been prior knowledge that AAV “was not as sensitive to pH changes upon freezing as the adenovirus at the concentrations studied.” Ex.1013, 1282. Thus, Evans’s successes in using pH values between 7.5

and 8.0 for its adenovirus formulations, as well as the known superior stability of AAV despite pH changes, would have provided a reasonable expectation that adjusting the pH of Evans's claim 5 virus formulations containing AAV particles within the range of 7.5 and 8.0 would maintain stability of the AAV formulations

167. Moreover, as I explained in Section V.D.2, selection of an appropriate pH for a therapeutic formulation had been a matter of routine optimization years before June 2004. *E.g.*, Ex.1018, Chapter 1, 12; Ex.1020, 235, 239. Indeed, long before June 2004, there had been a consensus among those skilled in the art that “[o]ptimization of pH is **a simple but very useful** solution for” addressing stability problems in pharmaceutical formulations. Ex. 1018, Chapter 1, 12 (emphasis added); *see also* Ex. 1008, 415. Indeed, for decades before 2004, formulators had been routinely using buffer systems “to help maintain an environment in which the drug is stable throughout the commercial shelf life of the product.” Ex. 1008, 415. Indeed, I have not seen any art reporting unique difficulties in adjusting the pH of AAV virus formulations.

168. Thus, in my opinion, Evans renders obvious “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0,” as recited in challenged claim 1.

d. “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate”

169. Claim 5 of Evans recites virus formulations containing a divalent cation “selected from the group consisting of MgCl_2 and CaCl_2 in an amount from about 0.1 mM to about 5 mM.” Ex.1003, claim 5.

170. A POSA would have been motivated to select MgCl_2 over CaCl_2 in view of Evans’s teachings. Throughout its disclosures, Evans discusses numerous embodiments of its stable viral compositions comprising MgCl_2 as the multivalent ions. *Id.* at 11:9-17:15, Example 1. Indeed, MgCl_2 appears as a key component dozens of times in various specific embodiments of stable viral compositions described in Evans. By contrast, Evans only mentions using CaCl_2 in two embodiments. *Id.* at 17:16-22, 22:23-24. Evans’s strong preference for using MgCl_2 in its stable viral compositions and actual successful use of MgCl_2 in its working examples would have motivated a POSA to choose to use MgCl_2 as the multivalent ions in Evans’s claim 5 formulation with a reasonable expectation of success. *Id.* at 11:9-17:15, Example 1.

171. Likewise, Liu used MgCl_2 in the storage buffer described in Example 17 and reported no visible adenovirus aggregation and no appreciable loss in infectious particles. Ex.1009, [00366], [00369], [00370]. Such prior knowledge,

along with Evans's teachings, renders MgCl_2 an obvious option for use in Evans's claim 5 formulation.

172. Thus, Evans renders obvious "excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate," as recited in challenged claim 1.

e. "wherein the ionic strength of the composition is greater than 200 mM"

173. Evans's claim 5 specifically recites virus formulations that contain "sodium chloride from about 25 mM to about 250 mM" and either magnesium chloride or calcium chloride "in an amount from about 0.1 mM to about 5 mM." Ex.1003, claim 4 (from which claim 5 depends), claim 5.

174. As I explained in section V.D.1, those skilled in the art would have been able to easily calculate the ionic strength of Evans's claim 5 formulations by using the well-established formula that takes half of the sum of the molar concentration of each ion multiplied by the valence squared. Taking the upper end of the NaCl concentration range in Evans's claim 5 formulations, 250 mM, and the upper end of the MgCl_2 concentration range in Evans's claim 5 formulations, 5 mM, the ionic strength of Evans's claim 5 formulation = $\frac{1}{2} \times (250 \text{ mM} \times (+1)^2 + 250 \text{ mM} \times (-1)^2 + 5 \text{ mM} \times (+2)^2 + 10 \text{ mM} \times (-1)^2) = 265 \text{ mM}$. This calculated ionic strength of 265

mM is within the ionic strength range of “greater than 200 mM” recited in claim 1 of the ’542 patent.⁴

175. A POSA would have been motivated to select the upper end of the NaCl concentration range in Evans’s claim 5 formulation in view of Evans’s teachings. For instance, Evans teaches that “[a] purpose of inclusion of a salt in the formulation is to attain the desired ionic strength or osmolarity.” *Id.* at 9:16-22. Evans further teaches that viral formulations containing a salt such as NaCl “at an ionic strength which is physiologically acceptable to the host” can “enhance viral stability.” *Id.* Evans further expressly instructs adding NaCl “at concentration within a range of **upwards of 250 mM NaCl.**” *E.g., id.* at 11:13-21 (emphasis added). These teachings collectively would have directed a POSA to the upper end of the NaCl concentration range in Evans’s claim 5 formulation, 250 mM NaCl and would have encouraged a POSA to add 250 mM NaCl to the viral formulation.

176. Evans further teaches that that “[a]n additional component which **further stabilizes** the added viral component comprise[s] the addition of at least one

⁴ I note that even if the lower end of the MgCl₂ concentration range from Evans’s claim 5 formulation (i.e., 0.1 mM) is used, the ionic strength would still be $[\frac{1}{2} \times (250 \text{ mM} \times (+1)^2) + 250 \text{ mM} \times (-1)^2 + 0.1 \text{ mM} \times (+2)^2 + 0.2 \text{ mM} \times (-1)^2] = 250.3 \text{ mM}$, which is above 200 mM.

salt of a divalent cation, including but not necessarily limited to MgCl_2 , CaCl_2 , and MnCl_2 .” *Id.* at 9:6-9. Evans teaches that “[t]he preferred divalent cations are MgCl_2 and CaCl_2 ,” and in the very first embodiment of the formulations described therein, Evans uses “ MgCl_2 as the divalent cation.” *Id.* at 3:26-30, 9:8-9. Evans further teaches that MgCl_2 can be used “in a range from about 0.1 mM to about 10 mM.” *Id.* at 11:13-19. Claim 5 of Evans also specifically requires the recited virus formulations to contain magnesium chloride or calcium chloride “in an amount from about 0.1 mM to about 5 mM.” *Id.*, claim 4 (from which claim 5 depends), claim 5. These teachings would have provided further motivation for a POSA to use MgCl_2 in combination with NaCl in amounts at the higher end of the range recited in Evans’s claim 5 composition to achieve a high ionic strength solution.

177. A POSA’s prior knowledge of improving viral formulation purity and stability by adding NaCl and MgCl_2 to achieve a total ionic strength of above 200 mM would have provided additional motivation for selecting the upper end of the NaCl concentration range in Evans’s claim 5 formulation and use it in combination with MgCl_2 to achieve a total ionic strength of above 200 mM. For instance, Wright identified ionic strength as a condition that may affect viral vector aggregation and stability. Ex.1007, 175. Liu had also demonstrated storing purified adenovirus particles in a “storage buffer (25 mM Tris, 300 mM NaCl, 5 mM MgCl_2 , 0.0025% polysorbate 80, 5% trehalose, pH 7.5).” Ex.1009, [00368]. The 300 mM NaCl and 5

mM MgCl₂ in the composition provide a total ionic strength of 315 mM, which is higher than 200 mM.⁵ *Id.* Liu reports that during the 7-day storage period, there were “no signs of settling or precipitation” of the virus particles in the storage buffer, and that there was “no significant decrease in particle number.” *Id.* at [00369], [00371]. Such prior use of NaCl and MgCl₂ with a total ionic strength of higher than 200 mM would have further motivated a POSA to select the high end of the concentration range for NaCl in Evans’s claim 5 formulation to achieve a high ionic strength solution.

178. A POSA also would have had a reasonable expectation of success in using NaCl at the high end of the about 25 mM to about 250 mM range in combination with MgCl₂ in Evans’s claim 5 formulation. As explained above, Evans explicitly teaches that NaCl can be “added at a concentration within a range of upwards of 250 mM” to achieve virus formulations with improved stability, including AAV virus formulations. Ex.1003, 11:13-21, 3:12-14. Evans further teaches that that “[a]n additional component which **further stabilizes** the added viral component comprise[s] the addition of at least one salt of a divalent cation, including but not necessarily limited to MgCl₂, CaCl₂, and MnCl₂,” and that MgCl₂ can be used

⁵ The total ionic strength = [$\frac{1}{2} \times (300 \text{ mM} \times (+1)^2) + 300 \text{ mM} \times (-1)^2 + 5 \text{ mM} \times (+2)^2 + 10 \text{ mM} \times (-1)^2$]] = 315 mM.

“in a range from about 0.1 mM to about 10 mM.” *Id.* at 9:6-9, 11:13-19 (emphasis added). Thus, Evans provides a reasonable expectation that adding NaCl at concentrations at the upper end of the about 25 mM to about 250 mM range in combination with MgCl₂, as described in claim 5 of Evans, would achieve stabilized formulations.

179. In addition, Liu demonstrated that adenovirus can be stored at about 4°C for 7 days in a storage buffer comprising 25mM Tris, 300mM NaCl, 5mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5, with “no signs of settling or precipitation” and “no significant decrease in particle number.” Ex.1009, [00366], [00369], Table 15. Because it was known that virus particle aggregation would result in agglomerates with increased size and reduced number of dispersed monomeric particles, Liu’s observation of “no signs of settling or precipitation” is indicative of satisfactory stability. Given the common knowledge that AAV is significantly more stable than adenovirus (Ex.1013, 1281, 1283), Liu’s successful use of a storage buffer with an ionic strength greater than 200 mM further provides a reasonable expectation that adding MgCl₂ in combination with NaCl at concentrations at the upper end of the about 25 mM to about 250 mM range described in Evans’s claim 5 would achieve stabilized rAAV formulations.

180. Moreover, Potter’s “improved protocol” for large-scale production of rAAV involved eluting the rAAV in a high-ionic strength (500 mM NaCl) buffer.

Ex.1011, 417-419, 429. And since Potter referred to these high-ionic strength rAAV compositions as “final virus stocks,” this further supports using a total ionic strength of above 200 mM in Evans’s claim 5 formulations containing AAV vector particles. *Id.* at 425

181. Selection of an appropriate ionic strength for a therapeutic AAV formulation would also have been a matter of routine optimization, especially in view of the clear guidance provided in Evans. During pharmaceutical formulation development, it had been a simple and routine practice for formulators to test various concentrations of excipients, calculate the total ionic strength, and determine stability of the virus in the composition. For instance, it would have been straightforward to select a few concentrations near the upper ends of the NaCl and MgCl₂ concentration ranges recited in Evans’s claims 4 and 5 and pick the combinations of concentrations that achieve optimal stability. After all, this type of practice had been routinely performed by formulators for decades. *See, e.g.*, Ex.1013, 1281-82 (reporting the selection of excipients “from a panel of pharmaceutically acceptable compounds known to promote the physical stability of protein and peptide-based therapeutics currently on the market”); Ex.1020, 239 (“Consideration of the mechanism of the reaction and the way in which the reaction rate is influenced by pH, buffer species and solvent permits selection of the optimum conditions for drug stability.”)

182. Thus, in my opinion, the claim limitation “wherein the ionic strength of the composition is greater than 200 mM” would have been obvious over Evans.

f. “wherein the purified AAV vector particles are stored in the composition without significant aggregation”

183. In my opinion, a POSA also would have reasonably expected Evans’s claim 5 formulations, regardless of removing empty capsids to achieve 1×10^{13} vg/ml virus or not, to have improved stability without aggregation.

184. As I explained in section IX.C.1.b, Evans’s claim 5 formulations comprise those containing AAV virus at a concentration of about 1×10^{13} total virus particles/ml, which may include empty AAV capsids. It was known that the size and surface characteristics of empty capsids “are similar to that of genome-containing vector particles” and that full vector particles and empty capsids presumably aggregate by a similar mechanism. Ex.1007, 175. As I explained above in sections V.C and IX.C.1.a, these teachings are consistent with the common understanding as of June 2004 that the nature of the viral genome packaged in the particles, the family classification of the virus, and its disease-causing properties (or lack thereof) do not impact the propensity of the particles to aggregate. Thus, a POSA would have expected Evans’s claim 5 formulations, including those containing 1×10^{13} cp/ml AAV, to be as stable as virus formulations containing about 1×10^{13} vg/ml AAV vector particles as recited in challenged claim 1.

185. In view of Evans's express teachings that its formulations, which include the claim 5 formulations, exhibit improved stability over long periods of time, a POSA would have reasonably expected that Evans's claim 5 formulations, regardless of removing empty capsids or not, have improved stability and prevent virus aggregation.

186. For example, Evans teaches that "[t]he present invention relates to stabilized virus formulations and related pharmaceutical products for use in gene therapy and/or vaccine applications." Ex.1003, 3:2-3. Evans further teaches that the preferred formulations "show improved viral stability when stored in about the 2-8 °C range and higher while also being compatible with parenteral administration." *Id.* at 3:4-7, 2:29-32 (disclosing "improved recombinant virus liquid formulations which show enhanced stability for longer periods of time at temperatures in the range of 2-8 °C"). Evans discloses that its virus formulations "allow[] for storage and eventual host administration of these liquid formulations over about a 1-2 year period with acceptable losses in virus infectivity." *Id.* at 4:21-25.

187. Evans further demonstrated stability upon short-term storage (up to 28 days), longer-term storage (up to 12 months), and freeze-thaw stability for several of its adenoviral formulations. *See, e.g., id.*, Examples 2-14. Thus, a POSA would have understood that Evans does not report any observed aggregation after storing their adenovirus formulations. Because it was known that AAV "is significantly

more stable than the adenovirus,” a POSA would have expected Evans’s claim 5 formulations comprising AAV vector particles at a concentration of “about 1×10^{13} vg/mL” to exhibit stability that is similar to or even greater than those tested adenovirus formulations in Evans and prevent AAV vector particle aggregation. Ex.1013, 1283.

188. A POSA would have been motivated to develop compositions with no aggregation. A POSA with prior knowledge of the undesirability of AAV aggregation would have had a strong motivation to develop formulations capable of storing AAV vector particles without aggregation. For instance, Huang acknowledges that AAV aggregates reduce the overall gene transfer efficiency. Ex.1005, S286. Wright similarly acknowledges that AAV vector particle aggregation is a known “issue for vector formulation and stability” and that a “potentially deleterious consequence of vector aggregation is inconsistency in the functional activity of AAV vectors following their administration *in vivo*.” Ex.1007, 175, 176.

189. Liu similarly teaches that “[t]he presence of viral vector particle aggregates is unfavorable since the clumping of the viral vector particles could result in an increased host immune response to the viral vector particles” and that “when viral vector particles are aggregated, it is more likely that more than one viral vector particle will infect any particular cell, which is undesirable.” Ex.1009, [00262]. In

view of these well-known detrimental consequences of AAV vector particle aggregation, a POSA would have been motivated to store AAV vectors in compositions with enhanced stability and no aggregation.

190. A POSA would have reasonably expected Evans's claim 5 formulation to achieve stable compositions comprising about 1×10^{13} vg/mL AAV vector particles without aggregation. As I explained above, Evans's own teachings and working examples and the known superiority of stability of AAV over adenovirus all support improved stability of formulations comprising about 1×10^{13} vg/mL AAV vector particles "without significant aggregation." Liu similarly reported that its high ionic strength adenovirus storage formulations showed "no signs of settling or precipitation" and "no significant decrease in particle number" following storage for 7 days at $\sim 4^{\circ}\text{C}$, which a POSA would have understood as showing no observable virus aggregation. *Id.* at [00366]-[00369], Table 15. Because AAV was known to be more stable than adenovirus, a POSA would have expected formulations comprising AAV particles to have even better stability and minimal aggregation.

191. Earlier successes reflected in Wright would have further reinforced the expectation of no "significant aggregation" in such AAV formulations. Specifically, Wright reported having generated highly purified AAV vector preparations at concentrations of 5×10^{13} capsid particles/ml that "are stable in a non-aggregated, monomeric state when stored at 2 to 8°C ." Ex.1007, 175. And as explained above,

those skilled in the art would have understood an AAV formulation with 5×10^{13} capsid particles/ml of virus to be as stable as an AAV formulation with a virus concentration of 5×10^{13} vg/ml, which is squarely within the claim scope of “greater than 1×10^{13} vg/ml.” Thus, Wright’s prior success of achieving stable AAV formulations containing AAV virus at a concentration almost 5-fold higher than that of Evans’s claim 5 formulation further strongly supports the expectation that a formulation developed from Evans’s claim 5 that comprises greater than 1×10^{13} vg/ml rAAV would have even better stability.

192. I have reviewed the prosecution history of the ’542 patent and found that the Patent Owner admitted during prosecution that a composition containing the recited mixture of components prevents AAV aggregation and, therefore, the purified AAV vector particles are necessarily stored in the composition “without significant aggregation”. Ex.1002, 241-42, 243. For instance, the Patent Owner stated that “the claims explicitly require that ‘aggregation of the purified AAV particles in the composition is prevented.’ Accordingly, Applicants’ unique mixture of components in the purified composition as claimed must be factored into the assessment of obviousness.” *Id.* at 241-42. The Patent Owner further stated that “the composition is one where ‘aggregation of the purified AAV particles in the composition is prevented’ and the causes of aggregation, and hence the proper formulation for preventing aggregation, were unknown prior to Applicants’

discovery.” *Id.* at 243. A POSA would have viewed these statements as basically stating that the claimed “storage . . . without significant aggregation” is a natural result of a composition meeting the recited mixture of components.

193. Thus, for all the reasons stated above, I conclude that challenged claim 1 would have been obvious over the prior art.

2. Claim 5 Would Have Been Obvious Over Evans in View of Huang and Mingozi

194. Claim 5 recites a composition of claim 1, “wherein the purified, recombinant AAV vector particles have an average particle size radius (Rh) of less than about 20 nm as measured by dynamic light scattering [DLS].” Ex.1001, 14:34-37. The ’542 patent merely teaches that “Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation.” Ex.1001, 9:25-27. Other than teaching that the recited radius range is exemplary of no “significant aggregation,” the patent does not identify anything critical about the recited radius range. In my opinion, therefore, this additional limitation merely describes a natural feature of the AAV formulations of claim 1 that prevent aggregation, and so those formulations do not have “significant aggregation.”

195. As I explained earlier in section V.C, it was well known that AAV vector particle aggregation would lead to formation of large interparticle aggregates or agglomerates with sizes bigger than monomeric virus. Ex.1007, 175; Ex.1005, S286. It was common knowledge that AAV vector particles have a diameter of

approximately 26 nm, which means that their particle radius is 13 nm and, therefore, below the recited “about 20 nm” limit. Ex.1007, 176 (“non-aggregated AAV particles (diameter of 0.026 μm)”). Thus, this claim essentially requires the AAV vector particles in the formulation to be monomeric or non-aggregates. To the extent claim 5 requires less aggregation than claim 1, a POSA would have been motivated to reduce any residual aggregation with a reasonable expectation of success for the reasons explained below.

196. A POSA would have been motivated to further reduce any residual aggregation of AAV particles in Evans’s formulations in view of the undesirability of aggregates in AAV formulations. As I explained above in section IX.C.1.f, it was known that aggregation during and after virus purification is “unfavorable” and “undesirable” and results in “deleterious consequences” such as “[r]educed yield,” an increased host immune response to the viral vector particles,” “inconsistency in the functional activity of AAV vectors following their administration *in vivo*,” and “less efficient [] gene transfer.” Ex.1007, 175, 176; Ex.1005, S286; Ex.1009, [00262]. In view of these teachings, a POSA would have been motivated to prevent aggregation from occurring during storage of AAV formulations such that the AAV vector particles have an Rh of less than about 20 nm as measured by dynamic light scattering.

197. In view of Evans’s teachings and prior successes, a POSA would have had a reasonable expectation of successfully developing compositions for storing AAV with no aggregation such that the average AAV Rh is less than about 20 nm measured by DLS. As explained above in section IX.C.1.f, Evans expressly teaches that its virus formulations, including its claim 5 formulation, are characterized by improved stability over long periods of time, which, as discussed above, indicates that those formulations did not have any observed aggregation issues. *E.g.*, Ex.1003, 3:2-3; 3:4-7; 2:29-32; 4:21-25.

198. Liu also taught storing high concentration of purified adenovirus in a storage buffer with a total ionic strength of above 200 mM and observing “no signs of settling or precipitation over the 7 day period” and “no significant decrease in particle number” during the entire tested extended period of time, meaning no observable aggregation of the adenovirus particles. Ex.1009, [00369], [00371]. Given that it was well known that AAV is significantly more stable than adenovirus, Ex.1013, 1283, a POSA would have expected even less aggregation for AAV if used in Liu’s Example 17 formulation.

199. Moreover, methods of reducing virus aggregation in viral formulations had been readily available as of 2004, and so it would have only taken routine optimization to further eliminate virus aggregation in Evans’s claim 5 composition. Indeed, it had been known that adjusting ionic strength, pH, buffer, and addition of

other known stabilizing agents could reduce virus aggregation. For instance, Wright explained that initial aggregation “could be reversed by adjusting buffer pH.” Ex.1007, 176. Specifically, when the pH of rAAV formulation was adjusted to pH 7.5, AAV vector particle aggregation was eliminated such that “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” Ex.1019, 585, 589. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex.1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex.1009, [00263].

200. Using these known tools, those skilled in the art had routinely eliminated virus aggregation in high concentration AAV formulations. For instance, Wright described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are *stable in a non-aggregated, monomeric state* when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added). Huang likewise discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5\text{-}10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. Thus, only routine optimization of known stability factors would have been required to prevent aggregation and obtain an average AAV Rh of $< 20\text{nm}$ as measured by DLS, which would have further supported a reasonable expectation of success in achieving the formulation recited in claim 5 of the '542 patent.

201. Thus, in my opinion, the composition of challenged claim 5 would have been obvious over Evans.

3. Claim 6 Would have Been Obvious Over Evans in View of Huang and Mingozi

202. Claim 6 recites a composition of claim 1, “wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.” Ex. 1001, 14:38-41. In my opinion, a POSA would have understood that this limitation reciting a recovery rate range after passing a 0.22 μm filter, like claim 5, describes a natural feature of the AAV formulations of claim 1 that prevent aggregation and so do not have “significant aggregation.”

203. As I explained earlier in Section V.C, it was common knowledge that AAV vector particle aggregation would lead to formation of large interparticle aggregates, which would result in loss of rAAV following a purification step that involves passing through a 0.2 μm membrane. As of June 2004, it had been routine to use 0.22 μm filters to obtain purified virus formulations and use recovery rate after passing the virus formulations through a 0.22 μm filter to indicate level of aggregation. For instance, Liu teaches that when its virus formulations comprising adenovirus vector particles were purified, the elute was “subjected to filtration using a 0.22 μm filter to yield a purified adenoviral vector particle composition.” Ex.1009, [00373]. It was also known that high virus particle recovery rates upon filtration by

a 0.22 μm filter indicates reduced aggregation levels. As Wright expressly teaches, “loss of rAAV following a 0.2- μm filtration step correlates with the extent of vector aggregation.” Ex.1007, 175. Huang similarly teaches that “the larger the aggregation,” “the less efficient the gene transfer.” Ex.1005, S286.

204. I reviewed the '542 patent and found that the patent does not identify anything critical about the recited recovery of at least about 90%. For instance, the patent states that “in various embodiments of the present invention, recovery is improved from less than about 80% to at least about 85%, 90%, 95% or more.” Ex. 1001, 9:1-4. A POSA would have understood this general discussion in the patent as suggesting a critical cutoff (if indeed one exists at all) that is greater than 80% recovery. In other words, the patent does not describe a cutoff value of 90% recovery that bears any relationship whatsoever to the concepts of “significant” or insignificant aggregation. Rather, based on the patent data, the limitation of “at least about 90%” appears to be completely arbitrary. Thus, a POSA would have reasonably expected that Evans’s claim 5 composition comprising AAV vector particles, which Evans teaches as having improved stability and without “significant aggregation,” to have at least 90% of the AAV particles in the stored composition recovered following filtration through a 0.22 μm filter.

205. To the extent claim 6 requires less aggregation than claim 1, As I explained above, Wright and Huang expressly teach the correlation between high

AAV virus particle recovery rate following a 0.2- μ m filtration step and minimized levels of vector aggregation. Ex.1007, 175 (“loss of rAAV following a 0.2- μ m filtration step correlates with the extent of vector aggregation.”); Ex.1005, S286 (teaching that “the larger the aggregation,” “the less efficient the gene transfer.”). In view of these teachings, a POSA would have been motivated to reduce any residual aggregation in Evans’s claim 5 formulation to maximize virus recovery through a 0.22 μ m filter.

206. A POSA would have had a reasonable expectation of successfully maximizing particle recovery after filtration in view of prior successes of reducing aggregation. For instance, Huang teaches developed formulations that “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” Ex.1005, S286. Liu also observed “no signs of settling or precipitation over the 7 day period” after purifying and storing its adenovirus particles in a high ionic strength buffer. Ex.1009, [00369]. A POSA would have expected similar results for AAV formulations, as it had been known that AAV “is significantly more stable than the adenovirus.” Ex.1013, 1283.

207. As I explained above section IX.C.2, reducing aggregation would have been a routine optimization of Evans’s claim 5 composition by, for example, adjusting ionic strength, pH, buffer, and other known stabilizing agents. For instance, as I explained, it was known that increasing ionic strength would improve

virus formulation stability and reduce aggregation. *See supra* § V.C. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex.1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex. 1009, [00263]. Adjusting pH to close to 7.5 had also been known to eliminate AAV particle aggregates. Ex.1019, 585, 589.

208. Wright similarly explained that initial aggregation “could be reversed by adjusting buffer pH” and described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are *stable in a non-aggregated, monomeric state* when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added), 176. Huang likewise discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5\text{-}10 \times 10^{13}$ genome copies/ml. Ex. 1005, S286. Thus, only routine optimization of known stability factors would have been required to improve the recovery of AAV from Evans’s claim 5 formulation following filtration through a 0.22 μm filter.

209. Thus, in my opinion, the composition of challenged claim 6 would have been obvious over the prior art.

D. Claim 2 Would Have Been Obvious over Evans and Wright in view of Huang and Mingozi

210. Claim 2 recites a composition of claim 1 “further comprising ethylene propylene oxide block copolymer Pluronic® F68.” Ex. 1001, 14:27-28. Evans

discloses “the Pluronic series of non-ionic surfactants (e.g., Pluronic 121).” As of 2004, Pluronic® F68 had become one of the most commonly used commercially available non-ionic surfactants among the “Pluronic series” discussed in Evans. Thus, Evans’s teachings of “Pluronic series of non-ionic surfactants” renders it obvious to use Pluronic® F68 in Evans’s claim 5 formulation.

211. A POSA would have been motivated to add the well-known non-ionic surfactant Pluronic® F68 in Evans’s claim 5 formulation in view of the common knowledge of the functionality of Pluronic® F68 in AAV formulations. For instance, Wright expressly teaches that “addition of the surfactants Polysorbate 80 or Pluronic® F68 . . . effectively prevent losses due to non-specific binding during [virus] vector sampling and transfer.” Ex. 1007, 175, 176. And Croyle successfully used Pluronic® F68 in its AAV formulations. Ex.1013, 1284 (Table 3), 1288 (identifying the “Pluronic” in Table 3 as Pluronic® F68).

212. A POSA also would have had a reasonable expectation of success in using Pluronic® F68 in Evans’s claim 5 formulation. For instance, Liu teaches that its stable storage compositions comprise “nonionic surfactants [that] are well known in the art,” including “Pluronic F68.” Ex.1009, [00189]. And Croyle further discloses that AAV compositions comprising Pluronic® F68 have an expiration date of 240 days when stored at 4°C, which a POSA would have understood as further demonstrating the suitability of Pluronic® F68 for long-term stable storage of AAV

formulations. Ex.1013, 1284 (Table 3, formulation comprising “0.01% Pluronic”), 1288 (identifying detergent as “Pluronic block copolymer F68”).

213. Thus, in my opinion, the composition of challenged claim 2 would have been obvious.

E. Claims 1, 2, 5, and 6 Are Obvious Over Frei in View of Huang and Mingozi

214. As I explain above, claims 1, 2, 5, and 6 of the '542 patent cover virus formulations that contain known and routinely used virus concentrations, multivalent salts, ionic strength, and pH ranges, and would have also been obvious to a POSA over Frei in view of Huang and Mingozi as of June 2004, as evidenced by Wright, Clark, Gatlin, Croyle, and Liu.

1. Claim 1 Would Have Been Obvious Over Frei in View of Huang and Mingozi

215. Frei expressly discloses a recombinant adenovirus composition, i.e., the D-1 formulation, that (1) contains a phosphate pH buffer that maintains the pH of the composition between 7.5 and 8.0; (2) contains a high virus concentration of 1.6×10^{13} particles/ml; (3) contains multivalent ions (MgCl_2) as a stabilizer; and (4) that stores the viral vector particles with a measured light scattering ratio that indicates no aggregation. A POSA would have arrived at challenged claim 1 simply by using rAAV in Frei's D-1 formulation and increasing the ionic strength of that formulation to above 200 mM. Those modifications would have been obvious to a POSA with

knowledge in the art as of June 2004 in view of teachings of Frei, Huang, and Mingozi.

a. **“A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particle**

216. Frei teaches that “[t]he present invention relates to compositions comprising viruses, especially viral vectors, having significantly improved stability.” Ex.1004, 1:15-16. According to Frei, “[t]he compositions of the present invention are useful in *maintaining the stability of viruses during storage*, and virus containing compositions of the present invention are particularly useful for therapeutic uses such as gene therapy.” *Id.* at 1:16-19 (emphasis added). “New methods for concentrating and *purifying virus preparations* are also provided” in Frei. *Id.* at 1:19-20 (emphasis added). Frei further teaches that “[t]he viruses are preferably recombinant viruses,” and that its virus formulations are “particularly well suited for stabilizing a recombinant virus, such as a live recombinant adenovirus (or ‘viral vector’), for therapeutic use in gene therapy.” *Id.* at 8:27-29.

217. Frei also reports results from experiments that involved storing several exemplary adenovirus compositions. *See, e.g., id.* Tables 1-5. For instance, Frei shows a formulation (Example D-1) comprising “stable concentrations of adenovirus [] prepared by concentrating DEAE pools in 30% glycerol” followed by “further purification by Superdex-200 gel filtration chromatography to obtain the

final formulation.” *Id.* at 22:17-31. As such, the D-1 formulation in Frei contains concentrated and purified adenovirus vector particles.

218. Based on these teachings, a POSA would have understood that Frei discloses compositions particularly suitable for the storage of purified, recombinant virus vector particles, comprising purified, recombinant virus vector particles, and further shows successful development of stable adenovirus compositions comprising purified recombinant adenovirus.

219. A POSA would have been motivated to use AAV in Frei’s disclosed adenovirus compositions such as its D-1 formulation because Frei expressly teaches the particular suitability of using rAAV in its compositions. For instance, Frei teaches that “[a] wide range of viruses,” including “adeno-associated viruses,” can be used in the compositions of the present invention” and that “[t]he methods of the instant invention are amenable to a wide range of viruses,” including “adeno-associated viruses.” *Id.* at 8:18-26, 16:23-26.

220. As I explained above in section IX.C.1.a, prior knowledge of the attractiveness and advantages of AAV vectors in gene therapy as reflected in Mingozi, Wright, and Clark would have further motivated a POSA to use AAV in Frei’s disclosed adenovirus compositions such as its D-1 formulation. *See supra* § IX.C.1.a; Ex.1006, 10497 (promoting AAV vectors as “attractive for clinical application and treatment of genetic disorders”); Ex.1007, 174-75 (acknowledging

AAV “is a promising vector for human gene transfer”); Ex.1012, S-9 (acknowledging “steadily increased” interest in “recombinant adeno-associated virus vectors (rAAV)” due to its superior ability to “mediate long-term, robust in vivo gene expression”).

221. A POSA would have reasonably expected to use rAAV vector particles in Frei’s D-1 formulation with success in view of Frei’s express teachings that AAV can be used in its virus formulations. Ex.1004, 8:18-21 (“A wide range of viruses can be used in the compositions of the present invention, including but not limited to . . . adeno-associated virus.”). In addition, because it was well known that AAV is “significantly more stable than the adenovirus,” a POSA would have reasonably expected Frei’s compositions to provide similar, if not better stability for storing AAV particles. Ex.1013, 1283.

222. Thus, in my opinion, Frei teaches and renders obvious “[a] composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particles,” as recited in claim 1.

b. “at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml”

223. Frei emphasizes that “[t]he formulation of the present invention has the additional advantage of having the ability to stabilize **high concentrations** of virus.” Ex.1004, 7:7-8 (emphasis added). Frei further demonstrates achieving multiple

exemplary adenovirus compositions with a virus concentration exceeding 1×10^{13} particles/ml. *Id.*, 22:25-23-20 (Example D-1: 1.6×10^{13} particles/ml; Example D-2: 1.5×10^{13} particles/ml; Example D-3: 1.3×10^{13} particles/ml; Example S-1: 1.66×10^{13} particles/ml).

224. A POSA would have understood that as long as more than 62.5% of the virus particles in the D-1 formulation contain vector genomes (or less than 37.5% of the virus particles are empty capsids), the disclosed 1.6×10^{13} *particles/ml* virus concentration in this formulation would be more than 1×10^{13} *vg/ml*.⁶ Frei's teachings that AAV can be used in its formulations would have rendered it obvious to similarly use AAV in the D-1 formulation and achieve a virus vector particle concentration that exceeds 1×10^{13} *vg/ml* as required by claim 1. Ex.1004, 8:18-21.

225. Even if less than 62.5% of the rAAV virus particles applied to the D-1 formulation contain vector genomes, and the rest are empty viral capsids, as I explained in section IX.C.1.b, the well-known desirability of delivering high concentrations of therapeutic AAV vectors in small volumes would have provided a

⁶ Assuming among 1.6×10^{13} particles in each ml of the composition, 1×10^{13} are virus particles contain vector genomes (vg), the percentage of virus particles containing vector genomes can be calculated as $(1 \times 10^{13}) / (1.6 \times 10^{13}) = 62.5\%$. The percentage of empty capsids is $100\% - 62.5\% = 37.5\%$.

POSA with strong motivation to remove the empty viral capsids and maintain the high virus concentration at a level of above 1×10^{13} vg/ml. *See supra* § IX.C.1.b; Ex.1005 (“To achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes.”); Ex.1006, 10497 (reporting sustained transgene expression in dogs from AAV vector doses of 10^{12} vg/kg).

226. Maximizing the portion of vector-genome-containing virus particles and maintaining a high concentration of AAV vector particles at a level exceeding 1×10^{13} vg/ml would have been particularly desirable for therapeutic virus formulations because AAV gene therapy was known to be routinely administered via parenteral administration, which was known to require administering limited volumes of only a few milliliters of drug product. *See supra* § IX.C.1.b; Ex.1008, 405, 417-418. Thus, a POSA would have understood that a more concentrated virus formulation is naturally more suitable for parenteral administration and has a greater potential to achieve improved clinical benefits.

227. A POSA would have had a reasonable expectation of success in achieving concentrations exceeding 1×10^{13} vg/ml in view of Frei’s teachings. For instance, Frei expressly highlights the ability to “stabilize high concentrations of virus” as an important “advantage” of “[t]he formulation of the present invention” and also provides “[n]ew methods for concentrating and purifying virus

preparations.” Ex.1004, 1:19-20, 7:7-8. Frei further specifically discloses using tangential flow filtration (“TFF”) as “a new method of stably concentrating an existing virus preparation,” “allowing one to readily select and prepare clinical dosages in a wide range of desired concentrations.” *Id.*, 16:9-12. In view of these teachings, a POSA would have reasonably expected to achieve stable AAV compositions having virus concentrations exceeding 1×10^{13} vg/ml when applying Frei’s D-1 formulation to AAV.

228. Indeed, as I explained in section IX.C.1.b, as of June 2004, well-known methods of removing empty capsids, concentrating genome-containing vectors, and generating high yields of AAV had been routinely reported and used in the field. For instance, Clark reported that large-scale cell line preparations can produce $> 1 \times 10^{14}$ AAV particles for formulation. *See supra* § IX.C.1.b; Ex.1012, S-12. Wright teaches using known techniques such as cesium chloride and iodixanol gradient ultracentrifugation to separate “genome-containing[] vector particles from the lighter empty capsids.” Ex.1007, 175. A POSA would have reasonably expected that such known methods, as well as the methods provided in Frei, can similarly remove empty capsids while maintaining the concentration of AAV when using AAV in Frei’s D-1 formulation.

229. Those skilled in the art also had already achieved AAV compositions exceeding a vector particle concentration of 1×10^{13} vg/ml. For instance, Huang

described AAV compositions having concentrations of $5-10 \times 10^{13}$ genome copies/ml, which a POSA would have understood as $5-10 \times 10^{13}$ vg/ml AAV, and taught that their formulation techniques “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” Ex.1005, S286. Mingozi also had prepared compositions of purified AAV-2 and AAV-5 having concentrations “ $>10^{13}$ vg/ml as determined by quantitative slot blot analysis,” and successfully utilized those rAAV compositions for gene transfer in mice. Ex.1006, 10497-98. These prior successes would have bolstered a POSA’s expectation of successfully achieving above 10^{13} vg/ml rAAV based on Frei’s teachings.

230. Thus, in my opinion, Frei renders obvious “at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml,” as recited in challenged claim 1.

c. “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0”

231. Frei discloses that its compositions are buffered to “maintain pH in a range from about 7 to about 8.5.” Ex.1004, Abstract. Frei explains that it is important to maintain pH to such a range in viral preparations “because at pH below 7.0 and above 8.5 the live virus particles are vulnerable to losing viability due to physical and chemical instability.” *Id.* at 2:6-8, 6:17-19.

232. Frei then demonstrates achieving multiple adenovirus formulations with pH within the recited range of between 7.5 to 8.0. *Id.* at 22:25-23:20 (Example D-1: pH 8; Example D-2: pH 7.8; Example D-3: pH 8; Example S-1: pH 8). The D-

1 composition in Frei contains “20 mM NaPi, 100 mM NaCl, 2 mM MgCl₂, 2% sucrose, 10% glycerol,” with “pH 8 at 2-10°C.” *Id.*, 22:27. It had been common knowledge that “NaPi” is the abbreviation of sodium phosphate, which is a mixture of mono- and dibasic-sodium phosphates” and one of the most commonly used pH buffers in pharmaceutical compositions. Ex.1024, Table A.2A.4. A POSA would also understand that the pH 8 of the D-1 formulation is within the range of “between 7.5 and 8.0” recited in challenged claim 1.

233. Thus, in my opinion, a POSA would understand that Frei directly teaches “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0,” as recited in challenged claim 1.

d. “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate”

234. Frei expressly teaches that “[p]harmaceutically acceptable divalent metal salt stabilizers, such as magnesium salts” “are used in preferred embodiments of the composition of the present invention” and that “[p]referably, the salt is a chloride salt or a magnesium salt, magnesium salt being particularly preferred.” Ex.1004, 5:31-34. Frei further demonstrates that its D-1 composition contains “2 mM MgCl₂.” *Id.* at 22:26. A POSA would have understood that the 2 mM MgCl₂ in the D-1 formulation is an excipient comprising magnesium divalent ions as required by the challenged claim 1.

235. Thus, Frei expressly teaches the claim limitation “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate.”

e. “wherein the ionic strength of the composition is greater than 200 mM”

236. Frei teaches that (1) virus formulations of its invention preferably may contain pharmaceutically acceptable divalent metal salt stabilizers such as magnesium salts; (2) the multivalent salts are preferably chloride salts or magnesium salts; and (3) “[p]referably, the salt (e.g., magnesium salt)” in its virus formulations “is present in an amount of from about 0.1 to 1 mg/ml.” Ex.1004, 5:31-36.

