CURRICULUM VITAE

Name:	Robert L. Garcea
Address:	2505 Pampas Court, Boulder, CO 80304
Place of Birth:	San Mateo, California
Marital Status:	Married (three children)

Education:

1970 A.B.	Harvard College (Chemistry)
1974 M.D.	University of California at San Francisco

Postdoctoral Training:

Internship and Residencies:

1974-1976	Intern and Resident (Pediatrics), Stanford University Medical School

Research Fellowships:

1972-1974	Pre-doctoral Fellow, Dept. of Biochemistry and Biophysics, University of California at San Francisco, (Prof. G. Tomkins)
1976-1977	Postdoctoral Fellow, Dept. of Pharmacology, Stanford Medical School, (Prof. H. Epstein)
1977-1979	Fellow in Biochemistry and Biophysics, University of California at San Francisco (Prof. Bruce Alberts)
1979-1982	Fellow in Hematology/Oncology, Children's Hospital Medical Center and Dana-Farber Cancer Institute (Boston, MA) (Prof. D. Nathan, Prof. T. Benjamin)

Licensure and Certification:

1975	California Medical Licensure No. G30171
1975	Diplomate National Boards of Medical Examiners
1980	Massachusetts License Registration No. 47071
1982	American Board of Pediatrics Certified No. 27997
1982	Board eligible Pediatric Hematology-Oncology
1993	Colorado Medical Licensure No. 32422

Academic Appointments:

1979-1982	Research Fellow in Pediatrics, Harvard Medical School
1982-1983	Instructor in Pediatrics, Harvard Medical School
1983-1988	Assistant Professor in Pediatrics, Harvard Medical School
1988-1993	Associate Professor in Pediatrics, Harvard Medical School
1993-2008	Professor in Pediatrics, University of Colorado School of Medicine (joint appointments in Cell & Developmental Biology (1994-08) and Microbiology (1999-08))
2008-2023	Professor, Molecular, Cellular, and Developmental Biology, University of Colorado Boulder
2024-	Emeritus Professor, University of Colorado Boulder
2024-	Senior Investigator in Vaccine Development

Hospital Appointments:

1979-1982	Clinical Fellow, Harvard Medical School
1982-1988	Assistant in Medicine, Children's Hospital (Boston)
1982-1985	Clinical Associate, Dana-Farber Cancer Institute
1985-1993	Assistant Physician, Dana-Farber Cancer Institute
1988-1993	Senior Associate in Medicine, Children's Hospital (Boston)
1993-2003	Chief, Section of Pediatric Hematology/Oncology/Bone Marrow Transplantation, University of Colorado School of Medicine, and Denver Children’s Hospital

Awards and Honors:

1966	National Merit Scholar
1967-1970	Harvard College Scholar
1970	Magna cum laude in Chemistry
1976-1978	Muscular Dystrophy Postdoctoral Fellow
1978-1979	Leukemia Society of America Postdoctoral Fellow
1980-1981	Leukemia Society of America Postdoctoral Fellow
1987-1989	American Cancer Society Junior Faculty Award
1989	American Society for Clinical Investigation
2006	UCDHSC Inventor of the Year
2006-08	Chair-Elect/Chair, American Society of Microbiology, Division S (DNA Viruses)
2012	Fellow, American Academy of Microbiology

Professional Societies:

1984-	American Society for Microbiology
1989-	American Society for Clinical Investigation (elected)

1991-	American Society for Virology
1992-	American Society of Hematology
1993-	Society for Pediatric Research (elected)

Committee Assignments:

1982-1992	Medical Records Committee, Dana-Farber Cancer Institute
1982-1992	Biosafety Committee, Dana-Farber Cancer Institute, (chairman 1988-92)
1983-1986	Library Committee, Dana-Farber Cancer Institute
1983-1993	Seminar Committee, Dana-Farber Cancer Institute
1985-1987	Chairman, Greater Boston Virology Dinner Club
1987,90,91	Admissions Committee, Committee on Virology, Harvard Medical School
1989,91	Seminar Committee, Committee on Virology
1990-1993	Instructor Promotions Committee, Boston Children's Hospital
1991-1993	ASCI Committee on Science Education (National)
1991-1993	Cell and Developmental Biology, Prelim Exam Committee
1993-1998	Trustee, Leukemia Society of America, (Rocky Mountain Chapter)
1993-	Member, University of Colorado Cancer Center (Program Director, Pediatric Oncology 1993-03)
1993-03	Member, Advisory Committee, Colorado Sickle Cell Center
1993-94	Member, Housestaff Selection Committee, TCH
1994-	Member, Program in Cell and Structural Biology, UCHSC
1994-03	Member, Dean's Advisory Board (Distinguished Lectures)
1994-	Member, Advisory Board, UCHSC Gene Therapy Program
1994-	Member, Program in Molecular Biology, UCHSC
1994-1996	Member, Biochemistry Chairperson Search Committee
1994-1996	Member, Radiation Therapy Chairperson Search Committee
1994	Member, Cell Biology Faculty Search Committee
1994-1998	Member, Molecular Biology Program Seminar Committee
1995	Member, TCH Medical Staff Physician Review
1995	Member, Research Retreat Committee, UCSOM
1995-	Member, Medical Scientist Training Program Faculty
1996	Member, Biochemistry Search Committee (junior faculty)
1998-	Member, Biomedical Sciences Program (UCHSC)
1998-	Member, Structural Biology Search Committee (UCHSC)