237. Moreover, Frei teaches that the virus compositions may also preferably include pharmaceutically acceptable monovalent metal salts such as sodium salts. *Id.* at 5:37-40. Frei teaches that “[p]referably, the salt is sodium chloride present in the amount of 0.6 to 10.0 mg/ml.” *Id.* at 6:5-6. Based on the molar mass of NaCl (58.44 g/mol), a POSA would have understood that Frei teaches preferably including up to about 171 mM NaCl (0.01 g/58.44 g/mol) in its virus formulations.

238. Frei further discloses that its D-1 composition contains “20 mM NaPi, 100 mM NaCl, 2 mM MgCl₂.” *Id.* at 22:25-26. NaPi is a sodium phosphate buffer made by mixing disodium hydrogen phosphate dihydrate (Na₂HPO₄•2H₂O) and monosodium dihydrogen phosphate monohydrate (NaH₂PO₄•H₂O). To calculate the ionic strength of the D-1 composition, I will conservatively assume that Frei’s NaPi

comprises all monosodium dihydrogen phosphate and no disodium hydrogen phosphate. This assumption underestimates the ionic strength of the buffer and provides the most conservative estimation. I note, however, that to maintain a pH of 8.0 in Frei's D-1 composition, a POSA would have been more likely to use a sodium phosphate buffer comprising mostly disodium hydrogen phosphate dihydrate. See Ex.1024, Table A.2A.4.

239. As I explained in section V.D.1, the Henderson-Hasselbalch equation can be used to calculate the concentrations of the H_2PO_4^- and HPO_4^{2-} anions in a sodium phosphate buffer. Applying the equation $\text{pH} = \text{pK}_a + \log([\text{A}^-]/[\text{HA}])$ to Frei's D-1 formulation having pH 8, using the known pK_a of H_2PO_4^- of approximately 7.2 (at 25 °C) (Ex.1021, 454), and assuming all of the 20 mM anion in the NaPi buffer is H_2PO_4^- , the concentration of HPO_4^{2-} , which is $[\text{A}^-]$ in the equation can be calculated as:

$$8 = 7.2 + \log([\text{HPO}_4^{2-}]/(20 \text{ mM}-[\text{HPO}_4^{2-}]))$$

$$\log([\text{HPO}_4^{2-}]/(20-[\text{HPO}_4^{2-}])) = 0.8$$

$$[\text{HPO}_4^{2-}]/(20-[\text{HPO}_4^{2-}])) = 6.3$$

$$(20-[\text{HPO}_4^{2-}])) \times 6.3 = [\text{HPO}_4^{2-}]$$

$$126.2 = 7.3 \times [\text{HPO}_4^{2-}]$$

$$[\text{HPO}_4^{2-}] \approx 17 \text{ mM}$$

The concentration of the dissociated H^+ from H_2PO_4^- would also be approximately 17 mM. The remaining $[\text{H}_2\text{PO}_4^-]$ would be approximately $20 \text{ mM} - [\text{HPO}_4^{2-}] = 3 \text{ mM}$. Thus, assuming the NaPi buffer in the D-1 formulation comprises NaH_2PO_4 only, the ionic strength of the sodium phosphate buffer is $\frac{1}{2} \times (20 \text{ mM} \times (+1)^2 + 3 \text{ mM} \times (-1)^2 + 17 \text{ mM} \times (-2)^2 + 17 \text{ mM} \times (+1)^2) \approx 54 \text{ mM}$. The total ionic strength calculated for the D-1 formulation is, thus, calculated as: $54 \text{ mM} + \frac{1}{2} \times (100 \text{ mM} \times (-1)^2 + 100 \text{ mM} \times (-1)^2 + 2 \times (+2)^2 + 4 \times (-1)^2) \approx 160 \text{ mM}$. Because the NaPi buffer should comprise more than zero percent Na_2HPO_4 in the first place, the total ionic strength of the D-1 formulation should be more than 160 mM. And as discussed earlier, because a POSA would have used a buffer comprising predominately disodium phosphate, the total ionic strength of the D-1 formulation would have been higher.

240. A POSA would have been motivated to increase the concentration of NaCl in the D-1 formulation to the upper end of Frei's preferred range of "0.6 to 10 mg/ml," i.e., 10 mg/ml, which is approximately 171 mM, in view of prior knowledge that increased ionic strength of virus compositions improve stability. *E.g.*, Ex.1009, Example 17; Ex.1003, 9:16-22, 11:13-21; Ex.1011, 417-419. When the NaCl concentration is adjusted to 171 mM in the D-1 formulation, the total ionic strength of the composition is calculated to be: $54 \text{ mM} + \frac{1}{2} \times (171 \text{ mM} \times (-1)^2 + 171 \text{ mM} \times (-1)^2 + 2 \times (+2)^2 + 4 \times (-1)^2) \approx 231 \text{ mM}$, which is above 200 mM. Thus, combining

Frei's teachings and the prior knowledge in the field, a POSA would have been motivated to increase the NaCl concentration in the D-1 formulation in Frei to achieve a total ionic strength falling within the scope of challenged claim 1.⁷

241. Specifically, Frei expressly promotes adding NaCl and MgCl₂ as preferred "salt stabilizers" in its virus formulations and teaches the advantages of adding them in amounts in the preferred ranges. Ex.1004, 5:31-6:6. Frei further teaches the additional benefits of adding NaCl to its viral formulation, stating "[i]n addition to stabilizing the composition, sodium chloride may suppress the rate and extent of the appearance of by-products of fermentation, resulting in a more pharmaceutically elegant presentation that may have reduced antigenicity potential due to protein aggregates." *Id.* at 6:7-10. Frei further acknowledges that "[t]he

⁷ As I explained, Frei also teaches that "[p]referably, the salt (*e.g.*, the magnesium salt) is present in an amount of from about 0.1 to 1 mg/mL." Ex.1004, 5:34-36. Based on the molecular mass of MgCl₂ (95.2 g/mol) used in the Frei D-1 formulation, the concentration of MgCl₂ can be calculated as 1mg/mL/95.2 $\approx$ 10 mM. Thus, modifying Frei's D-1 composition to comprise the high-end of the MgCl₂ range (*i.e.*, 1 mg/mL) would further increase the ionic strength by $[\frac{1}{2} \times (10 \times (+2)^2 + 20 \times (-1)^2)] - [\frac{1}{2} \times (2 \times (+2)^2 + 4 \times (-1)^2)] \approx 24$ mM.

addition of sodium chloride does not affect the pH of the formulation.” *Id.* at 6:10-11.

242. In addition, as I explained in sections V.D.1 and IX.C.1.e, before June 2004, a POSA had preferred using elevated ionic strength solutions to achieve improved purity and maintain the purified viral vectors in final virus formulations for improved stability. *E.g.*, Ex.1009, [00369], [00371] (reporting its purified formulations showed “no signs of settling or precipitation over the 7 day period” and that “no significant decrease in particle number over the 7 day test period”); *see also e.g.*, Ex. 1003, 9:16-22, 11:13-21 (teaching viral formulations with enhanced viral stability contain a salt “at an ionic strength which is physiologically acceptable to the host,” such as NaCl “added at concentration within a range of upwards of 250 mM NaCl”); Ex. 1011, 417-419 (disclosing an “improved protocol” for production of preclinical grade rAAV that involved eluting and storing the stocks in a high ionic strength (500mM NaCl) buffer); Ex.1007, 175 (identified ionic strength as a condition that may affect vector aggregation).

243. A POSA also would have had a reasonable expectation of success in using the higher end of Frei’s preferred NaCl concentration range because preparing virus formulations with enhanced ionic strength had been repeatedly reported by various research groups and become a matter of routine optimization as of June 2004. For instance, protocols for large-scale production of rAAV had been reported,

which involved eluting and storing the resulting rAAV stocks in a high-ionic strength buffer (500mM NaCl). Ex.1011, 417-419, 429. Adenovirus formulations comprising “25mM Tris, 300mM NaCl, 5mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5” also had been prepared and shown to have superior stability with “no signs of settling or precipitation” and “no significant decrease in particle number.” Ex.1009, [00366], [00369], Table 15. As explained in section IX.C.1.e (fn. 5), the total ionic strength of Liu’s storage composition is 315 mM, which is also above 200 mM.

244. As such, rAAV and adenovirus formulations having ionic strengths above 200 mM had been desired and achieved as of June 2004. These prior successes would have additionally provided a reasonable expectation of success in adjusting the ionic strength of the modified D-1 formulation in Frei to above 200 mM and achieving improved stability.

245. As I explained above, because it was well known that AAV is “significantly more stable than the adenovirus,” a POSA would have reasonably expected that substituting the adenovirus in Frei’s modified D-1 formulation with AAV particles would provide similar, if not better stability for storing those AAV particles. Ex.1013, 1283.

246. Moreover, selection of an appropriate ionic strength for a therapeutic AAV formulation also would have been a matter of routine optimization, especially

in view of the clear guidance provided in Frei. As I explained in section IX.C.1.e, during pharmaceutical formulation development, it had been a simple and routine practice for formulators to test various concentrations of excipients, calculate the total ionic strength, and determine stability of the virus in the composition. For instance, it would have been straightforward to select a few concentrations near the upper end of the NaCl concentration range (i.e., 10 mg/ml) disclosed in Frei and pick the combinations of concentrations that achieve optimal stability. After all, this type of practice had been routinely performed by formulators for decades. *See, e.g.*, Ex.1013, 1281-82 (reporting the selection of excipients “from a panel of pharmaceutically acceptable compounds known to promote the physical stability of protein and peptide-based therapeutics currently on the market”); Ex.1020, 216-221 (teaching calculating and adjusting ionic strength of drug solutions using well known excipients).

247. Thus, in my opinion, Frei renders obvious “wherein the ionic strength of the composition is greater than 200 mM,” as recited in claim 1.

f. “wherein the purified AAV vector particles are stored in the composition without significant aggregation”

248. Frei expressly teaches that its stable virus formulations prevented aggregation during storage. For instance, Frei teaches that the virus compositions of its invention are featured with “significantly improved stability” and can “maintain[] the stability of viruses during storage.” Ex.1004, 1:15-19, 4:29-30 (teaching that the

developed buffeted formulation “can preserve viral preparations with enhanced stability”). Frei further teaches that is formulations “are stable at typical refrigeration temperatures of *e.g.*, 2 °C to 8 °C, or higher, for substantial periods of time, preferably for several months or more.” *Id.* at 4:32-34.

249. Frei further tested the light scattering ratios of UV A320/A260 of multiple adenovirus formulations to particularly assess “the aggregation of the virus particles” in its formulations. *Id.* at 12:13-15. Frei discloses that the light scattering ratio (A320/A260) of its D-1 formulation is 0.22. *Id.* at 22:29. Because it was common knowledge that monomeric virus particles display a light scattering ratio (A320/A260) of about 0.22 to 0.30:1, a POSA would have understood that the light scattering ratio of the D-1 formulation is within the range of monomeric virus, and thus, indicates that the adenovirus particles in this formulation had no virus aggregation. *See* Ex.1015, 6:59-63. Frei also demonstrated stability after short- and long-term storage (1 week to 12 months) for multiple adenoviral compositions, albeit at lower virus concentrations. *See, e.g.*, Ex. 1004, Tables 1-5. In view of these teachings and the demonstrated stability of multiple virus formulations, a POSA also would have understood that Frei’s compositions are stable with no virus aggregation during storage. Indeed, as discussed above, Frei’s the D-1 formulation had been shown to have no observable aggregation by UV light scattering analysis.

250. Although Frei's D-1 formulation comprises virus at a concentration of 1.6×10^{13} total virus *particles/ml* and may contain empty AAV capsids, as I explained above in sections V.C and IX.C.1.f, it was known that full vector particles and empty capsids presumably aggregate by a similar mechanism, which is consistent with the common understanding as of June 2004 that the propensity of the particles to aggregate does not depend on the nature of the viral genome packaged in the particles, the family classification of the virus, or its disease-causing properties (or lack thereof). Ex. 1007, 175. Thus, a POSA would have expected that Frei's D-1 formulation, irrespective of whether it contained empty capsids or not, would be as stable as a virus formulation containing $> 1 \times 10^{13}$ vg/ml vector particles, as recited in challenged claim 1. Indeed, because AAV vectors were known to be more stable than adenovirus, a POSA would have expected obvious variants of Frei's D-1 formulation containing $> 1 \times 10^{13}$ vg/ml vector particles to have even better stability compared to the D-1 formulation.

251. Earlier successes in achieving stable formulations comprising high titer rAAV would have reinforced a POSA's expectation of successfully achieving stable formulations comprising concentrations exceeding 1×10^{13} vg/mL AAV vector particles with no aggregation. As I explained above, like Frei, Liu similarly reported that its high ionic strength adenovirus storage formulations showed "no signs of settling or precipitation" and "no significant decrease in particle number" following

storage for 7 days at $\sim 4^{\circ}\text{C}$. Ex. 1009, [00366]-[00369], Table 15. Wright also reported having generated highly purified AAV vector preparations at concentrations of 5×10^{13} capsid particles/ml that “are stable in a non-aggregated, monomeric state when stored at 2 to 8°C .” Ex. 1007, 175. As explained above, those skilled in the art would have understood an AAV formulation with 5×10^{13} capsid particles/ml of virus to be as stable as an AAV formulation with a virus concentration of 5×10^{13} vg/ml, which is squarely within the claim scope of “greater than 1×10^{13} vg/ml.” Thus, Wright’s prior success of achieving stable AAV formulations containing AAV virus at a concentration almost 5-fold higher than that of Frei’s D-1 formulation further strongly supports the expectation that obvious variants of Frei’s D-1 formation comprising greater than 1×10^{13} vg/ml rAAV would have no aggregation.

252. In addition, as I explained above in section IX.C.1.f, I found that the Patent Owner effectively admitted during prosecution of the ’542 patent that the claimed “storage . . . without significant aggregation” is a natural feature of a composition meeting the recited mixture of components. Thus, in my opinion, a rAAV formulation satisfying all requirements of claim 1 would have been obvious over Frei’s teachings in view of Huang and Mingozzi.

2. Claim 2 Would Have Been Obvious Over Frei in View of Huang and Mingozi

253. Claim 2 depends from claim 1 and further requires the AAV composition of claim 1 to contain “ethylene propylene oxide block copolymer Pluronic® F68.” ’542 patent (Ex.1001), 14:27-28. In my opinion, Frei teaches this limitation as well.

254. Specifically, Frei teaches that its compositions can further contain detergents as delivery-enhancing agents, which “enhance[] delivery of a therapeutic gene,” and exemplary detergents that can be used include “PLURONIC®-F68 detergent.” Ex.1004, 7:31-8:10.

255. As I explained in section IX.D, Pluronic® F68 was a commercially available non-ionic detergent already frequently used in pharmaceutical compositions as of June 2004. Even the inventors themselves admitted in an earlier publication that “addition of the surfactants Polysorbate 80 or Pluronic® F68 . . . effectively prevent losses due to non-specific binding during [virus] vector sampling and transfer.” Ex.1007, 175, 176. Thus, a POSA, in view of Frei’s teachings, would have been motivated to include Pluronic® F68 in a modified D-1 composition.

256. There would have been a reasonable expectation of success in using Pluronic® F68 in obvious variants of the D-1 composition in view of Frei’s teachings and the previously proven benefits of Pluronic® F68. First, as Frei teaches,

adding such detergents can enhance the delivery of a therapeutic gene by recombinant virus vectors. Ex.1004, 7:31-35. Second, it had been reported that prior AAV compositions comprising Pluronic® F68 were stable for at least 240 days when stored at 4 °C. Ex.1013, 1284, 1288. Such earlier successful uses of Pluronic® F68 in stable AAV compositions would have further provided a reasonable expectation of maintaining the stability of the modified D-1 composition from Frei after including Pluronic® F68 into the formulation.

257. Thus, in my opinion, the composition of claim 2 also would have been obvious.

3. Claim 5 Would Have Been Obvious Over Frei in View of Huang and Mingozi

258. Claim 5 also depends from claim 1 and further recites “wherein the purified, recombinant AAV vector particles have an average particle size radius (Rh) of less than about 20 nm as measured by dynamic light scattering [DLS].” Ex. 1001, 14:34-37. In my opinion, this claim also would have been obvious over Frei in view of Huang and Mingozi.

259. As I explained, even the '542 patent itself acknowledges that the recited particle size radius as measured by DLS is exemplary of no “significant aggregation.” Ex.1001, 9:25-27. Further as I explained in above in section IX.E.1.f, Frei assessed “the aggregation of the virus particles” in its formulations by the light scattering ratio of UV A320/A260 and reported that the light scattering ratio

(A320/A260) of the D-1 formulation is 0.22 and, therefore, within the range for monomeric virus particles, meaning there was no observable aggregation in the D-1 formulation. Ex.1004, 22:29; Ex. 1015, 6:59-63. Thus, a POSA would have understood that the aggregation level of Frei's D-1 formulation meets the requirement of the challenged claim 5.

260. To the extent claim 5 requires less aggregation than what Frei measured, as I explained above in section IX.C.2, a POSA would have been motivated to eliminate any potential aggregation in view of the known "deleterious consequences" of virus aggregation, such as "[r]educed yield," "an increased host immune response to the viral vector particles," "inconsistency in the functional activity of AAV vectors following their administration *in vivo*," and "less efficient [] gene transfer." Ex.1007, 175, 176; Ex.1005, S286; Ex.1009, [00262]. In view of these teachings, a POSA would have been motivated to prevent aggregation from occurring during storage of AAV formulations such that the AAV vector particles have an Rh of less than about 20 nm as measured by dynamic light scattering.

261. In view of Frei's teachings and prior successes, a POSA would have had a reasonable expectation of successfully developing compositions for storing AAV with no aggregation such that the average AAV Rh is less than about 20nm measured by DLS. As explained above in section IX.E.1.f, Frei teaches that its virus formulations are featured with "significantly improved stability" and can

“maintain[] the stability of viruses during storage” for “substantial periods of time, preferably for several months or more.” Ex.1004, 1:15-18, 4:29-30 (teaching that the developed buffeted formulation “can preserve viral preparations with enhanced stability”), 4:32-34. Thus, Frei provides a POSA with a reasonable expectation of success in achieving stable virus vector compositions that maintain monomeric particles and, therefore, have Rh values less than 20nm.

262. Frei further teaches that all its tested virus formulations had light scattering ratios reflecting the monomeric status of the virus particles and, therefore, exhibited enhanced stability. Ex.1004, Tables 1-5, 22:25-23:20, 22:10-12 (teaching that the salt-containing DEAE pool could be stored “for >10 days at 2-10°C[,], thus allowing for subsequent steps of virus concentration and/or gel filtration to be performed on separate days with substantial flexibility across a 10 day period.”). Given that it was well known that AAV is significantly more stable than adenovirus, Ex.1013, 1283, a POSA would have expected no aggregation for AAV if used in Frei’s formulations including the D-1 formulation.

263. Other prior successes of achieving high titer virus formulations with no observable aggregation would have reinforced the expectation that using AAV in Frei’s D-1 formulation would achieve no aggregation. As explained above, Liu stored purified adenovirus in a storage buffer with a total ionic strength above 200 mM and observed “no signs of settling or precipitation over the 7 day [storage]

period” and “no significant decrease in particle number” during the entire storage period, demonstrating no observable aggregation of the adenovirus particles. Ex.1009, [00369], [00371].

264. Moreover, methods of reducing virus aggregation in viral formulations had been readily available as of 2004, and it would have taken nothing more than routine optimization to prevent any potential virus aggregation in Frei’s D-1 composition. Indeed, it had been known that adjusting ionic strength, pH, buffer, and addition of other known stabilizing agents could reduce virus aggregation. For instance, Wright explained that initial aggregation “could be reversed by adjusting buffer pH.” Ex.1007, 176. Johnson further reported that when the pH of rAAV formulations was adjusted to pH 7.5, AAV vector particle aggregation was eliminated such that “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” Ex.1019, 585, 589. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex. 1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex. 1009, [00263].

265. Using these known tools, those skilled in the art had routinely eliminated virus aggregation in high concentration AAV formulations. For instance, Wright described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are *stable in a non-aggregated, monomeric state* when stored at 2 to

8°C.” Ex.1007, 175 (emphasis added). Huang likewise discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having 5-10 x 10¹³ genome copies/ml. Ex.1005, S286. Thus, only routine optimization of known stability factors would have been required to minimize any residual aggregation and obtain an average AAV Rh of < 20nm in Frei’s D-1 formulation, as measured by DLS, which would have further supported a reasonable expectation of success in achieving the formulation recited in claim 5 of the ’542 patent.

266. Accordingly, the composition of challenged claim 5 also would have been obvious over Frei in view of Huang and Mingozi.

4. Claim 6 Would Have Been Obvious Over Frei in View of Huang and Mingozi

267. Claim 6 depends from claim 1 and further requires that “recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 µm filter.” Ex.1001, 14:38-41. As I explained above in section IX.C.3, a POSA would have understood that this limitation reciting a recovery rate range after passing a 0.22 µm filter, like the particle radius limitation of claim 5, essentially merely requires preventing virus aggregation.

268. As I explained earlier in sections V.C and IX.C.3, 0.22 µm filters had been routinely used to obtain purified virus formulations, and it had been well known that the recovery rate after passing a virus formulation through a 0.22 µm filter

indicates aggregation levels—the higher the recovery rate, the lower the aggregation level. Ex.1009, [00373]; Ex.1007, 175 (“loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation”); Ex.1005, S286 (“the larger the aggregation,” “the less efficient the gene transfer.”). A POSA would have reasonably expected Frei’s D-1 formulation, which Frei reported to have no observable virus aggregation by light scattering data, to have at least 90% of the virus particles in the stored composition recovered following filtration through a 0.22 μm filter. Given that it was well known that AAV is significantly more stable than adenovirus, Ex. 1013, 1283, and that increasing ionic strength improves virus stability (*supra* § V.D.1), a POSA would have expected no aggregation for AAV if used in the obvious variants of Frei’s D-1 formulation discussed above.

269. To the extent claim 6 requires less aggregation than claim 1, as I explained above in section IX.C.3, Wright and Huang expressly teach the correlation between high AAV virus particle recovery rate following a 0.2-μm filtration step and minimized levels of vector aggregation. Ex.1007, 175 (“loss of rAAV following a 0.2-μm filtration step correlates with the extent of vector aggregation.”); Ex.1005, S286 (teaching that “the larger the aggregation,” “the less efficient the gene transfer.”). In view of these teachings, a POSA would have been motivated to reduce any residual aggregation in the obvious variants of Frei’s D-1 formulation discussed above to maximize virus recovery through a 0.22 μm filter.

270. A POSA would have had a reasonable expectation of maximizing particle recovery after filtration in view of prior successes of reducing virus aggregation. For instance, Huang teaches developing formulations that “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” Ex.1005, S286. Liu also observed “no signs of settling or precipitation over the 7 day [storage] period” after purifying and storing its adenovirus particles in a high ionic strength buffer. Ex. 1009, [00369]. A POSA would have expected similar, if not better, results for AAV formulations, as it had been known that AAV “is significantly more stable than the adenovirus.” Ex.1013, 1283.

271. As I explained above in section IX.C.3, to the extent a POSA would have needed to prevent any potential aggregation in the obvious variants of Frei’s D-1 composition discussed above, such modifications would have merely required the routine optimization of known formulation parameters, such as adjusting ionic strength, pH, buffer, and other known stabilizing agents. For instance, as I explained, it was known that increasing ionic strength would improve virus formulation stability and reduce aggregation. *See supra* § IX.C.3. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex.1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex.1009, [00263]. Adjusting pH to close to 7.5 had also been known to eliminate AAV particle aggregates. Ex.1019, 585, 589.

272. Wright similarly explained that initial aggregation “could be reversed by adjusting buffer pH” and described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are ***stable in a non-aggregated, monomeric state*** when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added), 176. Huang likewise discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5\text{-}10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. Thus, only routine optimization of known stability factors would have been required to improve the recovery of AAV following filtration through a 0.22 μm filter.

273. Thus, in my opinion, the composition of claim 6 would also have been obvious over Frei in view of Huang and Mingozi.

F. Claims 1, 2, 5, and 6 Are Obvious Over Liu in View of Huang and Mingozi

274. As I explained above, claims 1, 2, 5, and 6 of the '542 patent cover virus formulations that require known virus concentrations, multivalent ions, surfactants, and pH, which naturally prevent aggregation. In my opinion, the challenged claims would also have been obvious over Liu in view of Huang and Mingozi as of June 2004, based on the general knowledge in the art as evidenced by Wright, Clark, Gatlin, and Croyle.

1. Claim 1 Would Have Been Obvious Over Liu in View of Huang and Mingozi

275. Liu's Example 17 describes an adenovirus composition that (1) was used to store adenovirus particles for an extended period of time; (2) contains 1.62×10^{10} vector particle units of adenovirus; (3) contains a pH buffer that maintains a pH of the composition of 7.5; (4) contains magnesium multivalent ions as excipients; (5) has a total ionic strength of over 200 mM; and (6) showed no sign of aggregation during the storage time. As I further explain below, using rAAV in Liu's Example 17 formulation and increasing the virus concentration to $> 1 \times 10^{13}$ vg/ml would have been obvious to a POSA as of June 2004 in view of teachings of Liu, Huang, and Mingozi. In my opinion, therefore, a rAAV composition meeting the limitations of claim 1 would have been obvious over Liu in view of Huang and Mingozi.

a. "A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particle"

276. Liu relates to methods of "preparing viral vector particles and viral vector particle compositions" and provides purified viral compositions "comprising a significant number and concentration of viral vector particles (e.g., at least about 1×10^{12} PU)." Ex.1009, [0006].

277. Liu teaches that suitable viral vector particles include “adenoviral vector particles (including any virus of or derived from a virus of the adenoviridae), adeno-associated viral vector particles (AAV vector particles).” *Id.* at [0008]. Liu further teaches that the viral vector particles are “preferably” non-enveloped viral vector particles including “AAV vectors” and “[p]articularly preferred types of viral vector particles include adeno-associated viral vector particles and adenoviral vector particles.” *Id.* at [0011]. Liu also teaches that “[t]he viral vector particle desirably includes one or more heterologous nucleic acid sequences,” which a POSA would have understood as referring to recombinant viral vector particles. *Id.* at [0024]. Thus, Liu teaches that the preferred and desirable viral vector particles in its compositions can be recombinant AAV viral vector particles.

278. Liu’s Example 17 “demonstrates the ability of the storage compositions of the invention to effectively maintain a stable population of adenoviral vector particles during the viral vector particle production and/or purification processes.” *Id.* at [00365]. Liu shows that the produced adenoviral particles were purified and concentrated by microfiltration through a triple-filter clarification and subsequently by filter diafiltration via tangential flow filtration, and then stored in a storage buffer comprising “25mM Tris, 300mM NaCl, 5mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5).” *Id.* at [00366].

279. Liu discloses that the viral particles were maintained in the storage buffer for seven days. *Id.* at [00367]. Liu reports that “[v]isual inspection of the glass tubes showed no signs of settling or precipitation over the 7 day period” and that “[n]o significant change in the number of viral particles or infectious viral particles was observed at any of the times tested.” *Id.* at [00369], [00371]. Liu concludes that “[t]hese results demonstrate that viral vector compositions can be stably stored in the temporary storage buffers of the invention for ***extended periods of time.***” *Id.* at [00371] (emphasis added).

280. Indeed, Liu teaches that whereas the triple-microfiltration filter clarification used in Example 17 can occur any time during the purification process, “the diafiltration with the temporary storage buffer preferably occurs last in the series” at the end of the purification process. *Id.* at [00154]; [00197]. This is consistent with the sequence of purification steps in Example 17. Thus, a POSA would have understood that the composition in Liu Example 17 was used to store purified viral vector particles for an extended period of time. As such, Liu’s Example 17 discloses a composition for storage of concentrated and purified adenovirus vector particles.

281. Based on these teachings, a POSA would have understood that Liu discloses compositions suitable for the storage of purified, recombinant virus vector particles, comprising purified, recombinant adenovirus vector particles. Because Liu

also expressly teaches that the particularly preferred types of viral vector particles in its compositions include AAV vector particles and are desirably recombinant, a POSA would have been motivated to make versions of Liu's Example 17 composition comprising purified, recombinant AAV vector particles. *Id.* at [0011] ("Particularly preferred types of viral vector particles include adeno-associated viral vector particles"), [0024].

282. As I explained earlier in sections V.A, IX,C,1.a, and IX.E.1.a, Mingozi, Wright, and Clark, taught the attractiveness and advantages of AAV vectors in gene therapy, and would have further motivated a POSA to use AAV in Liu's disclosed adenovirus compositions such as its Example 17 composition. *See supra* §§ V.A, IX,C,1.a, and IX.E.1.a; Ex.1006, 10497 ("excellent safety profile combined with reduced potential for activation of inflammatory or cellular immune responses has made this vector system attractive for clinical application and treatment of genetic disorders."); Ex. 1007, 174-75 (teaching AAV has "shown significant promise for human gene therapy"); Ex 1012, S-9 (promoting AAV's ability "to mediate long-term, robust in vivo gene expression in numerous cell types").

283. A POSA would have reasonably expected success in using AAV vector particles in Liu's Example 17 composition in view of Liu's express teachings that AAV is one of the two particularly preferred viral vector particles to use in its

compositions. Ex.1009, [0011]. In addition, because it was well known that AAV is “significantly more stable than the adenovirus,” a POSA would have reasonably expected Liu’s compositions to provide similar, if not better stability for storing AAV particles. Ex.1013, 1283.

284. Thus, in my opinion, Liu teaches and renders obvious “[a] composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particles,” as recited in claim 1.

b. “at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml”

285. In Example 17, Liu placed 50 mL of the purified adenoviral vector particle composition into a sterile bag for storage. Ex.1009, [00367]-[00368]. Liu reports that the bag contained 1.62×10^{10} viral particle units (PU) on day 0. *Id.* at [00369], Table 15, [00126] (defining PU as “viral particle units” or “total viral vector particle particles”). A POSA would have understood that the virus concentration of Liu’s purified adenoviral vector particle composition was, therefore, $(1.62 \times 10^{10})/50 \text{ ml} = 3.24 \times 10^8$ (viral particles) vp/ml.

286. Even if Liu’s composition contained a portion of empty viral capsids, a POSA would have been motivated to remove them and increase the concentration of particles with vector genomes in view of Liu’s teachings and prior knowledge of the high doses and high concentrations of genome-containing particles that are

required for therapeutic gene transfer. Specifically, Liu itself expressly teaches eliminating empty capsids from the storage composition in Example 17, stating that an “advantage of the methods of the invention is in the reduction in the number of empty viral vector particles,” and “[o]ptimally, practically 0% of the adenoviral vector particles in the composition are empty capsids.” *Id.* at [00281]. This is consistent with the common knowledge that empty AAV capsids reduce the efficiency of AAV gene therapies. Ex. 1007, 175.

287. A POSA would have been motivated to increase the concentrations of genome-containing virus particles after removing empty capsids (if any) from Liu’s Example 17 formulation in view of prior knowledge of the desired high concentrations and doses required for gene therapy. For instance, Huang teaches that “[t]o achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes.” Ex. 1005, S286. Mingozi also reported that administering AAV vector average doses of 10^{12} vg/kg to dogs resulted in sustained transgene expression. Ex. 1006, 10497. As I explained in section V.B, average doses of 10^{12} vg/kg correspond to average doses of 3.2×10^{13} vector genomes for a 60 kg human.

288. In addition, AAV-based gene therapies are typically administered parenterally, which require the high doses of rAAV vector particles to be concentrated in a small volume of only a few milliliters. Ex. 1007, 175- 177; Ex.

1006, 10497; Ex. 1008, 405, 417-18. For instance, Gatlin teaches that a drug product administered parenterally generally requires small volumes. Ex. 1008, 405, (teaching using volumes as small as 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery); *see also* 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections), 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL” for intramuscular injection). Thus, a POSA would have been motivated to increase the genome-containing virus particle concentration in Liu’s Example 17 storage composition to prepare therapeutically useful amounts of gene therapy for parenteral administration.

289. A POSA would have been particularly motivated to increase the viral vector concentration in Liu’s Example 17 composition to $> 1 \times 10^{13}$ vg/ml in view of prior knowledge and achievement of AAV compositions at concentrations above 1×10^{13} vg/ml. For instance, Huang described gene transfer experiments with AAV compositions having concentrations of 5-10 x 10^{13} genome copies/ml. Ex.1005, S286 (emphasis added). As explained above, those skilled in the art would understand that genome copies/ml and virus genome/ml are virus concentration units used interchangeably to reflect the number of genome-containing particles. Huang, thus, teaches achieving gene transfer using compositions having AAV concentrations of 5-10 x 10^{13} vg/ml. Although Huang acknowledges that gene transfer efficiency was lower with compositions having these AAV concentrations

compared to corresponding compositions having lower concentrations, Huang reports developing multiple AAV formulations, which effectively reduced aggregation by up to 50%. *Id.*

290. Mingozi similarly reports preparing compositions containing purified AAV-2 and AAV-5 at concentrations of “> **10^{13}** vg/ml as determined by quantitative slot blot analysis,” and then using those compositions to achieve successful gene transfer in mice. Ex.1006, 10497-98 (emphasis added). Specifically, Mingozi reports that AAV doses averaging 10^{12} vg/kg resulted in sustained transgene expression in a dog model, which corresponds to doses of approximately 3.2×10^{13} vg for a 60 kg human. *Id.*, 10497. Likewise, Wright teaches that prior to June 2004, using dynamic light scattering, the inventors had observed “**highly purified** vector preparations at concentrations of 5×10^{13} cp/ml that are stable in a non-aggregated, monomeric state when stored at 2 to 8°C” without freeze-thaw cycles. Ex.1007, 175 (emphasis added). In view of these teachings, a POSA would have been motivated to increase the AAV concentration in Liu’s Example 17 composition to a level of > 1×10^{13} vg/ml.

291. A POSA also would have expected success in removing empty capsids and achieving such concentrations in view of Liu’s teachings and prior successes known to those skilled in the art. As explained above, Liu teaches that its purification methods provide an advantage of “reduction in the number of empty viral vector

particles,” and “[o]ptimally, practically 0% of the adenoviral vector particles in the composition are empty capsids.” Ex.1009, [00281]. Liu teaches achieving high-yield production of viral vector particles of about 1×10^{15} particle units/cell, their purification, concentration, and preparation in buffers to form storage compositions. *E.g., id.* at [0068], [00187], [00203], [00371] (emphasis added). Preparing such highly pure virus particles for parenteral administration in small volumes, as taught by Gatlin, would have been reasonably expected to result in a concentration of above 10^{13} vg/ml. Ex. 1008, 405 (teaching using volumes as small as 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery); *see also* 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections), 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL”).

292. Moreover, as I explained above in section IX.C.1.b, by June 2004, methods of generating high yields of AAV, removing empty capsids, and concentrating genome-containing vectors were well-known. For instance, Clark reported that stable cell lines can yield $> 1 \times 10^{14}$ AAV particles per large-scale preparation. Ex.1012, S-12. Wright taught the use of cesium chloride and iodixanol gradient ultracentrifugation for separating “genome-containing[] vector particles from the lighter empty capsids.” Ex.1007, 175. A POSA would have understood that such methods, in addition to Liu’s methods, could be used to successfully remove

empty capsids while maintaining the high concentration of purified AAV in the obvious variants in Liu's Example 17 composition discussed above.

293. Huang's and Mingozzi's successes in achieving AAV formulations having concentrations above 1×10^{13} vg/ml would have further provided a reasonable expectation of success. Huang described AAV compositions having concentrations of $5\text{-}10 \times 10^{13}$ genome copies/ml, which a POSA would understand as equivalent to achieving AAV concentrations of $5\text{-}10 \times 10^{13}$ vg/ml. Ex.1005, S286 (emphasis added). Huang further reported effectively reducing aggregation by up to 50% to maintain stability of such high concentration AAV formulations. *Id.* Mingozzi reported preparing compositions containing purified AAV-2 and AAV-5 at concentrations of " $>10^{13}$ vg/ml," and administering doses of 2.5×10^{12} vg to mice, which can be converted to roughly 4.8×10^{13} in humans, and achieving successful gene transfer in mice. Ex.1006, 10497-98 (emphasis added). These teachings would have further reinforced a POSA's reasonable expectation of success in achieving AAV formulations with concentrations of exceeding 1×10^{13} vg/ml based on Liu's Example 17 composition.

294. Thus, in my opinion, "a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml," as recited in challenged claim 1, would have been obvious.

c. “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0”

295. Liu discloses that the composition in Example 17 comprises a population of adenoviral vector particles in a storage buffer comprising 25mM Tris, 300mM NaCl, 5mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5. Ex.1009, [00366]. As I explained in section X.D.2, Tris had been a well-known pH buffer routinely used in pharmaceutical compositions. Ex.1008, 408, Table 17.3. In addition, the pH of the composition in Example 17 is 7.5, which is within the range of “between 7.5 to 8.0” recited in claim 1.

296. Thus, in my opinion, a POSA would understand that Liu directly teaches “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0,” as recited in challenged claim 1.

d. “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate”

297. Liu discloses that the composition in Example 17 comprises a population of adenoviral vector particles in a storage buffer comprising 25mM Tris, 300mM NaCl, **5mM MgCl₂**, 0.0025% polysorbate 80, 5% trehalose, pH 7.5. Ex. 1009, [00366]. As such, the composition in Example 17 contains excipients comprising magnesium multivalent ions.

298. Thus, in my opinion, a POSA would understand that Liu directly teaches “excipients comprising one or more multivalent ions selected from the group

consisting of citrate, sulfate, magnesium, and phosphate,” as recited in challenged claim 1.

e. “wherein the ionic strength of the composition is greater than 200 mM”

299. Liu discloses that the composition in Example 17 comprises “a population of adenoviral vector particles in a temporary storage buffer (25mM Tris, 300mM NaCl, 5mM MgCl₂, 0.0025% polysorbate 80, 5% trehalose, pH 7.5).” Ex. 1009, [00366].

300. A POSA would have understood that the total ionic strength of this composition, including the 300 mM NaCl and 5 mM MgCl₂, is $\frac{1}{2} \times (300 \text{ mM} \times (+1)^2 + 300 \text{ mM} \times (-1)^2 + 5 \text{ mM} \times (+2)^2 + 10 \text{ mM} \times (-1)^2) = 315 \text{ mM}$, which is greater than 200 mM.

301. Thus, in my opinion, Liu teaches the limitation “wherein the ionic strength of the composition is greater than 200 mM,” as recited in claim 1.

f. “wherein the purified AAV vector particles are stored in the composition without significant aggregation”

302. In Example 17, Liu tested the stability of the virus composition over a storage period of 7 days and reports that “[v]isual inspection of the glass tubes showed no signs of settling or precipitation over the 7 day period.” Ex.1009, [00369]. Liu further reports that “[n]o significant change in the number of viral particles or infectious viral particles was observed at any of the times tested.” *Id.*,

[00371]. Liu concludes that “[t]hese results demonstrate that viral vector compositions can be stably stored in the temporary storage buffers, during which the equipment of the automated closed production process can be assessed and repaired if required.” *Id.*

303. As I explained in section IX.C.1.f, a POSA would have understood that virus particle aggregation would result in large agglomerate sizes that may precipitate and reduce the numbers of evenly distributed monomeric particles in suspension. Thus, a POSA would understand that Liu’s stability test in Example 17 shows that within the tested 7 days, its composition containing 3.24×10^8 vp/ml adenovirus exhibited no “significant aggregation” as required by claim 1. Because AAV was known to be “significantly more stable than the adenovirus,” a POSA would have reasonably expected that obvious variants of Liu’s Example 17 composition comprising 3.24×10^8 vp/ml AAV particles would exhibit even greater stability than the adenovirus composition tested in Liu’s Example 17. Ex.1013, 1283.