1998-02	Member, SV40-Human Polyomavirus Serology Working Group (FDA/OVRR/CBER)
1999	Member, Biochemistry Search Committee (junior faculty)
1998,99	Member, Microbiology Search Committee
2000-02	Member, Pediatric Infectious Disease Chair Search Committee
2000,02	Organizing Committee, DNA Tumor Virus Meeting (Madison,WI)
2000-	Member, Program in Biomolecular Structure, UCHSC
2001	Co-Organizer, European Molecular Biology Organization (EMBO) Workshop on “The Structural Biology of Small DNA Tumor Viruses”
2002-09	External Advisor, Johns Hopkins SPORE for Cervical Cancer
2002	Member, Technology Transfer Search Committee (UCHSC)
2003	Member, International Scientific Committee, Symposium on Human Papillomavirus: vaccines and immunotherapies (Cambridge, U.K.)
2003	Chairperson/Organizer, Second International Workshop on the Structural Biology of Small DNA Tumor Viruses (Siena, IT)
2003	International Scientific Committee, ICGEB DNA Tumour Virus Meeting, (Trieste, IT)
2004-	Member, Colorado Center for AIDS Research
2005-09	Member, University of Colorado, Technology Transfer Business Advisory Board
2005	Chairperson/Co-Organizer, European Molecular Biology Organization (EMBO) Workshop on “The Structural Biology of Papovaviruses”
2006-present	Member, Pinnacles Group, CU Technology Transfer
2006,08	Co-chair, FASEB Research Conference on “Virus Assembly”
2007	Chairperson/Co-Organizer, Fourth International Workshop on the Structural Biology of Small DNA Tumor Viruses (Siena, IT)
2007	International Scientific Committee, Session Chair, ICGEB DNA Tumour Virus meeting, (Trieste, IT)
2007-08	Grand Challenges in Global Health, ESC Working Group on Vaccines

2008-	Member, Colorado Initiative in Molecular Biotechnology (BioFrontiers), University of Colorado Boulder
2008-09	International Scientific Committee, 2009 25th International Papillomavirus Conference, Malmo, Sweden
2009-10	International Scientific Committee, 2010 26th International Papillomavirus Conference, Montreal Canada
2010	Co-Organizer, 1st International meeting on "Emerging Oncogenic Viruses", WHO/IARC meeting, Puglia, IT.
2010-11	International Organizing Committee, 2011 27th International Papillomavirus Conference, Berlin.
2010-11	Member and Chair, MCDB Junior Faculty Search Committee
2011-	Member, University of Colorado Boulder, Committee on Research Misconduct
2012	International Scientific Organizing Committee, 28th International Papillomavirus Meeting, Puerto Rico
2012	Co-Organizer, 2nd International Meeting on "Emerging Oncogenic Viruses", WHO/IARC meeting, Puglia, IT.
2012	Member, BioFrontiers Junior Faculty Search Committee
2013	International Scientific Committee, Session Chair, DNA Tumour Virus meeting, (Birmingham, UK)
2013-14	International Scientific Committee, 29th International Papillomavirus Meeting, Seattle, WA
2014-21	Scientific Organizing Committee, "Emerging Oncogenic Viruses", WHO/IARC meetings, Puglia, IT.
2015-18	Member, Department Executive Committee, MCDB

Teaching Experience:

Harvard University Committee on Virology (1984-93)
Harvard Medical School Committee on Cell and Developmental Biology (1988-93)
Lecturer in Cell Biology 211 (Biology of the Cancer Cell), Harvard Medical School (1985,1987,1989)
Lecturer in Virology 101 (General Virology), Harvard Medical School (1987)
Lecturer in HST040 (Mechanisms of Microbial Pathogenesis), Harvard Medical School (1987-91)
Lecturer in Virology 202 (Topics in Virology), Harvard Medical School (1988)
Lecturer in Cell Biology 7604 (Cell Biology), UCHSC (1994-97)
Lecturer in Microbiology MICB7701/7627 (Molecular Virology), UCHSC (1996-06)
Lecturer in CSBI5001 (1998-99), IDPT7800 (1998-06) (Biomedical Sciences Core

Course in Cell Biology, UCHSC
Lecturer in Cancer Cell Biology 7600 (2005-06) UCHSC
Lecturer in Infectious Disease Core Course IDPT6004 (2006) UCHSC
Lecturer in Science in Medicine, MCDB4100, (2007-10) CU Boulder
Lecturer in Graduate MCDB Core MCDB5230, (2010-13) CU Boulder
Lecturer in Scientific Conduct MCDB5776, (2009) CU Boulder
Course Director, Virology, MCDB4750, (2010-21) CU Boulder
Member Ph.D. Thesis Committee (UCHSC: Grimison, Mueller, Kosten, Chromy, Clase, Fletcher, Lilyestrom, Ortega, Bishop, Medina, Howard, Kovacs, Townsend, Kean. CU Boulder: Busha, Takeshita, Cohen, Stickel, Riemondy, Pugach, Gadek, Dong, Gilchrist, Stabell)
Ph.D. Advisor: (UCHSC: Chromy, Kean; CU Boulder: Heiser, Langberg, O'Hara, Peters)

Patents Pending:

U.S.S.N. 07/695,647 : R. L. Garcea and D.J. Bergsagel, Assay for polyomavirus in humans and uses thereof (awarded 12/92).

U.S.S.N.6165471: R.L. Garcea, M.McCarthy, J. Suzich, R. Rose: Homogeneous Human papillomavirus capsomere containing compositions, methods for manufacture, and use thereof as diagnostic, prophylactic or therapeutic agents (awarded 12/00).