304. A POSA would have expected virus aggregation in a composition containing a concentration of 3.24×10^8 **vg/ml** AAV virus particles to be at a similarly negligible level as the obvious variants of Liu’s Example 17 composition comprising 3.24×10^8 **vp/ml** AAV virus particles. This is because, as discussed above, empty capsids and full vector particles were understood to “aggregate by a similar

mechanism,” which is consistent with the common knowledge that the propensity of the particles to aggregate does not depend on the nature of the viral genome packaged in the particles. Ex.1007, 175.

305. A POSA likewise would have expected obvious variants of Liu’s composition comprising AAV particles to have prevented aggregation at even higher virus concentrations, in view of prior successes of achieving reduced or non-observable aggregation for high concentration AAV formulations. As I explained, Wright had already reported that highly purified AAV vector preparations at concentrations of 5×10^{13} *cp/ml* “are stable in a non-aggregated, monomeric state when stored at 2 to 8°C” without a freeze-thaw cycle. Ex. 1007, 175. Because empty capsids and genome-containing particles were known to contribute equally to concentration-induced aggregation, a POSA would have expected that an AAV composition containing 5×10^{13} vg/ml AAV would be similarly stable in a non-aggregated, monomeric state during storage, meeting the recited “without significant aggregation” limitation in challenged claim 1. *Id.*

306. To the extent the obvious variants of Liu’s composition discussed above do not prevent aggregation, a POSA would have been motivated to further minimize any potential aggregation in view Liu’s teachings of the undesirability of virus aggregation. For instance, Liu teaches that “[t]he presence of viral vector particle aggregates is unfavorable since the clumping of the viral vector particles

could result in an increased host immune response to the viral vector particles.” Ex.1009, [00262]. Liu further teaches that when there is virus aggregation, “it is more likely that more than one viral vector particle will infect any particular cell, which is undesirable.” *Id.*

307. Liu’s teachings are consistent with the POSA’s prior knowledge of the virus aggregation problem reflected in Huang and Wright. For instance, Huang acknowledges that AAV aggregates reduce the overall gene transfer efficiency. Ex.1005, S286. Wright also acknowledged that AAV vector particle aggregation is a known “issue for vector formulation and stability” and that a “potentially deleterious consequence of vector aggregation is inconsistency in the functional activity of AAV vectors following their administration *in vivo*.” Ex. 1007, 176. Thus, a POSA would have been motivated to store AAV vectors in compositions that prevent particle aggregation.

308. A POSA would have reasonably expected to achieve stable formulations comprising about 1×10^{13} vg/mL AAV vector particles with no aggregation. As I explained above, Liu’s own teachings and working examples and the known superior stability of AAV over adenovirus all support improved stability of formulations comprising about 1×10^{13} vg/mL AAV vector particles with no observable aggregation.

309. Liu reported that its high ionic strength adenovirus storage formulations showed “no signs of settling or precipitation” and “no significant decrease in particle number” following storage for 7 days at $\sim 4^{\circ}\text{C}$, which a POSA would have understood as showing no observable virus aggregation. Ex.1009, [00366]-[00369], Table 15. Liu further teaches achieving high-yield production of viral vector particles of about 1×10^{15} particle units/cell, their purification and concentration, and incorporating them into storage compositions. *E.g.*, *id.*, [0068], [00187], [00203], [00371]. Importantly, Liu teaches that if virus aggregation occurs in the composition, the aggregation can be reduced by “addition of surfactants.” *Id.* at [00263]. Thus, by showing achievement of virus formulations without observable aggregation, teaching methods to achieve higher yields of virus particles, and providing tools to reduce aggregation, Liu provides a reasonable expectation of achieving obvious variants of its Example 17 composition comprising AAV concentrations of 1×10^{13} vg/ml with no aggregation.

310. Earlier successes reflected in Wright would have further reinforced the expectation of no “significant aggregation” in such AAV formulations. Specifically, Wright reported having generated highly purified AAV vector preparations at concentrations of 5×10^{13} capsid particles/ml that “are stable in a non-aggregated, monomeric state when stored at 2 to 8°C .” Ex.1007, 175. And as explained above, those skilled in the art would have understood an AAV formulation with 5×10^{13}

capsid particles/ml of virus to be as stable as an AAV formulation with a virus concentration of 5×10^{13} vg/ml, which is squarely within the claim scope of “greater than 1×10^{13} vg/ml.” Thus, Wright’s prior success of achieving stable AAV formulations containing AAV virus at a concentration almost 5-fold higher than that of challenged claim 1 further strongly supports the expectation that a formulation developed from Liu’s Example 17 composition that comprises greater than 1×10^{13} vg/ml rAAV would have no aggregation.

311. Thus, for all the reasons stated above, I conclude that challenged claim 1 would have been obvious over the prior art.

2. Claim 2 Would Have Been Obvious Over Liu In View Of Huang And Mingozi

312. Claim 2 recites a composition of claim 1 “further comprising ethylene propylene oxide block copolymer Pluroinic® F68.” Ex.1001, 14:27-28. In my opinion, this additional limitation also would have been obvious.

313. For instance, Liu specifically teaches that “[i]n another preferred embodiment, the temporary storage composition further comprises a nonionic surfactant,” including “nonionic surfactants [] well known in the art,” such as “Pluronic F68.” Ex.1009, [00189].

314. As I explained in section IX.D, Pluronic® F68 was a well-known commercially available non-ionic detergent frequently used in pharmaceutical compositions as of June 2004. Even the inventors themselves admitted in an earlier

publication that “addition of the surfactants Polysorbate 80 or Pluronic® F68 . . . effectively prevent losses due to non-specific binding during [virus] vector sampling and transfer.” Ex.1007, 175, 176. Thus, a POSA, in view of Liu’s teachings, would have been motivated to include Pluronic® F68 in Liu’s Example 17 composition.

315. There would have been a reasonable expectation of success in using Pluronic® F68 in Liu’s Example 17 composition in view of previously proven benefits of Pluronic® F68. It had been reported that prior AAV compositions comprising Pluronic® F68 were stable for at least 240 days when stored at 4 °C. Ex.1013, 1284, 1288. Such earlier successful uses of Pluronic® F68 in stable AAV compositions would have further provided a reasonable expectation of maintaining the stability of the Liu Example 17 composition after including Pluronic® F68 into the formulation.

316. Thus, in my opinion, the composition of claim 2 would have been obvious.

3. Claim 5 Would Have Been Obvious Over Liu in View of Huang and Mingozi

317. Claim 5 also depends from claim 1 and further recites “wherein the purified, recombinant AAV vector particles have an average particle size radius (Rh) of less than about 20 nm as measured by dynamic light scattering [DLS].” Ex. 1001,

14:34-37. In my opinion, this claim also would have been obvious over Liu in view of Huang and Mingozzi.

318. As I explained already in section V.C, even the '542 patent itself similarly acknowledges that the recited particle size radius as measured by DLS is exemplary of no “significant aggregation.” Ex.1001, 9:25-27. Thus, for the reasons I discussed above for section IX.F.1.f, a POSA would have reasonably expected that AAV particles stored in the obvious variants of Liu’s Example 17 composition discussed above with no aggregation also have an Rh of less than about 20 nm measured by DLS.

319. To the extent modifications to Liu’s composition, beyond those described above, would be required to achieve the features of claim 5, a POSA would have been motivated to make such changes to minimize any potential aggregation. As I explained above in section IX.C.2, Liu as well as other prior art, acknowledged that virus aggregation is “unfavorable,” “undesired,” and has “deleterious consequences” such as “[r]educed yield,” “an increased host immune response to the viral vector particles,” “inconsistency in the functional activity of AAV vectors following their administration *in vivo*,” and “less efficient [] gene transfer.” Ex.1009, [00262]; Ex.1007, 175, 176; Ex.1005, S286. In view of these teachings, a POSA would have been motivated to prevent aggregation from occurring during storage of AAV formulations such that the AAV vector particles have an Rh of less than about

20 nm as measured by dynamic light scattering. A POSA would have reasonably expected success in minimizing particle size, based on Liu's teachings, availability of known aggregation reduction tools, and prior successes reflected in the art. As I explained, Liu taught that its aggregation measuring experiments did not detect any sign of substantial virus aggregation in the tested adenovirus formulation and "demonstrate that viral vector compositions can be stably stored in the temporary storage buffers of the invention for extended periods of time." Ex.1009, [00369], [00371]. A POSA would have understood that obvious variants of the tested adenovirus formulations comprising AAV would have prevented aggregation because AAV "is significantly more stable than the adenovirus." Ex.1013, 1283. Liu also teaches reducing potential aggregation by addition of surfactants, further supporting a reasonable expectation of success in eliminating any potential residual aggregation to achieve monomeric AAV vector particles with an average Rh of less than about 20 nm. Ex.1009, [00263].

320. Indeed, as I explained above in section V.C, methods of reducing virus aggregation in viral formulations had been readily available as of 2004, and it would have only taken routine optimization to prevent virus aggregation in obvious variants of Liu Example 17 composition comprising concentrations exceeding 1×10^{13} vg/ml AAV vector particles. Specifically, it had been known that adjusting ionic strength, pH, buffer, and addition of other known stabilizing agents could reduce virus

aggregation. For instance, Wright explained that initial aggregation “could be reversed by adjusting buffer pH.” Ex.1007, 176. Johnson further reported that when the pH of an rAAV formulation was adjusted to pH 7.5, AAV vector particle aggregation was eliminated such that “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” Ex.1019, 585, 589. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex.1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex.1009, [00263].

321. Using these known tools, those skilled in the art had routinely eliminated virus aggregation in high concentration AAV formulations. For instance, Wright described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are *stable in a non-aggregated, monomeric state* when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added). Huang likewise discussed a successful “30-50% reduction in the size of aggregates” in high-titer formulations having 5-10 x 10^{13} genome copies/ml. Ex. 1005, S286. Thus, only routine optimization of known stability factors would have been required to minimize any residual aggregation and obtain an average AAV Rh of < 20nm as measured by DLS, which would have further supported a reasonable expectation of success in arriving at the obvious variants of Liu’s Example 17 formulation discussed above.

322. Thus, in my opinion, the composition of challenged claim 5 also would have been obvious over Frei in view of Huang and Mingozi.

4. Claim 6 Would Have Been Obvious Over Liu in View of Huang and Mingozi

323. Claim 6 recites a composition of claim 1, wherein “recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.” Ex. 1001, 14:38-41. As I explained above in sections IX.C.3 and IX.E.4, a POSA would have understood that this limitation reciting a recovery rate range after passing a 0.22 μm filter, like claim 5, essentially merely requires preventing aggregation. In my opinion, this claim also would have been obvious over Liu in view of Huang and Mingozi.

324. Liu teaches that “[a]n eluate from the size-exclusion chromatography resin was obtained and subjected to filtration using a 0.22 μm filter to yield a purified adenoviral vector particle composition” with improved stability. Ex. 1009, [00373]. It also had been well known that recovery rates after passing the virus formulations through a 0.22 μm filter indicate the level of aggregation—the higher the recovery rate, the lower the aggregation level. Ex.1007, 175 (“loss of rAAV following a 0.2- μm filtration step correlates with the extent of vector aggregation”); Ex.1005, S286 (“the larger the aggregation,” “the less efficient the gene transfer.”). A POSA would have reasonably expected that obvious variants of Liu’s Example 17 composition,

which Liu reported to have no observable virus aggregation, will have at least 90% of the virus particles in the stored composition recovered following filtration through a 0.22 μm filter. Given that it was well known that AAV is significantly more stable than adenovirus, Ex.1013, 1283, a POSA would have expected even better stability for AAV if used in Liu's Example 17 formulation.

325. To the extent claim 6 requires less aggregation than claim 1, as I explained above in sections IX.C.3 and IX.E.4, Wright and Huang expressly teach the correlation between high AAV virus particle recovery rate following a 0.2- μm filtration step and minimized level of vector aggregation. Ex.1007, 175 (“loss of rAAV following a 0.2- μm filtration step correlates with the extent of vector aggregation.”); Ex.1005, S286 (teaching that “the larger the aggregation,” “the less efficient the gene transfer.”). In view of these teachings, a POSA would have been motivated to prevent aggregation in the obvious variants of Liu's Example 17 formulation discussed above that contain AAV in order to maximize virus recovery through a 0.22 μm filter.

326. A POSA would have had a reasonable expectation of successfully maximizing particle recovery after filtration through a 0.22 μm filter in view of Liu's teachings and prior successes of reducing aggregation. For instance, after purifying and storing its adenovirus particles in a high ionic strength buffer, Liu observed “no signs of settling or precipitation over the 7 day period” and “[n]o significant change

in the number of viral particles or infectious viral particles [] at any of the times tested.” Ex.1009, [00369], [00371]. A POSA would have expected similar results for AAV formulations, as it had been known that AAV “is significantly more stable than the adenovirus.” Ex.1013, 1283. Huang also teaches having developed formulations that “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” Ex.1005, S286.

327. As I explained above, preventing aggregation would have merely been a matter of routine optimization of the obvious variants of Liu’s Example 17 formulation discussed above, by adjusting, for example, ionic strength, pH, buffer, and other known stabilizing agents. For instance, as I explained, it was known that increasing ionic strength would improve virus formulation stability and reduce aggregation. *See supra* § IX.C. It was also known that adding a free radical scavenger or a chelating agent could further be used to enhance stability and reduce aggregation. Ex.1003, 7:10-12. “[A]ddition of surfactants” also was known to “reduce aggregation.” Ex. 1009, [00263]. Adjusting pH to close to 7.5 had also been known to eliminate AAV particle aggregates. EX.1019, 585, 589.

328. Wright similarly explained that initial aggregation “could be reversed by adjusting buffer pH” and described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are ***stable in a non-aggregated, monomeric state*** when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added), 176. Huang likewise

discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5-10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. Thus, only routine optimization of known stability factors would have been required to improve the recovery of AAV following filtration through a 0.22 μ m filter.

329. Thus, in my opinion, the composition of claim 6 would also have been obvious over Liu in view of Huang and Mingozi.

G. Claims 1, 2, 5, and 6 Are Obvious Over Lochrie in View of Huang Mingozi, Johnson, and Liu

330. As I explained above, claims 1, 2, 5, and 6 of the '542 patent cover virus formulations that require known virus concentrations, multivalent ions, surfactants, and pH, and naturally achieve reduced aggregation. In my opinion, the challenged claims would have been obvious over Lochrie in view of Huang, Mingozi, Johnson, and Liu as of June 2004, based on the general knowledge in the art as evidenced by Wright, Clark, Gatlin, and Croyle.

1. Claim 1 Would Have Been Are Obvious Over Lochrie in View of Huang, Mingozi, Johnson, and Liu

331. As I further elaborate below, Lochrie's Example 2 describes an adenovirus composition that (1) was used to store purified rAAV virions at a concentration of 4×10^{12} vg/ml; (2) uses NaH_2PO_4 as a pH buffer to maintain the pH of the composition at 7.4; (3) contains magnesium multivalent ions as excipients; (4) uses phosphate as a multivalent ion excipient; and (5) has a total ionic strength of

almost 200 mM. As I further explain below, modest modifications of Lochrie's Example 2 composition would have been obvious to a POSA with knowledge in the art as of June 2004 in view of the teachings of Huang, Mingozzi, Johnson, and Liu. In my opinion, a rAAV composition meeting the limitations of claim 1 also would have been obvious over Lochrie in view of Huang, Mingozzi, Johnson, and Liu.

a. **“A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particle”**

332. Lochrie teaches “efficient and commercially viable methods for producing stocks of rAAV virions with reduced amounts of empty capsids” and generates “a **stock** of rAAV virions substantially free of empty AAV capsids.” Ex. 1010, 5:2-4, 7:1-3 (emphasis added). A POSA would have understood Lochrie's discussions of generating “stocks” of purified rAAV virions as teaching compositions for storage of purified rAAV vector particles.

333. Lochrie's Example 2 further describes the production and purification of rAAV virions that were subsequently “formulated” in 20mM NaH₂PO₄, 150mM NaCl, 5% sorbitol, and 0.1% Tween-80, at pH 7.4, with the rAAV vector particles at a concentration of 4×10^{12} vg/ml. *Id.* at 28:22-29:14. These teachings establish that Lochrie's stock solutions containing purified rAAV virions are compositions for rAAV storage.

334. Thus, in my opinion, Lochrie teaches and renders obvious “[a] composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising: purified, recombinant AAV vector particles,” as recited in claim 1.

b. “at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml”

335. Lochrie’s Example 2 composition contains rAAV vector particles at a concentration of 4×10^{12} vg/ml. *Id.* at 28:22-29:14.

336. As discussed above, a POSA would have been motivated to increase the concentration of Lochrie’s composition to $>1 \times 10^{13}$ vg/mL to prepare therapeutically useful amounts of AAV particles for parenteral administration in view of prior knowledge of desired high AAV concentrations and doses for gene therapy. See § IX.F.1.b (citing Huang, Mingozi, Clark, Gatlin).

337. For instance, Huang teaches that “[t]o achieve high level of gene transfer and ensure the safety of vector administration it is desirable to deliver high doses of vector in small volumes.” Ex.1005, S286. Mingozi also reported that administering AAV vector doses averaging 10^{12} vg/kg to dogs resulted in sustained transgene expression, which can be converted to roughly 3.2×10^{13} vg for a 60 kg human. Ex.1006, 10497.

338. In addition, AAV-based gene therapies are typically administered parenterally, which require the high doses of rAAV vector particles to be

concentrated in a small volume of only a few milliliters. Ex.1007, 175, 176, 177; Ex.1006, 10497; Ex.1008, 405, 417-18. For instance, Gatlin teaches that a drug product administered parenterally generally requires small volumes. Ex.1008, 405, (teaching using volumes as small as 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml for intradermal delivery); *see also* 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections), 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL” for intramuscular injection). Thus, a POSA would have been motivated to increase the AAV particle concentration in Lochrie’s Example 2 formulation to prepare therapeutically useful amounts of of AAV particles for parenteral administration.

339. A POSA would have been particularly motivated to increase the AAV concentration in Lochrie’s Example 2 formulation to exceed 1×10^{13} vg/ml in view of the prior knowledge and achievement of AAV compositions at concentrations above 1×10^{13} vg/ml. For instance, Huang described AAV compositions having concentrations of 5-10 x 10^{13} genome copies/ml. Ex.1005, S286 (emphasis added). As explained above, those skilled in the art would understand that genome copies/ml and virus genome/ml are virus concentration units used interchangeably to reflect the number of genome-containing particles. Huang, thus, teaches achieving AAV concentrations of 5-10 x 10^{13} vg/ml. Although Huang acknowledges that gene transfer efficiency was lower with compositions having these AAV concentrations,

Huang reports developing multiple AAV formulations, which effectively reduced aggregation by up to 50%. *Id.*

340. Mingoizzi similarly reports preparing compositions containing purified AAV-2 and AAV-5 at concentrations of “> **10^{13}** vg/ml as determined by quantitative slot blot analysis,” and then using those compositions to achieve successful gene transfer in mice. Ex.1006, 10497-98 (emphasis added). Specifically, Mingoizzi reports that AAV doses averaging 10^{12} vg/kg resulted in sustained transgene expression in a dog model, which corresponds to doses of approximately 3.2×10^{13} vg for a 60 kg human. *Id.* at 10497. Likewise, Wright teaches that using dynamic light scattering, it had been observed that “**highly purified** vector preparations at concentrations of 5×10^{13} cp/ml [] are stable in a non-aggregated, monomeric state when stored at 2 to 8°C” without freeze-thaw cycles. Ex.1007, 175 (emphasis added).

341. In view of these teachings, a POSA would have been motivated to increase the AAV concentration in Lochrie’s Example 2 composition to a level exceeding 1×10^{13} vg/ml. A POSA would have reasonably expected success in achieving such concentrations, since Lochrie itself provides methods for generating high yields of AAV, removing empty capsids, and concentrating genome-containing vectors. Specifically, Lochrie teaches that “[t]he present invention involves reducing the numbers of, or eliminating, empty capsids contained within purified stocks of

AAV virions, with minimal loss to packaged capsids contained therein.” Ex.1010, 5:15-17, 17:9-11 (“Once stocks of AA V virions are produced, a number of methods, detailed below, can be used to determine infectious titers and to purify packaged genomes away from empty capsids”). Lochrie further acknowledges that “rAAV virions can be purified from the host cell using a variety of conventional purification methods, such as column chromatography, CsCl gradients, and the like” before empty capsids are recovered while maintaining high concentration of genome-containing virus particles. *Id.* at 24:5-25. These teachings would have provided a reasonable expectation for a POSA to concentrate AAV particles and achieve a high concentration rAAV formulation exceeding 1×10^{13} vg/ml.

342. Liu similarly teaches that purification methods provide an advantage of “reduction in the number of empty viral vector particles,” and “[o]ptimally, practically 0% of the adenoviral vector particles in the composition are empty capsids.” Ex.1009, [00281]. Liu further teaches achieving high-yield production of viral vector particles of about 1×10^{15} particle units/cell, their purification and concentration, and incorporating them into storage compositions. *E.g., id.*, [0068], [00371], [00187], [00203]. Preparing such highly pure virus particles for parenteral administration in small volumes, as taught by Gatlin, would have been reasonably expected to result in a concentration of above 10^{13} vg/ml. Ex.1008, 405 (teaching using volumes as small as 3 ml for intramuscular, 1 ml for subcutaneous, and 0.2 ml

for intradermal delivery); *see also* 417 (reporting volumes of “0.5 to 2.0 mL (target 1 mL or less)” for subcutaneous injections), 418 (reporting “volumes of 0.5 mL or less” for intradermal injections and “up to 5 mL”).

343. Moreover, as I explained above in sections IX.C.1.b and IX.E.1.b, by June 2004, methods of generating high yields of AAV, removing empty capsids, and concentrating genome-containing vectors were well-known. For instance, Clark reported that stable cell lines can yield between $> 1 \times 10^{14}$ AAV particles per large-scale preparation. Ex.1012, S-12. Wright taught the use of cesium chloride and iodixanol gradient ultracentrifugation for separating “genome-containing[] vector particles from the lighter empty capsids.” Ex.1007, 175. A POSA would have understood that such methods, in addition to Lochrie’s methods, could also be used to successfully increase the concentration of purified AAV in Lochrie’s Example 2 composition.

344. Huang’s and Mingozi’s successes in achieving AAV formulations having concentrations above 1×10^{13} vg/ml would have further provided a reasonable expectation of success. Huang described AAV compositions having concentrations of $5\text{--}10 \times 10^{13}$ genome copies/ml, which a POSA would understand as equivalent to achieving AAV concentrations of $5\text{--}10 \times 10^{13}$ vg/ml. Ex.1005, S286. Huang further reported effectively reducing aggregation by up to 50% to maintain stability of such high concentration AAV formulations. *Id.* Mingozi reported preparing

compositions containing purified AAV-2 and AAV-5 at concentrations of “>**10¹³** vg/ml,” administering doses of 2.5×10^{12} vg to mice, which can be converted to roughly 4.8×10^{13} in humans, and achieving successful gene transfer in mice. Ex.1006, 10497-98 (emphasis added). These teachings would have further reinforced a POSA’s reasonable expectation of success in achieving AAV formulations with concentrations of exceeding 1×10^{13} vg/ml based on Lochrie’s Example 2 composition.

345. Thus, in my opinion, “a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml,” as recited in challenged claim 1, would have been obvious.

c. “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0”

346. Lochrie discloses in Example 2 that the purified and eluted rAAV was “formulated in 20 mM NaH₂PO₄, 150 mM NaCl, 5% sorbitol, and 0.1 % Tween- 80, at pH 7.4.” Ex.1010, 29:1-9.

347. As I explained above in section IX.G.1.b, a POSA would have been motivated to increase Lochrie’s virus concentration to exceed 10^{13} vg/ml to prepare therapeutically useful amounts of AAV particles. It had been reported that undesired virus aggregation is associated with virus concentration and may start to occur when the virus particles reach concentrations exceeding 1×10^{13} vg/ml. Ex.1007, 175, 176 (acknowledging “concentration limitations associated with aggregation” and

“reporting aggregation “at concentrations $\geq 10^{14}$ capsid particles (cp)/ml,” which can correspond to “ $\geq 10^{13}$ vg/ml”).

348. It was also known that buffer pH is one of the factors that could be adjusted to impact virus aggregation. *Id.* at 176 (teaching that “[i]nitial aggregation could be reversed by adjusting buffer pH”). Thus, a POSA would have been motivated to adjust the pH to eliminate any potential aggregation when the AAV concentration in Lochrie’s Example 2 is increased to exceed 1×10^{13} vg/ml.

349. A POSA would have been particularly motivated to increase the pH of Lochrie’s Example 2 formulation to 7.5 in view of Johnson’s teachings. Johnson reports that at pH 7.2 and all lower tested pHs AAV particles “were aggregated and were not evenly distributed in the field but were found in clumps,” and “[m]any aggregates were found containing thousands of particles.” Ex.1019, 589. However, at pH 7.5, “the virus particles occurred singly and were evenly distributed” and “no aggregates were seen.” *Id.*

350. Based on these results, Johnson concludes that “[t]he greatest effect of pH appeared to be its influence on the aggregation of the viral particles” and that “increasingly large aggregates of particles [were shown] as the pH was lowered” from 7.5. *Id.* at 589-90. Based on these teachings, a POSA would have been motivated to prevent any concentration-induced aggregation by increasing the pH of Lochrie’s composition to 7.5.

351. A POSA would have reasonably expected success in raising the pH of Lochrie's composition by a tenth of a pH unit in view of Johnson's success of achieving "singly and []evenly distributed" AAV virus with "no aggregation" at pH 7.5. Ex.1010, 29:1-9; Ex.1019, 589-90.

352. Moreover, as I explained above in section X.D.2, using conventional buffers to maintain the pH of therapeutic virus compositions at pH 7.5 had been a matter of routine optimization by June 2004. For instance, it had been common knowledge that "[o]ptimization of pH is a **simple but very useful** solution for" solving stability problems in pharmaceutical formulations. Ex.1018, Chapter 1, 12 (emphasis added); Ex.1020, 335 ("Buffers are used to maintain the pH of a medicinal at an optimal value"), 230 ("The evidence for enhanced stability of systems when these are maintained within a narrow range of pH ... is abundant"). Frei achieved multiple stable adenovirus formulations with pH between 7.5 to 8; *Id.*, 25:25-26:20. Liu also taught purifying and storing adenovirus formulations in elevated ionic strength buffers "at pH 7.5" and achieving stability for 7 days. Ex. 1009, [00366], [00369], Table 15. Evans similarly taught that the pH range of its stable virus formulations is preferably from about 7.5 to about 8.5, and further claimed purified virus vector formulations "at a pH from about 7.5 to about 8.5." Ex.1003, 36:15-18. All these earlier teachings support the routineness of adjusting the pH of Lochrie's Example 2 composition to 7.5.

353. Thus, in my opinion, a POSA would understand that “a pH buffer, wherein the pH of the composition is between 7.5 and 8.0,” as recited in challenged claim 1, would have been obvious.

d. “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate”

354. Lochrie’s Example 2 composition contains 20 mM NaH_2PO_4 . Ex.1010, 29:7-9. A POSA would have understood that NaH_2PO_4 , which comprises a phosphate multivalent ion, can act as an excipient. Ex.1022, 457 (describing that the functional categories of the NaH_2PO_4 excipient include not only a buffering agent but also an “emulsifying agent”).

355. Thus, in my opinion, Lochrie teaches and renders obvious “excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate,” as recited in challenged claim 1.

e. “wherein the ionic strength of the composition is greater than 200 mM”

356. Lochrie’s Example 2 composition contains “20 mM NaH_2PO_4 , 150 mM NaCl, 5% sorbitol, and 0.1 % Tween-80.” Ex.1010, 28:22-29:14.

357. As I explained in section V.D.1, the Henderson-Hasselbalch equation can be used to calculate the concentrations of the H_2PO_4^- and HPO_4^{2-} anions in a sodium phosphate buffer. Applying the equation $\text{pH} = \text{pK}_a + \log([A^-]/[\text{HA}])$ to Lochrie’s Example 2 formulation having pH 7.4, using the known pK_a of H_2PO_4^- of

approximately 7.2 (at 25 °C) and the 20 mM H_2PO_4^- anion, the concentration of HPO_4^{2-} , which is $[\text{A}^-]$ in the equation can be calculated as:

$$7.4 = 7.2 + \log([\text{HPO}_4^{2-}]/(20 \text{ mM}-[\text{HPO}_4^{2-}]))$$

$$\log([\text{HPO}_4^{2-}]/(20- [\text{HPO}_4^{2-}])) = 0.2$$

$$[\text{HPO}_4^{2-}]/(20-[\text{HPO}_4^{2-}]) = 1.58$$

$$1.58 \times (20-[\text{HPO}_4^{2-}]) = [\text{HPO}_4^{2-}]$$

$$31.6 = 2.58 \times [\text{HPO}_4^{2-}]$$

$$[\text{HPO}_4^{2-}] = 31.6/2.58 \approx 12 \text{ mM}$$

358. The concentration of the dissociated H^+ from H_2PO_4^- would also be 12 mM. The remaining $[\text{H}_2\text{PO}_4^-]$ would be $20 \text{ mM} - [\text{HPO}_4^{2-}] \approx 8 \text{ mM}$. Thus, the ionic strength of in sodium phosphate buffer is $\frac{1}{2} \times (20 \text{ mM} \times (+1)^2 + 8 \text{ mM} \times (-1)^2 + 12 \text{ mM} \times (-2)^2 + 12 \text{ mM} \times (+1)^2) \approx 44 \text{ mM}$. The total ionic strength calculated for Example 2 composition is, thus, calculated as: $44 \text{ mM} + \frac{1}{2} \times (150 \text{ mM} \times (-1)^2 + 150 \text{ mM} \times (-1)^2) \approx 194 \text{ mM}$.

359. When the pH of Lochrie's composition is increased to 7.5, as discussed in section XXX above, $[\text{A}^-]$ in the equation can be calculated as:

$$7.5 = 7.2 + \log([\text{HPO}_4^{2-}]/(20 \text{ mM}-[\text{HPO}_4^{2-}]))$$

$$\log([\text{HPO}_4^{2-}]/(20-[\text{HPO}_4^{2-}])) = 0.3$$

$$[\text{HPO}_4^{2-}]/(20-[\text{HPO}_4^{2-}]) = 2$$

$$2 \times (20-[\text{HPO}_4^{2-}]) = [\text{HPO}_4^{2-}]$$

$$40 = 3 \times [\text{HPO}_4^{2-}]$$

$$[\text{HPO}_4^{2-}] = 40/3 \approx 13 \text{ mM}$$

360. The concentration of the dissociated H^+ from H_2PO_4^- would also be 13 mM. The remaining $[\text{H}_2\text{PO}_4^-]$ would be $20 \text{ mM} - [\text{HPO}_4^{2-}] = 7 \text{ mM}$. Thus, the ionic strength of in Lochrie's sodium phosphate buffer at pH 7.5 is $\frac{1}{2} \times (20 \text{ mM} \times (+1)^2 + 7 \text{ mM} \times (-1)^2 + 13 \text{ mM} \times (-2)^2 + 13 \text{ mM} \times (+1)^2) \approx 46 \text{ mM}$. The total ionic strength calculated for Lochrie's Example 2 composition at pH 7.5 is, thus, calculated as: $46 \text{ mM} + \frac{1}{2} \times (150 \text{ mM} \times (-1)^2 + 150 \text{ mM} \times (-1)^2) \approx 196 \text{ mM}$.

361. As such, the calculated total ionic strength for Lochrie's Example 2 at pH 7.4 and pH 7.5 are both below but close to 200 mM.

362. As discussed above, because undesired virus aggregation was known to be associated with virus concentration and may start to occur when the virus particles reached high concentrations, e.g., at concentrations exceeding 1×10^{13} vg/ml, a POSA would have been motivated to prevent such aggregation after increasing the virus concentration in Lochrie's Example 2 composition to exceeding 1×10^{13} vg/ml. And ionic strength was one of the known variables to modify to reduce aggregation. Ex.1007, 175 (listing buffer ionic strength among the known "conditions that may affect aggregation").

363. A POSA would have been motivated to increase the ionic strength of Lochrie's composition in view of the stability improving capacity of increased ionic

strength. For instance, Liu teaches “increas[ing] the ionic strength of the viral vector particle composition to a level such that the concentration of non-viral component polynucleotides and/or proteins are reduced.” Ex.1009, [00181].

364. A POSA would have further been motivated to increase the ionic strength of Lochrie’s Example 2 composition at pH 7.5 to above 200 mM in view of Liu’s teachings. Liu not only recommended using 0.5-1.2 M NaCl to provide a high ionic strength during is purification steps but also used 300 mM NaCl in a virus storage composition for high concentration adenovirus, with a total ionic strength of 315 mM. Ex.1009, [0223], [0366]. Liu’s express recommendation and successful use of greater than 200 mM NaCl to store highly concentrated virus formulations would have motivated a POSA to similarly increase the NaCl concentration to above 200 mM, e.g., Liu’s ionic strength of ~300 mM, for obvious variants of Lochrie’s Example 2 composition comprising $> 1 \times 10^{13}$ vg/ml for improved stability and reduced aggregation.

365. A POSA also would have reasonably expected to prevent virus aggregation by using NaCl at a concentration of above 200 mM, e.g., 300 mM, in view of Liu’s success. As explained above, upon using 300 mM NaCl and achieving a total ionic strength of 315 mM, Liu reported that the composition showed “no signs of settling or precipitation over the 7 day [storage] period” and “[n]o significant change in the number of viral particles or infectious viral particles” within the

extended periods of storage. Ex.1009, [00369], [00371]; Liu concludes “viral vector compositions can be stably stored in the [high ionic strength] buffers of the invention for extended periods of time.” *Id.* at [00371]. Because AAV “is significantly more stable than the adenovirus,” a POSA would have reasonably expected AAV particles to achieve even greater stability in such buffers than the adenovirus tested in Liu. Ex.1013, 1283.

366. Adjusting ionic strength for a therapeutic AAV formulation would have been a matter of routine optimization for a POSA. During pharmaceutical formulation development, it had been a simple and routine practice for formulators to test various concentrations of excipients, calculate the total ionic strength, and determine stability of the virus in the composition. For instance, it would have been straightforward to increase the concentration of NaCl from 150 mM to 300 mM NaCl and pick the combinations of concentrations with other excipients that achieve optimal stability and prevent aggregation. Indeed, this type of practice had been routinely performed by formulators for decades. *See, e.g., id.* at 1281-82 (reporting the selection of excipients “from a panel of pharmaceutically acceptable compounds known to promote the physical stability of protein and peptide-based therapeutics currently on the market”); Ex.1020, 216-221 (teaching calculating and adjusting ionic strength of drug solutions using well known excipients).

367. Thus, in my opinion, Lochrie renders obvious the limitation “wherein the ionic strength of the composition is greater than 200 mM,” as recited in claim 1.

f. “wherein the purified AAV vector particles are stored in the composition without significant aggregation”

368. After the concentration of AAV in Lochrie’s Example 2 is increased to $> 1 \times 10^{13}$ vg/ml, a POSA would have been motivated to prevent any potential aggregation that may occur.

369. As I explained in section IX.C.1.f, Liu, Huang and Wright all expressly acknowledged that virus vector particle aggregation is associated with reduced gene transfer efficiency *in vivo*, and thus “deleterious,” “unfavorable,” and “undesired.” Ex.1009, [00262] (teaching that “[t]he presence of viral vector particle aggregates is unfavorable since the clumping of the viral vector particles could result in an increased host immune response to the viral vector particles” and that “when viral vector particles are aggregated, it is more likely that more than one viral vector particle will infect any particular cell, which is undesirable.”); Ex.1005, S286 (“the larger the aggregation,” “the less efficient the gene transfer.”); Ex.1007, 176 (“potentially deleterious consequence of vector aggregation is inconsistency in the functional activity of AAV vectors following their administration *in vivo*”); *see also supra* § IX.C.1.f. Thus, a POSA would have been motivated to minimize aggregation when storing AAV vector particles in the obvious variants of Lochrie Example 2 composition as discussed above.

370. A POSA also would have reasonably expected to prevent AAV vector particle aggregation in the obvious variants of Lochrie's Example 2 composition discussed above. First, as mentioned in section V.D, it was known that adjusting "buffer ionic strength and pH" could reduce aggregation. Ex.1007, 175. Liu and Johnson further showed that using increased ionic strength and increasing the pH of the composition to 7.5 had successfully minimized aggregation in adenovirus or AAV compositions. Ex.1019, 589-590; Ex.1009, [00369]. Second, Huang reported that its formulations led to a 30-50% reduction in the size of aggregates at high AAV concentrations Ex.1005, S286. Even the inventors themselves had already reported that highly purified AAV vector preparations at concentrations of 5×10^{13} vp/ml "are stable in a non-aggregated, monomeric state when stored at 2 to 8°C" without freeze-thaw circles. Ex.1007, 175. Thus, a POSA would also have reasonably expected that the obvious variants of Lochrie's Example 2 composition discussed above, with adjusted ionic strength and pH, would have no AAV vector particle aggregation.

371. Thus, for all the reasons stated above, I conclude that challenged claim 1 would have been obvious over the prior art.

2. Claim 2 Would Have Been Obvious Lochrie in View of Huang, Mingozi, Johnson, and Liu

372. Claim 2 recites a composition of claim 1 "further comprising ethylene propylene oxide block copolymer Pluronic® F68." Ex.1001, 14:27-28. In my opinion, this additional limitation also would have been obvious.

373. As I explained in section IX.D, Pluronic® F68 was a well-known commercially available non-ionic detergent/surfactant frequently used in pharmaceutical compositions as of June 2004. Even the inventors themselves admitted in an earlier publication that “addition of the surfactants Polysorbate 80 or Pluronic® F68 . . . effectively prevent losses due to non-specific binding during [virus] vector sampling and transfer.” Ex.1007, 175, 176. Liu similarly teaches that “[i]n another preferred embodiment, the temporary storage composition further comprises a nonionic surfactant,” including “nonionic surfactants [] well known in the art,” such as “Pluronic F68.” Ex.1009, [00189]. Thus, a POSA would have been motivated to include Pluronic® F68 in Lochrie’s Example 2 composition.

374. There would have been a reasonable expectation of success in using Pluronic® F68 in Lochrie’s Example 2 composition in view of previously proven benefits of Pluronic® F68. It had been reported that prior AAV compositions comprising Pluronic® F68 were stable for at least 240 days when stored at 4 °C. Ex.1013, 1284, 1288. Such earlier successful uses of Pluronic® F68 in stable AAV compositions would have further provided a reasonable expectation of maintaining the stability of Lochrie’s Example 2 composition after including Pluronic® F68 into the formulation.

375. Thus, in my opinion, the composition of claim 2 would have been obvious.

3. Claim 5 Would Have Been Obvious Over Lochrie in View of Huang, Mingozzi, Johnson, and Liu

376. Claim 5 recites a composition of claim 1, “wherein the purified, recombinant AAV vector particles have an average particle size radius (Rh) of less than about 20 nm as measured by dynamic light scattering [DLS].” Ex. 1001, 14:34-37. As I explained above, the ’542 patent does not show anything critical about the recited radius range, and a POSA with prior knowledge of AAV vector particle sizes would have understood that this additional limitation merely requires that there is no AAV vector particle aggregation in the claimed composition. *Supra*, § IX.F.3. Thus, for the reasons I discussed above for section IX.G.1.f, a POSA would have reasonably expected that AAV particles stored in the obvious variants of Lochrie’s Example 2 composition discussed above also have no aggregation, and thus, comprise vector particles having an Rh of less than about 20nm measured by DLS.

377. To the extent modifications to Lochrie’s composition, beyond those described above, would be required to achieve the features of claim 5, a POSA would have been motivated to make such changes to prevent any potential aggregation. As I explained above in section IX.C.2, Liu as well as other prior art acknowledged that virus aggregation is “unfavorable,” “undesired,” and has “deleterious consequences” such as “[r]educd yield,” “an increased host immune response to the viral vector particles,” “inconsistency in the functional activity of AAV vectors following their administration *in vivo*,” and “less efficient [] gene transfer.” Ex.1009, [00262];

Ex.1007, 175, 176; Ex.1005, S286. In view of these teachings, a POSA would have been motivated to prevent aggregation from occurring during storage of AAV formulations such that the AAV vector particles have an Rh of less than about 20 nm as measured by dynamic light scattering.

378. A POSA would have reasonably expected success in minimizing particle size, based on known variables impacting aggregation and prior successes reflected in the art. As I explained, Liu taught that its aggregation measuring experiments did not detect any sign of substantial virus aggregation in the tested adenovirus formulation and “demonstrate that viral vector compositions can be stably stored in the temporary storage buffers of the invention for extended periods of time.” Ex.1009, [00369], [00371]. A POSA would have understood that obvious variants of the tested adenovirus formulations comprising AAV would have no aggregation because AAV “is significantly more stable than the adenovirus.” Ex.1013, 1283. Liu also teaches reducing potential aggregation by addition of surfactants, further supporting a reasonable expectation of success in eliminating any potential residual aggregation to achieve monomeric AAV vector particles with an average Rh of less than about 20 nm. Ex.1009, [00263].

379. By adjusting well known variables including pH, buffer ionic strength, addition of surfactant, a free radical scavenger or a chelating agent, those skilled in the art had routinely eliminated virus aggregation in high concentration AAV

formulations. For instance, Wright described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are ***stable in a non-aggregated, monomeric state*** when stored at 2 to 8°C.” Ex.1007, 175 (emphasis added). Huang likewise discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5\text{-}10 \times 10^{13}$ genome copies/ml. Ex. 1005, S286. Thus, only routine optimization of known stability factors would have been required to improve the recovery of AAV following filtration through a 0.22 μm filter.

380. Thus, in my opinion, the composition of challenged claim 5 would have been obvious over Lochrie in view of Huang and Mingozi.

4. Claim 6 Would Have Been Obvious Over Lochrie in View of Huang, Mingozi, Johnson, and Liu

381. Claim 6 recites a composition of claim 1, “wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.” Ex.1001, 14:38-41. As I explained above in sections IX.C.3 and IX.E.4, a POSA would have understood that this limitation reciting a recovery rate range after passing a 0.22 μm filter, like the Rh limitation of claim 5, essentially merely requires no virus aggregation. In my opinion, this claim also would have been obvious over Lochrie in view of Huang, Mingozi, Johnson, and Liu.

382. As I explained earlier in sections V.C and IX.C.3, 0.22 μm filters had been routinely used to obtain purified virus formulations, and it had been well known

that recovery rates after passing the virus formulations through a 0.22 μm filter indicate the level of aggregation—the higher the recovery rate, the lower the aggregation level. Ex.1009, [00373]; Ex.1007, 175 (“loss of rAAV following a 0.2- μm filtration step correlates with the extent of vector aggregation”); Ex.1005, S286 (“the larger the aggregation,” “the less efficient the gene transfer.”). A POSA would have reasonably expected that obvious variants of Lochrie’s Example 2 formulation, which Lochrie reported to have no observable virus aggregation, have at least 90% of the virus particles in the stored composition recovered following filtration through a 0.22 μm filter.

383. To the extent claim 6 requires less aggregation than claim 1, as I explained above in sections IX.C.3 and IX.E.4, Wright and Huang expressly teach the correlation between high AAV virus particle recovery rate following a 0.2- μm filtration step and minimized level of vector aggregation. Ex.1007, 175 (“loss of rAAV following a 0.2- μm filtration step correlates with the extent of vector aggregation.”); Ex.1005, S286 (teaching that “the larger the aggregation,” “the less efficient the gene transfer.”). In view of these teachings, a POSA would have been motivated to prevent aggregation in obvious variants of Lochrie’s Example 2 formulation to maximize virus recovery through a 0.22 μm filter.

384. A POSA would have had a reasonable expectation of successfully modifying Lochrie’s Example 2 formulation as described above, and maximizing

particle recovery after filtration in view of reported prior successes of reducing aggregation. For instance, after purifying and storing its adenovirus particles in a high ionic strength buffer, Liu observed “no signs of settling or precipitation over the 7 day period” and “[n]o significant change in the number of viral particles or infectious viral particles [] at any of the times tested.” Ex.1009, [00369], [00371]. A POSA would have expected similar results for AAV formulations, as it had been known that AAV “is significantly more stable than the adenovirus.” Ex.1013, 1283. Huang also teaches having developed formulations that “could lead to a 30-50% reduction in the size of aggregates at high vector concentrations.” Ex.1005, S286.

385. As I explained above, reducing aggregation would have been a matter of routine optimization of the obvious variants of Lochrie’s Example 2 formulation described above, such as by adjusting ionic strength, pH, buffer, and other known stabilizing agents. In addition to adjusting pH and ionic strength based on teachings of Johnson and Liu as discussed in sections IX.G.1.c and IX.G.1.d, adding a surfactant, a free radical scavenger and/or a chelating agent were also known to be able to enhance stability and reduce aggregation. Ex.1003, 7:10-12; Ex.1009, [00263] (“[A]ddition of surfactants” also was known to “reduce aggregation.”).

386. Wright similarly described “highly purified vector preparations at concentrations of 5×10^{13} cp/ml that are ***stable in a non-aggregated, monomeric state*** when stored at 2 to 8 °C.” Ex.1007, 175 (emphasis added), 176. Huang likewise

discussed the successful “30-50% reduction in the size of aggregates” in high-titer formulations having $5-10 \times 10^{13}$ genome copies/ml. Ex.1005, S286. Thus, only routine optimization of known stability factors would have been required to improve the recovery of AAV from the obvious variants of Lochrie’s Example 2 formulation discussed above following filtration through a 0.22 μm filter.

387. Thus, in my opinion, the composition of challenged claim 6 was obvious.

X. DECLARATION

388. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

A handwritten signature in black ink, appearing to read "Mansoor M. Amiji", is written on a light-colored rectangular background.

Date: February 22nd, 2023

Mansoor M. Amiji, R. Ph., Ph.D

Exhibit A

CURRICULUM VITAE

MANSOOR M. AMIJI, PhD, FAAPS, FCRS

CONTACT INFORMATION

Campus Address:

Northeastern University
360 Huntington Avenue
140 The Fenway Building, Room 156
Boston, Massachusetts 02115
Office #: (617) 373-3137
Fax #: (617) 373-8886
E-mail: m.amiji@northeastern.edu
Personal Website: <http://www.northeastern.edu/amijilab>
LinkedIn: <https://www.linkedin.com/in/mansoor-amiji-46109629/>
ResearchGate: <https://www.researchgate.net/profile/Mansoor-Amiji>
Google Scholar: <https://scholar.google.com/citations?user=0BKYPYMAAAAJ&hl=en>

Residential Address:

195 Richie Road
Attleboro, Massachusetts 02703
Home #: (508) 222-3034
Cell #: (617) 839-9679

EDUCATION AND TRAINING

September, 1984 - June, 1988: Undergraduate Student in the College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA.

September, 1986 - May, 1988: Undergraduate Honors Student Research Project Entitled "*Preparation and Characterization of Doxorubicin-Dextran Conjugates*" – Major Advisor: Professor Mehdi Boroujerdi.

June, 1988: Bachelor of Science Degree in Pharmacy (*magna cum laude*).

July, 1988: Registered Pharmacist – Licensed to practice in Massachusetts (license # 20415)

August, 1988 - July, 1992: Doctoral Student in the Department of Industrial and Physical Pharmacy, College of Pharmacy, Purdue University, West Lafayette, Indiana.

August, 1988 - June, 1989: Teaching Assistant in the Department of Industrial and Physical Pharmacy.

July, 1989 - June, 1992: Research Assistant in the Department of Industrial and Physical Pharmacy.

July, 1989 - June, 1992: Doctoral Dissertation Research Entitled "*Surface Modification of Biomaterials with Water-Soluble Polymers: A Steric Repulsion Approach*" - Major Advisor: Professor Kinam Park.

July, 1992: Doctor of Philosophy Degree in Pharmaceutics and Pharmaceutical Sciences.

PROFESSIONAL AND ACADEMIC POSITIONS

August, 1992 – December, 1992: Senior Research Scientist, Columbia Research Laboratories, Madison, WI.

January, 1993 – June, 1999: Assistant Professor, Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Bouve College of Health Sciences, Boston, MA.

July, 1999 – April, 2006: Associate Professor (with tenure), Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

June, 2000 – December, 2000: Visiting Research Scholar. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA. (Sabbatical leave appointment in Institute Professor Robert Langer's laboratory).

May, 2006 – Present: Full Professor (primary), Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, Boston, MA.

February, 2010 – April, 2016: Bouve College Distinguished Professor, Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

September, 2012 – July, 2018: Affiliate Faculty Member, Department of Chemical Engineering, College of Engineering, Northeastern University, Boston, MA.

September, 2013 – Present: Affiliate Faculty Member, Department of Biomedical Engineering, College of Engineering, Northeastern University, Boston, MA.

January, 2014 – September, 2017: Distinguished Adjunct Professor, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

April, 2016 – Present: University Distinguished Professor, Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

March, 2017 – August, 2018: Distinguished Adjunct Professor, Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia.

August 2018 – Present: Full Professor (secondary). Department of Chemical Engineering, College of Engineering, Northeastern University, Boston, MA.

ADMINISTRATIVE AND LEADERSHIP POSITIONS

July, 1995 – June 2000: Pharmaceutics Group Leader, Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

September, 2002 – June, 2004: Education and Outreach Coordinator, Molecular Biotechnology Initiative at Northeastern University, Boston, MA.

July, 2002 – Present: Director, Laboratory of Biomaterials and Advanced Nano-Delivery Systems (BANDS) at Northeastern University, Boston, MA

July, 2003 – December, 2016: Co-Director, Nanomedicine Education and Research Consortium (NERC) at Northeastern University, Boston, MA.

July, 2005 – December, 2008: Associate Chairman, Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

January, 2009 – January, 2010: Interim Chairman, Department of Pharmaceutical Sciences, School of Pharmacy. Bouve College of Health Sciences, Northeastern University, Boston, MA.

February, 2010 – April, 2016: Chairman, Department of Pharmaceutical Sciences, School of Pharmacy, Bouve College of Health Sciences, Northeastern University, Boston, MA.

RESEARCH INTERESTS

The primary focus of research in my laboratory is on the development of biocompatible materials from natural and synthetic polymers, target-specific drug and gene delivery systems for cancer and infectious diseases, and nanotechnology applications for medical diagnosis, imaging, and therapy. Specific projects that we are currently pursuing include:

- Preparation and characterization of polymeric membranes and microcapsules with controlled permeability properties for medical and pharmaceutical applications.
- Role of hypoxia, mitochondrial dysfunction, extracellular vesicles, and immunity in the tumor microenvironment in development of therapeutic resistance, angiogenesis, and metastasis.
- Intracellular and sub-cellular delivery systems for drugs and genes using target-specific, long-circulating, biodegradable polymeric nanoparticles.
- Localized delivery of cytotoxic and anti-angiogenic drugs, siRNA, and genes for solid tumors in novel biodegradable polymeric nanoparticles.
- Local administration of drugs and nucleic acid-containing nano-vectors immobilized on stents for the treatment of arterial diseases (e.g., coronary restenosis).
- Target-specific drug, gene, and vaccine delivery systems for diseases of the gastro-intestinal tract.
- Systemic and local delivery of DNA, mRNA, siRNA, microRNA, and CRISPR-Cas9 constructs for genetic engineering and therapeutic applications.
- Novel oil-in-water nanoemulsion formulations for drug delivery through the gastrointestinal tract and across the blood-brain barrier.
- Systemic and mucosal vaccination using novel immune-modulatory strategies and delivery systems.
- Intranasal and trans-nasal administration of biodegradable formulations, including sustained release implants, to enhance brain delivery of small molecules, peptides, proteins, siRNA, mRNA, and genes for treatment of chronic neurodegenerative diseases and brain tumors.
- Functionalized inorganic nanoparticles - including gold, silver, iron oxide, metal alloys, and core-shell nanostructures - for biosensing, imaging, and targeted therapeutic applications.

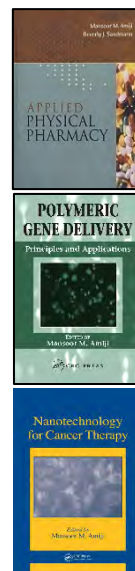
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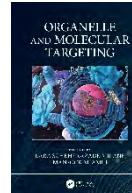
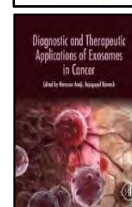
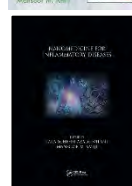
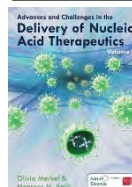
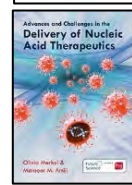
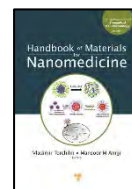
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Attarwala, H. and Amiji, M.M. *In vitro* evaluations of nanoparticle-in-emulsion formulations for gene delivery and transfection in macrophages. *Proceeding of the International Symposium on the Controlled Release of Bioactive Materials* (2010).

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Barchet, T. and Amiji, M.M. Advances in siRNA delivery: preliminary work in the development of an siRNA nanoemulsion delivery system. *The AAPS Journal*, 12: S2, (2010).

Xu, J. and Amiji, M.M. Gene delivery and transfection in human pancreatic cancer cells using epidermal growth factor receptor-targeted gelatin nanoparticles. *The AAPS Journal*, 12: S2, (2010).

INTRAMURAL AND EXTRAMURAL RESEARCH SUPPORT (Total = \$55M; as PI = \$40M)

Current Funding

Source: National Institute of Neurological Disorders and Stroke, National Institutes of Health R01 Grant (R01-NS108968)
Sub-contract from Massachusetts Eye and Ear Infirmary

Title: Direct CNS Delivery System for BDNF Antagonists using Heterotopic Mucosal Grafting for the Treatment of Parkinson's Disease

Amount: \$2,546,316 (total); NU Sub-contract: \$1,279,394

Duration: January, 2019-December, 2023

Role: Principal Investigator on NU Sub-Contract (PI of the Grant: Dr. Benjamin Bleier, Mass Eye and Ear, Boston, MA)

Source: National Science Foundation, D-ISBN: TRACK 2 Grant (IIS- 2039945)

Title: Collaborative Research: Financial Network Disruptions in Illicit and Counterfeit Medicines (FIND-M)

Amount: \$169,999 (total)

Duration: September 2020 – August 2023 (with NCE)

Role: Co-Principal Investigator (PI: Prof. Nikos Passas, College of Social Sciences and Humanities, Northeastern University, Boston, MA)

Source: Glaxo-Smith Kline Vaccines, Rockville, MD

Title: Pharm Sci Industrial Graduate Fellowship Program (designated to Mr. Shashank Bhangde)

Amount: \$266,000 (total)

Duration: October 2020 – September 2023

Role: Principal Investigator

Source: Moderna Therapeutics, Inc., Cambridge, MA

Title: Academic-Industrial Post-Doctoral Fellowship (designated to Dr. Mir Javid Iqbal)

Amount: \$275,000

Duration: November 2020 – October 2023 (with NCE)

Role: Principal Investigator

Source: National Institutes of Health, National Cancer Institute R21 Grant (1R21 CA246413-01A1)

Title: Towards *In Vivo* Liquid Biopsy of Circulating Tumor Cells

Amount: \$475,000 (total)

Duration: January 2021 – December 2023

Role: Co-Investigator (PI of the Grant: Dr. Mark Niedre, Department of Bioengineering, College of Engineering, Northeastern University, Boston, MA)

Source: Massachusetts Life Sciences Center (MLSC) and Eli Lilly & Company, Cambridge, MA – 2021 Novel Therapeutic Delivery Program

Title: CNS Delivery of Nucleic Acid Therapeutics using MIND

Amount: \$750,000 (MLSC) and \$554,000 (Eli Lilly)

Duration: July 2021 – June 2023

Role: Principal Investigator

Source: Moderna Therapeutics, Inc., Cambridge, MA

Title: Academic-Industrial Post-Doctoral Fellowship (designated to Dr. Dimitrios Bitounis)

Amount: \$275,000

Duration: July 2021 – June 2023

Role: Principal Investigator

Source: National Institutes of Health, National Institute of Aging (1R21-AG074472-01)

Title: Intranasal Gene Delivery for Alzheimer's Disease

Amount: \$445,124 (total), NU Share: \$196,265

Duration: August 2021 – July 2023

Role: Co-Principal Investigator (Multi-PI Grant with Dr. Jonghan Kim, Department of Nutrition Science, Zuckerberg College of Health Sciences, University of Massachusetts at Lowell, Lowell, MA)

Source: National Institute of Alcohol Abuse and Alcoholism (NIAAA) of the National Institutes of Health Grant R21-AA029260-01.

Title: Role of Intestinal Proteostasis Mediator HSP90 in Alcoholic Liver Disease

Amount: \$475,000 (total); NU Share 47,500

Duration: September 2021 – August 2023

Role: PI of NU Sub-Contract (PI: Pranoti Mandrekar, University of Massachusetts Medical School, Department of Medicine, Worcester, MA)

Source: Glaxo-Smith Kline Vaccines, Rockville, MD

Title: Pharm Sci Industrial Graduate Fellowship Program (designated to Mr. Apurva Krishna)

Amount: \$266,000 (total)

Duration: January 2022 – December 2024

Role: Principal Investigator

Source: Northeastern University-Dana Farber Cancer Institute Joint Research Program on Cancer Drug Development

Title: MIND Delivery of Stapled Peptides for the Treatment of Pediatric Brain Tumors

Amount: \$50,000 (total)

Duration: August 2022 – July 2023

Role: Principal Investigator (in Collaboration with Dr. Loren Walensky, Dana-Farber Cancer Institute)

Source: Northeastern University's Center for Research Innovation 2022 SPARK Fund Program

Title: Oral RNA Tx - A Platform for Oral RNA Delivery for Therapeutics and Vaccination

Amount: \$50,000 (total)

Duration: September 2022 – August 2023

Role: Principal Investigator

Source: National Institutes of Health, National Cancer Institute (1R01-CA269233-01A1)

Title: TGX-1214 - Combination Strategy for the Treatment of Advanced Pancreatic Cancer

Amount: \$2,998,487 (total); NU Share: \$1,180,240

Duration: February, 2023 – January, 2028

Role: Co-Principal Investigator (Multi-PI Grant with Dr. Gerardo Mackenzie, Department of Nutrition Sciences, University of California Davis)

Past Funding

Source: National Institutes of Health, Biomedical Research Support Grant

Title: Prevention of Protein Adsorption on Poly(Ethylene Oxide)-Modified Surfaces

Amount: \$2,000 (total).

Duration: June, 1993 - May, 1994

Role: Principal Investigator

Source: Northeastern University, 1995 Research and Scholarship Development Fund

Title: Development of Blood-Compatible Hemodialysis Membranes from Chitosan

Amount: \$10,000 (total)

Duration: July, 1995 - December, 1996 (with 6-months no-cost extension)

Role: Principal Investigator

Source: Bristol-Myers Squibb Pharmaceutical Research Institute, Kenilworth, NJ.

Title: Development of Novel Thermogelling System for Localized Delivery of Taxol®

Amount: \$8,000 (total)

Duration: July, 1996 - June, 1997

Role: Principal Investigator.

Source: Roche Laboratories, Inc., Nutley, NJ.

Title: HPLC Assay Development for Mycophenolic Acid and Mycophenolic Acid Glucuronide

Amount: \$10,000 (total)

Duration: July 1996 - December, 1996

Role: Co-Principal Investigator (PI: Prof. Rafaat Seifeldin, Department of Pharmacy Practice, Northeastern University)

Source: Northeastern University, Honors Program Research Funds

Title: Undergraduate Honors Students Research Initiatives

Amount: \$5,000.

Duration: July 1997 - June 1999.

Role: Principal Investigator.

Source: Pharmaceutical Research and Manufacturers of America Foundation, Undergraduate Pharmaceuticals Research Fellowship. (On behalf of Ms. Phung-Kim Lai, B.S. student in the class of 1999).

Title: Novel Thermogelling Paclitaxel Formulation for Intra-Tumoral Delivery

Amount: \$5,000 (total)
Duration: January 1999 - December 1999
Role: Principal Investigator.

Source: Northeastern University, 1999 Research and Scholarship Development Fund
Title: Stomach-Specific Antibiotic Delivery for *H. pylori* Infection
Amount: \$10,000 (total)
Duration: July 1999 - June 2000
Role: Principal Investigator

Source: EOS Pharmaceuticals Corporation, Woburn, MA (through National Institutes of Health, Small Business Innovation Research Program, Phase I Grant)
Title: Stomach-Specific Anti-*H. pylori* Therapy
Amount: \$50,000 (total, ~ 45% of the total SBIR funding)
Duration: July, 2000 – June, 2001
Role: Principal Investigator

Source: American Association of Pharmaceutical Scientists and American Foundation for Pharmaceutical Education, 2000-2001 "Gateway" Research Scholar Program. (On behalf of Mr. Chi-Sing Nip, Pharm.D. student, Class of 2001)
Title: Perm-selective Membranes from Chitosan for Extracorporeal Removal of β -2-Microglobulin
Amount: \$5,000 (total)
Duration: July 2000 – June 2001
Role: Principal Investigator

Source: Braintree Laboratories, Inc., Braintree, MA
Title: Analysis of Phosphate Binding to Cationic Polymers
Amount: \$31,000 (total)
Duration: December 2000 – June 2001
Role: Principal Investigator

Source: Pharmaceutical Research and Manufacturers of America Foundation, Undergraduate Pharmaceutics Research Fellowship. (On behalf of Ms. Erica J. Waugh, Pharm.D. student in the class of 2002)
Title: Novel Biodegradable Long-Circulating Nanoparticles for Tumor-Selective Drug Delivery
Amount: \$5,000 (total)
Duration: January 2001 – December 2001
Role: Principal Investigator

Source: Braintree Laboratories, Inc., Braintree, MA
Title: Chitosan-Iron Complexes as Polymeric Phosphate Binders
Amount: \$15,000 (total)
Duration: January 2002 – June 2002
Role: Principal Investigator

Source: Northeastern University, Undergraduate Research Fund. (On behalf of Ms. Nikita Mody, Pharm.D. student in the class of 2004)
Title: Tumor-Selective Gene Delivery Systems
Amount: \$3,000 (total)
Duration: January 2002 – June 2002
Role: Principal Investigator

Source: Northeastern University, 2002 Research and Scholarship Development Fund
Title: Immobilized Enzyme System for Detection and Detoxification of Chemical Warfare Agents
Amount: \$5,006 (total)
Duration: July 2002 - June 2003
Role: Principal Investigator

Source: Alfred P. Sloan Foundation, New York, NY
Title: Multi-Track Professional Masters Degree Program in Biotechnology
Amount: \$75,000 (total)
Duration: July, 2002 – June 2004 (with one year no-cost extension)
Role: Co-Investigator (PI: Prof. William Detrich, Department of Biology, Northeastern University).

Source: Boston Scientific Corporation. Watertown, MA
Title: Drug Interactions with Poly(vinyl alcohol) Embolic Microspheres: Part I
Amount: \$33,256 (total)
Duration: February 2003 – December 2003
Role: Principal Investigator

Source: National Cancer Institute, National Institutes of Health (R01-CA95522-01A2)
Title: Long-Circulating Tumor-Selective DNA Delivery Systems
Amount: \$744,100 (total)
Duration: July 2003 – June 2007 (with one-year NCE)
Role: Principal Investigator

Source: National Science Foundation, Major Research Instrumentation Grant (MRI-0320638)
Title: Acquisition of Scanning Electron Microscope for Nanoscience and Biotechnology
Amount: \$477,846 (total)
Duration: July 2003 – June 2004
Role: Co-Investigator (PI: Prof. Donald Heiman, Department of Physics, Northeastern University)

Source: Roger Williams Medical Center, Providence, RI. Subcontract of the Department of Defense Grant DAMD17-03-0716
Title: Drug Encapsulation in Poly(Epsilon-Caprolactone) Nanoparticles
Amount: \$20,119 (total)
Duration: October 2003 – September 2005 (with one year no-cost extension)
Role: Principal Investigator

Source: Boston Scientific Corporation. Watertown, MA
Title: Drug Interactions with Poly(vinyl alcohol) Embolic Microspheres: Part II
Amount: \$44,865 (total)
Duration: February 2004 – December 2004
Role: Principal Investigator

Source: American Foundation for Pharmaceutical Education, Undergraduate Gateway Research Scholarship. (On Behalf of Ms. Stephanie Whalen, PharmD Class of 2006)
Title: Novel Gold Nanoparticle Sensor for Non-invasive Glucose Measurements
Amount: \$5,000 (total)
Duration: June 2004 – December 2004
Role: Principal Investigator

Source: Boston Scientific Corporation, Natick, MA
Title: Preparation and Characterization of Drug-Loaded SIBS Copolymer Microsphere Formulations
Amount: \$100,019 (total)
Duration: October 2004 – December 2005 (with no-cost extension)
Role: Principal Investigator

Source: Novavax, Inc., Malvern, PA.
Title: Nanocarrier Formulation Optimization Studies
Amount: \$60,543 (total)
Duration: May 2005 – December 2005
Role: Principal Investigator

Source: American Foundation for Pharmaceutical Education, Undergraduate Gateway Research Scholarship. (On Behalf of Mr. Zeu Hong Tzeng, PharmD Class of 2007)
Title: Thiolated Gelatin Nanoparticles for Intracellular siRNA Delivery
Amount: \$5,000 (total)
Duration: July 2005 – June 2006
Role: Principal Investigator

Source: Northeastern University, 2005 Research and Scholarship Fund
Title: Functionalized Gold Nanoparticles as Novel Mitochondria-Targeted Vectors
Amount: \$15,000 (total)
Duration: July 2005 – June 2006
Role: Co-Investigator (PI: Prof. Volkmar Weissig, Department of Pharmaceutical Sciences, Northeastern University)

Source: Transport Pharmaceuticals, Inc., Framingham, MA
Title: Evaluation of Formulation Properties Used in Iontophoretic Drug Delivery Device
Amount: \$58,875 (total)
Duration: August 2005 – July 2006
Role: Principal Investigator

Source: Boston Scientific Corporation. Malborough, MA
Title: Drug Interactions with Poly(vinyl alcohol) Embolic Microspheres: Part III
Amount: \$125,208 (total)
Duration: March 2006 – February 2007
Role: Principal Investigator

Source: Transport Pharmaceuticals, Inc., Framingham, MA
Title: Evaluation of Formulation Properties Used in Iontophoretic Drug Delivery Device: Part II
Amount: \$72,952 (total)
Duration: August 2006 – July 2007
Role: Principal Investigator

Source: Parsalus Pharmaceuticals, Inc., Boston, MA
Title: Preliminary Micellar Characterization Studies of Lipopeptide Molecules
Amount: \$12,372 (total)
Duration: October 2007 – January 2008
Role: Principal Investigator

Source: The Michael J. Fox Foundation for Parkinson's Research

Title: Feasibility of Intranasal Delivery of GDNF for Parkinson's Disease
Amount: \$74,800 (total)
Duration: November 2007 – December 2008
Role: Co-Investigator (PI: Prof. Barbara Waszczak, Department of Pharmaceutical Sciences, Northeastern University)

Source: Microfluidics, Inc., Newton, MA
Title: HPLC Studies of Drug Encapsulation in Polymeric Nanoparticle Formulations
Amount: \$13,549 (total)
Duration: May 2008 – July 2008
Role: Principal Investigator

Source: BioCure, Inc., Norcross, GA
Title: Cellular Uptake and Trafficking Properties of siRNA-Loaded Polymeric Nano-Vesicles
Amount: \$28,414 (total)
Duration: July 2008 – June 2009
Role: Principal Investigator

Source: National Cancer Institute of the National Institutes of Health, Nanotechnology Characterization Laboratory, Frederick, MD.
Title: Multifunctional Nanoemulsions for Brain Tumor Imaging and Therapy
Amount: Undisclosed (complete preclinical characterization studies)
Duration: July 2006 – December 2009
Role: Principal Investigator

Source: National Cancer Institute of the National Institutes of Health, Nanotechnology Characterization Laboratory, Frederick, MD.
Title: Engineered Gelatin-Based Nano-vectors for Pancreatic Cancer Gene Therapy
Amount: Undisclosed (complete preclinical characterization studies)
Duration: July 2007 – December 2009
Role: Principal Investigator

Source: Physical Sciences, Inc., Andover, MA. Sub-Contract of the National Cancer Institute STTR Phase I Application in response to RFA "Image Guided Cancer Interventions" (STTR R41/R42) (1R41-CA132256-01).
Title: Endoscopically Guided OCT Imaging for Early Cancer Screening
Amount: 87,933 (total)
Duration: May 2008 – December 2009
Role: Principal Investigator of NU Sub-contract (PI: Dr. Nick Iftemia, Physical Sciences, Inc., Andover, MA)

Source: BioCure, Inc., Norcross, GA. Sub-contract funding from the National Cancer Institute Phase I SBIR contract.
Title: Targeted Multifunctional Polymersomes for Cancer Therapy
Amount: \$66,958 (total)
Duration: September 2009 – June 2010
Role: Principal Investigator

Source: Physical Sciences, Inc., Andover, MA. Sub-Contract of the National Cancer Institute STTR Phase I Application in response to RFA "Image Guided Cancer Interventions" (STTR R41/R42) (1R41-CA135911-01).
Title: Enhanced Contrast Optical Imaging for Screening of Early Stage Pancreatic Cancer
Amount: \$115,071 (total)
Duration: August 2008 – July 2010
Role: Principal Investigator of NU Sub-contract (PI: Dr. Nick Iftemia, Physical Sciences, Inc., Andover, MA)

Source: National Cancer Institute/National Science Foundation, Interdisciplinary Graduate Education and Training (IGERT) Grant (DGE-0504331)

Title: IGERT-Nanomedical Science and Technology

Amount: \$3,320,168 (total)

Duration: October 2005 – September 2010

Role: Co-Investigator (PI: Prof. Srinivas Sridhar, The Nanomedicine Education and Research Consortium (NERC), Northeastern University)

Source: National Cancer Institute of the National Institutes of Health. R01 proposal in response to NCI's Alliance for Nanotechnology in Cancer RFA "Cancer Nanotechnology Platform Partnership Grant" program (1R01-CA119617-01)

Title: Nanotherapeutic Strategy for Multidrug Resistant Tumors

Amount: \$1,329,399 (total)

Duration: October 2005 – July 2011 (with no-cost extension)

Role: Principle Investigator

Source: National Cancer Institute of the National Institutes of Health. ARRA Administrative Supplement for "Cancer Nanotechnology Platform Partnership Grant" (1R01-CA119617-S1)

Title: Nanotherapeutic Strategy for Multidrug Resistant Tumors

Amount: \$200,000 (total)

Duration: August 2009 – July 2011 (with no-cost extension)

Role: Principle Investigator

Source: Forsyth Institute, Boston, MA. Sub-Contract of the National Institute of Dental and Craniofacial Research's R21 Proposal in Response to Program Announcement PA-03-107 "NIH Exploratory/Developmental Research Grant Program" (1R21-DE018782-01A2).

Title: Nanoparticle-Based Antimicrobial Photochemotherapy in Biofilms

Amount: \$140,092 (Sub-contract total)

Duration: August 2009 – July 2011

Role: Principal Investigator on NU Subcontract (PI: Dr. Nikos Soukos, Forsyth Institute, Boston, MA)

Source: Medix Corporation, Mexico City, Mexico

Title: Stomach-Specific Non-Antibiotic *H. pylori* Therapy

Amount: \$399,759 (total)

Duration: October 2008 – May 2012 (with NCE)

Role: Principal Investigator

Source: Nemucore Medical Innovations, Inc., Sub-Contract of NCI Phase 1 SBIR grant.

Title: EGFR-Targeted Nanoemulsions for Imaging and Therapy of Ovarian Cancer

Amount: \$ 79,310

Duration: September 2010 – August 2012

Role: Principle Investigator on NU Sub-Contract

Source: National Cancer Institute of the National Institutes of Health, R21 Proposal in response to RFA PAR-07-034 "Nanoscience and Nanotechnology in Biology and Medicine". (1R21-CA135594)

Title: Nano-Delivery of Mitochondria-Targeted Ceramide to Overcome Tumor Drug Resistance

Amount: \$355,232 (total)

Duration: January 2010 – December 2012 (with NCE)

Role: Principal Investigator

Source: National Institute of Diabetes, and Digestive Diseases, and Kidney Diseases (NIDDK) of the National Institutes of Health. R01 proposal in response to RFA “*Nanoscience and Nanotechnology in Biology and Medicine*” program (1R01-DK080477).

Title: Oral Gene Therapy with NiMOS for Inflammatory Bowel Disease

Amount: \$1,334,500 (total)

Duration: March 2008 – February 2013 (with NCE)

Role: Principle Investigator

Source: National Institute of Neurological Disorders and Stroke of the National Institutes of Health, R21 Proposal in response to RFA PAR-08-232 “*NINDS Exploratory/Developmental Projects in Translational Research (R21)*”. (1R21-NS066984)

Title: Multifunctional Nanoemulsions for Modulation of BBB Transport

Amount: \$429,000 (total)

Duration: February 2010 – April 2013 (with NCE)

Role: Principal Investigator

Source: Northeastern University-Dana Farber Cancer Institute Joint Research Program Cancer Drug Development

Title: Evaluating Synergy between Inhibition of Replication and Promotion of Apoptosis in the Treatment of Ovarian Cancer

Amount: \$100,000 (total)

Duration: July 2012 – June 2014

Role: Principal Investigator (in Collaboration with Dr. Michael Goldberg, Dana-Farber Cancer Institute)

Source: Merrimack Pharmaceuticals, Inc., Cambridge, MA

Title: *In Vivo* Imaging Study

Amount: \$35,000 (total)

Duration: August 2014 – June 2015

Role: Principal Investigator

Source: Novartis Vaccine and Diagnostics, Cambridge, MA

Title: Pharm Sci Industrial Graduate Fellowship Program (designated to Ms. Ruchi Shah)

Amount: \$159,300 (total)

Duration: May 2012 – August 2015

Role: Principal Investigator

Source: Northeastern University Tier-1 Grant

Title: A Cyber-Physical Platform for Rapid Development of Nano-Delivery Systems

Amount: \$50,000 (total)

Duration: July 2014 – December 2015

Role: Principal Investigator (in Collaboration with Dr. Ravi Sundaram, College of Computer and Information Science, Northeastern University)

Source: National Cancer Institute of the National Institutes of Health proposal in response to NCI’s Alliance for Nanotechnology in Cancer RFA CA-09-012 “*Center of Cancer Nanotechnology Excellence (CCNE) U54 Grant*” program (1U54-CA151881. Project #3).

Title: Multi-Modal Therapy for Pancreatic Cancer with Targeted Nanovectors

Amount: \$1,420,972 (total project funding); \$13.9 million (total CCNE funding)

Duration: September 2010 – December 2015 (with NCE)

Role: Principal Investigator of Project #3 (CCNE PI: Prof. Vladimir Torchilin, Department of Pharmaceutical Sciences, Northeastern University)

Source: National Cancer Institute of the National Institutes of Health proposal in response to the NCI's Alliance for Nanotechnology in Cancer RFA CA-09-013 "*Cancer Nanotechnology Platform Partnership U01 Grant*" program (1U01-CA151452)

Title: Combinatorial-Designed Nano-Platforms to Overcome Tumor Drug Resistance

Amount: \$2,317,537 (total)

Duration: September 2010 – August 2016 (with NCE)

Role: Principal Investigator

Source: National Science Foundation, Interdisciplinary Graduate Education and Training (IGERT) Grant (DGE-0965843)

Title: IGERT-Nanomaterial Science and Technology

Amount: \$3,178,512 (total)

Duration: September 2010 – August 2016 (with NCE)

Role: Co-Principal Investigator (PI: Prof. Srinivas Sridhar, The Nanomedicine Education and Research Consortium (NERC), Northeastern University)

Source: Nemucore Medical Innovations, Inc., Wellesley, MA. Sub-Contract of NCI proposal in response to Request for Application "*Academic-Industrial Partnerships for Translation of In Vivo Imaging Systems for Cancer Investigations (R01)*" PAR-10-169 (1R01-CA158881)

Title: Integrated Image-Guided Targeted Therapy for Refractory Ovarian Cancer

Amount: \$3,138,368 (total), NU share: \$823,893

Duration: April 2011 - December 2016 (with NCE)

Role: Principal Investigator

Source: Northeastern University-Houston Methodist Research Institute Collaboration Grant

Title: Non-Viral Telomerase Gene Therapy in Progeria

Amount: \$75,000 (total)

Duration: August 2015 – July 2016

Role: Principal Investigator (in Collaboration with Dr. John P. Cooke, Houston Methodist Research Institute, Houston, TX)

Source: Moderna Therapeutics, Inc. Cambridge, MA

Title: Oral mRNA Delivery and Transfection with Multicompartmental Systems

Amount: \$93,543 (total)

Duration: July 2016 – April 2017

Role: Principal Investigator

Source: National Cancer Institute of the National Institutes of Health, R21 Proposal in response to PAR13-146 "*NCI Exploratory/Developmental Research Grant Program (NCI Omnibus R21)*". (R21 proposal CA-179652-A1).