U.S.S.N.7279306: Robert Garcea and Richard Schlegel: Stable (fixed) forms of viral capsid proteins, and viral capsid protein fusions, preferably papillomavirus L1 proteins and uses thereof. (awarded 10/07)

U.S.S.N.7763259: Robert L. Garcea and Renee Finnen: Therapeutic and prophylactic vaccine for the treatment and prevention of papillomavirus infection. (awarded 7/10).

U.S.S.N.10751408: Theodore W. Randolph, Robert Garcea, Alan W. Weimer: Compositions and methods for making and using thermostable immunogenic formulations with increased compatibility of use as vaccines against one or more pathogens (awarded 8/20).

U.S.S.N.11185580: Robert L. Garcea and Dennis G. Macejak: Compositions, methods and uses for improved human papilloma virus constructs. (awarded 11/21).

U.S.S.N. 11273127: Theodore W. Randolph, Robert Garcea, Kimberly J. Hassett: Compositions, methods and uses for thermally stable multi-targeted antigens (awarded 3/15/2022, published 6/9/2022)

U.S.S.N.0175907A1: Theodore Randolph, Robert Garcea, Kimberly J. Hassett: Compositions, methods, and uses for thermally stable human papillomavirus formulations. (awarded 2/4/2022, published 11/24/2022).

U.S.S.N 11,806,432 B2: Theodore Randolph and Robert Garcea: Compositions, methods and uses for thermally stable multi-targeted antigens. (published 11/7/2023).

Private Enterprise Collaborations:

1992-98	Senior Scientific Advisory Council, NeXstar, Inc. (Boulder, CO)
2003-04	Consultant, Sirna Therapeutics (Boulder CO)
2004-06	Member, Scientific Advisory Board, Centre for Nanostructural Bioengineering, the University of Queensland, Australia
2009-12	Tysabri Advisory Panel, Biogen-Idec and Elan
2011-12	Scientific Advisory Board, Aura Biosciences
2012-17	PML Consortium Scientific Advisory Board
2014	Co-Founder, VitriVax Inc.
2022-	Board Member, VitriVax Inc.

Referee and Review:

1985-present Ad hoc, Virology, Journal of Virology, Journal of Clinical Investigation, Microbiological Reviews, Archives of Virology, Journal of General Virology, Journal of Mol. Biology, Cancer Research, American J. of Pathology, Proc. Natl. Acad. Sci., Virus Research, Nature Biotechnology, Wellcome Trust, DOE, NSF, Biophysical Journal, J. Infect. Dis., Oncogene, J. Emerging Inf. Dis., Italian Association for Cancer Research, FEBS Letters, Human Gene Therapy, PLoS Biology, PLoS Pathogens, mSphere, eLife, Nature Communications.

1990-1993	Biomedical Research Grant Review Committee (Dana-Farber Cancer Institute)
1993/4, 2007	NCI Site Visit Special Review Committee
1994-96	Member, Colorado Cancer League, Scientific Advisory Board

1995-96	Ad hoc, Virology Study Section, NIH
1996-00	Member, Virology Study Section, NIH
1996-98	Member, BRS/HHMI Research Review Committee (UCSOM)
2000-21	Member, Editorial Board, Journal of Virology
2001-15	Member, Editorial Board, Virology
2014	Ad hoc, Virology B Study Section, NIH.
2015-19	Member, Virology A Study Section NIH

Publications:

1. Garcea R., and Gorman G. A difference in male territorial display in two sibling species of *Anolis*. Copeia 1968; **2**:419-20.

2. White J.D., Bremner J.B., Dimsdale J.J., and Garcea R.L. Darzen's condensation of α-halolactones. Glycidic lactones as intermediates in acetogenin synthesis. J. Amer. Chem. Soc. 1972; **93**:281-2.

3. Baxter J.D., Rousseau G.G., Benson M.C., Garcea R.L., Ito J., and Tomkins G.M. The role of DNA and specific cytoplasmic receptors in glucocorticoid action. Proc. Nat. Acad. Sci. (USA) 1972; **69**:1982-6.

4. Simons S.S., Baxter J.D., Garcea R.L., and Tomkins G.M. The role glucocorticoid receptors in steroid induced protein synthesis. In: Extracellular Matrix Influences on Gene Expression, H.C. Slavkin and R.C. Greulich (eds.), Academic Press, 1975; pp. 37-45.

5. Simons S.S., Martinez H.H., Garcea R.L., Baxter J.D., and Tomkins G.M. The nature of acceptor sites of glucocorticoid receptor-steroid complexes. J. Biol. Chem. 1976; **251**:334-43.

6. Schachat F.H., Harris H.E., Garcea R.L., Lapointe J.W., and Epstein H.F. Studies on two body-wall myosins in wild type and mutant nematodes. In: Molecular Biology of Eukaryotic Systems, ICN-UCLA Symposia, J.N. Abelson, G. Wilcox, and C.F. Fox (eds.), Academic Press, 1977; Vol. 8, pp.373-380.

7. Schachat F.H., Garcea R.L., and Epstein H.F. Myosins exist as homodimers of heavy chains: Demonstration with specific antibody purified by nematode mutant myosin affinity chromatography. Cell 1978; **15**:405-11.

8. Garcea R.L., Schachat F.H., and Epstein H.F. Coordinate synthesis of two myosins in wild

type and mutant nematode muscle during larval development. Cell 1978; **15**:421-28.

9. Mackenzie J., Garcea R.L., Zengel J.M., and Epstein H.F. Muscle development in *Caenorhabditis elegans*: Mutants exhibiting retarded sarcomere construction. Cell 1978; **15**:751-62.