Title: Targeted Platinates/siRNA Combination Therapy for Resistant Lung Cancer

Amount: \$427,625 (total)

Duration: April 2014 – March 2017 (with NCE)

Role: Principal Investigator

Source: National Institute of General Medical Sciences, National Institutes of Health R01 grant in response to RFA "PAR-10-142 *Interface of the Life and Physical Sciences*" (1R01-GM098117)

Title: Impact of Lipids on Compound Absorption: Mechanistic Studies and Modeling

Amount: \$2,315,953 (total); Co-PI Share: \$238,330

Duration: July 2012 – June 2017

Role: Co-Investigator (PI: Rebecca Carrier, Department of Chemical Engineering, Northeastern University)

Source: Northeastern University Tier-1 Grant
Title: Engineering a Sprayable Multifunctional Wound Dressing
Amount: \$50,000 (total)
Duration: July 2016 – December 2017
Role: Principal Investigator (in Collaboration with Dr. Nasim Annabi, Department of Chemical Engineering in the College of Engineered at Northeastern University)

Source: National Institute of Diabetes, and Digestive Diseases, and Kidney Diseases (NIDDK) of the National Institutes of Health. (1 R01 DK098655)
Title: Hepatic Insulin Resistance and Metabolic Disease
Amount: \$2,427,993 (total), NU share: \$212,447
Duration: April 2013 – March 2018
Role: PI of NU Sub-Contract (PI: Morris F. White, Children's Hospital/Harvard Medical School, Boston, MA)

Source: National Cancer Institute of the National Institutes of Health, R56 "Bridge" (R56-CA198492) Grant on the NCI Nanotechnology for Cancer IRCN (U01) Submission
Title: Integrated Nano-Therapeutics to Overcome Tumor Plasticity and Resistance
Amount: \$300,000 (total)
Duration: September 2017 – August 2018
Role: Principal Investigator

Source: Targagenix, Inc., Stony Brook, NY - Sub-Contract of NCI SBIR Contract
Title: Nanoemulsion Formulation and IND Enabling Studies of a Novel Cancer Stem Cell Cytotoxic Agent
Amount: \$330,000 (total)
Duration: November 2015 – October 2018
Role: Principal Investigator

Source: Takeda Vaccines, Cambridge, MA
Title: Determination of Stability of Inactivated Zika Viral Vaccine Formulation
Amount: \$79,751 (total)
Duration: February 2018 – October 2018
Role: Principal Investigator

Source: National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health, R21 Proposal in response to PA-16-040 "Exploratory/Developmental Bioengineering Research Grants (EBRG) [R21]". (R21 proposal EB-023025-01).
Title: Oral Gene Delivery to Improve Iron Overload Disorders
Amount: \$419,425 (total)
Duration: June 2016 – March 2019 (with NCE)
Role: Co-Principal Investigator (In Collaboration with Dr. Jonghan Kim, Department of Pharmaceutical Sciences, School of Pharmacy at Northeastern University)

Source: Northeastern University-Dana Farber Cancer Institute Joint Research Program on Cancer Drug Development
Title: MicroRNA-Based Reprogramming of Tumor-Associated Macrophages in Ovarian Cancer
Amount: \$100,000 (total)
Duration: August 2017 – July 2019
Role: Principal Investigator (in Collaboration with Dr. Michael Goldberg, Dana-Farber Cancer Institute)

Source: Glaxo-Smith Kline Vaccines, Cambridge, MA
Title: Pharm Sci Industrial Graduate Fellowship Program (designated to Mr. Rushit Lodaya)

Amount: \$195,000 (total)
Duration: October 2015 – December 2019
Role: Principal Investigator

Source: Dicerna Pharmaceuticals, Inc. Cambridge, MA
Title: Pharm Sci Industrial Graduate Fellowship Program (designated to Ms. Dongyu Chen)
Amount: \$195,000 (total)
Duration: October 2015 – December 2019
Role: Principal Investigator

Source: National Cancer Institute of the National Institutes of Health, R21 Proposal in response to PAR13-146 "NCI Exploratory/Developmental Research Grant Program (NCI Omnibus R21)". (R21 proposal CA213114-01A1).
Title: Reprogramming Tumor-Associated Macrophages in PDAC with MicroRNA Nano-Vectors
Amount: \$427,625 (total)
Duration: August 2017 – July 2020 (with NCE)
Role: Multi-Principal Investigator (with Prof. Gerardo Mackenzie, University of California at Davis, Davis, CA)

Source: Northeastern University 2020 "Fast Track" Funding
Title: Nasal Exosome Content Modulation for Anti-Viral Therapeutic Effects
Amount: 30,000 (total)
Duration: June 2020 – May 2021
Role: Principal Investigator

Source: Bessor Pharmaceuticals, Inc., Framingham, MA
Title: Systemic Liposomal Peptide (BP-1002) Formulation and Characterization
Amount: \$31,484 (total)
Duration: February 2021 – May 2021
Role: Principal Investigator

Source: National Institute of Diabetes, and Digestive Diseases, and Kidney Diseases (NIDDK) of the National Institutes of Health.
Title: Heat Shock Protein 90 in Alcoholic Liver Disease: Targeting Macrophage Function
Amount: \$1,570,350 (total), NU Share: \$75,000
Duration: August 2015 – July 2021 (with NCE)
Role: PI of NU Sub-Contract (PI of the Grant: Pranoti Mandrekar, University of Massachusetts Medical School, Department of Medicine, Worcester, MA)

Source: Physical Sciences, Inc., Andover, MA. Sub-Contract of the National Institute of Mental Health SBIR Phase I Application (1R43-MH121179-01).
Title: Multi-channel Functional Imaging Tool for Neuropsychological Drug Development
Amount: \$155,000 (total), NU Sub-contract: \$53,108
Duration: September 2019 – September 2021 (with NCE)
Role: PI of NU Sub-Contract (PI of the Grant: Youbo Zhou, Physical Sciences, Inc., Andover, MA)

Source: Morningside Technology Advisory, LLC, Newton, MA
Title: CNS Delivery of Nilotinib using MIND: Phase 1 & 2
Amount: \$148,500 (total)
Duration: November 2020 to December 2021
Role: Principal Investigator

Source: National Institutes of Health, Office of the Director (R21-OD027052-01)

Sub-contract from Jackson Laboratory, Inc.

Title: Development and Validation of a Novel Cas13a and Nanoparticle Guide-RNA Delivery System that Allows Precise Ablation of Host Macrophage Populations in a Humanized Mouse Model

Amount: \$475,000 (total); NU Sub-contract: \$65,000

Duration: April 2019 – March 2022 (with NCE)

Role: Principal Investigator on NU Sub-Contract (PI of the Grant: Dr. Michael Wiles, Jackson Labs, Bar Harbor, ME)

Source: Northeastern University Tier-1 Grant

Title: Evaluating New Detection Modalities for Covert Pharmaceutical Authentication

Amount: \$50,000 (total)

Duration: July 2021 – June 2022

Role: Co-Investigator (PI: Prof. Laura Lewis, Department of Chemical Engineering, College of Engineering, Northeastern University, Boston, MA)

Source: Takeda Vaccines, Inc., Cambridge, MA (designated to Dr. Kohal Das)

Title: Academic-Industrial Post-Doctoral Fellowship

Amount: \$387,656

Duration: August 2019 – July 2022

Role: Principal Investigator

PATENTS AND INVENTION DISCLOSURES

Amiji, M.M. *"Biocompatible Articles and Method of Making Same"*. United States Patent Number 5,885,609. Issued: March 1999.

Amiji, M.M. *"Drug Delivery Using pH-Sensitive Semi-Interpenetrating Network Hydrogels"*. United States Patent Number 5,904,927. Issued: May 1999.

Langer, R.S., Lynn, D.M., Putnam, D., Amiji, M.M., and Anderson, D.G. *"Biodegradable Poly(Beta-Amino Esters) and Uses Thereof"*. United States Patent Number 6,998,115. Issued: February, 2006.

Langer, R.S., Lynn, D.M., Putnam, D., Amiji, M.M., and Anderson, D.G. *"Biodegradable Poly(Beta-Amino Esters) and Uses Thereof"*. European Patent Application. (Serial number 019775410-2115-US0131270, Filed: October, 2001).

Amiji, M.M. and Taqieddin, E.S. *"Hybrid Immobilized Catalytic System with Controlled Permeability"* United States Patent Application US2004/0266026, Pending (Filed: January, 2003).

Amiji, M.M., Shenoy, D.B., and van Vlerken, L.E. *"Nanoparticulate Delivery Systems for Treating Multi-drug Resistance"*. United States Patent Application, US2006/0257493, Pending (Filed: April, 2006).

Amiji, M.M. and Tiwari, S.K. *"Novel Nanoemulsion Formulations"* United States Patent Application US2007/0148194, Pending (Filed: November, 2006).

Hirt, T., Lu, Z., Meir, W., and Amiji, M. *"Mucoadhesive Vesicles for Drug Delivery"*. PCT and United States Patent Application PCT/US2008/12/157,144. Pending (Filed: August, 2008).

Hanson, R., Amiji, M., and Weissig, V. *"Precision-Guided Nanoparticle System for Drug Delivery"* PCT and United States Patent Application PCT/US2008/01766, Pending (Filed: February, 2008).

Amiji, M. and Iyer, A.K. *"Multi-Functional Self-Assembling Polymeric Nanosystems"*. PCT and United States Patent Application US200961/246,355 and WO 2010042823-A1 Pending (Filed: September, 2009).

Amiji, M., Kalariya, M., Jain, S., and Attarwala, A. *"Multi-Compartmental Macrophage Delivery"*. PCT and United States Patent Application US 20130243689-A1 and WO 2011119881-A1, Pending (Filed: March, 2011).

Amiji, M., Ganta, S., and Tsai, P.-C. *"Multimodal Diagnostic Technology for Early Stage Cancer Lesions"*. PCT and United States Patent Application US 20130224120-A1 and WO 2011119822-A1, Pending (Filed March, 2011).

Amiji, M. and Iyer, A.K. *"Biodegradable Polymeric Buffers"*. PCT and United States Patent Application WO 2014008283-A3, Pending (Filed: July, 2013).

Amiji, M. and Singh, A. *"Releasable Magnetic Cell Capture Technology"*. PCT and United States Patent Application WO 2014110578-A1, Pending (Filed: January, 2014).

Amiji, M., Trivedi, M., and Singh, A. *"Mitochondrial Reprogramming by Non-Viral Nucleic Acid Delivery"*. Provisional United States Patent Application (Filed: September, 2014).

Amiji, M., Singh, A., Nascimento, A.V., and Su, M.J. *"Cellular Reprogramming by Modulation of Extracellular Vesicle (Exosome) Composition"*. Provisional United States Patent Application (Filed: September, 2014).

Amiji, M., Bleier, B., and Padmakumar, S., *"Biodegradable Implant for Sustained Trans-Nasal Delivery of Therapeutic Agents to the Brain"* United States Patent Application, Pending (Filed: July, 2021).

PRESENTATIONS

American Association of Pharmaceutical Scientists Annual Meeting, Orlando, FL. Poster presentation entitled *"Adsorption Isotherms of Doxorubicin on Oxidized Dextran"*. November, 1988.

Controlled Release Society Annual Meeting, San Francisco, CA. Podium presentation entitled *"Mucoadhesive Hydrogels Effective at Neutral pH"*. March, 1989.

Society for Biomaterials Annual Meeting, Santa Fe, NM. Podium presentation entitled *"The Minimum Amount of Biologically Active Fibrinogen Necessary for Surface-Induced Platelet Activation"*. April, 1989.

NIH Conference on Cardiovascular Science and Technology, Bethesda, MD. Podium presentation entitled *"Mechanism of Surface Passivation by Albumin"*. May 1990.

Surfaces in Biomaterials Foundation First Annual Meeting, Minneapolis, MN. Podium presentation entitled *"Mechanism Study on the Prevention of Surface-Induced Platelet Activation by Adsorbed Albumin"*. March, 1991.

Society for Biomaterials Annual Meeting, Scottsdale, AZ. Podium presentation entitled *"Prevention of Protein Adsorption and Platelet Adhesion by Steric Repulsion"*. May, 1991.

American Chemical Society National Meeting, Washington, DC. Podium presentation entitled *"Surface Passivating Effect of PEO/PPO/PEO Triblock Copolymers"*. August, 1992.

American Chemical Society National Meeting, Washington, DC. Podium presentation entitled *"Analysis on the Surface Adsorption of PEO/PPO/PEO Triblock Copolymers"*. August, 1992.

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- American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Podium presentation entitled *"Prevention of Protein Adsorption on Surfaces by PEO/PPO/PEO Triblock Copolymers"*. November, 1992.
- Society for Biomaterials Annual Meeting, Boston, MA. *"Adsorption Behavior of PEO/PPO/PEO Triblock Copolymers on DDS-Glass"*. April, 1994.
- Society for Biomaterials Annual Meeting, Boston, MA. Podium presentation entitled *"Development of Poly(ethylene oxide)-Chitosan Blend Membranes for Hemodialysis"*. April, 1994.
- American Chemical Society Annual Meeting, Washington, DC, Podium presentation entitled *"Chitosan-Poly(ethylene oxide) Semi-IPN as a pH-Sensitive Drug Delivery System"*. August, 1994.
- American Association of Pharmaceutical Scientists Annual Meeting, Orlando, FL. Podium presentation entitled *"Pyrene Fluorescence Study of Insulin Denaturation and Aggregation at Hydrophobic Interfaces"*. November, 1994.
- Controlled Release Society Annual Meeting, Seattle, WA. Poster presentation entitled *"Chitosan-Poly(Ethylene Oxide) Hydrogels for pH-Sensitive Oral Drug Delivery"*. August, 1995.
- American Chemical Society's Conference on Formulations and Drug Delivery, Boston, MA. Podium presentation entitled *"Site-specific Oral Delivery of Antibiotics using pH-Sensitive Hydrogels"*. October, 1995.
- American Association of Pharmaceutical Scientists, Eastern Regional Meeting, New Brunswick, NJ. Poster presentation entitled *"Site-Specific Delivery of Antibiotics for the Treatment of Helicobacter pylori Infection in Peptic Ulcer Disease"*. June, 1995.
- Fifth World Biomaterials Congress, Toronto, Ontario, Canada. Podium presentation entitled *"Surface Modification of Chitosan Hemodialysis Membranes with Anionic Polysaccharides"*. June, 1996.
- Surfaces in Biomaterials '96 Symposium, Chandler, AZ. Poster presentation entitled *"Modification of Chitosan Membrane Surfaces by Complexation-Interpenetration of Anionic Polysaccharides"* September, 1996.
- Nineteenth Annual Undergraduate Research Seminar, Morgantown, WV. Poster presentation entitled *"Factors Influencing Stomach-Specific Antibiotic Delivery for H. pylori Infection"*. October, 1997.
- First International Symposium on Advanced Biomaterials, Montréal, Quebec, Canada. Podium presentation entitled *"Chitosan Surface Modification with Anionic Poly(Ethylene Glycol) Derivative for Improved Blood Compatibility."* October, 1997.
- American Association of Pharmaceutical Scientists Annual Meeting, Boston, MA. Poster presentation entitled *"The Role of Gastric pH and Mucin Permeability on Localized Antibiotic Delivery for H. pylori Infection"*. November, 1997.
- American Association of Pharmaceutical Scientists Annual Meeting, Boston, MA. Poster presentation entitled *"Mucoadhesive Chitosan Microspheres for Stomach-Specific Antibiotic Delivery"*. November, 1997.
- American Association of Pharmaceutical Scientists Annual Meeting, Boston, MA. Poster presentation entitled *"Surface Modification of Chitosan by Polyelectrolyte Complexation-Interpenetration to Improve Biocompatibility"*. November, 1997.
- Society for Biomaterials Annual Meeting, Providence, RI. Podium presentation entitled *"Novel Thermogelling Paclitaxel Formulation for Localized Delivery"*. May, 1999.
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Society for Biomaterials Annual Meeting, Providence, RI. Podium presentation entitled *"Surface Modification of Chitosan Microspheres to Improve Biocompatibility"*. May, 1999.

Controlled Release Society Annual Meeting, Boston, MA. Poster presentation entitled *"Membranes Formed by Physical Interpenetration of Chitosan with PEO/PPO/PEO Triblock Copolymers"*. June, 1999.

American Association of Pharmaceutical Sciences Annual Meeting. Indianapolis, IN. Poster presentation entitled *"Preparation and Characterization of Cross-linked Chitosan Microspheres for Delivery of Tetracycline Locally in the Stomach"*. November, 2000.

American Association of Pharmaceutical Sciences, Pharmaceutical Congress of the Americas, Orlando, FL. Poster presentation entitled *"Intratumoral Administration of Paclitaxel in a Thermogelling Pluronic® F-127 Formulation"*. March, 2001.

American Association of Pharmaceutical Sciences, Pharmaceutical Congress of the Americas, Orlando, FL. Poster presentation entitled *"Biodegradable Chitin-Paclitaxel Microparticle Formulations for Localized Drug Delivery"*. March, 2001.

American Association of Pharmaceutical Scientists, Pharmaceutical Congress of the Americas, Orlando, FL. Poster presentation entitled *"Permselective Membranes Prepared by Physical Interpenetration of Chitosan with PEO/PPO/PEO Triblock Copolymers"*. March, 2001.

American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Poly(epsilon-caprolactone) Nanoparticles for Intracellular Delivery of Tamoxifen"*. April, 2001.

American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Poly(ethylene glycol)-Modified Gelatin Nanoparticles as Long-Circulating Intracellular Delivery Vehicle"*. April, 2001.

American Association of Pharmaceutical Scientists Annual Meeting, Denver, CO. Poster presentation entitled *"Poly(epsilon-caprolactone) Nanoparticles for Intracellular Delivery of Tamoxifen"*. October, 2001.

American Association of Pharmaceutical Scientists Annual Meeting, Denver, CO. Poster presentation entitled *"Perm-Selective Alginate-Chitosan Hybrid Microcapsules for Enzyme Immobilization"*. October, 2001.

American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Long-Circulating, pH-Sensitive Poly(beta-amino ester) Nanoparticles for Tumor-Selective Paclitaxel Delivery"*. April, 2002.

American Association of Pharmaceutical Scientists Annual Meeting, Toronto, Ontario, Canada. Poster presentation entitled *"Cellular Uptake, Trafficking, and DNA Transfection Studies with Poly(ethylene glycol)-Modified Gelatin Nanoparticles"*. November, 2002.

American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"In Vitro Evaluation of DNA Delivery to Tumor Cells Using Poly(ethylene glycol)-Modified Gelatin Nanoparticles"*. April, 2003.

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- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Effect of Chemical Cross-linking of Chitosan Microspheres on Gastric Residence and Local Tetracycline Concentrations in Fasted Gerbils for Local Treatment of H. pylori Infection"*. April, 2003.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Chitosan Stabilized Colloidal Gold Complexes: Cationic Probes for Intracellular DNA Trafficking and Delivery"*. April, 2003.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Plasmid DNA Encapsulation in a Hybrid Nanoparticles-in-Microsphere System for Oral Delivery"*. April, 2004.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Preparation of Thiolated Gelatin Nanoparticles for Rapid Intracellular Delivery in Response to Glutathione"*. April, 2004.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Polymeric Nanoparticles for Targeted and Controlled Tamoxifen Delivery in Breast Cancer: In Vitro and In Vivo Investigations"*. April, 2004.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Biodistribution and Targeting Potential of Poly(ethylene glycol)-Modified Gelatin Nanoparticles in Tumor-Bearing Mice"*. April, 2004.
- American Association of Pharmaceutical Scientists Annual Meeting, Baltimore, MD. Poster presentation entitled *"Polymeric Nanoparticles for Targeted and Controlled Tamoxifen Delivery in Breast Cancer: In-vitro and In-vivo Investigations"*. November, 2004.
- American Association of Pharmaceutical Scientists Annual Meeting, Baltimore, MD. Poster presentation entitled *"Preparation of Thiolated Gelatin Nanoparticles for Rapid Intracellular Delivery in Response to Glutathione"*. November, 2004.
- American Association of Pharmaceutical Scientists Annual Meeting, Baltimore, MD. Poster presentation entitled *"Biodistribution and Targeting Potential of Poly(ethylene glycol)-Modified Gelatin Nanoparticles in Tumor-Bearing Mice"*. November, 2004.
- American Association of Pharmaceutical Scientists Annual Meeting, Baltimore, MD. Poster presentation entitled *"Nanoparticles-in-Microsphere Hybrid Systems for Oral DNA Vaccine Delivery"*. November, 2004.
- Materials Research Society Fall National Meeting, Boston, MA. *"Biomedical Applications of Gold Nanoparticles Functionalized Using Hetero-Bifunctional Poly(ethylene glycol) Spacer"*. December, 2004.
- Materials Research Society Fall National Meeting, Boston, MA. *"Biodegradable Polymeric Nanoparticles for Tumor-Selective Tamoxifen Delivery: In Vitro and In Vivo Studies"*. December, 2004.
- Materials Research Society Fall National Meeting, Boston, MA. *"Biodistribution and Tumor-Targeting Potential of Poly(ethylene glycol)-Modified Gelatin Nanoparticles"*. December, 2004.
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- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Polymeric Nanoparticles for Targeted and Controlled Tamoxifen Delivery in Breast Cancer: In-Vitro and In-Vivo Investigations"*. April, 2005.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Plasmid DNA Encapsulation in a Hybrid Nanoparticles-in-Microsphere System for Oral Delivery"*. April, 2005.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Improved Oral Delivery of Hydrophobic Drugs with Novel Nanoemulsion Formulations"* April, 2005.
- Nano Science and Technology Institute's Nano2005 Conference and Trade Show, Anaheim, CA. *"Biomedical Applications of Gold Nanoparticles Functionalized Using Hetero-bifunctional Poly(ethylene glycol) Spacer"*. May, 2005.
- Nano Science and Technology Institute's Nano2005 Conference and Trade Show, Anaheim, CA. *"Super-paramagnetic Iron Oxide-Gold Core-Shell Nanoparticles for Biomedical Applications"*. May, 2005.
- American Association of Pharmaceutical Scientists Annual Meeting, Nashville, TN. Poster presentation entitled *"Formulation Optimization for the Nanoparticles-In-Microsphere Hybrid Oral Delivery System Using Factorial Design"*. November, 2005.
- American Association of Pharmaceutical Scientists Annual Meeting, Nashville, TN. Poster presentation entitled *"Formulation Development and In Vivo Biodistribution Studies of Poly(ethylene glycol)-Modified Thiolated Gelatin Nanoparticles"*. November, 2005.
- American Association of Pharmaceutical Scientists Annual Meeting, Nashville, TN. Poster presentation entitled *"Novel Nanoemulsions for Improved Oral Delivery of Hydrophobic Drugs"*. November, 2005.
- American Association of Pharmaceutical Scientists Annual Meeting, Nashville, TN. Poster presentation entitled *"Nanoemulsion Formulations for Improved CNS Drug Delivery"*. November, 2005.
- American Association of Pharmaceutical Scientists Annual Meeting, Nashville, TN. Poster presentation entitled *"Application of Statistical Factorial Design for the Preparation of Poly(styrene-b-isobutylene-b-styrene) Triblock Copolymer Microspheres"*. November, 2005.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Intracellular Delivery of Saquinavir in Biodegradable Polymeric Nanoparticles for HIV/AIDS"* April, 2006.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Improved Oral Delivery of Saquinavir in Nanoemulsion Formulations for HIV/AIDS"*. April, 2006.
- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Development, Characterization and Transfection of the Plasmid DNA Encapsulated in Nanoparticles-in-Microsphere Formulations for Oral Delivery"*. April, 2006.
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- American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. Poster presentation entitled *"Tumor-Targeted Delivery of Hydrophobic Drugs in pH-Sensitive Poly(ethylene oxide)-Modified Poly(beta-amino ester) Nanoparticles"*. April, 2006.
- Society for Biomaterials Annual Meeting, Pittsburgh, PA. Podium presentation entitled *"Modulation of Intracellular Ceramide Using Polymeric Nanoparticles to Overcome Multidrug Resistance in Tumor Cells"*. April, 2006.
- Society for Biomaterials Annual Meeting, Pittsburgh, PA. Poster presentation entitled *"Development of the Nanoparticles-in-Microsphere Hybrid Formulations for Oral Delivery of Plasmid DNA"*. April, 2006.
- Society for Biomaterials Annual Meeting, Pittsburgh, PA. Poster presentation entitled *"Novel Nanoemulsions for Improved Oral Delivery of Hydrophobic Drugs"*. April, 2006.
- Nano Science and Technology Institute's Nano2006 Conference and Trade Show, Boston, MA. Podium presentation entitled *"Modulation of Intracellular Ceramide Using Polymeric Nanoparticles to Overcome Multidrug Resistance in Tumor Cells"*. May, 2006.
- Nano Science and Technology Institute's Nano2006 Conference and Trade Show, Boston, MA. Poster presentation entitled *"Formulation Optimization for the Nanoparticles-in-Microsphere Hybrid Oral Delivery Systems Using Factorial Design"*. May, 2006.
- Nano Science and Technology Institute's Nano2006 Conference and Trade Show, Boston, MA. Poster presentation entitled *"Novel Nanoemulsions for Improved Oral Delivery of Poorly Soluble Drugs"*. May, 2006.
- Nano Science and Technology Institute's Nano2006 Conference and Trade Show, Boston, MA. Poster presentation entitled *"Characterization and In Vivo Biodistribution Studies with Poly(ethylene glycol)-Modified Thiolated Gelatin Nanoparticles"*. May, 2006.
- Controlled Release Society Annual Meeting, Vienna, Austria. Poster presentation entitled *"Poly(Styrene-*b*-Isobutylene-*b*-Styrene) Triblock Copolymer Microspheres for Sustained Release Drug Delivery"*. July, 2006.
- Society of Neuroscience 2006 Annual Meeting, Atlanta, GA. Poster presentation entitled *"Brain Delivery of Proteins by the Intranasal Route of Administration Using Cationic Liposomes"*. October, 2006.
- The First National Cancer Institute's Alliance in Nanotechnology Principal Investigator Meeting. San Diego, CA. Poster presentation entitled *"Multifunctional Nanosystems to Overcome Drug Resistance in Cancer"*. October, 2006.
- The First National Cancer Institute's Alliance in Nanotechnology Principal Investigator Meeting. San Diego, CA. Poster presentation entitled *"Modulation of Multidrug Resistance in Cancer with Polymer Blend Nanoparticles"*. October, 2006.
- American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Tumor-Targeted Delivery of Hydrophobic Drugs in pH-Sensitive Poly(ethylene oxide)-Modified Poly(beta-amino ester) Nanoparticles"*. November, 2006.
- American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Development and Characterization of Nanoemulsion Formulations Containing Multimodal Therapeutics for Brain Tumor"*. November, 2006.
- American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Biodegradable Polymeric Nanoparticles for Intracellular Saquinavir Delivery in HIV/AIDS"*. November, 2006.
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American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Oral Plasmid DNA Administration and Transfection Using Nanoparticles-in-Microsphere Formulations"*. November, 2006.

American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Modulation of Multidrug Resistance in Cancer with Polymer Blend Nanoparticles"*. November, 2006.

American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Tumor-Targeted sFlt-1 Gene Delivery Using Long-Circulating Thiolated Gelatin Nanoparticles"*. November, 2006.

American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. Poster presentation entitled *"Improved Oral Delivery of Saquinavir in Nanoemulsion Formulations for HIV/AIDS"*. November, 2006.

Controlled Release Society Annual Meeting, Long Beach, CA. Poster presentation entitled *"Gastrointestinal Gene Delivery in Mice using Polymeric Nanoparticles-in-Microsphere Oral System"*. July, 2007.

Controlled Release Society Annual Meeting, Long Beach, CA. Poster presentation entitled *"Design and Development of a Polymer-Blend Nanoparticle Drug Delivery System to Overcome Multidrug Resistance in Cancer"*. July, 2007.

Controlled Release Society Annual Meeting, Long Beach, CA. Poster presentation entitled *"Paclitaxel and Ceramide Combination Therapy in Biodegradable Polymeric Nanoparticles to Overcome Multidrug Resistance in Cancer"*. July, 2007.

The Second National Cancer Institute's Alliance in Nanotechnology Principal Investigator Meeting. Chapel Hill, NC. Poster presentation entitled *"Nanoparticulate Therapeutic Strategy for Intracellular Ceramide Modulation to Lower Apoptotic Threshold and Overcome Multidrug Resistance in Cancer"*. October, 2007.

American Association of Pharmaceutical Scientists Annual Meeting, San Diego, CA. Poster presentation entitled *"Modulation of Intracellular Ceramide Metabolism with Biodegradable Polymeric Nanoparticle-Encapsulated Tamoxifen to Overcome Multidrug Resistance in Cancer."* November, 2007.

American Association of Pharmaceutical Scientists Annual Meeting, San Diego, CA. Poster presentation entitled *"Multifunctional Polymer Blend Nanoparticles for Temporal-Controlled Release of Combination Therapeutics to Overcome Multidrug Resistance of Cancer"*. November, 2007.

American Association of Pharmaceutical Scientists Annual Meeting, San Diego, CA. Poster presentation entitled *"Development of Novel Biodegradable Polymeric Nanoparticles-in-Microsphere Formulation for Local Plasmid DNA Delivery in the Gastrointestinal Tract"*. November, 2007.

American Association of Pharmaceutical Scientists Annual Meeting, San Diego, CA. Poster presentation entitled *"HER2/neu Receptor-Targeted Engineered Gelatin Nanovectors for Gene Delivery and Transfection in Pancreatic Cancer Cells"*. November, 2007.

American Chemical Society Annual Meeting, New Orleans, LA. Podium presentation entitled *"Gene Delivery and Transfection Studies with Lipopolyplexes in Human Endothelial and Smooth Muscle Cells"*. April, 2008.

Nano Science and Technology Institute's Nano2008 Conference and Trade Show, Boston, MA. Podium presentation entitled *"Gene Delivery and Transfection Studies with Lipopolyplexes in Human Endothelial and Smooth Muscle Cells"*. June, 2008.

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- Nano Science and Technology Institute's Nano2008 Conference and Trade Show, Boston, MA. Podium presentation entitled *"Epidermal Growth Factor Receptor-Targeted Engineered Gelatin Nanovectors for Gene Delivery and Transfection in Pancreatic Cancer Cells"*. June, 2008.
- Nano Science and Technology Institute's Nano2008 Conference and Trade Show, Boston, MA. Poster presentation entitled *"Non-condensing Calcium Alginate Microspheres for Macrophage-Selective Gene Delivery and Transfection"*. June, 2008.
- Nano Science and Technology Institute's Nano2008 Conference and Trade Show, Boston, MA. Poster presentation entitled *"Multifunctional Nanoparticulate System for Simultaneous EGFR Gene Silencing and Enhancement of Apoptosis in Pancreatic Cancer Cells"*. June, 2008.
- Controlled Release Society Annual Meeting, New York, NY. Podium presentation entitled *"Gene Delivery and Transfection Studies in Smooth Muscle Cells with Lipopolyplexes Immobilized in Gelatin-Coated Stainless Steel Substrates."* July, 2008.
- Controlled Release Society Annual Meeting, New York, NY. Poster presentation entitled *"Cellular Trafficking Studies of Ceramide-Loaded Poly(ethylene Oxide)-Modified Poly(epsilon-caprolactone) Nanoparticles with Raman Spectroscopy."* July, 2008.
- American Association of Pharmaceutical Scientists Annual Meeting, Atlanta, GA. Poster presentation entitled *"Epidermal Growth Factor Receptor-Targeted Gelatin-Based Nanoparticles for Reporter and Therapeutic Gene Delivery in Human Pancreatic Cancer Cells"*. November, 2008.
- The Fourth National Cancer Institute's Alliance in Nanotechnology Principal Investigator Meeting. Manhattan Beach, CA. Poster presentation entitled *"Polymer Blend Nanoparticulate System for Combination Paclitaxe/Lonidamine Co-Therapy in Overcoming Multidrug Resistance in Breast and Ovarian Cancer via Exploitation of the Warburg's Effect"*. October, 2009.
- The Fourth National Cancer Institute's Alliance in Nanotechnology Principal Investigator Meeting. Manhattan Beach, CA. Poster presentation entitled *"Inhibition of ABCD1 (MDR-1) Expression by siRNA Nanoparticulate Delivery System to Overcome Drug Resistance in Osteosarcoma"*. October, 2009.
- American Association of Pharmaceutical Scientists Annual Meeting, Los Angeles, CA. Poster presentation entitled *"In Vitro Studies with Estradiol-Loaded Omega-3 Fatty Acid-Containing Oil-in-Water Nanoemulsion Formulations for the Treatment of Coronary Restenosis"*. November, 2009.
- American Association of Pharmaceutical Scientists Annual Meeting, Los Angeles, CA. Poster presentation entitled *"The Effect of Curcumin in Enhancing Oral Absorption and Anti-Tumor Therapeutic Efficacy of Paclitaxel Administered in Nanoemulsion Formulations"*. November, 2009.
- American Association of Pharmaceutical Scientists Annual Meeting, Los Angeles, CA. Poster presentation entitled *"TNF- α Gene Silencing Using Nanoparticles-in-Microsphere Oral Delivery System in an Inflammatory Bowel Disease Model"*. November, 2009.
- Controlled Release Society Annual Meeting, Portland, OR. Poster presentation entitled *"Tuftsin-Modified Alginate Nanoparticles as a Non-Condensing Macrophage-Targeted Gene Delivery System for Anti-Inflammatory Therapy."* July, 2010.

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- Controlled Release Society Annual Meeting, Portland, OR. Poster presentation entitled *"Preliminary Evaluations of Combination Ceramide/Estradiol Therapy in Coronary Restenosis with Omega-3 Fatty Acid-Containing Nanoemulsion Formulations"*. July, 2010.
- Controlled Release Society Annual Meeting, Portland, OR. Poster presentation entitled *"In Vitro Evaluations of Nanoparticle-In-Emulsion Formulations for Gene Delivery and Transfection in Macrophages"*. July, 2010.
- American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA. Poster presentation entitled *"Preliminary Evaluations of Combination Ceramide/Estradiol Therapy in Atherosclerosis with Omega-3 Fatty Acid-Containing Nanoemulsion Formulations"*. November, 2010.
- American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA. Podium and poster presentations entitled *"Tuftsin-Modified Alginate Nanoparticles as a Non-Condensing Macrophage-Targeted Gene Delivery System for Anti-Inflammatory Therapy"*. November, 2010.
- American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA. Poster presentation entitled *"Advances in siRNA Delivery: Preliminary Work in the Development of an siRNA Nanoemulsion Delivery System"*. November, 2010.
- American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA. Poster presentation entitled *"Advances in siRNA Delivery: Preliminary Work in the Development of an siRNA Nanoemulsion Delivery System"*. November, 2010.
- American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA. Poster presentation entitled *"Gene Delivery and Transfection in Human Pancreatic Cancer Cells using Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. November, 2010.
- American Association of Pharmaceutical Scientists, National Biotechnology Conference. San Francisco, CA. Poster presentation entitled *"Therapeutic Gene Delivery and Transfection in Human Pancreatic Cancer Cells Using Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. May, 2011.
- Nano Science and Technology Institute's Nano2011 Conference and Trade Show, Boston, MA. Podium presentation entitled *"Label-Free Raman Micro-Spectral Imaging of the Micro-Environment of Panc-1 Spheroids"*. June, 2011.
- Nano Science and Technology Institute's Nano2011 Conference and Trade Show, Boston, MA. Podium presentation entitled *"Therapeutic Gene Delivery and Transfection in Human Pancreatic Cancer Cells Using Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. June, 2011.
- Controlled Release Society Annual Meeting, National Harbor, MD. Poster presentation entitled *"Label-Free Imaging of Panc-1 Human Pancreatic Tumor Spheroids by Raman Microspectroscopy"*. July, 2011.
- Controlled Release Society Annual Meeting, National Harbor, MD. Poster presentation entitled *"Therapeutic Gene Delivery and Transfection in Human Pancreatic Cancer Cells Using Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. July, 2011.
- 17th International Workshop on Single Molecule Spectroscopy and Ultrasensitive Analysis in the Life Sciences, Berlin, Germany. Poster presentation entitled *"Label-Free and Sub-Micron Imaging of Tumor Micro-Environment In Vitro"*. September, 2011.

The Fifth National Cancer Institute's Alliance in Nanotechnology for Cancer Annual Principal Investigators Meeting, Boston, MA. Poster presentation entitled *"Combinatorial Library Approach Using Functionally Variant Hyaluronic Acid-Based Self-Assembling Nanosystems for Tumor-Targeted Drug and Oligonucleotide Delivery"*. September, 2011.

The Fifth National Cancer Institute's Alliance in Nanotechnology for Cancer Annual Principal Investigators Meeting, Boston, MA. Poster presentation entitled *"Evaluations of Dextran-Based Nanoparticle-Mediated Drug and siRNA Delivery"*. September, 2011.

The Fifth National Cancer Institute's Alliance in Nanotechnology for Cancer Annual Principal Investigators Meeting, Boston, MA. Poster presentation entitled *"Multimodal Therapeutic Approach for Pancreatic Cancer: Delivery of Combination wt-p53 Gene and Gemcitabine in Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. September, 2011.

Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) 38th Annual Meeting. Reno, NV. Posium presentation entitled *"Label-Free Sub-Micron Imaging of Biological Systems"*. October, 2011.

American Association of Pharmaceutical Scientists Annual Meeting, Washington, DC. Poster presentation entitled *"Combinatorial Library Approach Using Functionally Variant Hyaluronic Acid-Based Self-Assembling Nanosystems for Tumor-Targeted Drug and Oligonucleotide Delivery"*. October, 2011.

American Association of Pharmaceutical Scientists Annual Meeting, Washington, DC. Poster presentation entitled *"Squalane oil Multiple Emulsion Formulations for Enhanced Immune Response to Peptide-Based Melanoma Vaccine"*. October, 2011.

American Association of Pharmaceutical Scientists Annual Meeting, Washington, DC. Poster presentation entitled *"Macrophage-Targeted Tuftsin-Modified Alginate Nanoparticles for Anti-Inflammatory Gene Therapy in the Treatment of Rheumatoid Arthritis"*. October, 2011.

American Association of Pharmaceutical Scientists Annual Meeting, Washington, DC. Poster presentation entitled *"Multimodal Therapeutic Approach for Pancreatic Cancer: Delivery of Combination wt-p53 Gene and Gemcitabine in Epidermal Growth Factor Receptor-Targeted Gelatin Nanoparticles"*. October, 2011.

Connective Tissue Oncology Society (CTOS) Annual Meeting, Chicago, IL. Podium presentation entitled *"Dextran-Based Nanoparticulate Delivery System to Overcome Multidrug Resistance in Osteosarcoma"*. October, 2011.

Eight Annual Workshop on FT-IR Spectroscopy in Microbiological and Medical Diagnostics. Berlin, Germany. Poster presentation entitled *"Raman-Active Gold Nanoparticles as Beacons in Cervical Cancer Cells"*. October, 2011.

International Society of Pharmaceutical Engineers Annual Meeting. Dallas, TX. Podium presentation entitled *"Macrophage-Targeted Tuftsin-Modified Alginate Nanoparticles for Anti-Inflammatory Gene Therapy in the Treatment of Rheumatoid Arthritis"*. November, 2011.

American Association of Pharmaceutical Scientists, National Biotechnology Conference. San Diego, CA. Poster presentation entitled *"Macrophage-Targeted Tuftsin-Modified Alginate Nanoparticles for Anti-Inflammatory Gene Therapy in the Treatment of Experimental Arthritis"*. May, 2012.

INVITED PRESENTATIONS AND TUTORIALS

Massachusetts College of Pharmacy and Allied Health. Division of Pharmaceutical Sciences. Boston, MA. *"Formulation of Controlled Release Dosage Forms"*. May, 1996.

Advanced Magnetics, Inc., Cambridge, MA. *"Poly(Ethylene Glycol)-Modified Biomaterial Surfaces"*. January, 1998.

Innovative Imaging Systems, Inc., North Billerica, MA. *"Polymers for Controlled Drug Delivery Systems"*. August, 1998.

Kuwait University, Faculty of Pharmacy, Safat, Kuwait. *"Medical and Pharmaceutical Applications of Chitosan"*. February, 1999.

Tufts University, Department of Chemical Engineering and Bioengineering and Center for Biotechnology Engineering. Medford, MA. *"Chitosan-Based Biomaterials and Drug Delivery Systems"*. March, 2000.

Massachusetts Institute of Technology (MIT), Department of Chemical Engineering, Cambridge, MA. *"Polymeric Site-Specific Drug Delivery Systems"*. September, 2000.

Zycos, Inc., Lexington, MA. *"Cationic Interpenetrating Network Hydrogels for DNA Delivery"*. December, 2000.

Northeastern University, Department of Pharmaceutical Sciences, Boston, MA. *"Novel Biodegradable pH-Responsive Polymers for Intracellular Delivery"*. March, 2001.

Northeastern University, School of Pharmacy, Boston, MA. *"Polymeric Delivery Systems for Drugs and Genes"*. March, 2002.

Cambridge Scientific, Inc., Cambridge, MA. *"Polymeric Drug and Gene Delivery Systems"*. March, 2002.

Marine Polymer Technologies, Inc., Topsfield, MA. *"Chitosan-Based Biomaterials and Drug Delivery Systems"*. April, 2002.

Northeastern University, Biotechnology Academic Steering Committee, Boston, MA. *"Polymeric Biomaterials and Drug Delivery Systems"*. January, 2003.

Northeastern University, Department of Biology, Boston, MA. *"Polymeric Biomaterials and Delivery Systems"*. April, 2003.

Northeastern University, Technology Transfer and Biotechnology Symposium, Boston, MA. *"Polymeric Biomaterials and Drug Delivery Systems"*. April, 2003.

Catalyst Oncology, Inc., Providence, RI. *"Tumor-Targeted Polymeric Nanoparticle Delivery Systems"*. December, 2003.

Archemix, Inc., Cambridge, MA. *"Nanotechnology for Drug Delivery"*. May, 2004.

Novartis Institute for Biomedical Research, Cambridge, MA. *"Polymeric Technologies for Delivery of Drugs and Genes"*. August, 2004.

University of MA at Lowell, Lowell, MA. *"Polymer-Based Technologies for Targeted Delivery of Drugs and Genes"*. February, 2005.

Spherics, Inc., Lincoln, RI. *"Polymeric Delivery Systems for Drugs and Genes"*. April, 2005.

Northeastern University, Department of 2005 Pharmaceutical Sciences Research Showcase, Boston, MA. *"Nanotechnology for Tumor-Targeted Delivery of Drugs and Genes"*. May, 2005.

Nano Science and Technology Institute's Nano2005 Conference and Trade Show, Anaheim, CA. *"Nanotechnology for Medical Diagnosis, Imaging, and Therapy – a Tutorial"*. May, 2005.

Nano Science and Technology Institute's Nano2005 Conference and Trade Show, Anaheim, CA. *"Tumor Targeted Nanocarriers for Drug and Gene Delivery"*. May, 2005.

Pfizer Central Research, Groton, CT. *"Polymeric Biomaterials and Targeted Delivery Systems"*. September, 2005.

Universidad Metropolitana (UMET), Department of Science and Technology, National Science Foundation Sponsored XIV Undergraduate Research Symposium. San Juan, PR. *"Nanotechnology for Medical Imaging and Therapy"*. September, 2005.

Strategic Research Institute, Inc., Cambridge, MA. Nanomedicine: Commercialization of Drug Discovery, Delivery, and Diagnostics Conference. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. October, 2005.

Nano Science and Technology Institute's Tutorial, Washington, DC *"Nanotechnology for Cancer Therapeutics – A Tutorial"* October, 2005.

National Institute of Standards and Technology, Gaithersburg, MD. *"Nanotechnology for Cancer Imaging and Therapy"* December, 2005.

Alkermes, Inc., Cambridge, MA. *"Multi-Functional Nanotechnology for Imaging and Therapy"*. April, 2006.

Nano Science and Technology Institute's Tutorial, Washington, DC *"Nanotechnology for Cancer Therapeutics – A Tutorial"* May, 2006.

Nano Science and Technology Institute's Nano2006 Conference and Trade Show, Boston, MA. *"Nanotechnology in Drug Delivery: An Overview"*. May, 2006.

2006 Cancer Nanotechnology Conference, Paris, France. *"Nanotechnology for Tumor-Targeted Drug and Gene Delivery"*. May, 2006.

Northeastern University, Department of 2006 Pharmaceutical Sciences Research Showcase, Boston, MA. *"Multi-functional Nanosystems for Drug Delivery and Imaging"*. May, 2006.

National Cancer Institute, Center for Cancer Research, Nanobiology "Think Tank" Meeting, Frederick, MD. *"Multi-functional Nanosystems to Overcome Drug Resistance in Cancer"*. June, 2006.

Microfluidics, Inc., Newton, MA. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. June, 2006.

Wyeth Pharmaceuticals, Inc., Andover, MA. *"Nanotechnology and the Promise of Molecular Medicine"*. July, 2006.

Emory University, School of Medicine, Department of Ophthalmology, Atlanta, GA. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. July, 2006.

New Jersey Center for Biomaterials, Rutgers – the States University of New Jersey, Piscataway, NJ. *"Polymers for Tumor-Targeted Delivery and Modulation of Multidrug Resistance"*. August, 2006.

Accelrys Nanobiotechnology Seminar Series, Cambridge, MA. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. August, 2006.

Swiss House of Advanced Research and Education (SHARE), Consulate of Switzerland, Boston, MA. *"Nanotechnology for Medical Diagnosis and Treatment"*. September, 2006.

American Academy of Nanomedicine 2nd Annual Meeting, Washington, DC. *"Nanotechnology for Targeted Drug and Gene Delivery"*. September, 2006.

Epic Therapeutics, Inc., Norwood, MA. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. September, 2006.

Twelfth Samsung International Symposium on Molecular Medicine, Seoul, Korea. *"Multi-functional Nanosystems for Tumor-Targeted Drug and Gene Delivery"*. September, 2006.

Nanotechnology 2006 Conference, Rensselaer Polytechnic Institute, Troy, NY. *Multi-functional Nanosystems for Targeted Drug and Gene Delivery"*. September, 2006.

Missouri Nanotechnology Alliance – Third Annual Meeting, Columbia, MO. *"Multi-functional Nanosystems for Tumor-Targeted Drug and Gene Delivery"*. October, 2006.

Cambridge Healthtech Institute's Targeted Nanodelivery Conference, Baltimore, MD. *"Multi-functional Nanosystems to Overcome Drug Resistance in Cancer"*. October, 2006.

Oncogene Science – A Bayer Healthcare Company, Cambridge, MA *"Nanomedicine: Realizing the Potential for Early Cancer Diagnosis and Molecular Therapy"*. October, 2006.

Northeastern University, Department of Chemistry and Chemical Biology, Boston, MA. *"Multi-functional Nanosystems for Imaging and Drug Delivery"*. November, 2006.

Second Annual Conference on Biomaterials and Advanced Drug Delivery, New Jersey Center for Biomaterials. Rutgers's - The State University of New Jersey, New Brunswick, NJ. *"Multi-functional Nanosystems for Drug and Gene Delivery"*. November, 2006.

American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology Annual Meeting, Tucson, AZ. *"Nanotechnology for Medical Imaging and Therapy"*. December, 2006.

National Institutes of Health, National Cancer Institute's, Drug Development and Therapeutics Committee, Bethesda, MD. *"Multifunctional Nanosystems to Overcome Drug Resistance in Cancer"*. January, 2007.

Millennium Pharmaceuticals, Inc., Cambridge, MA. *"Nanotechnology Applications in Translational Oncology"*. January, 2007.

Harvard-MIT Health Science and Technology Program, MIT, Cambridge, MA. *"Nanotechnology Applications in Cancer Therapy"*. February, 2007.

Third Annual Conference on Biomaterials and Advanced Drug Delivery New Jersey Center for Biomaterials, Rutgers's - The State University of New Jersey, New Brunswick, NJ. *"Polymer Libraries for Tumor-Targeted Delivery and Modulation of Multidrug Resistance"*. March, 2007.

Strategic Research Institute's Second Annual Nanomedicine Conference, Washington, DC. *"Nanotechnology for Tumor-Targeted Delivery of Drugs and Genes"*. March, 2007.

Materials Research Society, 2007 Spring Meeting, San Francisco, CA. *"Pre-Clinical In Vivo Efficacy and Safety Studies – a Tutorial"*. April, 2007.

Materials Research Society, 2007 Spring Meeting, San Francisco, CA. *"Multifunctional Nanosystems for Targeted Drug and Gene Delivery"*. April, 2007.

American Association of Pharmaceutical Scientists, Northeast Regional Discussion Group Annual Meeting, Rocky Hill, CT. *"Micro- and Nanotechnology for Oral Drug and Gene Delivery"*. April, 2007.

Genzyme Pharmaceuticals, Inc. – Drug and Biomaterials R&D, Waltham, MA. *"Nanotechnology Applications in Translational Oncology"*. April, 2007.

Vertex Pharmaceuticals, Inc., Cambridge, MA. *"Nanotechnology Applications in Translational Oncology"*. April, 2007.

Thayer School of Engineering, Dartmouth College, Hanover, NH. *"Cancer Nanomedicine: Potential for Targeted Delivery and Molecular Medicine"*. May, 2007.

2007 Association for Research in Vision and Ophthalmology Annual Meeting, Fort Lauderdale, FL. *"Nanotechnology in Advanced Drug Delivery: An Overview"*. May, 2007.

2007 Biotechnology Industry Organization Annual Conference, Cancer Nanotechnology for Early Diagnosis and Therapy Symposium, Boston, MA. *"Multifunctional Nanosystems for Tumor-Targeted Drug and Gene Delivery"*. May, 2007.

Northeastern University, Department of Pharmaceutical Sciences 2007 Research Showcase, Boston, MA. *"Systemic and Oral Therapeutic Gene Delivery with Non-Viral Vectors"*. May, 2007.

2007 Cancer Nanotechnology Conference, Paris, France. *"Multifunctional Nanosystems for Tumor-Targeted Drug and Gene Delivery"*. June, 2007.

Wellman's Photomedicine Center, MA General Hospital, Boston, MA. *"Nanotechnology for Cancer Imaging and Therapy"*. July 2007.

National Cancer Institute, National Institutes of Health. Frederick, MD. *"Multifunctional Nanosystems to Overcome Drug Resistance in Cancer"*. July 2007.

Cerulean (Tempo) Pharmaceuticals, Inc., Cambridge, MA. *"Nanotechnology Applications in Translational Oncology"*. September 2007.

Tufts University – New England Medical Center, Molecular Oncology Research Institute, Boston, MA. *"Nanotechnology Applications in Translational Oncology"*. September 2007.

Merck Research Laboratories, Boston, MA. *"Nanotechnology Applications in Translational Oncology"*. September 2007.

2007 Boston Society for Advanced Therapeutics, Annual Meeting, Harvard Medical School, Boston, MA. *"Nanotechnology for Targeted Delivery of Drugs and Genes"*. September 2007.

Second Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Chapel Hill, NC. *"Multifunctional Nanoparticles to Overcome Tumor Drug Resistance"*. October 2007.

Northeastern University, University's Board of Trustee's Annual Meeting, Boston, MA. *"Nanomedicine: Opportunity for Targeted Imaging and Drug Delivery"*. October, 2007.

Dartmouth College, Thayer School of Engineering, 8th Annual Nanomaterials Conference, Hanover, NH. *"Nanotechnology for Cancer Specific Drug and Gene Delivery"*. October, 2007.

The Fifth Annual Nanomedicine and Drug Delivery Symposium (NanoDDS), Boston, MA *"Multifunctional Nanotherapeutic Strategies for Drug and Gene Delivery"*. November, 2007.

University of Central Florida, Nano Science and Technology Center, Orlando, FL. *"Nanotechnology Applications in Translational Oncology"*. November, 2007.

Dartmouth Medical School, Hanover, NH. *"Nanotechnology Applications in Translational Oncology"*. December, 2007.

University of South Carolina, Department of Pharmaceutical Sciences, College of Pharmacy, Columbia, SC. *"Nanomedicine: Opportunity for Translation of Molecular Therapies"*. February, 2008.

Medical University of South Carolina, School of Pharmacy, Charleston, SC. *"Nanomedicine: Opportunity for Translation of Molecular Therapies"*. February, 2008.

IQPC, The Future of Nanotechnology for Targeted Drug Delivery Conference, Boston, MA. *"Nanotechnology Applications in Translational Oncology"*. February, 2008.

Novartis Institutes for Biomedical Research, Cambridge, MA. *"Advances in Oral Drug and Gene Delivery Systems"*. April, 2008.

Museum of Science, Boston, MA. *"Nanomedicine: Realizing the Potential for Targeted Cancer Therapy"*. April, 2008.

Boston University, College of Engineering, 2008 Emerging Technology and Best Practices Seminar Series Nanotechnology in Medicine: From Diagnostics to Therapeutics Program, Boston, MA. *"Advances in Nanotechnology for Drug and Gene Delivery"*. April, 2008.

Microfluidics Corporation, Newton, MA. *"Nanotechnology for Targeted Imaging and Drug Delivery"*. April, 2008.

Atrium Medical Corporation, Hudson, NH. *"Advances in Nanotechnology for Drug and Gene Delivery"*. April, 2008.

First European Conference for Clinical Nanomedicine, Basel, Switzerland. *"Polymeric Nanosystems for Targeted Delivery of Drugs and Genes"*. May, 2008.

Mayo Clinic, Department of Biomedical Engineering, Rochester, MN. *"Nanotechnology Applications in Translational Oncology"*. September, 2008.

Pfizer – Research Technology Center, Cambridge, MA. *"Nanomedicine: Opportunity for Translation of Molecular Therapies"*. September, 2008.

Bio-Rad, Inc., Hercules, CA. *"Advances in Nanotechnology for Non-Viral Gene Delivery"*. October, 2008.

Purdue University, School of Pharmacy, West Lafayette, IN. *"Multifunctional Nanosystems for Targeted Drug and Gene Delivery"*, November, 2008.

American Association of Pharmaceutical Scientists 2008 National Meeting, Atlanta, GA. *"Advances in Nanotechnology for Drug and Gene Delivery"*, November, 2008.

Materials Research Society, 2008 Fall Meeting, Boston, MA. *"Multifunctional Nanosystems for Cancer-Targeted Imaging and Drug Delivery"*. December, 2008.

Xavier University of Louisiana, College of Pharmacy, New Orleans, LA. *"Multifunctional Nanosystems for Targeted Drug and Gene Delivery"*. December, 2008.

Arqule, Inc., Waltham, MA. *"Nanotechnology Applications in Translational Oncology"*. January, 2009.

Indo-US Science and Technology Forum-Sponsored Cancer Nanotechnology Symposium, New Delhi, India. *"Multifunctional Nanosystems to Overcome Tumor Drug Resistance"*. February, 2009.

Panacea Biopharmaceuticals, Inc. New Delhi, India. *"Advances in Nanotechnology for Non-Viral Gene Delivery"*. February, 2009.

Institute of Genomics and Integrative Biology, New Delhi, India. *"Multi-functional Nanosystems for Targeted Drug and Gene Delivery"*. February, 2009.

Cadila Pharmaceuticals, LTD, Ahmedabad, India. *"Multi-functional Nanosystems for Targeted Drug and Gene Delivery"*. February, 2009.

Microfluidics Corporation, Newton, MA. *"Multifunctional Nanosystems for Targeted Imaging and Drug Delivery"*. February, 2009.

Strem Chemicals, Inc., Newburyport, MA. *"Nanotechnology Applications in Translational Oncology"*. February, 2009.

Microfluidics Corporation, Newton, MA. Webinar on *"Multifunctional Nanomedicine: Opportunity for Targeted Drug and Gene Delivery"*. May, 2009.

Massachusetts General Hospital, Department of Orthopedic Surgery and Orthopedic Oncology, Boston, MA. *"Multifunctional Nanosystems to Overcome Tumor Drug Resistance"*. July, 2009.

Microfluidics Corporation, Newton, MA. Roundtable Discussions with Scientific Board and Directors. *"Nanomedical Technologies in Early Disease Diagnosis, Imaging and Therapy"*. July, 2009.

2009 American Association of Colleges of Pharmacy Annual Meeting, Boston, MA. *"Nanomedical Technologies in Early Diagnosis, Imaging and Therapy"*. July, 2009.

Novartis Institute of Biomedical Research, Vaccine and Diagnostics Division, Cambridge, MA. *"Advances in Oral Non-Viral Gene Delivery Systems"*. July, 2009.

2009 Nano Business Alliance Conference, Chicago, IL. *"Nanotechnology in Early Diagnosis and Targeted Therapy"*. September, 2009.

Roche Pharmaceuticals Partnering Event, Cambridge, MA. *"Nanotechnology in Early Diagnosis and Targeted Therapy"*. September, 2009.

Fifth Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Manhattan Beach, CA. *"Multifunctional Nanoparticles to Overcome Tumor Drug Resistance"*. October 2009.

First Annual Conference of the American Society for Nanomedicine, Bolger Center, Potomac, MD. *"Multifunctional Nanosystems for Cancer Diagnosis and Therapy"*. October, 2009.

University of Wisconsin at Madison, School of Pharmacy, Madison, WI. *"Nanotechnology in Early Diagnosis and Targeted Therapy"*. November, 2009.

University of Illinois at Chicago, College of Pharmacy, Chicago, IL. *"Nanomedicine: Opportunity for Early Diagnosis and Targeted Therapy"*. January, 2010.

Langer Lab Seminar Series, Massachusetts Institute of Technology, Cambridge, MA. *"Nanotechnology in Early Diagnosis and Targeted Therapy"*. February, 2010.

University of Nebraska Medical Center, College of Pharmacy, Omaha, NE. *"Nanotechnology in Early Diagnosis and Targeted Therapy"*. March, 2010.

Canadian Society for Pharmaceutical Sciences 2010 Annual Meeting, Vancouver, British Columbia, Canada. *"Nanotechnology Applications in Cancer Diagnosis and Therapy"* June, 2010.

National Cancer Institute, Center for Cancer Research, Nano-Biology Program, Frederick, MD. *"Nanotechnology Applications in Cancer Diagnosis and Therapy"* June, 2010.

Stanford University, School of Medicine, Nano-Biotechnology Program Seminar Series, Stanford, CA. *"Multifunctional Nanosystems for Early Diagnosis and Targeted Therapy"*. June 2010.

American Chemical Society's National Meeting, Boston, MA. *"Multifunctional Nanosystems for Tumor Imaging and Therapy"*. August, 2010.

Second Annual Conference of the American Society for Nanomedicine, NIAID-Sponsored Symposium on Nanotechnology for HIV/AIDS. Bolger Center, Potomac, MD. *"Nanotechnology Advances in the Prevention and Treatment of HIV/AIDS"*. October, 2010.

Avila Therapeutics, Inc., Waltham, MA. *"Nanotechnology for Disease Diagnosis and Targeted Therapy"*. November, 2010.

2010 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Bethesda, MD. *"Combinatorial-Designed Nano-Platforms to Overcome Tumor Drug Resistance"*. November, 2010.

Center for Medicine and Innovative Technologies (CIMIT)-Wellcome Trust Joint Workshop on PTSD and TBI. Boston, MA. *"Strategies for Overcoming the Blood-Brain Barrier in CNS Therapies"*. January, 2011.

Fifteenth International Symposium on Recent Advances in Drug Delivery Systems, University of Utah, Salt Lake City, UT. *"Multifunctional Nanosystems for Targeted Delivery of Molecular Therapies"*. February, 2011.

Keynote Presentation at the 2011 GRASP Annual Meeting, MA College of Pharmacy and Health Sciences, Boston, MA. *"Multifunctional Nanosystems for Molecular Medicine"*. June, 2011.

Fox Chase Cancer Center, Philadelphia, PA. *"Multifunctional Nanosystems to Overcome Tumor Drug Resistance"*. July, 2011.

2011 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Tutorial Presentation, Boston, MA. *"Nucleic Acid Therapeutics: Using DNA and Small Interfering RNA for Cancer"*. September 2011.

2011 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Boston, MA. *"Taming the Beast: Nanotechnology Solutions for Tumor Aggression"* September, 2011.

Eight Lohmann Therapie Systems (LTS) Academy Meeting, Bonn, Germany. *"Multi-functional Nanomedicines: From Diagnostics to Targeted Delivery"*. September, 2011.

Indiana University, Department of Biochemistry and Molecular Biology, Indianapolis, IN. *"Multi-functional Nanomedicines: From Cancer Diagnostics to Targeted Delivery"*. October, 2011.

University of Missouri at Columbia, Oncology Grand Rounds, Columbia, MO. *"Multi-functional Nanomedicines: From Cancer Diagnostics to Targeted Delivery"*. October, 2011.

American Association of Pharmaceutical Scientists (AAPS) 2012 annual meeting. Special symposium on *"Nano-Delivery Systems for Vaccines"*. Washington, DC. *"Multi-Compartmental Delivery Systems for Cancer Vaccination"*. October, 2011.

Carolina Center for Cancer Nanotechnology Excellence Symposium, University of North Carolina. Chapel Hill, NC. *"Multifunctional Nanosystems: From Diagnostic Imaging to Targeted Therapies"*. January, 2012.

Second International Conference on Nanotechnology at Bio-Medical Interface. Amrita Centre for Nanosciences and Molecular Medicine, Kochi, Kerala State, India. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. February, 2012.

Wayne State University, Department of Pharmaceutical Sciences, Applebaum College of Pharmacy and Allied Health. Detroit, MI. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. March, 2012.

2012 Nano-Bio International Collaborative Conference, University of South Florida, Tampa, FL. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. March, 2012.

Tufts University, Center for Translational Science Institute (CTSI) Roundtable Discussions, Boston, MA. *"Delivery Strategies for CNS Therapies"*. March, 2012.

Tufts University School of Medicine, Cancer Center Seminar Program. Boston, MA. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. April, 2012.

Canadian Society for Pharmaceutical Sciences 2012 Annual Meeting, Toronto, Ontario, Canada. *"Multi-Compartmental Lipid Delivery Systems for Cancer Vaccination"*. June, 2012.

2012 Nano Science and Technology Institute's Cancer Nanotechnology Symposium. Santa Clara, CA. *"Translational Cancer Nanomedicine: Multimodal Strategies to Overcome Tumor Drug Resistance"*. June, 2012.

University of Illinois at Urbana-Champaign 2012 BioSensing, BioActuation, and BioNanotechnology Summer Institute. Urbana-Champaign, IL. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. August, 2012.

2012 American Association of Pharmaceutical Scientists Annual Meeting *"Tumor-Targeting Symposium"*. Chicago, IL. *Translational Cancer Nano-Medicine: Multimodal Strategies to Overcome Tumor Drug Resistance* October, 2012.

2012 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Houston, TX. *"Combinatorial-Designed Self-Assembled Nano-Systems for Tumor-Targeted RNAi/Drug Therapy"* November, 2012.

University of Toronto, Leslie Dan School of Pharmacy, Toronto, Ontario, Canada. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. November, 2012.

Merck Research Laboratories, Rahway, NJ. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. November, 2012.

American Society of Health-Systems Pharmacists (ASHP) Midyear Meeting, *Spotlight on Science* Plenary Talk, Las Vegas, NV. *"Nanotechnology in Medicine: Very Tiny Solutions for Big Challenges"*. December, 2012.

National Cancer Institute, National Institutes of Health, Special symposium on "Dysregulated Endocytosis in Cancer". Bethesda, MD. *"Nanotechnology for Tumor-Targeted Drug and Nucleic Acid Delivery"*. January, 2013.

Harvard University, School of Engineering and Applied Sciences, Cambridge, MA. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. January, 2013.

University of Connecticut, School of Pharmacy, Department of Pharmaceutical Sciences. Storrs, CT. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. April, 2013.

Ferris State University, College of Pharmacy's 60th Annual Spring Pharmacy Seminar Series Keynote Presentation, Big Rapids, MI. *"Nanotechnology in Medicine: Very Tiny Solutions for Bio-Medical Challenges"*. May, 2013.

University of Missouri Kansas City, School of Pharmacy, Department of Pharmaceutical Sciences. Kansas City, MO. *"Translational Cancer Nano-Medicine: From Diagnostic Imaging to Targeted Therapies"*. May, 2013.

First International Translational Nanomedicine Conference. Plenary Presentation. Northeastern University, Boston, MA. *"Translational Nano-Medicine: Diagnostics and Therapeutics for Cancer and Inflammatory Diseases"*. July, 2013.

University of Massachusetts Medical School, Program in Molecular Medicine, Worcester, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases"*. September, 2013.

2013 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Bethesda, MD. *"Combinatorial-Designed Nano-Platforms: Opportunity for Targeted Delivery of Drugs and siRNA"* September, 2013.

Tufts University, Department of Biomedical Engineering, College of Engineering, Medford, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases"*. March, 2014.

Johnson & Johnson Innovation Center, Cambridge, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Cancer, Pain, and Inflammatory Diseases"*. April, 2014.

2014 Cambridge Healthtech Institute (CHI) Biologics Formulation and Delivery Summit, Keynote Presentation, Cambridge, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases"*. May, 2014.

2014 Oligonucleotide and Peptide Therapeutics Symposium – TIDES, Providence, RI. *“CNS Delivery of Peptide Therapeutics”*. May, 2014.

Second International Translational Nanomedicine Conference. Plenary Presentation. Northeastern University, Boston, MA. *“Nanotechnology for CNS Delivery of Biological Therapeutics”*. July, 2014.

Second Annual Workshop on Micro- and Nano-Technologies for Medicine: Emerging Frontiers and Applications. MIT-Harvard Science and Technology Program and the Wyss Institute. Cambridge, MA. *“Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases”*. July, 2014.

2014 Annual National Cancer Institute's Nanotechnology in Cancer Alliance's Principal Investigators Meeting, Bethesda, MD. *“Combinatorial-Designed Nano-Platforms: RNAi and Drug Co-Therapy in Resistant Tumors”* October, 2014.

Houston Methodist Research Institute's George and Angelina Kostas Research Center for Cardiovascular Nanomedicine Inaugural Symposium. Houston, TX. *“Targeted Therapeutic Delivery for Endothelial Dysfunction in Cardiovascular Diseases”*. October, 2014.

University of Michigan, College of Pharmacy, Department of Pharmaceutical Sciences, Ann Arbor, MI. *“Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases”*. November, 2014.

Purdue University, School of Pharmacy and Pharmacal Sciences, Twelfth Annual Garnet E. Peck Symposium, West Lafayette, IN. *“Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Diseases”*. February, 2015.

Henry Stuart Talks, London, UK. *“Nanotechnology for CNS Delivery of Biological Therapeutics”*. March, 2015.

Takeda Pharmaceuticals, New Frontier Science, Cambridge, MA. *“Nanotechnology for Systemic and Local GI Delivery”*. March, 2015.

Microfluidics, Inc., Westwood, MA. A webinar presentation on *“Nano-Emulsions for CNS Delivery of Biological Therapeutics”*. April, 2015.

New England Structural Biology Association (NESBA) Annual Symposium, Bentley University, Waltham, MA. Keynote presentation entitled *“Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases”*. May, 2015.

King Abdulaziz University, Faculty of Pharmacy, Jeddah, Saudi Arabia. Presentation to the graduating class of Doctor of Pharmacy students on *Pharmacy Career Day 2015* entitled *“Translational Nano-Medicine: Opportunities in Graduate Education and Research”*. May, 2015.

King Abdulaziz University, Faculty of Pharmacy, Jeddah, Saudi Arabia. Research presentation to the faculty and students entitled *“Translational Nano-Medicine: Tiny Solutions for Big Biomedical Challenges”*. May, 2015.

Tufts University Cancer Center 2015 Research Retreat, Cummings School of Veterinary Medicine at Tufts, Grafton, MA. *“Multimodal Nanotechnology Solutions for Drug Resistant Tumors”*. June, 2015.

Eight European Summit for Clinical Nanomedicine and Targeted Medicine, Basel, Switzerland. *“Macrophage-Targeted Nano-Delivery Systems for Anti-Inflammatory Therapy”*. June, 2015.

Third Annual Workshop on Micro- and Nano-Technologies for Medicine: Emerging Frontiers and Applications. MIT-Harvard Science and Technology Program and the Wyss Institute. Cambridge, MA. *"Advances in the Delivery of Cancer Vaccines and Therapeutics"*. July, 2015.

Northeastern University and Houston Methodist Research Institute Collaborative Meeting, Boston, MA. *"Targeted Nucleic Acid Delivery in Inflammatory Diseases"*. August, 2015.

Bioscience Seminar Program, Morsani College of Medicine, USF Health, University of South Florida, Tampa, FL. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Disease"*. September, 2015.

Houston Methodist Research Institute's George and Angelina Kostas Research Center for Cardiovascular Nanomedicine Second Annual Symposium. Houston, TX. *"Targeted Delivery of Nucleic Acids for Inflammatory Diseases"*. October, 2015.

Forsyth Institute, Cambridge, MA. *"Advances in Nanotechnology for Inflammatory Diseases"*. January, 2016.

Dicerna Pharmaceuticals, Inc., Cambridge, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Disease"*. February, 2016.

Eight Bangalore India Nano Conference. Bangalore, Karnataka State, India. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Disease"*. March, 2016.

2016 Annual SPIE Conference. Special symposium on Multifunctional Nanoparticles for Biomedical Research, Baltimore, MD. *"Multifunctional Nanoparticles for Nucleic Acid Therapy"*. April, 2016.

2016 American Association of Pharmaceutical Scientists, National Biotechnology Conference, Boston, MA. *"Targeted Nano-Therapeutics for Drug Resistant Tumors"*. May, 2016.

2016 Pharmaceutical Sciences Research Showcase, Northeastern University, Boston, MA. *"Exosome-Mediated Reprogramming of the Tumor Microenvironment"*. May, 2016.

Engineering Conference International. Symposium on Nanotechnology in Medicine: From Molecules to Humans, Henstein, Austria. *"Translational Nano-Medicine: Targeted Therapeutics for Cancer and Inflammatory Disease"*. July, 2016.

Fourth Annual Workshop on Micro- and Nano-Technologies for Medicine: Emerging Frontiers and Applications. MIT-Harvard Science and Technology Program and the Wyss Institute. Cambridge, MA. *"Macrophage Reprogramming in Cancer and Inflammatory Diseases"*. July, 2016.

Third International Ovarian Cancer Symposium and International Symposium on Tumor Microenvironment and Therapy Resistance, University of Oklahoma Health Sciences Center, Oklahoma City, OK. *"Targeted Nano-Therapeutics for Drug Resistant Tumors"*. August, 2016.

2016 Asian Polymer Association's International Conference on Advanced Polymers, Biomaterials, Bioengineering and Nano-Drug Delivery. Flic-En-Flac, Mauritius. *"Combinatorial-Designed Polymeric Nanosystems for Targeted Drug Delivery"*. September, 2016.

University of Porto, Porto, Portugal. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Disease"*. October, 2016.

Brown University, Department of Molecular Pharmacology, Physiology, and Biotechnology. Providence, RI. *"Targeting Nucleic Acid Base Therapeutics for Cancer and Inflammatory Diseases"*. November, 2016.

2017 Systems Oncology Conference, Amrita Institute of Medical Sciences, Amrita Institute, Kochi, Kerala, India. *"Targeted Nano-Therapeutics for Drug Resistant Tumors"*. March, 2017.

University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton, Alberta, Canada. Invited presentation entitled *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. March, 2017.

Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. May, 2017.

Keynote presentation at the Ninety-first American Chemical Society Colloid and Surface Science Symposium. City College of New York, New York, NY. *"Combinatorial-Designed Nano-Systems for Delivery of Nucleic Acid"*. July, 2017.

Fifth Annual Workshop on Micro- and Nano-technologies for Medicine: Emerging Frontiers and Applications. MIT-Harvard Science and Technology Program and the Wyss Institute. Cambridge, MA. *"Advances in CNS Delivery of Biological Therapeutics"*. July, 2017.

Keynote presentation at the Applied Pharmaceutical Nanotechnology (APN) 2017 Conference, Broad Institute, Massachusetts Institute of Technology, Cambridge, MA. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. October, 2017.

Seminar presentation in the Department of Biochemistry and Molecular and Cellular Biology, Georgetown University Medical Center, Washington, DC. *"Delivery of Nucleic Acid Therapeutics for Refractory Tumors"*. October, 2017.

Seminar presentation in the Department of Chemistry, Kennedy College of Sciences, University of Massachusetts at Lowell, Lowell, MA. *"Advances in Systemic and Oral Nucleic Acid Therapeutic Delivery"*. November, 2017.

Plenary presentation at the End-2-Cancer Symposium on Emerging Nanotechnology and Drug Delivery for Cancer. University of Oklahoma Health Sciences Center, Oklahoma City, OK. *"Reprogramming the Tumor Microenvironment by Exosomal Transfer of Nucleic Acids"*. December, 2017.

Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia. *"Nanotechnology for the Treatment of Resistant Tumors"*. March, 2018.

Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia. *"Nanotechnology for CNS Delivery of Biological Therapies"*. March, 2018.

Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia. *"Nanotechnology in Cardiovascular Medicine"*. March, 2018.

American Chemical Society and American Association of Pharmaceutical Scientists - 2018 Drug Design and Delivery Symposium Webinar Series. *"Advanced Nano-Delivery Systems: Facilitating Tumor Delivery and Mitigating Resistance"*. May, 2018.

Stony Brook University Institute of Chemical Biology and Drug Discovery (ICB & DD) 2018 Symposium on "Frontiers of Nanomedicine: Drug Delivery, Therapeutics, and Diagnosis" Keynote Presentation. Stony Brook, NY *"Integrated Nano-Medicine for Cancer and Inflammatory Diseases"*. October, 2018.

University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology, Worcester, MA. *"Targeted Delivery of Nucleic Acid Therapy for Cancer and Inflammatory Diseases"*. November, 2018.

Chapman University, College of Pharmacy. Irvine, CA. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Disease"*. November, 2018.

University of Santiago de Compostela, "Frontiers in Science" Seminar Series, Keynote Presentation. Santiago de Compostela, Spain. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. January, 2019.

Astra Zeneca, Waltham, MA. *"Advances in Nano-Delivery of Nucleic Acid Therapies"*. August, 2019.

Seventeenth Annual Nanomedicine and Drug Delivery Technology Symposium (NanoDDS). Massachusetts Institute of Technology, Cambridge, MA. *"Macrophage Reprogramming with Nano-Delivery for Cancer and Inflammatory Diseases"*. September, 2019.

University of Nebraska Medical Center, College of Pharmacy. Omaha, NE., *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. October, 2019.

Sant Johns University, College of Pharmacy and Health Sciences, Queens, NY. *"Targeted Delivery of Biological Therapies for Cancer and Inflammatory Diseases"*. October, 2019.

2019 American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX. *"Role of Protein Corona on Oligonucleotide Delivery and Transfection with Lipid Nanoparticles"*. November, 2019.

Keynote Presentation at the 2019 Advancing Drug Development Forum, Boston, MA. *"Advances in the Delivery of Therapeutics to the Brain"*. December, 2019.

2020 Blood-Brain Barrier Summit - Enhancing Drug Delivery Across the Blood-Brain Barrier to Develop More Clinically Meaningful Therapeutics for Neurodegenerative Diseases, Boston, MA. *"CNS Delivery of Biological Therapeutics for Chronic Pain and Neuroinflammation"*. August, 2020.

Eli Lilly and Company, Cambridge Innovation Center, Cambridge, MA. *Endogenous Targeting Strategies for Nanoparticle Delivery Systems*. October, 2020.

2020 Spring and Fall Virtual Meeting of the Materials Research Society, Oligonucleotide Symposium. Boston, MA. *"Role of Protein Corona on Oligonucleotide Delivery and Transfection with Lipid Nanoparticles"*. December, 2020.

Northeastern University, Bouve College of Health Sciences, Boston, MA. *"The Science of COVID-19 Vaccines"*. December, 2020.

University of Delhi, Department of Chemistry, 2021 Professor Amarnath Maitra Memorial Virtual Lecture. Delhi, India. *"Translational Nano-Medicine: Targeted Therapeutics for Pain, Cancer, and Inflammatory Diseases"*. February, 2021.

Senda Biosciences, Inc., Cambridge, MA. *"Multi-Compartmental Oral Nucleic Acid Delivery Systems"*. March, 2021.

Abbvie Bioresearch Center, Worcester, MA. *"Peritoneal Macrophage Reprogramming and Tropism in Inflammatory Diseases and Cancer"*. April, 2021.

Boehringer Ingelheim Pharmaceuticals, External Innovation Group, Ridgefield, CT. *"Drug Delivery Research and Education at Northeastern University"*. September, 2021.

2021 Nanomedicine Meets the Tumor Microenvironment (NANOTME-2021) Virtual Conference, The University of Twente, Twente, The Netherlands. *"Modulation of the Tumor Microenvironment with microRNA Nano-Vectors"*. September, 2021.

2021 Professor Ambikanandan Misra Memorial Virtual Lecture – Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Baroda, India. *"Translational Nano-Medicines: Targeted Therapeutic Delivery for the Treatment of Chronic Pain and Cancer"*. September, 2021.

Life Sciences Collaborative, Boston Group Meeting, Boston, MA. *"Life Sciences Entrepreneurship and Industrial Partnerships at Northeastern University"*. September, 2021.

Generation Bio, Inc., Cambridge, MA. *"Advances in Non-Viral Nucleic Acid Delivery Systems"*. October, 2021.

2021 GMP Symposium: Moving Forward into the PK World: New Drugs, New Tools, New Questions. Paris, France. *"Vaccine Formulation and Delivery Strategies to Improve Global Access"*. October, 2021.

University of Rhode Island, Department of Chemical Engineering. Kingston, RI. *"Translational Nano-Medicine: Targeted Therapeutic Delivery for Pain, Cancer, and Inflammatory Diseases"*. November, 2021.

University Institute of the Pharmaceutical Sciences, UGC Networking Resource Centre, Panjab University, Chandigarh, India. *"Translational Nano-Medicine: Targeted Therapeutic Delivery for Cancer and Inflammatory Diseases"*. January, 2022.

The Controlled Release Society – Indian Local Chapter Annual Meeting, Mumbai, India. *"Advances in Non-Viral Nucleic Acid Delivery Systems"*. February, 2022.

The American Society of Neurochemistry, 2022 Annual Meeting, Symposium on "Nano-Delivery Systems for CNS Therapeutics" Roanoke, VA. *"Nose-to-Brain Delivery of Nucleic Acid Therapeutics"*. April, 2022.

Northeastern University and the University of Ben Gurion at Negev Collaborative Symposium, Boston, MA. *"Strategies for Delivery of Nucleic Acid Therapeutics in Cancer and Inflammatory Diseases"*. May, 2022.

Hanson Wade 2022 Non-Viral RNA Delivery Systems Summit. Workshop on CNS Delivery of RNA Therapeutics, Boston, MA. *"RNA Delivery to the Brain: Challenges and Opportunities"*. June, 2022.

The Controlled Release Society, 2022 Annual Meeting and Exposition, Montreal, Quebec, Canada. *"Overcoming Biological Barriers in Nucleic Acid Delivery"*. July, 2022.

INTELLECTUAL CONSULTANCY

GelTex Pharmaceuticals, Inc., Waltham, MA. A medium sized pharmaceutical company that is marketing non-absorbable polymeric materials as therapeutic agents. I have consulted on pharmaceutical product development with GelTex's polymeric materials from May 1997 to June 2000.

FzioMed, Inc., San Luis Obispo, CA. A start-up medical device company intending to market polymeric materials to prevent post-surgical adhesions and thrombus formation. I have consulted on improving blood compatibility of the polymeric materials developed by FzioMed from October 1996 to April 1999.

EOS Pharmaceutical Corporation, Natick, MA. A start-up contract formulation company with domestic and international clients. I have consulted EOS Pharmaceuticals on novel bioadhesive polymeric materials for drug delivery from June 1997 to September 2001.

Biopolymer Technologies International, Inc., Westborough, MA. A start-up biotechnology company specializing in the development of polymer-based therapeutics and nutraceuticals. I have consulted for Biopolymer Technologies International from December 1997 to July 1999.

Braintree Laboratories, Inc., Braintree, MA. A medium sized pharmaceutical company developing various products, including phosphate binders, poly(ethylene glycol)-based laxatives, and gastric lavage agents. I have consulted for Braintree Laboratories from September 1999 to June 2003.

Catalyst Oncology, Inc., Providence, RI. A biotechnology start-up company, supported by the Slater Foundation, to develop novel target-specific anticancer drugs. I have consulted Catalyst Oncology on anticancer drug delivery issues from December 2003 to December 2004.

Cytogel, Inc., Stonington, CT. Cytogel is a small pharmaceutical company focusing on hydrogel drug delivery technologies. I have consulted Cytogel on their hydrogel delivery platforms from September, 2004 to April 2005.

LifeScan, Inc., Milpitas, CA. LifeScan is a Johnson and Johnson subsidiary developing diagnostic systems for diabetes. I have been consulting LifeScan on their diabetes monitoring technologies from August, 2005 to May 2006.

Boston Scientific Corporation, Malborough, MA. A large-cap medical device company that manufactures drug-coated stents, catheters, and embolic microspheres. I have consulted Boston Scientific on drug delivery technologies from October, 2003 to September, 2006.

Novavax, Inc., Columbia, MD. A medium-sized pharmaceutical company that focuses on women's healthcare market. I have consulted Novavax on their micellar nanoparticle and other delivery platforms from February, 2004 to December, 2006.