10. Garcea R.L., and Alberts B.M. Comparative studies of histone acetylation in nucleosomes, nuclei and intact cells: Evidence for special factors which modify acetylase action. J. Biol. Chem. 1980; **255**:11454-463.

11. Tekamp P., Garcea R.L., and Rutter W.J. Transcription and in vitro processing of yeast 5S rRNA. J. Biol. Chem. 1980; **255**:950-61.

12. Garcea, R.L., and Benjamin, T.L. Host-range transforming gene of polyoma virus plays a role in virus assembly. Proc. Natl. Acad. Sci. (USA) 1983; **80**:3613-3617.

13. Garcea, R.L., and Benjamin, T.L. Isolation and characterization of polyoma nucleoprotein complexes. Virology 1983; **130**:65-75.

14. Garcea, R.L., Ballmer-Hofer, K., and Benjamin, T.L. Characterization of the virion assembly defect of polyoma hr-t mutants: underphosphorylation of the major capsid protein, VP1, prior to viral DNA encapsidation. J. Virol. 1985; **54**:311- 316.

15. Leavitt, A.D., Roberts, T.M., and Garcea, R.L. Polyoma virus major capsid protein VP1: purification after high level expression in *Escherichia coli*. J. Biol. Chem. 1985; **260**:12803-12809.

16. Kaplan, D.R., Whitman, M., Schaffhausen, B., Raptis, L., Garcea, R.L., Pallas, D., Roberts, T.M., and Cantley, L. Phosphatidylinositol metabolism and polyoma-mediated transformation. Proc. Natl. Acad. Sci. (USA) 1986; **83**:3624-3628.

17. Salunke, D.M., Caspar, D.L.D., and Garcea, R.L. Self-assembly of the purified polyomavirus capsid protein VP1. Cell 1986; **46**:895-904.

18. Bianchi, D.W., and Garcea, R.L. Successful pregnancy in an adolescent with metastatic undifferentiated sarcoma: A case report. J. Reprod. Med. 1987; **32**:76-78.

19. Zullo, J.N., Stiles, C.D., and Garcea, R.L. Induction of c-myc and c-fos by polyomavirus: distinct roles for the capsid protein VP1 and the viral early proteins. Proc. Natl. Acad. Sci. (USA) 1987; **84**:1210-1214.

20. Garcea, R.L., Salunke, D.M., and Caspar, D.L.D. Site-directed mutation affecting polyomavirus capsid self-assembly *in vitro*. Nature (London) 1987; **329**:86-87.

21. Moreland, R.B., Langevin, G.L., Singer, R.H., Garcea, R.L., and Hereford, L.M. Amino acid sequences determining the nuclear localization of yeast histone 2B. Mol. Cell Bio. 1987; **7**:4048-4057.

22. Garcea, R.L., Talmage, D.A., Harmatz, A., Freund, R., and Benjamin, T.L. Separation of host-range from transformation functions of the hr-t gene of polyoma virus. Virology 1989; **168**:312-319.

23. Salunke, D.M., Caspar, D.L.D., and Garcea, R.L. Polymorphism in the assembly of polyomavirus capsid protein VP1. Biophys. J. 1989; **56**:887-900.

24. Freund, R., Garcea, R.L., Sahli, R., and Benjamin, T.L. A single amino acid substitution in polyomavirus VP1 correlates with plaque size and hemagglutination behavior. J. Virol. 1991; **65**: 350-355.

25. Moreland, R.B., Montross, L., and Garcea, R.L. Characterization of the DNA binding properties of the polyomavirus capsid protein VP1. J. Virol. 1991; **65**: 1168-1176.

26. Montross, L., Watkins, S., Moreland, R. B., Mamon, H., Caspar, D.L.D., and Garcea, R. L. Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. J. Virol. 1991; **65**: 4991-4998.

27. Moreland, R. B., and Garcea, R.L. Characterization of a nuclear localization sequence in the polyomavirus capsid protein VP1. Virology 1991; **185**: 513-518.

28. Bergsagel, D.J., Finegold, M. J., Butel, J. S., Kupsky, W. J., and Garcea, R.L. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. N. Engl. J. Med. 1992; **326**: 988-993.

29. Horvath, C. J., Simon, M. A., Bergsagel, D. J., Pauley, D. R., King, N. W., Garcea, R. L., and Ringler, D. J. Simian virus 40 (SV40)-induced disease in rhesus monkeys with simian acquired immunodeficiency syndrome. Am. J. Path. 1992; **140**: 1431-1440.

30. Sahli, R., Freund, R., Dubensky, T., Garcea, R., Bronson, R., and Benjamin, T. Defect in entry and altered pathogenicity of a polyoma virus mutant blocked in VP2 myristylation. Virology 1993; **192**: 142-153.

31. Rose, R. C., Bonnez, W., Reichman, R.C., and Garcea, R. L. Expression of the human papillomavirus type 11 (HPV-11) L1 protein in insect cells: *In vivo* and *in vitro* assembly of virus-like particles (VLPs). J. Virol. 1993; **67**: 1936-1944.

32. Delos, S.E., Montross, L., Moreland, R.B., and Garcea, R.L. Expression of the polyomavirus VP2 and VP3 proteins in insect cells: co-expression with the major capsid protein VP1 alters VP2/VP3 subcellular localization. Virology 1993; **194**: 393-398.

33. Li, M., and Garcea, R.L. Identification of the threonine phosphorylation sites on the polyomavirus major capsid protein VP1: relationship to the activity of middle T antigen. J. Virol. 1994; **68**: 320-327.