Scientia Advisors, LLC, Cambridge, MA. Scientia Advisors is an international management and strategy consulting firm with a concentration in biotechnology and life sciences. I provide intellectual consulting on polymeric drug delivery technologies. I have consulted Scientia Advisors from July, 2008 to May, 2009.

Cequent Pharmaceuticals, Inc., Cambridge, MA. Cequent Pharmaceutical is small company interested in development of proprietary Transkingdom RNA interference technology. I provide consulting service in formulation development and oral delivery. I have consulted Cequent Pharmaceuticals from May, 2007 to December, 2008.

Marine Polymer Technologies, Inc., Burlington, MA. A medium sized biotechnology/medical device company developing poly(acetyl-D-glucosamine)-based products. I have been consulting Marine Polymer on drug formulation and delivery technologies from September 2002 to June, 2007.

Genzyme Pharmaceuticals – Biomaterials and Drug Development Group, Waltham, MA. Genzyme is a medium sized pharmaceutical company with interest in polymeric drug development. I provide intellectual consultation on polymeric biomaterials and drug delivery systems. I provided consultation on drug delivery and nanotechnology from August, 2007-July, 2008.

Grayhead Associates, Wellesley, MA. Grayhead Associates is an international management consulting firm that assists its clients to capitalize on their technological resources and entrepreneurial spirit. I have been consulting Greyhead Associates on biomedical nanotechnology and drug development technologies from October, 2005 to May, 2009.

Vertex Pharmaceuticals, Inc., Cambridge, MA. Vertex is a medium sized pharmaceutical company developing a number of proprietary therapeutics in cancer, inflammation, and infectious diseases. I provide intellectual consultation on drug delivery and nanotechnology. I have been consulting Vertex from January, 2008 to April, 2010.

BioCure, Inc., Norcross, GA. BioCure is a medium-sized biomedical device company developing polymer nanotechnology for imaging and drug delivery applications. I have been consulting BioCure on their nanotechnology research portfolio from August 2006 to June, 2010.

Cerulean Pharmaceuticals, Inc., (aka Tempo Pharmaceuticals Inc.), Cambridge, MA. Cerulean Pharmaceutical is a startup company interested in development of proprietary multi-modal polymeric nanoparticle technology. I provide consulting service in nanoparticle formulations and preclinical studies. I have been consulting Cerulean Pharmaceuticals from September, 2008 to June, 2016.

Takeda Pharmaceuticals, Inc., Cambridge, MA. Takeda is a large multi-national pharmaceutical industry interested in development of nucleic acid therapeutics. I serve as a consultant in the area of nucleic acid formulations for both oral and systemic delivery for variety of disease targets from May, 2015 to December 2016.

Sun Pharmaceuticals Advanced Research Centre (SPARC), Baroda, India. SPARC is a research and development of arm of multinational Sun Pharmaceuticals, Inc. I am involved in consultation on oral and systemic controlled release drug delivery systems for various therapeutic areas from February, 2016 to December, 2017.

Summit Street Medical, LLC, Wallingford, CT. Summit Street is a start-up company involved in repurposing existing therapeutics using novel drug formulation and delivery devices. I am involved in consultation on systemic drug delivery systems for various therapeutic areas from September, 2017 to June, 2018.

Thompson-Reuters Group (aka RTG), Washington, DC. RTG provides expert witness, intellectual consulting, and professional presentation services to many diverse groups of clients. I serve as a consultant in the area of pharmaceutical product development, biomaterial science and applications, and nano-medical technologies from February, 2004 to present.

IMS Expert Services, Pensacola, FL. IMS provides expert witness and intellectual consulting services to many diverse groups of clients. I serve as a consultant in the area of pharmaceutical product development, biomaterial science and applications, and nano-medical technologies from June, 2011 to present.

Rubin-Anders Consulting, Boston, MA. Rubin-Anders provides expert witness and intellectual consulting services to many diverse groups of clients. I serve as a consultant in the area of pharmaceutical product development, biomaterial science and applications, and nano-medical technologies from March, 2013 to present.

Krystal Biotech, Inc., Pittsburgh, PA. Krystal Biotech is a clinical stage gene therapy company focusing on development of gene therapy solutions for rare diseases using viral vectors. I provide strategic consulting on novel routes of administration and formulation development. The consultancy period is from May, 2021 to December, 2021.

Brixton Biosciences and EyeCool Therapeutics, Inc., Cambridge, MA. Brixton and EyeCool and both start-up companies developing a proprietary nerve block technology for use in pain and other therapeutic applications. I have served as a consultant to Brixton in the development of the technologies and preclinical testing from November, 2019 to present.

Cipla, LTD, Mumbai, India. Cipla is a large multinational pharmaceutical company headquartered in Mumbai, India. I provide intellectual constancy on novel product development strategies specifically focusing on biologics and vaccines. The consultancy period is from June, 2021 to present.

Pykus Therapeutics, Inc., Cambridge, MA. Pykus is an early-stage biopharmaceutical company developing treatment strategies for acute and chronic eye diseases, including non-surgical method to treat retinal detachment and age-related macular degeneration. I consult on formulation strategies for ocular delivery. The consultancy period is from August, 2021 to present.

UbiquiTx, Inc., Cambridge, MA. UbiquiTx is a startup company leveraging parentship between Cornell University and MIT on development of mRNA therapeutics targeting the ubiquitin-proteosome pathway in cancer and other diseases. I consult on the mRNA formulation and delivery strategies. The consultancy period is from April, 2022 to present.

Tornado Biosciences, San Francisco, CA. Tornado Bio is a startup company in the Bay Area focusing on development of immuno-oncology therapeutics with mRNA technology. I am consulting on the formulation development and characterization of delivery strategies. The consultancy period is from April, 2022 to present.

TEACHING EXPERIENCES

Courses Currently Teaching (2003 – Present)

Doctor of Pharmacy Program

Pharmaceutics 2 (4 SH): Theoretical course on the physicochemical properties of the drug product and their influence on development of pharmaceutical formulations and delivery offered under the semester calendar (for 16 weeks). I coordinate and teach 60% of this course to 4th year students. Approximate class size is about 150 students each year.

Capstone Course (4 SH): A final year course for PharmD students focusing on their cumulative educational experience at Northeastern University. Student groups participate in laboratory research, review of published papers around a thematic area, or a creative exercise (e.g., creating a new CE program).

Directed Study (4 SH): An elective course for professional pharmacy students interested in performing research under a faculty advisor.

Graduate Program in Pharmaceutical Sciences:

Advanced Drug Delivery Systems (3 SH): A graduate course for MS and PhD students in Pharmaceutical Sciences and other departments across campus focusing on novel drug delivery systems such as polymeric and lipid nano-formulations for drug, gene, vaccine, and siRNA delivery. The course is taught to 50-60 students each Fall and I coordinate the course and invite speakers from the industry to give specific presentations and case studies.

Pharmaceutical Science Internship (1 SH): Coordinator of industrial internship opportunities for graduate students in Pharmaceutical Science. Each year in the Summer, I assist 3-5 MS and PhD students in securing an internship position and evaluating their progress.

Graduate Program in Chemical Engineering:

Targeted Drug Delivery Systems (3 SH): A graduate course for MS and PhD students for Chemical Engineering and other departments across campus focusing on novel drug delivery systems such as polymeric and lipid nano-formulations for drug, gene, vaccine, and siRNA delivery. The course is taught to about 10 students each Fall and I coordinate the course and invite speakers from the industry to give specific presentations and case studies.

Courses Previously Taught

Pharm.D. Program:

Pharmaceutical Calculations (PCT 1240, 4 QH): Basic prescription interpretation and calculations for second year pharmacy students.

Dosage Forms (PMD 1323, 4 QH): Theoretical course on pharmaceutical product development, characterization, and quality control issues taught to the third year pharmacy students.

Dosage Forms Laboratory (PCT 1300, 2 QH): A complementary laboratory course for Dosage Forms on prescription compounding skills.

Physical Pharmacy (PMD 1400, 4 QH): Theoretical course on the physicochemical properties of the drug product and their influence on development of pharmaceutical formulations and delivery.

Physical Pharmacy Laboratory (PCT 1320, 2 QH): A complementary laboratory course to Physical Pharmacy on analytical and experimental methods for testing of pharmaceutical products for quality control.

Biopharmaceutics and Pharmacokinetics (PMD 1410, 4 QH): Introduction to biopharmaceutics and pharmacokinetic principles, application of compartmental and non-compartmental modeling for analysis of data, and interpretation of pharmacokinetic data in clinical pharmacy practice.

Graduate Program in Pharmaceutical Sciences:

Advanced Physical Pharmacy (PCT 3200, 2 QH): A graduate level physical pharmacy course that with emphasis on physicochemical characterization of drug products.

Drug Design, Evaluation, and Development (PSC G210, 2 SH): A graduate level comprehensive course on drug discovery, development, and evaluation from an industrial perspective. I teach about 20% of the course to approximately 50-60 MS and PhD students in the Fall semester each year.

Pharmaceutical Science Seminar (PSC G200, 1 SH): A course on graduate seminar and journal club presentations for development of scientific skills for MS and PhD students in the department. I coordinate and teach 100% of the course for approximately 20-30 MS and PhD students in the Fall semester each year.

Interdisciplinary Graduate Programs:

Introduction to Biotechnology (2 SH): An interdisciplinary graduate course offered to students in the Professional MS program in Biotechnology in the Fall semester each year. I provide 10% effort to this course.

Introduction to Nanomedicine Science and Technology (2 SH): An interdisciplinary graduate course offered under the Nanomedicine Science and Technology IGERT program in the Fall semester of each year. I provide 20% effort to this course.

Nanosystems Design for Biomedical Applications (2 SH): An interdisciplinary graduate course offered under the Nanomedicine Science and Technology IGERT program in the Spring semester of each year. I provide 20% effort to this course.

Seminars in Nanomedicine (1 SH): An interdisciplinary graduate course offered under the Nanomedicine Science and Technology IGERT program in the Fall semester of each year. I coordinate this seminar course and provide 100% effort.

TEACHING-RELATED ACCOMPLISHMENTS

Master of Science (MS) Degree in Pharmaceutical Engineering: Working with colleagues in the Department of Chemical Engineering and the Department of Pharmaceutical Sciences, I have assisted in developing a proposal for establishment of a non-thesis MS in an interdisciplinary field of Pharmaceutical Engineering. The program will offer core courses in Pharmaceutical Sciences, Pharmaceutical Engineering, and laboratory as well as capstone course requirement as well as electives in Regulatory Affairs, Statistics and Experimental Design, and Biomanufacturing. Anticipated start-date for the program is Fall, 2023.

Professional Science Master's (PSM) Degree Program in Biomedical Nanotechnology: Working with Pharmaceutical Sciences, School of Law, and College of Business faculty, I have developed a new Professional Science Masters program in Biomedical Nanotechnology. The program offers terminal MS degree with didactic scientific, patent law, and entrepreneurship courses along with practical internship experience. The first class was admitted in Fall, 2013.

Experiential ("Non-Traditional") PhD Program at Northeastern University: For students who have completed an MS degree in Pharmaceutical Sciences or related field and are currently working in industry, I started implemented a new 'non-traditional' PhD program that waives their didactic coursework. With permission from the corporate sponsors, students enroll in the PhD program and start thesis project under a faculty mentor, while still keeping their "day job" with the industry. The program was started in 2009 and graduated the first cohort of PhD students in 2012. The Experiential PhD program has now expanded across Northeastern University through the Provost Office (<https://catalog.northeastern.edu/graduate/phdprograms/experiential-phd/>).

IGERT Training Grants for Doctoral Training in Nanomedicine - Phase 1 (2005-2010) and Phase 2 (2010-2015): With funding from the National Cancer Institute and the National Science Foundation (NSF), we have established an Interdisciplinary Graduate Education, Research and Training (IGERT) program. I am involved in the development and implementation of the Nanomedicine Science and Technology doctoral program. The program will admit interdisciplinary fellows who will take didactic courses, participate in internship opportunity, and carry out dissertation research in nanomedicine-related project. Phase 1 program started in the Fall, 2005 with a total of \$4.0M in funding and Phase 2 program started in Fall 2010 with a total of over \$3.5M in funding from the NSF.

Multi-Track Professional Science Masters (PSM) Degree Program in Biotechnology: With \$75K funding support from the Alfred P. Sloan Foundation, I have worked with faculty members from the Biology and Chemical Engineering Departments at Northeastern University to develop and implement an interdisciplinary Professional MS degree program in Biotechnology with tracks in Molecular Biotechnology, Pharmaceutical Biotechnology, and Engineering Biotechnology. The program started in the Fall of 2003.

Applied Physical Pharmacy Textbook: In collaboration with Professor Beverly Sandmann of Butler University, I have authored "*Applied Physical Pharmacy*" textbook specifically geared to pharmacy students and practitioners. The book contains physical chemical concepts in drug product design with examples that are relevant to practice. The textbook was published in November 2002. In collaborations with Professors Thomas Cook from Tauro College of Pharmacy and W. Cary Mobley from University of Florida College of Pharmacy, a second edition of the textbook is in development for anticipated publication in early 2014.

Problem-Based Undergraduate Pharmaceutics Textbook: In collaboration with other pharmaceutics faculty members across the U.S. and Canada, I have authored cases for a textbook entitled "*Cases in Pharmaceutics for Problem-Based Learning and Problem Solving*" edited by Wendy C. Duncan-Hewitt and David L. Mount of the University of Toronto, Canada.

Pharmaceutics Laboratory Exercises Database and CD-ROM: I have contributed eight pharmaceutics laboratory exercises to the database and CD-ROM initiative of David L. Mount and others.

Pharmaceutics Instructional Materials on the World-Wide Web: With funding from the Dean's "Excellence in Teaching" initiative, I have developed pharmaceutics instructional materials on the Internet. These pages can be accessed through the World-Wide Web at "<http://www.pharmsci.neu.edu/Courses/courses.html>".

Computer-Aided Pharmaceutics Instruction: I am involved in various aspects of promoting and developing computer aids and tutorial programs for undergraduate pharmaceutics courses. In addition, I strongly advocate the use of pharmacy resources that are available on the Internet.

NAPLEX Review: In the Spring quarter of every year, I provide an extensive review of Calculations, Physical Pharmacy, and Dosage Forms to the senior students who are planning to take the National Board of Pharmacy Licensing Examination (NAPLEX). In addition, I also provide a review of compounding skills to those students taking their NAPLEX exam in states that have a wet-lab section (e.g., New York, Maryland, Wisconsin, etc.).

RESEARCH ADVISING

Visiting Scientist and Research/Clinical Fellow

Mr. Srinivas Ganta	PhD Candidate	University of Auckland Auckland, New Zealand July, 2007 – October, 2007
Dr. Pauline Pei Li	Associate Professor	Hong Kong Polytechnic University, Hong Kong, August, 2007
Dr. Wei Duan	Associate Professor	Daikin University, School of Medicine Melbourne, Victoria, Australia, May, 2008
Dr. Cristina Dehelean	Professor	Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, November, 2008
Ms. Jose das Neves	PhD Candidate	University of Porto, College of Pharmacy, Porto, Portugal September, 2009 – March, 2010
Ms. Sharareh Adeli	PhD Candidate	Tehran University of Medical Sciences, Tehran, Iran. November, 2009 – March, 2010
Dr. Satheesh Elangovan	Research Fellow	The Forsyth Dental Institute, Boston, MA November, 2009 – May, 2010
Ms. Meghna Talekar	PhD Candidate	University of Auckland Auckland, New Zealand July, 2011 – December, 2011
Dr. Florence Gattacceca	Assistant Professor	Montpellier University, Faculty of Pharmacy Montpellier, Cedex, France August, 2012 – August, 2013 June, 2016 – September, 2016

	Associate Professor	Aix- Marseille University, Faculty of Pharmacy Marseille, France July, 2018 – September, 2018
Ms. Ana Vanessa Nascimento	PhD Candidate	University of Porto, College of Pharmacy, Porto, Portugal August, 2012 – October, 2014
Ms. Minah Iqbal	MS in Biotechnology Student	Columbia University New York, NY January, 2015 – May, 2015
Dr. Sundus Tewfik	Professor	London Metropolitan University London, United Kingdom March, 2016 – April, 2016
Dr. Michelle Miyake	Clinical ENT Fellow	Faculdade de Ciências Médicas Santa Casa de São Paulo São Paulo, SP, Brazil January, 2017 – December 2017
Ms. Smrithi Padmakumar	PhD Candidate	Amrita Institute of Medical Sciences Kochi, Kerala, India September, 2017 – March, 2018
Ms. Sevde Altuntas	PhD Candidate	Tobb University of Economics and Technology Ankara, Turkey November, 2017 – August, 2018
Ms. Flavia Sousa	PhD Candidate	University of Porto, College of Pharmacy, Porto, Portugal February, 2018 – August, 2018
Dr. Alan D. Workman	Clinical ENT Fellow	University of Pennsylvania Perelman School of Medicine Philadelphia, PA June, 2020 – December, 2021
Dr. Melis Debreli Coskun	Post-Doctoral Associate	University of Massachusetts at Lowell Zuckerberg College of Health Science Lowell, MA May, 2021 - Present
Dr. Andy Chua	Clinical ENT Fellow	Sengkang General Hospital Singapore September, 2022-Present

Research Faculty and Post-Doctoral Associate

Dr. Curtis F. Crasto	Post-Doctoral Associate	July, 2002 – May, 2004, under the Nanomedicine Consortium
Dr. Dinesh B. Shenoy	Associate Research Scientist	June, 2003 – December, 2005
Dr. Sandip K. Tiwari	Post-Doctoral Associate	August, 2004 – June, 2006
Dr. Tushar K. Vyas	Post-Doctoral Associate	December, 2005 – December, 2006
Dr. Aliasgar Shahiwala	Post-Doctoral Associate	August, 2006 – August, 2007
Dr. Harikrishna Devalapally	Post-Doctoral Associate	November, 2005 – February, 2008
Dr. Christina Kriegel	Post-Doctoral Associate	September, 2008 – June, 2010
Dr. Srinivas Ganta	Post-Doctoral Associate Associate Research Scientist	April, 2008 – February, 2010 March, 2010 – November, 2010
Dr. Sampath Abeylath	Post-Doctoral Associate	March, 2010 – May, 2011
Dr. Tatyana Chernenko	Post-Doctoral Associate	May, 2010 – March, 2012
Dr. Ming Chen	Associate Research Scientist	July, 2011 – March, 2012
Dr. Qiong-Lin Zhou	Research Assistant Professor	December, 2010 – August, 2012
Dr. Srinivas Reddy Boreddy	Associate Research Scientist	August, 2012 – January, 2013
Dr. Dattatri Nagesha	Post-Doctoral Associate Coordinator of the IGERT Nanomedicine Program	July, 2004 – May, 2013
Dr. Arun K. Iyer	Post-Doctoral Associate Associate Research Scientist Research Assistant Professor	May, 2008 – December, 2008 December, 2010 – June, 2012 July, 2012 – January, 2014
Dr. Sanjib Bhattacharya	Post-Doctoral Associate	April, 2014 – June, 2014
Dr. Malav Trivedi	Post-Doctoral Associate	July, 2014 – December, 2014
Dr. Amit Singh	Associate Research Scientist	May, 2011 – January, 2015
Dr. Meghna Talekar	Post-Doctoral Associate	April, 2013 – April, 2015
Dr. Than-Huyen Tran	Post-Doctoral Associate	December, 2013 – October, 2015
Dr. George Matthiolampakis	Post-Doctoral Associate	August, 2014 – May, 2016
Dr. Gulzar Ahmad	Post-Doctoral Associate	December, 2015 – November, 2017

Dr. Neha Parayath	Post-Doctoral Associate	April, 2016 – May, 2018
Dr. Harkiranpreet Dhaliwal	Post-Doctoral Associate	August, 2016 – October, 2018
Dr. Maie Taha	Post-Doctoral Associate	June, 2019 – August, 2020
Dr. Renuka Khatik	Post-Doctoral Associate	December, 2020 – January, 2021
Dr. Smrithi Padmakumar	Post-Doctoral Associate	May, 2019 – April, 2021
Dr. Shallu Kutlehria	Post-Doctoral Associate	December, 2020 – October, 2021
Dr. Haneyeh Shahbazian	Post-Doctoral Associate	June, 2021 – October, 2021
Dr. Ahmed Radwan	Medical Fellow Visiting Scholar	March, 2016 – August, 2018 September, 2018 - Present
Dr. Kohal Das	Academic-Industrial Postdoc Fellowship Supported by Takeda Vaccines	November, 2019 – May, 2022
Dr. Saeideh Nozohouri	Post-Doctoral Associate Collaborations with Eli Lilly on MLSC Project	January, 2022 – October, 2023
Dr. Di Huang	Post-Doctoral Associate Associate Research Scientist	April, 2020 – June, 2022 June, 2022 - Present
Dr. Anisha D'Souza	Post-Doctoral Associate	April, 2021 – Present
Dr. Mir Javid Iqbal	Post-Doctoral Associate Fellowship Supported by Moderna Therapeutics	July, 2021 – Present
Dr. Dimitrios Bitounis	Post-Doctoral Associate Fellowship Supported by Moderna Therapeutics	February, 2022 – Present
Dr. Valentina Di Francesco	Post-Doctoral Associate	February, 2022 – Present
Dr. Sindhu Pillai	Post-Doctoral Associate Collaboration with Dr. Ken Anderson, Dana Farber Cancer Center	November 2022 - Present
Dr. Zhongkun Zhang	Post-Doctoral Associate Collaboration with Dr. Ken Anderson, Dana Farber Cancer Center	November 2022 - Present

Doctoral Students

Mr. Radi Hejazi	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery (Graduated, May 2003) Thesis Title: <i>Stomach-Specific Delivery of Tetracycline-Chitosan Microspheres for the Treatment of Helicobacter pylori Infection.</i>
Ms. Goldi Kaul	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery

Thesis Title: *Long-Circulating Poly(Ethylene Glycol)-Modified Gelatin Nanoparticles for Tumor-Targeted Gene Delivery.*

Ms. Sushma Kommareddy Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
(Graduated, May 2006)

Thesis Title: *Gelatin-Based Nanoparticulate Vectors for Tumor-Targeted Therapeutic Gene Delivery*

Mr. Mayank Bhavsar Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
(Graduated, August 2007)

Thesis Title: *Oral Gene Therapy for Inflammatory Bowel Disease Using Nanoparticles-in-Microsphere Hybrid Delivery System*

Ms. Lilian van Vlerken Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
IGERT Nanomedicine Fellow
(Graduated, February 2008)

Thesis Title: *Modulation of Multidrug Resistance in Cancer Using Polymer-Blend Nanoparticles*

Mr. Luis Brito Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
IGERT Nanomedicine Fellow
(Graduated, December 2008)

Thesis Title: *Local Endothelial Nitric Oxide Synthase Gene Delivery and Transfection with Lipopolyplexes for the Treatment of Coronary Restenosis*

Ms. Lara Jabr-Milane Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
IGERT Nanomedicine Fellow
(Graduated, June 2010)

Thesis Title: *Tumor Hypoxia, Warburg's Effect, and Drug Resistance: Modulation of Aerobic Glycolysis Using Combination Paclitaxel/Lonidamine Therapy Delivered in Targeted Polymeric Nanoparticles*

Mr. Mayur Kalariya Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Alkermes
(Graduated, July 2012)

Thesis Title: *Multi-Compartmental Delivery Systems for Peptide and DNA Vaccines for Melanoma Immunotherapy*

Ms. Shanthi Ganesh Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Novartis
(Graduated, August 2012)

Thesis Title: *Hyaluronic Acid-Based Self-Assembled Multifunctional Nanosystems to Overcome Drug Resistance in Lung Cancer*

Ms. Lipa Shah Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Novartis
(Graduated, May 2013)

Thesis Title: *Multifunctional Nanoemulsions for Systemic Delivery of Analgesic Peptides to the CNS*

Ms. Jing Xu Ph.D. in Pharmaceutical Science - Interdisciplinary Option
(Graduated, June 2013)

Thesis Title: *Self-Emulsifying Adjuvant Systems Containing Alpha-Tocopherol for Subunit Vaccines*

Ms. Dongyu Chen PhD in Pharmaceutical Sciences – Pharmaceutics and Drug Delivery
Dicerna Pharmaceuticals - Industrial Graduate Fellow
(Graduated, June 2019)

Thesis Title: *Role of Protein Corona on Tumor-Targeted Delivery of dsRNA using Lipid Nanoparticles*

Ms. Grishma Pawar PhD in Pharmaceutical Sciences – Pharmaceutics and Drug Delivery
(Graduated, June 2019)

Thesis Title: *"Trans-nasal Mucosal Delivery of BDNF Antagonist Oligonucleotides Using Heterotopic Mucosal Engrafting for Parkinson's Disease"*

Mr. Christopher Francis Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Pfizer
(Graduated, April 2020)

Thesis Title: *Non-Viral CRISPR/Cas9 Based Gene Editing in Hepatocytes for Wilson's Disease*

Mr. Aatman Doshi Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Astra Zeneca
(Graduated, July 2021)

Thesis Title: *Targeted Delivery of Low-Dose Liposomal STING Agonist to CD103+ Dendritic Cells in Tumor Immunotherapy*

Mr. Srujan Gandham Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Moderna
(Graduated, July 2021)

Thesis Title: *Modulation of Extracellular Vesicular microRNA in Multidrug Resistant Ovarian Cancer*

Ms. Archita Menon Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
American Heart Association Graduate Fellowship Recipient
(Graduated, July 2021)

Thesis Title: *The Role of Iron in Doxorubicin-Induced Cardiotoxicity – Risk Factors and Therapeutic Strategies*

Mr. Kevin Craig Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
Experiential (Industrial) PhD Fellow supported by Dicerna
(Graduated, August 2021)

Thesis Title: *Macrophage-Targeted Oligonucleotide Therapeutics to Downregulate Inflammatory Cytokines in Non-Alcoholic Steatohepatitis (NASH)*

Ms. Angela Nocera Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery
(Graduated, January 2022)

Thesis Title: *Role of Cystatins in the Formation and Maintenance of Chronic Rhinosinusitis with Nasal Polyps: Mechanistic Understanding and RNAi Therapeutic Strategy*

Mr. Gregory Jones	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery Experiential (Industrial) PhD Fellow supported by Frequency Therapeutics (Graduated, July 2022)
Thesis Title: <i>Pharmacokinetic and Pharmacodynamic Modeling of Iron Transport to Guide the Rational Development of Novel Nano-Chelator Formulations for the Treatment of Iron Overload Disorders</i>	
Ms. Lauren Gauthier	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery Experiential (Industrial) PhD Fellow supported by Takeda Pharmaceuticals
Thesis Title: <i>Investigating Relevant Nonclinical Toxicity Species for Immune-Modulatory Drugs</i>	
Ms. Shwetha Iyer	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery Experiential (Industrial) PhD Fellow supported by Novartis
Thesis Title: <i>Sustained Intraocular Delivery of Anti-VEGF Antibody for Age-Related Macular Degeneration</i>	
Mr. Dhaval Oza	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery Experiential (Industrial) PhD Fellow supported by Alnylam
Thesis Title: <i>Peritoneal Macrophage Tropism and Migration in Acute Injury and Therapeutic Strategy for Acetaminophen-Induced Liver Injury</i>	
Ms. Kanika Suri	Ph.D. in Bioengineering Experiential (Industrial) PhD Fellow supported by Takeda Pharmaceuticals
Thesis Title: <i>Oral microRNA Therapy for the Treatment of Inflammatory Bowel Disease Targeting Inflammation and Fibrosis</i>	
Ms. Megha Suresh	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery Experiential (Industrial) PhD Fellow supported by Abbvie
Thesis Title: <i>Myeloid Cell-Targeted Delivery of Immunomodulators for the Treatment of Idiopathic Pulmonary Fibrosis</i>	
Mr. Shashank Bhangde	Ph.D. in Pharmaceutical Science – Pharmaceutics and Drug Delivery GSK Vaccines - Industrial Graduate Fellow
Thesis Title: <i>Novel Self-Emulsifying Vaccine Adjuvant and Immunomodulator Combination for Rapid Pandemic Response</i>	
Mr. Apurva Krishna	Ph.D. in Bioengineering GSK Vaccines - Industrial Graduate Fellow
Thesis Title: <i>Development and Evaluation of STING Agonist Adjuvants for Systemic and Mucosal Vaccination</i>	
Ms. Taylor Hickman	PhD in Pharmaceutical Sciences – Biomedical Sciences Experiential (Industrial) PhD Fellow supported by Takeda Pharmaceuticals
Thesis Title: <i>Critical Understanding and Mitigation Strategies for Cellular Immunotherapy-Associated Neurotoxicity (CANS) in Cancer</i>	
Mr. Prashant Agarwal	Ph.D. in Chemical Engineering Experiential (Industrial) PhD Fellow supported by Amgen Pharmaceuticals

Thesis Title: *Minimally Invasive Nasal Depot (MIND) Procedure for Delivery of EGFR Targeted PROTACS in the Brain for Glioblastoma Treatment*

Ms. Raquel Sevilla PhD in Pharmaceutical Sciences – Biomedical Sciences
Experiential (Industrial) PhD Fellow supported by Merck

Thesis Title: *Role of Intercellular Communication through Tunneling Nanotubes in Triple Negative Breast Cancer Resistance and Metastasis*

Master's Students

Mr. Vijaykumar R. Patel M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, September 1995).

Thesis Title: *Chitosan-Poly(Ethylene Oxide) Semi-Interpenetrating Polymer Network as a pH-Sensitive Drug Delivery System.*

Ms. Amira Ahmed M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, September 1997).

Project Title: *Novel Drug Delivery Systems for the Treatment of H. pylori Infection.*

Ms. Sweta Shah M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, September 1999).

Project Title: *Stomach-Specific Antibiotic Therapy for H. pylori Infection.*

Ms. Sarah Nsereko M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, June 2001).

Thesis Title: *Chitin Microparticles for Localized Delivery of Paclitaxel: In Vitro and In Vivo Studies.*

Mr. Jugminder S. Chawla M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, April 2002).

Thesis Title: *Poly(epsilon-caprolactone) Nanoparticles for Tumor-Targeted Delivery of Tamoxifen: In Vitro Studies.*

Mr. Ehab Taqieddin M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, April 2002).

Thesis Title: *Enzyme Immobilization in Perm-selective Chitosan-Alginate Hybrid Microcapsules.*

Ms. Anupama Potineni M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, April 2002).

Project Title: *Poly(Ethylene Oxide)-Modified Poly(Beta-Amino Ester) Nanoparticles: Long-Circulating pH Sensitive Biodegradable System for Paclitaxel Delivery.*

Mr. Srinivasan Namala M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, June 2003).

Project Title: *Iontophoresis: A Tool to Enhance Transdermal Drug Delivery*

Ms. Pallavi Devurkar M.S. in Biomedical Sciences – Pharmaceutics Specialization
(Graduated, June 2003).

Project Title: *In Vitro Evaluation of Chitosan Microspheres for Stomach-Specific Drug Delivery*

Ms. Lilian van Vlerken	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, April 2005; Matriculated into the PhD program)
Project Title: <i>Combination Therapy of Ceramide and Paclitaxel Delivered in Poly(Ethylene Oxide)-Modified Poly(Epsilon-Caprolactone) Nanoparticles as a Potential Strategy for Overcoming Tumor Multidrug Resistance</i>	
Mr. Gurinder S. Saini	M.S. in Interdisciplinary Studies – Materials Science Specialization (Co-Advisor, under the Nanomedicine Consortium) (Graduated, January 2006)
Thesis Title: <i>Preparation and Characterization of Superparamagnetic Iron Oxide-Gold Core-Shell Nanoparticles for Biomedical Applications</i>	
Ms. Lipa Shah	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, December 2006, Matriculated into the PhD program)
Thesis Title: <i>Biodegradable Polymeric Nanoparticles for Intracellular Saquinavir Delivery in HIV/AIDS</i>	
Ms. Ankita Desai	M.S. in Biotechnology – Pharmaceutical Science Track (Graduated, December 2006)
Project Title: <i>In Vitro Evaluations of Multi-functional Nanoemulsion Formulations for Brain Tumor Therapy</i>	
Ms. Jasneet Oberai	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, May 2007)
Project Title: <i>Novel Nanoemulsions with Temperature-Responsive Drug Delivery</i>	
Ms. Shraddha Babaria	M.S. in Biotechnology – Pharmaceutical Science Track (Graduated, August 2007)
Project Title: <i>Cationic Liposomes for Intranasal Gene Delivery to the Brain</i>	
Ms. Sunita Yadav	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, December 2007, Matriculated into the PhD program)
Thesis Title: <i>Multifunctional Nanotherapeutic Strategy for MDR-1 Gene Silencing and Chemotherapy Administration to Overcome Multidrug Resistance in Cancer</i>	
Ms. Dipti Deshpande	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, December 2007, Matriculated into the PhD program)
Thesis Title: <i>Biodegradable Nanoparticle System for Intracellular Administration of Paclitaxel and Ceramide in Coronary Restenosis</i>	
Ms. Aparna Chavali	M.S. in Biotechnology – Pharmaceutical Science Track (Graduated, May 2008)
Project Title: <i>p53 Gene Therapy for Cancer</i>	
Ms. Sunaina Pai	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, August 2008)
Thesis Title: <i>In Vitro Evaluations of Multifunctional Nanoparticles for Simultaneous EGFR Gene Silencing and Drug Delivery in Pancreatic Cancer</i>	
Ms. Sindhura Ganga	M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization (Graduated, August 2008)

Mr. Shardool Jain M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
 (Graduated, August 2008; Matriculated into the PhD program)

Ms. Padmaja Magadala
M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, October 2008)

Ms. Pooja Sane

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2009)

Ms. Saradha Chandrasekhar M.S. in Biotechnology – Pharmaceutical Science Track
(Graduated, May 2009)

Mr. Niraj Patel M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, July 2009)

Mr. Chinmay Bakshi M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, July 2009)

Ms. Sandra Chadwick

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2009)

Ms. Anisha Korde

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2010, Matriculated into the PhD program)

Ms. Pei-Chin Tsai M.S. in Biotechnology – Pharmaceutical Science Track
(Graduated, December 2010)

Ms. Shruti Shah M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization

Thesis Title: *Hypoxia in Tumor Angiogenesis and Metastasis: Evaluation of VEGF and MMP Over-expression and Down-Regulation of HIF-1 α with RNAi in Hypoxic Tumor Cells*

Mr. Husain Attarwala M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2010, Matriculated into the PhD program)

Thesis Title: *In Vitro Evaluations of Macrophage-Targeted Anti-Inflammatory Gene Delivery and Transfection using Nanoparticle-in-Emulsion Formulations*

Mr. Milind Chalishazar M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2011)

Project Title: *Isolation and Evaluation of Human Melanoma Exosomes in Multiple Emulsion Formulation for Prophylactic and Therapeutic Vaccination*

Ms. Ruchi Shah M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2011, Matriculated into the PhD program)

Project Title: *Inhibition of Hypoxia-Inducible Factor-1 Activation in Pancreatic Tumor Spheroids with 2-Methoxyestradiol-Containing Polymeric Nanoparticles*

Mr. Hardip Gopani M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2011)

Thesis Title: *Combination Chemo- and Hyperoxia Therapy using Nanoemulsion Delivery Systems*

Mr. Deep Shah M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2012)

Project Title: *Establishment and Characterization of an Adjuvant Arthritis Model in Lewis Rats*

Ms. Kinjal Sankhe M.S. in Pharmaceutical Sciences– Pharmaceutics Specialization
(Graduated, May, 2012)

Project Title: *In Vivo Evaluations of Endothelial Regenerative Effects of Estradiol-Encapsulated Nanoemulsions in Wild-type C57BL/6J and ApoE^{-/-} Knockout Mice*

Ms. Lavanya Thapa M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2012)

Project Title: *Evaluation of Melanoma Exosomes-Containing W/O/W Multiple Emulsion Vaccine Formulation in B16F10-Tumor Bearing C57BL6/J Mice*

Mr. Kamaljeet Singh Sandhu M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2012)

Project Title: *TNF- α Gene Silencing using Hyaluronic Acid-Based Self-Assembled Nanoparticles in Macrophages for the Treatment of Inflammatory Conditions Associated with Type 1 Diabetes*

Ms. Darshna Patel M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2012)

Thesis Title: *In Vitro Evaluations of Ceramide Co-Therapy in Non-Targeted and EGFR-Targeted Biodegradable Polymeric Nanoparticles for Enhancing Therapeutic Efficacy in Ovarian Cancer*

Ms. Sravani Kathireddy M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization

(Graduated, May 2013)

Project Title: *Multi-functional Nanoemulsions for Targeted Estradiol Delivery in the Treatment of Endothelial Dysfunction in Atherosclerosis*

Mr. Ganesan Venkatesan M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2013)

Project Title: *“Click” Synthesis and Characterization of Functionalized Hyaluronic Acid-Based Macrostructures for Self-Assembled Nanoparticles*

Ms. Ankita Raikar M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2013)

Thesis Title: *HIF-1 α Activation in 3D Tumor Spheroids and Evaluation of 2-Methoxyestradiol Therapy using Targeted Nanoparticle Formulations*

Mr. Aatman Doshi M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, August 2013)

Thesis Title: *In Vitro Evaluations of Control and Lyp-1 Peptide-Modified Nano-Particulate Bisphosphonate Delivery for Ablation of Tumor-Associated Macrophages (TAMs)*

Mr. Srujan Gandham M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2014)

Thesis Title: *In Vitro Evaluations of Hexokinase-2 Inhibition with 2-Bromopyruvate Encapsulated in Targeted Nanocarrier Formulations using 3D Spheroid Models of Aerobic Glycolysis*

Mr. Adwait Oka M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2014)

Thesis Title: *Targeted siRNA Delivery Strategy with Water-in-Oil-in-Water Multiple Emulsion for Modulation of Tumor-Associated Macrophage Polarity in Immunotherapy of Cancer*

Mr. Qijun (Oscar) Ouyang M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2015)

Project Title: *Re-Polarization of Tumor Associated Macrophages with MicroRNA Nanovectors*

Ms. Grishma Pawar M.S. in Pharmaceutical Sciences – Pharmacology Specialization
(Graduated, May 2015 – Matriculated into the PhD program)

Project Title: *CNS Delivery of BDNF in Thermogelling Polymer Depot in Sprague-Dawley Rats*

Mr. Parin Shah M.S. in Pharmaceutical Sciences – Pharmacology Specialization
(Graduated, May 2015)

Project Title: *MicroRNA-Based Transfection and Epigenetic Changes in Lung Tumor Model*

Ms. Mei-Ju Su M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2015).

Thesis Title: *Tumor Exosome-Mediated Macrophage Reprogramming in a Co-Culture Model and Evaluation of MicroRNA Delivery with Hyaluronic Acid-Based Nanoparticles*

Ms. Dandan Ling M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2016).

Project Title: *miR-34a/Let-7a Combination Therapy in Refractory Lung Cancer using Targeted Hyaluronic Acid Nanoparticles*

Ms. Megha Suresh

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2016).