34. Hendrix, R.W., and Garcea, R.L. Capsid assembly of dsDNA viruses. Seminars in Virology 1994; **5**: 15-26.

35. Li, M., Delos, S.E., Montross, L., and Garcea, R.L. Polyomavirus VP1 phosphorylation: Coexpression with the VP2 capsid protein modulates VP1 phosphorylation in Sf9 insect cells. Proc. Natl. Acad. Sci. (USA)1995; **92**:5992-5996.

36. Lednicky, J., Garcea, R.L., Bergsagel, D.J., and Butel, J. S. Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. Virology 1995; **212**:710-717.

37. Delos, S.E., Cripe, T. P., Leavitt, A.D., Greisman, H., and Garcea, R.L. Expression of the polyomavirus minor capsid proteins, VP2 and VP3, in *Escherichia coli*: In vitro interactions with recombinant VP1 capsomeres. J. Virol. 1995; **69**:7734-7742.

38. Li, M., Lyon, M.K., and Garcea, R.L. In vitro phosphorylation of the polyomavirus major capsid protein VP1 on serine 66 by casein kinase II. J. Biol. Chem. 1995; **270**: 26006-26011.

39. Cripe, T.P., Delos, S.E., Estes, P.A., and Garcea, R.L. In vivo and in vitro association of Hsc70 with the polyomavirus capsid proteins. J. Virol. 1995; **69**:7807-7813.

40. Carbone, M., Rizzo, P., Procopio, A., Giuliano, M., Pass, H.I., Gebhardt, M.C., Mangham, C., Hansen, M. Malkin, D.F., Bushart, G., Pompetti, F., Picci, P, Levine, A.S., Bergsagel, J.D., and Garcea, R.L. SV40-like sequences in human bone tumors. Oncogene 1996; **13**:527-535.

41. Li, M., Cripe, T. P., Estes, P. A., Lyon, M. K., Rose, R. C., and Garcea, R. L. Expression of the human papillomavirus type 11 L1 capsid protein in *Escherichia coli*: Characterization of protein domains involved in DNA binding and capsid assembly. J. Virol. 1997; **71**:2988-2995.

42. Garcea, R.L. and Liddington, R.C. Structural biology of polyomaviruses. In Structural Biology of Viruses, Chiu, W., Burnett, R.M., and Garcea, R.L. (eds.), Oxford Press, 1997; pp. 187-208.

43. Chiu, W., Burnett, R.M., and Garcea, R.L. (eds.). Structural Biology of Viruses, (1997) Oxford University Press.

44. Garcea, R.L. and Estes, P.A. Purification of papovavirus virus-like particles (VLPs) from Sf9 insect cells, In Cell Biology: A Laboratory Handbook, J. E. Celis (ed.), Academic Press, 1998, Vol. 1, 521-527.

45. Wang, J. and Garcea, R.L. (1998) Simian virus 40 DNA sequences in human brain and bone tumors. In: Simian Virus 40 (SV40): A Possible Human Polyomavirus. Brown F. and Lewis A.M. (eds), Dev. Biol. Stand., Basel Karger, 1998; vol 94, I (3): 13-21.

46. Li. M., Beard, P., Estes, P.A., Lyon, M.K., and Garcea, R.L. Intercapsomeric disulfide bonds in papillomavirus assembly and disassembly. J. Virol. 1998; **72**:2160-2167.

47. Rose, R.C., White, W., Li, M., Suzich, J., Lane, C., and Garcea, R.L. Human papillomavirus type 11 (HPV-11) recombinant L1 capsomeres induce virus-neutralizing antibodies. J. Virol. 1998; **72**:6151-6154.

48. Szomolanyi-Tsuda, E., Le, Q.P., Garcea, R.L., and Welsh, R.M. T cell-independent immunoglobulin G responses in vivo are elicited by live-virus infection, but not by immunization with viral proteins or virus-like particles. J. Virol. 1998; **72**:6665-6670.

49. Lednicky, J.A. and Garcea. R.L. Detection of SV40 DNA sequences in human tissue. In Methods in Molecular Biology, "SV40 Protocols" ed. Leda Raptis, Humana Press Inc., Totowa, NJ., Vol. 165 (2000) pp.257-267.

50. Schwartz, R., Garcea, R.L., and Berger, B. "Local Rules" theory applied to polyomavirus polymorphic capsid assemblies. Virology 2000; **268**:461-470.

51. Chen, X., Garcea, R.L., Casini, G., Goldberg, I., and Harrison, S.C. Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. Mol. Cell 2000; **5**:557-567.

52. Szomolanyi-Tsuda, E., Brien, J.D., Dorgan, J.E., Welsh, R.M., and Garcea, R.L. The role of CD40-CD154 interaction in antiviral T cell-independent IgG responses. J. Immunol. 2000;

164:5877-82.

53. Garcea, R.L. SV40: A human pathogen? (2001) Disease Markers 17:149-151, ed. C.M. Steel.

54. Szomolanyi-Tsuda, E., Brien, J.D., Dorgan, J.E., Garcea, R.L., Woodland, R.T., and Welsh, R.M. Antiviral T cell-independent type 2 antibody responses induced in vivo in the absence of T and NK cells. Virology 2001; 280:160-168.

55. Chen, X., Casini, G. Harrison, S.C., and Garcea, R.L. Papillomavirus capsid protein expression in Escherichia coli: Purification and assembly of HPV11 and HPV16 L1 J. Mol. Bio. 2001: 307: 173-182.

56. Malkin, D., Chilton-MacNeill, S., Meister, L.A., Sexsmith, E., Diller, L., and Garcea, R.L. Tissue-specific expression of SV40 virus and mechanisms of loss of heterozygosity of p53 in a child with Li-Fraumeni Syndrome. Oncogene 2001; 20: 4441-4449.

57. Yuan, H., Estes, P.A., Chen, Y., Newsome, J., Olcese, V.A., Garcea, R.L., and Schlegel, R. Immunization with a pentameric L1 fusion protein protects against papillomavirus infection. J. Virol. 2001; 75: 7848-7853.

58. Finnen, R.L., Erickson, K.D., Chen, X.S., and Garcea, R.L. Interactions between papillomavirus L1 and L2 capsid proteins. J. Virol. 2003; **77**:4818-4826.

59. Öhlschläger, P., Osen, W., Dell, K. Faath, S., Garcea, R. L., Hochmus, I., Müller, M., Pawlita, M. Schäfer, K. Sehr, P, Staib, C., Sutter, G, Gissmann, L. Human papillomavirus (HPV) type 16 L1 capsomeres induce L1-specific cytotoxic T lymphocytes and tumor regression in C57BL/6 mice. J. Virol. 2003; **77**:4635-4645.

60. Garcea, R.L., and Imperiale, M.J. Simian virus 40 infection of humans. J. Virol. 2003;**77**:5039-5045.

61. Carter, J.J., Madeleine, M.M., Wipf, G.C., Garcea, R.L., Pipkin, P.A., Minor, P.D., Galloway, D.A., Lack of serologic evidence for prevalent SV40 infection in humans. J. Natl. Cancer Inst.2003; **95**:1522-1530.

62. Chromy, L.R., Pipas, J.M., and Garcea, R.L. Chaperone-mediated *in vitro* assembly of polyomavirus capsids. Proc. Natl. Acad. Sci. 2003; **100**:10477-10482.

63. Casini, G.L., Heine, D., Graham, D., Garcea, R.L., and Wu, D.T. *In vitro* papillomavirus

capsid assembly analyzed by light scattering. Virology 2004; **325**:320-327.

64. Garcea, R.L., and Gissmann, L. Virus-like particles (VLPs) as vaccines and vessels for delivery of small molecules. Current Opinions in Biotechnology, 2004; **15**:1-5.

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Selected Oral Presentations:

8/82 "Intermediate steps in polyoma virion assembly are controlled by host-range transforming gene products", Cold Spring Harbor Tumor Virus Symposium (NY).

8/83 "Characterization and control of polyoma VP1 post-translational modifications: a role for small T antigen", Imperial Cancer Research Fund Tumor Virus Meeting (Cambridge, UK).

7/85 "Self-assembly of the polyomavirus VP1 protein", Biocenter Seminar, (Basel, Switzerland).

6/87 "*In vitro* self-assembly of the polyomavirus VP1 protein", Gordon Research Conference, Animal Cells and Viruses (NH).

8/87 "*In vitro* assembly of the polyoma capsid protein VP1 as an assay for mutations affecting capsid formation", Imperial Cancer Research Fund Tumor Virus Meeting (Cambridge, UK).

6/89 “Structure-function relationships of the polyomavirus VP1 protein", Gordon Research Conference, Animal Cells and Viruses (NH).

8/89 “Cloning the polyomavirus cell surface receptor, Imperial Cancer Research Fund Tumor Virus Meeting (Cambridge, U.K.)

7/90 "Polyoma capsid assembly", FASEB conference on Viral Assembly (VT).

5/91 "Human brain tumors and papovaviruses", American Society for Microbiology (TX).

7/94 'Polyomavirus Assembly", FASEB conference on Viral Assembly (VT)

7/95 "Polyomavirus VP1 phosphorylation: dependence on VP2 expression and the casein kinase II (CKII) signal transduction pathway", Imperial Cancer Research Fund Tumor Virus Meeting (Cambridge, UK).

1/97 “SV40-like sequences in human brain and bone tumors”, NIH/NCI/NCID conference on “Simian Virus 40 (SV40): A Possible Human Polyomavirus.” (Bethesda, MD).

6/97 “Papovavirus Structure, Assembly, and Disassembly”, Gordon Research Conference, Viruses and Cells, (NH).

9/98 “Structural Biology of Papillomaviruses and Prospects for Vaccine Development”, Distinguished Speaker, National Jewish Research Center, (Denver, CO).

6/99 “Structure and Assembly of Papovaviruses”, Symposium on the Mechanisms of Viral Morphogenesis, American Society for Microbiology General Meeting,(Chicago, IL)

6/00 “A Comparison of Polyoma and Papillomavirus Structure and Assembly”, FASEB conference on Virus Assembly (VT).

7/00 “3.5Å Resolution Structure of the HPV16 L1 Capsid Protein”, 18th International Papillomavirus Conference (Barcelona).

10/00 “SV40 and Brain Tumors”, Symposium on “Viruses and Cancer: New Associations”, Fred Hutchinson Cancer Research Center (Seattle, WA).

3/01 “Tissue Specific Expression of SV40 in Tumors Associated with the Li-Fraumeni Syndrome”, National Cancer Institute Workshop on “Viruses and Human Cancer” (Bethesda)

4/01 “Association of SV40 with Human Brain Tumors”, Malignant Mesothelioma--Therapeutic Options and Role of SV40: An Update, (Chicago).

5/01 “A Comparison of Polyoma and Papillomavirus Structure and Assembly”, Deutsches Krebsforschungszentrum (Heidelberg, DE).

5/01 “Regulating Virus Capsid Assembly and Disassembly”, EMBO Workshop on the Structural Biology of Small DNA Tumor Viruses, (Siena, Italy).