Thesis Title: *Hetero-Cellular 3-D Spheroids of Pancreatic Tumor Cells for MicroRNA-34 Delivery using Hyaluronic Acid-Based Nanoparticles*

Ms. Charul Avachat

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2017)

Thesis Title: *In Vitro Evaluations of BDNF Plasmid DNA Delivery and Transfection using Cationic Liposomes in Parkinson's Disease Model*

Ms. Krina Shah

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2017)

Thesis Title: *Pancreatic Tumor Cell-Fibroblast Heterocellular 3D Spheroid as a Model of Hypoxia and the Role of Nanoparticle-Mediated MicroRNA Therapy*

Ms. Tanjeela Jahan

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, May 2020)

Project Title: *Mitochondrial Network Density in Regulating Apoptosis and Neuronal Cell Death in Neurodegenerative Diseases*

Mr. Saket Dolare

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2021)

Thesis Title: *Mitochondrial Network Density and Cellular Apoptosis Modulation in Drug Resistant Triple Negative Breast Cancer Cells*

Ms. Rutuja Dighe

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization
(Graduated, December 2021)

Thesis Title: *Role of Intercellular Communication through Tunneling Nanotubes in Ovarian Cancer Cells and Therapeutic Resistance*

Mr. Hongyu Li

M.S. in Pharmaceutical Sciences – Pharmaceutics Specialization

Thesis Title: *Exosomal Transfer of Sub-Cellular Structures in Reprogramming Tumor Cells towards Resistant Phenotype*

Membership in Graduate Students Thesis Advisory Committees

Mr. Mukur Gupta

M.S. in Industrial Pharmacy, MA College of Pharmacy and Health Sciences
(Graduated, June 2001).
Ph.D. in Industrial Pharmacy, MA College of Pharmacy and Health Sciences
(Graduated, February, 2004)

Mr. Anurag Singhal

M.S. in Pharmaceutics (Graduated, June 2002)

Ms. Prachi Parulkar

M.S. in Pharmaceutics (Graduated, August 2004)

Ms. Solani Bhardwaj

M.S. in Chemical Engineering (Graduated, December 2007)

Ms. Shweta Raini

M.S. in Pharmaceutics (Graduated, May 2009)

Ms. Aditi Jhaveri

M.S. in Pharmaceutics (Graduated, May 2009)

Mr. Kelton Barnsley

M.S. in Chemistry (Graduated, November 2014)

Ms. Roshani Patil	M.S. in Biomedical Engineering (Graduated, May 2019)
Ms. Chia-Yu Wang	M.S. in Chemical Engineering (December, May 2020)
Ms. Bhavya Singh	M.S. in Chemical Engineering (December, May 2021)
Mr. Jayesh Vora	Ph.D. in Pharmaceutics (Graduated, June 1994)
Ms. Kamelia Behnia	Ph.D. in Pharmaceutics (Graduated, September 1996)
Mr. Daniel J. Magiera, III	Ph.D. in Biomedical Sciences (Graduated, June 1997)
Ms. Sandhya Ramanathan	Ph.D. in Pharmaceutics (Graduated, September 1997)
Ms. Imran Vural	Ph.D. in Biomedical Sciences (Graduated, June 1998)
Ms. Sujata Vaidyanathan	Ph.D. in Pharmaceutics (Graduated, September 1998)
Mr. Ramin Darvari	Ph.D. in Pharmaceutics (Graduated, December 2001)
Mr. Ram Rammohan	Ph.D. in Biomedical Sciences (Graduated, June 2002)
Mr. Jose DaSilva	Ph.D. in Biomedical Sciences (Graduated, June 2002)
Mr. Ananth Srinivas Chakilam	Ph.D. in Pharmaceutics (Graduated, December 2004)
Mr. Sarathi Vijay Bodapatti	Ph.D. in Pharmaceutics (Graduated, August 2007)
Ms. Suman Dandamudi	Ph.D. in Pharmaceutics (Graduated, January 2008)
Ms. Mattia Migliore	Ph.D. in Pharmacology/Nanomedicine (Graduated, May 2008)
Ms. Paula Lampton	Ph.D. in Biology/Nanomedicine (Graduated, December 2008)
Ms. Heather Brodtkin	Ph.D. in Chemistry/Nanomedicine (Graduated, May 2009)
Ms. D. Ece Gamsiz	Ph.D. in Chemical Engineering (Graduated, December 2009)
Mr. J. Adam Hendricks	Ph.D. in Chemistry/Nanomedicine (Graduated, December 2009)
Ms. Shifalika Tangutoori	Ph.D. in Pharmaceutics (Graduated, April 2010)
Ms. Tatyana Chernenko	Ph.D. in Chemistry/Nanomedicine (Graduated, April 2010)
Ms. Agnes Rafalko	Ph.D. in Chemistry/Nanomedicine (Graduated, May 2011)
Ms. Tao Wang	Ph.D. in Pharmaceutics (Graduated, December 2011)
Mr. Claudio Falcao	Ph.D. in Pharmaceutics (Graduated, December 2011)
Ms. Fulden Buyukozturk	Ph.D. in Chemical Engineering (Graduated, August 2012)
Mr. Robert Riehle	Ph.D. in Pharmaceutics/Nanomedicine (Graduated, May 2013)
Mr. Sean Essex	Ph.D. in Pharmaceutics (Graduated, August 2013)
Ms. Mary Katharine Balaconis	Ph.D. in Bioengineering (Graduated, December 2013)
Ms. Jennifer Monahan-Fore	Ph.D. in Chemistry/Nanomedicine (Graduated, December 2013)
Mr. Michael Cuccarese	Ph.D. in Chemistry/Nanomedicine (Graduated, March 2014)
Ms. Jennifer Woodring	Ph.D. in Chemistry/Nanomedicine (Graduated, December 2014)
Mr. Helal Al-Suleimani	Ph.D. in Pharmaceutical Science (Graduated, December 2017)
Ms. Loraine Speciner	Ph.D. in Bioengineering (Graduated, March 2018)
Ms. Jennifer Morales	Ph.D. in Bioengineering (Graduated, December 2018)
Mr. Bumjun Kim	Ph.D. in Chemical Engineering (Graduated, November 2018)
Ms. Murui Han	Ph.D. in Pharmaceutical Science (Graduated, December 2018)
Ms. JuOae Chang	Ph.D. in Pharmaceutical Science (Graduated, May, 2019)
Ms. Wenjun De	Ph.D. in Pharmaceutical Science (Graduated, July 2019)
Mr. Armin Vedadghavami	Ph.D. in Bioengineering (Graduated, February 2022)
Mr. Jacob Hebert	Ph.D. in Chemical Engineering (Graduated, July 2022)
Mr. Bryce Johnson	Ph.D. in Pharmaceutical Sciences
Mr. Ian Smith	Ph.D. in Chemical Engineering
Mr. Ronodeep Mitra	Ph.D. in Chemical Engineering
Ms. Sabrina Marnoto	Ph.D. in Chemical Engineering
Mr. Fernando Ivich	Ph.D. in Bioengineering
Mr. Joshua Price	Ph.D. in Bioengineering
Ms. Sadiqua Shadbar	Ph.D. in Chemistry and Chemical Biology

Mr. Man-Hon (Johnny) Lam (B.S., Class of 1994)

Mr. Joseph M. Goreham (B.S., Class of 1995)

Mr. Peter B. Ng (B.S., Class of 1995)

Ms. Rakhee H. Tailor (B.S., Class of 1995)

Ms. Mai-Ki Ly (B.S., Class of 1995)

Mr. Ketankumar Patel (B.S., Class of 1996)

Ms. Susanne Verrico (B.S., Class of 1996)

Ms. Laurie Galvin (B.S., Class of 1996)

Ms. Fiona Duncan (B.S., Class of 1997)

Ms. Ekata V. Shah (Pharm.D., Class of 1997)

- Recipient of Dean's Undergraduate Research Achievement Award, April 1995

Ms. Rina Qaqish (Pharm.D., Class of 1997)

Ms. Gity Roostai-Mills (B.S., Class of 1998)

Ms. Roula Qaqish (Pharm.D., Class of 1998)

- Recipient of Dean's Undergraduate Research Achievement Award, April 1996
- Currently Clinical Science Manager, Abbott Laboratories Eastern Division, Baltimore, MD

Ms. Phung-Kim Lai (B.S., Class of 1999)

- Recipient of Dean's Undergraduate Research Achievement Award, April 1996
- Recipient of the PhRMA Foundation's Undergraduate Research Fellowship, January 1999

Ms. Tragiang Nguyen (B.S., Class of 1999)

Ms. Trinh Tran (B.S., Class of 1999)

Ms. Bich-Thuy Tran (B.S., Class of 1999)

Mr. Chad McQueen (Pharm.D., Class of 2000)

- Recipient of Dean's Undergraduate Research Achievement Award, April 1999

Ms. Angela L. Silvia (Pharm.D., Class of 2000)

- Recipient of Dean's Undergraduate Research Achievement Award, April 1999

Mr. Kristian Jackson (B.S., Class of 2000)

- Recipient of the University of Connecticut Research Fellowship, June 1998

Mr. Pulin Patel (B.S., Class of 2000)

- Pursued graduate studies in Pharmaceutical Chemistry at the University of Kansas, Lawrence, KS

Mr. Derick Anderson (Pharm.D., Class of 2001)

- Recipient of Dean's Undergraduate Research Achievement Award, April 2000

Ms. Kwai-Dzy Mak (Pharm.D., Class of 2001)

Mr. Chi-Sing Nip (Pharm.D., Class of 2001)

- Recipient of the AAPS-AFPE "Gateway" Research Scholarship, June 2000

Ms. Erica J. Waugh (Pharm.D., Class of 2002)

- Recipient of the PhRMA Foundation's Undergraduate Research Fellowship, January 2001
- Recipient of Dean's Undergraduate Research Achievement Award, April 2002

Ms. Nikita Mody (Pharm.D., Class of 2004)

- Recipient of Northeastern University Provost's Undergraduate Research Award, January 2002
- Recipient of Dean's Undergraduate Research Achievement Award, April 2003

Ms. Stephanie Whalen (Pharm.D., Class of 2006)

- Recipient of the AFPE "Gateway" Research Scholarship, June 2004
- Recipient of Northeastern University Provost's Undergraduate Research Award, November 2004

Ms. Sarah Rogers (Pharm.D., Class of 2008)

- Recipient of Northeastern University Provost's Undergraduate Research Award, November 2004

Mr. Zeu Hong Tzeng (Pharm.D., Class of 2007)

- Recipient of the AFPE "Gateway" Research Scholarship, July 2005

- Recipient of Northeastern University Provost's Undergraduate Research Award, January 2007

Ms. Michelle Drown (Pharm.D., Class of 2009)

- Recipient of Northeastern University's Undergraduate Research Award, August 2005

Ms. Erin Curran (Pharm.D., Class of 2007)

Ms. Christina Guerra (Pharm.D., Class of 2012)

- Recipient of Northeastern University Provost's Undergraduate Research Award, September 2007

Ms. Shubha Bhat (Pharm.D., Class of 2012)

Mr. Ravi Patel (BS, Chemistry, Class of 2014)

Ms. Erica Diamantides (Pharm.D., Class of 2015)

Ms. Kristin Hong (Pharm.D., Class of 2015)

Ms. Faryal Mir (BS, Biology/Pre-Med Program, Class of 2014)

Ms. Kendall Donohoe (Pharm.D., Class of 2018)

Ms. Rachael Heiss (BS, Mechanical Engineering., Class of 2019)

Ms. Sneha Hingorany (BS, Biology/Pre-Med Program, Class of 2019)

Ms. Reema Patel (Pharm.D., Class of 2020)

Ms. Mina Nayeri (Pharm.D., Class of 2019)

Ms. Priyanka Talagadadevi (BS, Biomed Engr and Physics, Class of 2020)

Ms. Alyssa Bilotta (BS, Chemistry, Class of 2017)

Mr. Youngwoo Cho (Pharm.D., Class of 2020)

- Recipient of Northeastern University Provost's Undergraduate Research Award, September 2018

Ms. Casey Spellman (BS, Biochemistry, Class of 2020)

Ms. Suha Yacoob (BS, Chemical Engineering, Class of 2021)

Ms. Alicia Sobeneau (BS, Molecular and Cell Biology, Class of 2022)

Ms. Molly Haag (BS, Biochemistry, Class of 2022)

Ms. Hiba Hussein (BS, Biology/Pre-Med Program, Class of 2025)

Ms. Ira Hysi (BS, Chemical Engineering, Class of 2025)

High School Summer Research Scholars

Ms. Phung-Kim Lai (Summer, 1994)

Ms. Luoisy Raymond (Summer, 1995)

Mr. Bao-Tuan Nguyen (Summer, 1995)

Mr. Kong-Jie Kah (Summer, 1997)

- Through Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Jeremy L. England (Summer, 1998)

- Research Science Institute, Center for Excellence in Education, McLean, VA

- Semi-Finalist in 1999 Intel High School Talent Search Competition

Ms. Iris Wei (Summer, 1999)

- Research Science Institute, Center for Excellence in Education, McLean, VA

- Semi-Finalist in the 2000 Intel High School Talent Search Competition

Mr. Brad M. Rosen (Summer, 2000)

- Research Science Institute, Center for Excellence in Education, McLean, VA

- Semi-Finalist in the 2001 Intel High School Talent Search Competition

Ms. Natalie Karabel (Summer, 2001)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Feng Tu (Summer, 2002)

- Research Science Institute, Center for Excellence in Education, McLean, VA

- Semi-Finalist in the 2002 Siemens-Westinghouse High School Talent Search Competition

Ms. Maria Elena DeObaldia (Summer, 2003)

-
- Research Science Institute, Center for Excellence in Education, McLean, VA
 - Winner of 2004 USA TODAY's All-USA High School Academic First Team

Ms. Joline Fan (Summer, 2004)

- Research Science Institute, Center for Excellence in Education, McLean, VA
- Finalist in the 2004 Siemens-Westinghouse High School Talent Search Competition

Mr. Harold Au (Summer, 2004)

- Research Science Institute, Center for Excellence in Education, McLean, VA
- Gold Award Winner at the 2005 Singapore Science and Engineering Fair.
- Award Winner at the 2005 Shanghai Science Expo, Shanghai, China.

Ms. Yi-Meng (Sally) Tan (Summer, 2005)

- Research Science Institute, Center for Excellence in Education, McLean, VA
- Semi-Finalist in the 2005 Siemens-Westinghouse High School Talent Search Competition

Ms. Thilini Ariyawansa (Summer, 2006)

- Research Science Institute, Center for Excellence in Education, McLean, VA
- Winner of 2007 USA TODAY's All-USA High School Academic First Team

Ms. Elizabeth Lawler (Summer, 2006)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Lili Ge (Summer, 2006)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Ana Lyons (Summer, 2007)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Zhi-Guang Ng (Summer, 2007)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Jamie Kang (Summer, 2007)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Marissa Dickson (Summer, 2007)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Jia-Wei Lim (Summer, 2008)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Mark-Alex Espanol (Summer, 2008)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Rachael Le (Summer, 2008)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Xiaojun Chen (Summer, 2009)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Tien An (Summer, 2009)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Gary Lee Lim (Summer, 2010)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Manasi Malik (Summer, 2010)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Fay Khudairi (Summer, 2010)

- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Debra Van Egeren (Summer, 2011)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Rahul Shankar (Summer, 2011)

- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Kruti Vora (Summer, 2011)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Jennifer Makovkina (Summer, 2011)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Ruiyi Gao (Summer, 2012)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Jennifer Flaherty (Summer, 2012)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Nathan Kondamuri (Summer, 2012)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Minerva Tili (Summer, 2013)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Juan Paniagua (Summer, 2013)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Sejal Batra (Summer, 2013)

Mr. Rachit Singh (Summer, 2013)
- Research Science Institute, Center for Excellence in Education, McLean, VA
- Semi Finalist in the 2014 Intel Talent Search Competition

Ms. Batelhem Gemechu (Summer, 2014)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Nathan Pan-Doh (Summer, 2014)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Michelle Campeau (Summer, 2014)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Ruchir Rastogi (Summer, 2014)
- Research Science Institute, Center for Excellence in Education, McLean, VA
- Semi Finalist in the 2014 Intel Talent Search Competition

Ms. Michelle Gee (Summer, 2015)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Yasmeen Elaywan (Summer, 2015)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Anirudh Jain (Summer, 2015)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Yue (Jerry) Zhang (Summer, 2015)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Caroline Quinn (Summer, 2016)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Fred Eberstadt (Summer, 2016)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Dina Shehata (Summer, 2016)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Yilin (Alan) Huang (Summer, 2016)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Sophia Luo (Summer, 2017)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Timothy Tran (Summer, 2017)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Junyi (Michelle) He (Summer, 2017)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Sreenikitha (Nikki) Emani, (Summer, 2017)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Hassan Osman (Summer, 2018)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Yi-Yong Tan (Summer, 2018)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Dinesh Sangadi (Summer, 2018)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Pamina Meija (Summer, 2018)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Roc Bellostas (Summer, 2019)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Mr. Edward Pham (Summer, 2019)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Lauren Murphy (Summer, 2019)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Felix Xu (Summer, 2020)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Raina Alshawabkeh (Summer, 2020)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Me. Daniel Becker (Summer, 2021)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Alice Han (Summer, 2021)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Murtaza Khalil (Summer, 2022)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Mr. Sarper Paker (Summer, 2022)
- Young Scholars Program, Center for STEM Education, Northeastern University, Boston, MA

Ms. Puja Chopade (Summer, 2022)
- Research Science Institute, Center for Excellence in Education, McLean, VA

Ms. Josephine Hibou (Summer, 2022)
- Research Science Institute, Center for Excellence in Education, McLean, VA

SERVICE

Professional

- Membership in Professional and Scientific Societies:
 - American Society for Cell and Gene Therapy (2022-Present)
 - American Association for the Advancement of Science (2022-Present)

- American Association of Pharmaceutical Scientists (1987-Present).

Member of the Polymers in Drug Delivery SIG

Member of the Nanotechnology SIG

- The Controlled Release Society (1993-Present).

Scientific Advisory Board Member (2012-2014)

Member of Polymeric Biomaterial Discussion Group

- American Association of Colleges of Pharmacy (1993-Present).

Member of the Council of Faculties.

Member of the Pharmaceutics SIG

Member of the Laboratory Instructors SIG

- The Society for Biomaterials (1989-2010).

Member of the Task Force on the Impact of Biomaterials on Graduate Education.

Member of the Polymeric Materials Special Interest Group.

- Grant Reviewer:

- National Institutes of Health (Partial List)

National Heart, Lung, and Blood Diseases Institute (NHLBI) 2004 Program of Excellence in Nanotechnology – Special Emphasis Panel [ZHL1-CSR-K (F1)(R)].

Center for Scientific Review (CSR) November 2004 Gene and Drug Delivery (GDD) Study Section – SBIR/STTR Programs Special Emphasis Panel [ZRG1-BST-Z (10)].

National Institute of Allergy and Infectious Diseases (NIAID) 2005 Challenge Grants: Biodefense Product Development – Special Emphasis Panel [ZA11-TS-M (M5)(R)].

Center for Scientific Review (CSR) July 2005 Gene and Drug Delivery (GDD) Study Section Meeting – Temporary Member.

National Heart, Lung, and Blood Diseases Institute (NHLBI) October 2005 – Special Review Committee for P01 Program Proposals.

Center for Scientific Review (CSR), November 2005 Gene and Drug Delivery (GDD) Study Section – SBIR/STTR Programs Special Emphasis Panel [ZRG1 BST-Z 10(B)].

National Cancer Institute (NCI), March 2006 - Ruth L. Kirschstein NRSA Fellowships (F32/F33) in Cancer Nanotechnology Research (RFA-CA-06-010) Special Emphasis Panel [ZCA1 RTRB-Z(M1)(R)].

Center for Scientific Review (CSR), April, 2006 Gene and Drug Delivery (GDD) Study Section – SBIR/STTR Programs Special Emphasis Panel [ZRG1 BST-Z (10) (B)].

National Heart, Lung, and Blood Diseases Institute (NHLBI) May, 2006 – Special Review Committee for P01 Program Proposals.

Center for Scientific Review (CSR), June, 2006 – Special Emphasis Panel on Proposals in Response to RFA “Biology of RNA Interference” [2006/10 ZRG1 BST-Z (52) (R)].

Center for Scientific Review (CSR), March, 2007 – Special Emphasis Panel on Proposals in Response to RFA “Small Business: Orthopedics” [ZRG1 MOSS (10)].

Center for Scientific Review (CSR), July, 2007 – Cancer Drug Development and Therapeutics SBIR/STTR ONC-V (13) [2007/10 ZRG1 ONC-V (13) B].

Center for Scientific Review (CSR), October 2007 – ZRG1 NANO-M (01) “Nanotechnology” Study Section – Temporary Member.

National Institute of Biomedical Engineering and Bioimaging (NIBIB), July 2008 - ZEB1 OSR-E (01) S “Special Emphasis Panel/Scientific Review Group 2008/10 on T32/K99/K01 Training Grants”.

National Institute of General Medical Sciences (NIGMS), August 2008 2008/10 ZGM1 MBRS-0 (NP) “Support of Competitive Research (SCORE) Minority Biomedical Research Support in Neurophysics” Special Emphasis Panel.

National Cancer Institute (NCI), October, 2008, 2009/01 ZRG1 ONC-X (14) B “Experimental Cancer Therapeutics SBIR/STTR” Special Emphasis Panel.

National Institute of Biomedical Imaging and Bioengineering (NIBIB) Special Emphasis Panel. 2009/01 ZEB1 OSR-B (J1) S. November 2008 Support for Scientific Conference (R13) Grant Application Review Panel.

Center for Scientific Review (CSR), January, 2009 – Cancer Immunopathology and Immunotherapy Study Section (CII), *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), April, 2009 – 2009/05 ZRG1 BST-G (10) B - Assays, Detectors, and Devices SBIR/STTR Panel, *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), June 2009 - 2009/10 ZRG1 BST-M (58) R, RFA OD-09-003 Challenge Grants Panel 4, *Ad Hoc* Reviewer.

National Cancer Institute (NCI), July 2009, 2009/01 ZRG1 OTC-X 14 B, Experimental Cancer Therapeutics, SBIR Special Emphasis Panel.

Center for Scientific Review (CSR), October, 2009, 2010/01 BTSS Bioengineering Technology and Surgical Sciences Study Section. *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), February, 2010, 2010/05 BTSS Bioengineering Technology and Surgical Sciences Study Section. *Ad Hoc* Reviewer.

National Cancer Institute (NCI), June 2010 ZCA1 SRLB-Q C1 B, Multifunctional Therapeutics Phase II Special Emphasis Panel, Reviewer.

National Institute of Drugs of Abuse (NIDA), July, 2010 ZDA1 JXR-D (10) Special Emphasis Panel, Reviewer.

Center for Scientific Review (CSR), September 2010, 2011/01 ZRG1 BST-N (03) Member Conflicts: Bioengineering Sciences and Technologies, *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), September 2010, 2011/01 ZRG1 IMST-K (03) Member Conflict: Enabling Bioanalytical and Imaging Technologies, *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), February, 2011, 2011/01 BTSS Bioengineering Technology and Surgical Sciences Study Section. *Ad Hoc* Reviewer.

Center for Scientific Review (CSR), September, 2011, 2011/01 BTSS Bioengineering Technology and Surgical Sciences Study Section. *Permanent Member*.

Center for Scientific Review (CSR), May, 2012, 2012/10 PAR11-301-303: Pediatric Drug Formulations and Drug Delivery (ZRG1 ETTN-S (50) R) Special Emphasis Panel, *Co-Chair*.

National Institute of Biomedical Imaging and Bioengineering (NIBIB), July 2013, 2013/10 ZEB1 OSR-E (01) S. NIBIB K Training Meeting (Teleconference) Special Emphasis Panel, *Member*.

Center for Scientific Review (CSR), June, 2016 and Feb, 2017 – Cancer Immunopathology and Immunotherapy Study Section (CII), *Ad Hoc* Reviewer.

- Department of Defense, United States Army Medical Research and Material Command, Congressionally-Directed Medical Research Program
 - Traumatic Brain Injury, Drug Development Program (2022)
 - Breast Cancer Research Program Review Panel (2006-Present)
 - Breast Cancer Concept Award Review Panel (2010-Present)
 - Breast Cancer Training Program Review Panel (2010-2012)
 - Peer Reviewed Medical Research Program – Pre-applications for Inflammatory Bowel Disease-1 (2013)
- United States Department of Agriculture
 - Investigator Initiated Proposals.
 - SBIR/STTR Proposals.
- United States Food and Drug Administration
 - Intramural Proposals on Nanotechnology
- American Chemical Society, Washington, DC
 - Petroleum Research Funds
- Susan G. Komen Foundation for Breast Cancer Grant Program, Dallas, TX
- American Association for Advancement of Science, Life Sciences Discovery Fund, Washington, DC
- Georgia Cancer Coalition Grant Program, Atlanta, GA
- University of Missouri Intramural Grant Program, Columbia, MO
- University of Kansas Intramural Grant Program, Lawrence, KS
- Medical Research Council of United Kingdom, London, UK
- The Wellcome Trust of United Kingdom, London, UK
- The Leenaards Foundation Prize, Lausanne, Switzerland
- The Netherlands Organisation for Scientific Research – The Netherlands
- National Medical Research Council, Ministry of Health, Singapore
- Singapore Science and Engineering Research Council (SERC) – an Agency of Science, Technology and Research Singapore (A*STAR), Singapore City

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- Israel Science Foundation, Tel Aviv, Israel
 - Czech Science Foundation, Prague, Czech Republic
 - Hong Kong Innovation and Technology Support Programme, Wanchai, Hong Kong
 - Hong Kong Research Grants Council, Wanchai, Hong Kong
 - Skolkovo Foundation, Moscow, Russia
 - Danish Research Council, Copenhagen, Denmark
 - Kuwait Foundation for the Advancement of Science, Kuwait City, Kuwait
 - Omani Research Council, Muscat, Oman
 - South African Medical Research Council, Durban, South Africa
 - Scientific Foundation of Ireland, Dublin, Ireland
 - Membership in Industrial and Academic Scientific Advisory Boards:
 - Bessor Pharma, Inc., Framingham, MA (Scientific Advisory Board member)
 - Targagenix, Inc., Stony Brook, NY (Scientific Advisory Board member)
 - Nemucore Medical Innovations, Wellesley, MA (Founder and Scientific Advisory Board member)
 - Blue Ocean Biomanufacturing, Inc., Worcester, MA (Founder and Scientific Advisory Board member)
 - OnSite Therapeutics, Inc., Lowell, MA (Scientific Advisory Board member)
 - Cerulean Pharmaceuticals, Inc., Cambridge, MA (Scientific Advisory Board member)
 - Aten Porus Life Sciences Private, LTD, Bangalore, India (Scientific Advisory Board member)
 - Controlled Release Society (Scientific Advisory Board member)
 - Center for Nanomedicine and Drug Delivery, Xavier University of Louisiana, New Orleans, LA (Scientific Advisor)
 - International Symposium on Recent Advances in Drug Delivery Systems, University of Utah, Salt Lake City, UT (Scientific Advisor)
 - Journal Editorships:
 - Editor for the Americas, *Drug Delivery and Translational Research* (Springer)
 - Associate Editor, *OpenNano* (Elsevier)
 - Associate Editor, *International Journal of Green Nanotechnology: Biomedicine* (Taylor & Francis)
 - Associate Editor, *Nanomedicine: Nanotechnology, Biology, and Medicine* (Elsevier) (2008-2015)
 - Membership in Journal Editorial Boards:
 - Bioengineering
 - Drug Design, Development and Therapy
 - Expert Opinion on Drug Delivery
 - Journal of Biopharmaceutics and Biotechnology
 - Journal of Controlled Release
 - Journal of Nano Education
 - Nature Scientific Reports
 - Nanotechnology, Science and Applications
 - Pharmaceutical Formulations and Quality
 - Recent Advances in Drug Delivery and Formulations
 - Recent Patents on Drug Delivery and Formulations
 - Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry
 - The Open Drug Delivery Journal
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- Tissue Barriers

- Reviewer for Scientific Journals (Partial List):

- ACS Nano
- Advanced Drug Delivery Reviews
- Angewandte Chemie, International Edition
- Bioconjugate Chemistry
- Biomacromolecules
- Biomaterials
- Cancer Chemotherapy and Pharmacology
- Cancer Letters
- Cancer Research
- Carbohydrate Polymers
- European Polymer Journal
- European Journal of Pharmaceutics and Biopharmaceutics
- European Journal of Pharmaceutical Sciences
- Expert Opinion on Drug Delivery
- Expert Opinion on Biological Therapy
- Gene Therapy
- International Journal of Cancer
- International Journal of Pharmaceutics
- Journal of Applied Polymer Sciences
- Journal of the American Chemical Society
- Journal of Biomaterial Science, Polymer Edition
- Journal of Biomedical Materials Research
- Journal of Controlled Release
- Journal of Liposome Research
- Journal of Pharmacy and Pharmacology
- Journal of Pharmaceutical Sciences
- Journal of Pharmacology and Experimental Therapeutics
- Life Sciences
- Macromolecular Biosciences
- Molecular Cancer Therapeutics
- Molecular Pharmaceutics
- Nature Communications
- Nature Nanotechnology
- Nature Reviews Drug Discovery
- Pharmaceutical Research
- Polymer International
- Science
- Science Advances
- Science Translational Medicine
- Small
- STP Pharma Sciences

- Participation in Short Courses:

- Scanning Probe Microscopy Seminar and Workshop. Woburn, MA.
- Particle Technology Seminar and Workshop. Natick, MA.
- Application of HTML for Developing Instructional Materials. Boston, MA.
- Absolute Macromolecular Characterization with Light Scattering. Boston, MA.

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- Scientific and Engineering Applications of Macintosh® Computers. Boston, MA.
 - Surface Characterization of Biomedical Materials. Phoenix, AZ.
 - Hydrogels in Medicine and Pharmacy. Indianapolis, IN.
 - American Association of Pharmaceutical Scientists (AAPS), Northeast Regional Discussion Group Planning Committee Member (2001-2005).
 - Organizer of the National Cancer Institute/Nano Science and Technology Institute Special Symposium on *"Nanotechnology for Cancer Prevention, Diagnosis, and Treatment"* (2005-Present).
 - Instructor for Nano Science and Technology Institute's Tutorial Session on *"Nanotechnology for Medical Imaging and Therapy"* (2005 – Present).
 - Scientific Organizing Committee Member and Session Chair of the *"Cancer Nanotech Conference: Detecting and Treating Cancer"*. Paris, France. (2005-2007).
 - Organizer of the *"2009 Indo-US Cancer Nanotechnology Symposium"*, sponsored by the Indo-US Science and Technology Forum, New Delhi, India. February 2009.
 - Organizer of the Materials Research Society, Spring 2012 Meeting, San Francisco, CA. Symposium on *"Nanomedicine for Molecular Imaging and Therapy"* March, 2012.

University, College, School, & Department

- Faculty Delegate to the United States Pharmacopeia (USP) Convention (2003-Present).
- Faculty Advisor – American Association of Pharmaceutical Scientist – Northeastern University Student Chapter (2009-2015)
- Faculty Advisor - Beta Tau Chapter, Rho Chi Pharmaceutical Honor Society (1993-1999).
- Coordinator of the American Association of Pharmaceutical Scientists, Visiting Scientist Program (1993-1999).
- Presentations for Pharmacy Continuing Education Programs.
 - *Helicobacter pylori* and Peptic Ulcer Disease
 - Advances in Drug Delivery Systems: A Primer for Pharmacists
 - Nanomedicine: Realizing the Potential of Targeted and Molecular Therapies
 - Pharmaceutical Calculations: A Primer for Pharmacists and Pharmacy Technicians
- Participation in School of Pharmacy/Department Open Houses and Student Orientations.
- Participation in Pharmacy Alumni Activities.
- Pharmacy Student's Academic Advising/Portfolio Reviewer.
- Committee Assignments (Partial List):

University:

- University Task Force on the Future of COVID Testing Facility (2020-Present)

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- Provost's Excellence in Research and Creative Endeavor Award Committee (2020-Present)
 - Senate Committee for the Department of Chemical Engineering Chair Search (2020-2021)
 - Provost's Task Force on Promotion of Research Faculty (2020-2022)
 - University Distinguished Professorship Committee (2016-2019)
 - University Patent Committee (2002-2009, 2012-2018)
 - Biotechnology Academic Steering Committee (2002-2010)
 - Senate Search Committee for Bouve College Dean (2011-2012)
 - Provost's Tenure Advisory Committee (2008-2010)
 - Academic Computing Advisory Committee (2004-2006)
 - Senate Library Advisory Committee (2001-2007)
 - Education Technology Faculty Advisory Committee (2004-2006)
 - Senate Committee for Evaluation of Dean of Nursing (2004-2006)
 - Senate Committee for Evaluation of Dean of Libraries (2003-2004)
 - Executive Committee of the Biotechnology Initiative (2002-2004)
 - Senate Committee for the Department of Pharmacy Practice Chair Search (2001-2002)
 - University Instructional Technology Task Force (2000-2001)
 - Senate Committee for Department of Pharmaceutical Sciences Chair Search (1996-1997)

Bouvé College:

- Bouve Dean's Leadership Team (2011-2016).
- Bouve Administrative Committee (2005 – 2011).
- Associate Dean for Research Search Committee (2010-2011).
- George D. Behrakis Endowed Professor Search Committee (2002-2004).
- College Technology Committee (1998-2000).
- College Diversity Committee, (1994-1995).
- College Computer Advisory Committee, (1995-1998).
- Graduate Education and Research Task Force (1998-1999).
- Graduate Committee for Biomedical Sciences Program (1998-2000).

School of Pharmacy:

- Pharmacy Professional Affairs Committee (2020-Present).
- Pharmacy Executive Committee (2008-2016).
- Pharmacy Steering Committee (2001-2004, 2005-2016).
- Pharmacy Curriculum Committee (1998-2002, 2007-2016).
- Pharmacy Re-Accreditation/Self-Study Committee, (1995-1996, 2008-2010).
- Pharmacy Professional Affairs Committee (2006-2009)
- Pharmacy Transfer Students Admission Committee, (1994-2001, 2003-2007).
- Graduate Committee of the School of Pharmacy (2002-2006).
- Pharmacy Honors and Awards Committee, (1994-2001).
- Pharmacy Progression Requirements Committee, (1996-2000).
- Pharmacy Laboratory Renovation Committee (1997-1999).
- Doctor of Pharmacy Tracking Admission Committee (1998-2000).
- Doctor of Pharmacy Curriculum Working Group (1996-1999)*.

Departmental of Pharmaceutical Sciences:

- Merit Review Committee, (1994-1996, 1997-2000, 2002-2003, 2007-2008).
- Faculty Search Committees, (1994-1996, 1998-1999, 2002-2003*, 2007-2008, 2019-2020*, 2020-2021).
- Pharmaceutical Sciences Workload Policy Committee (2005-2007*)
- BS in Pharm. Sciences Program Evaluation Committee (2001-2003, 2009-2010).

Community

- Provide Math and Science Tutoring to Middle and High School Students.
- Discuss Pharmacy as a Career Choice to Middle and High School Students and Parents.
- Participate in Fund Raising Activities for Local Schools and Charitable Community Organizations.

AWARDS AND HONORS

- Purdue University, College of Pharmacy, Distinguished Alumni Award – 2019.
- Clarivate Analytics/Web of Science Highly Cited Author (Top 1%) in Pharmacology & Toxicology – 2014 & 2018.
- Northeastern University, School of Pharmacy Distinguished Alumni Award – 2016.
- Northeastern University, University Distinguished Professorship Award – 2016.
- Elected into the College of Fellows, Controlled Release Society – 2014.
- Phi Lambda Sigma, Pharmacy Leadership Society, Honorary Member – 2014.
- Controlled Release Society - Tsuneji Nagai Award – 2012.
- Elected Fellow of the American Association of Pharmaceutical Scientists (AAPS), 2007.
- American Association of Pharmaceutical Scientists (AAPS) - Meritorious Manuscript Award, 2007.
- Nano Science and Technology Institute (NSTI) Fellowship Award for Outstanding Contributions towards the Advancement, in Nanotechnology, Microtechnology, and Biotechnology, 2006.
- Cited in Academic Pharmaceutical Scientists Who's Who, 2004.
- Eurand Award for Outstanding Research in Oral Drug Delivery, Third Prize, 2003.
- Special Faculty Recognition Award from the Doctor of Pharmacy Students, Class of 1999.
- Recipient of Rho Chi Honor Society Advisor Appreciation Award, 1997, 1998, & 1999.
- Recipient of Dean's Excellence in Teaching Award, 1996.
- Cited in Who's Who in Science and Engineering, 1996.
- Elected Member of Sigma Xi - The Scientific Research Society, 1996.
- Elected Member of Rho Chi - Pharmaceutical Honor Society, 1987.
- Recipient of Dean's Undergraduate Research Achievement Award, 1987.
- Recipient of Burroughs Wellcome Academic Scholarship Award, 1987.
- Recipient of Dean Leroy Keagle Memorial Scholarship Award, 1986.

Last Updated: February, 2023

Exhibit B

Exhibit B

EXHIBIT	DESCRIPTION
1001	US9051542
1002	US9051542 Prosecution History
1003	PCT Publication of International Application No. PCT/US01/07194, "ADENOVIRUS FORMULATIONS" ("Evans")
1004	PCT Publication of International Application No. PCT/US99/01873, "COMPOSITIONS COMPRISING VIRUSES AND METHODS FOR CONCENTRATING VIRUS PREPARATIONS" ("Frei")
1005	Huang, J., et al. "Aggregation of AAV vectors, its impact on liver-directed gene transfer and development of vector formulations to prevent and dissolve aggregation and enhance gene transfer efficiency." Mol. Therapy 1 (2000): 286-291. ("Huang")
1006	Mingozzi, Federico, et al. "Improved hepatic gene transfer by using an adeno-associated virus serotype 5 vector." Journal of virology 76.20 (2002): 10497-10502. ("Mingozzi")
1007	Wright, J. Fraser, et al. "Recombinant adeno-associated virus: formulation challenges and strategies for a gene therapy vector." Current opinion in drug discovery & development 6.2 (2003): 174-178. ("Wright")
1008	Gatlin, L. A., and CA Brister Gatlin. "Formulation and administration techniques to minimize injection pain and tissue damage associated with parenteral products." Injectable drug development: Techniques to reduce pain and irritation (1999): 401-425. ("Gatlin")
1009	PCT Publication of International Application No. PCT/US02/35049, "VIRAL VECTOR PRODUCTION METHODS AND COMPOSITIONS" ("Liu")
1010	PCT Publication of International Application No. PCT/US02/37944, "METHODS FOR PRODUCING STOCKS OF RECOMBINANT AAV VIRIONS" ("Lochrie")
1011	Potter, Mark et. Al. "Streamlined Large-Scale Production of Recombinant Adenovirus-Associated Virus (rAAV) Vectors"

	Methods in Enzymology, Vol. 346, Gene Therapy Methods (M Ian Phillips, ed. 2002) (“Potter”)
1012	Clark, K. Reed. "Recent advances in recombinant adeno-associated virus vector production." Kidney international 61.1 (2002): S9-S15. (“Clark”)
1013	Croyle, M. A., X. Cheng, and J. M. Wilson. "Development of formulations that enhance physical stability of viral vectors for gene therapy." Gene therapy 8.17 (2001): 1281-1290. (“Croyle”)
1015	US6261823
1016	Freireich, Emil J. "Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man." Cancer Chemother. Rep. 50 (1966): 219-244.
1017	U.S. Food and Drug Administration, “Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” July 2005
1018	Carpenter, John F., and Mark C. Manning. "Rational Design of Stable Protein Formulation." Effects of conformation on the chemical stability 13 (2002): 85-107.
1019	Johnson et al., “Effect of Environmental pH on Adenovirus-Associated Virus” in Proceedings of The Society For Experimental Biology And Medicine 150, 585-590 (1975)
1020	Gennaro, Alfonso R. “Remington: The Science and Practice of Pharmacy, Nineteenth Edition, Volume I.” PA, Mack Publishing Co. (1995).
1021	Gennaro, Alfonso R. “Remington: The Science and Practice of Pharmacy, Nineteenth Edition, Volume II.” PA, Mack Publishing Co. (1995).
1022	Handbook of Pharmaceutical Excipients, Second Edition Wade, Ainley, and P. Weller. " Handbook of Pharmaceutical Excipients, Second Edition." American Pharmaceutical Association, Pharmaceutical Press London (1994).
1024	Solutions, Stock. “Common buffers, media, and stock solutions.” John and Wiley and Sons: Hoboken, NJ, USA (2001).