5/02 “Human Papillomaviruses: Structure, Assembly, and Vaccine Development”, Scripps Research Institute (LaJolla, CA)

12/02 “Polyoma and Papillomaviruses: Structure, Assembly, and Vaccine Development”, Johns Hopkins Medical School, Department of Pathology, (Baltimore, MD).

4/03 “Chaperone-Mediated *In Vitro* Assembly of Polyomavirus Capsids”, Second International Workshop on the Structural Biology of Small DNA Tumor Viruses (Siena, Italy)

11/03 “Polyoma and Papillomaviruses: Structure, Assembly, and Vaccine Development”, University of Michigan Department of Microbiology and Immunology (Ann Arbor, MI).

6/04 “Polyoma and Papillomaviruses: Structure, Assembly, and Vaccine Development”, Department of Molecular Genetics, University of Rome, La Sapienza, Italy.

7/04 “Papovavirus capsid assembly and disassembly mediated in vitro by hsp70 chaperones”, FASEB Conference on Virus Assembly (VT).

11/04 “The Structural Biology of Papillomaviruses”, International Workshop on Cervical

Cancer, National Center for Biological Sciences, Bangalore, India.

11/04 "The Structure and Assembly of Papovaviruses", National Institute of Immunology, New Delhi, India.

3/05 "Another Virus in the Vaccine: Science, Sociology, and Politics in the Eradication of Polio", Pediatric Grand Rounds, Denver Children's Hospital.

4/05 "Chaperoning Papovavirus Assembly and Disassembly", EMBO Workshop on the Structural Basis of Papovavirus Biology, (Siena, Italy).

5/05 "Structural Biology of Papillomaviruses in Relation to Vaccine Development", University of Colorado Cancer Center Seminar Series.

6/05 "Chaperone-Mediated Assembly and Disassembly of Polyomaviruses", the Maurice Hilleman-Merck Research Laboratories Lecturer, Symposium Lecture, American Society for Virology 24th Annual Meeting, (College Station, PA).

9/05 "Re-engineering Clinical Studies of Human Polyomaviruses", Third International Conference on Polyomaviruses and Human Diseases, (Brown University, USA).

10/05 "A Chaperone Protein Equilibrium: Papovavirus Assembly *versus* Disassembly", Department of Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder.

12/05 "Viruses and Recombinant Virus-Like Particles as Platforms and Vessels for Nanotechnology", Mark Oliphant Conference, Plenary Lecturer, BioNano: The Next Frontier 2005, (Brisbane, Australia).

4/06 "Assembly and Disassembly of Papovaviruses", Department of Biochemistry, Robert Wood Johnson Medical School, NJ.

4/06 "A Chaperone Protein Equilibrium: Papovavirus Assembly *versus* Disassembly", the Immunology/Virology Program, University of Massachusetts School of Medicine, MA.

12/06 "Structural Biology of Virus-like Particles", HPV Symposium, Deutsches Krebsforschungzentrum (DKFZ), Heidelberg, DE.

8/07 "Confounding Biologic Variables in Modeling Virus Assembly", Second International Mathematical Virology Workshop, International Centre for Mathematical Sciences (ICMS), Edinburgh, Scotland.

9/07 "Structural Biology of Papillomaviruses in Relation to Vaccine Development", Department of Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder.

9/07 "Serologic Evidence of Lymphotropic Polyomavirus-like Infection of Humans", IV International Conference on Polyomaviruses and Human Diseases, Barcelona, Spain.

11/07 "New Vaccines for the Prevention and Treatment of Human Papillomavirus Infection", Butcher Symposium on Genetics and Biotechnology, Westminister (CO).

3/08 "Structural Biology of Papillomaviruses in Relation to Vaccine Development", Department of Pathology, Harvard Medical School, Boston MA.

3/08 "Structural Biology of Papovaviruses in Relation to Vaccine Development", Structural Biology Program, University Florida, Gainesville, FL.

5/08 “Structural Biology of Papovaviruses in Relation to Vaccine Development”, Dept. of Oncologic Pathology, Karolinska Institute, Stockholm, SE.

6/08 “Next Generation HPV Vaccines: Prophylactic and Therapeutic”, American Society of Microbiology General Meeting, Div. S Symposium, Boston MA.

11/08 “HPV Capsomere Subunit Vaccines”, International Symposium on HPV-Associated Cancers, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India.

11/08 “Design and Development of Human Papillomavirus Vaccines”, National Institute of Immunology, New Delhi, India.

11/08 “New Vaccines for the Prevention and Treatment of Human Papillomavirus Infection”, Eurogin 2008, Nice, France.

2/09 “Structural Biology of Papovaviruses in Relation to Vaccine Development”, Dept. of Neuroscience and Cell Biology, Univ. TX Medical Branch, Galveston.

2/09 “Structural Biology of Papillomaviruses in Relation to Vaccine Development”, Gordon Conference, Physical Virology, Galveston, TX.

4/09 “Structural Biology of Papovaviruses in Relation to Vaccine Development”, Dept. of Chemistry, Colorado School of Mines, Golden, CO

5/09 “Polyomavirus Assembly at PML-NB Nuclear ‘Work-Benches’”, Sixth International Virus Assembly Symposium, Crete, Greece.

11/09 “Chaperoning Papovavirus Assembly and Disassembly”, Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville, VA.

3/10 “Papovavirus Assembly: Chaperones and Nuclear Virus Factories”, Centro Nacional de Biotechnologia, Madrid, Spain.

6/10 “Epidemiology of Human Polyomaviruses”, 1st WHO/IARC meeting on “Emerging Oncogenic Viruses”, Bevagna, Manduria, IT.

7/10 “Nuclear Virus Factories in Polyomavirus-Infected Cells”, DNA Tumor Virus Meeting, Madison, WI.

8/10 “Nuclear Virus Factories in Polyomavirus-Infected Cells”, 3rd International Mathematical Virology Workshop, Ambleside, UK.

10/10 “Next Generation Prophylactic Human Papillomavirus (HPV) Vaccines”, Wuhan International Symposium on Vaccines, Wuhan, PRC.

7/11 “Capsomere Vaccines Protect Mice from Vaginal Challenge with Human Papillomavirus”, ICGEB DNA Tumour Virus Meeting, Trieste, IT

10/11 “Nuclear Virus Factories in Polyomavirus-Infected Cells”, Department of Microbiology and Immunology, Louisiana State University, Shreveport, LA.

11/11 “Polyomavirus Assembly: Chaperones and Nuclear Virus Factories”, Dept. Virology, Pasteur Institute, Paris, Fr.

1/12 “Nuclear Virus Factories in Polyomavirus-Infected Cells”, Department of Microbiology, University of Colorado School of Medicine, Dept. of Microbiology. (student invited speaker).

5/12 “Nuclear Virus Factories in Polyomavirus-Infected Cells”, 2nd WHO/IARC meeting on

"Emerging Oncogenic Viruses", Bevagna, Manduria, IT.

6/12 "Nuclear Factories for Polyomavirus Assembly", FASEB Conference on Virus Structure and Assembly, Saxtons River, VT.

11/12 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Novartis Institute for Infectious Diseases, Emeryville, CA.

5/13 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Viruses and Cells Gordon Conference, Lucca IT.

6/13 "Virion Assembly Factories in Polyomavirus-Infected Cells", Conference on Progressive Multifocal Leukoencephalopathy, New York Academy of Sciences, NYC.

9/13 "Polyomaviruses", This Week in Virology Podcast #250, American Society of Microbiology.

10/13 ""The Structural Biology of Papillomaviruses in Relation to Vaccine Development", Henry F. Epstein Memorial Symposium, UTMB, Galveston, TX.

12/13 "Next Generation Prophylactic Human Papillomavirus (HPV) Vaccines", International Center for Genetic Engineering and Biotechnology, Trieste, IT.

6/14 "Murine Polyomavirus Activation of Intracellular Signaling Pathways Upon Binding to Cell Surface Ganglioside and Integrin Receptors", Emerging Oncogenic Viruses, Manduria , IT.

10/14 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Queen's University, Kingston, Ontario, Canada.

10/14 "Nuclear Virus Factories in Polyomavirus-Infected Cells", ASM Conference on Viral Manipulation of Host Cells, Washington, DC.

11/14 "HPV Viral Structure and Assembly", Plenary Speaker, First ICGEB Workshop on Human Papillomavirus: From Basic Biology to Cervical Cancer Prevention, Rosario, AR.

11/14 "New Approaches to Prophylactic HPV Vaccines for Cervical Cancer Prevention", First ICGEB Workshop on Human Papillomavirus: From Basic Biology to Cervical Cancer Prevention, Rosario, AR.

03/15 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Northwestern University School of Medicine, Department of Microbiology, Chicago, IL.

04/15 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Heidelberg Combined Program Seminar Series, German Cancer Center (DKFZ), Heidelberg, Germany.

04/15 "Nuclear Virus Factories in Polyomavirus-Infected Cells", University of Southern California School of Medicine, Department of Biochemistry, Los Angeles, CA

07/15 "Mouse Polyomavirus (MPyV) Activates Intracellular Signaling Pathways Upon Binding to Cell Surface Gangliosides and the □4-Integrin Receptor", ICGEB DNA Tumour Virus Meeting, Trieste, IT.

08/15 "Polyomavirus Pathogenesis: Man to Mouse", Keynote Speaker, 11th Smögen Summer Symposium on Virology, Swedish Society for Virology, Smögen, Sweden.

05/16 "Gangliosides and the Alpha-4-Integrin Receptor Mediate Signaling Events Required

for Mouse Polyomavirus Infection", Microbiology Ph.D. Program Student Invited Speaker, University of Colorado Denver.

06/16 "Gangliosides and the Alpha-4-Integrin Receptor Mediate Signaling Events Required for Mouse Polyomavirus Infection", Emerging Issues in Oncogenic Virus Research, Manduria, IT.

07/16 "Polyomavirus Entry: Receptors, Pathways, and Pathogenesis", FASEB Conference on Virus Structure and Assembly, Steamboat Springs, US.

11/16 ""HPV Viral Structure and Assembly", Plenary Speaker, Second ICGEB Workshop on Human Papillomavirus: From Basic Biology to Cervical Cancer Prevention, Hong Kong, China.

11/16 "New Approaches to Prophylactic HPV Vaccines for Cervical Cancer Prevention", Second ICGEB Workshop on Human Papillomavirus: From Basic Biology to Cervical Cancer Prevention, Hong Kong, China.

9/17 "HPV Virion Structure and Assembly", Third ICGEB Workshop on Human Papillomavirus, Sao Paulo, Brazil.

9/17 "New Approaches to Prophylactic HPV Vaccines for Cervical Cancer Prevention", Third ICGEB Workshop on Human Papillomavirus, Sao Paulo, Brazil.

11/17 "Polyomavirus Pathogenesis: Man to Mouse, Denver University Dept. of Biological Sciences.

11/17 "Highly Thermostable Single-Dose Vaccines", International Conference on Vaccine Research and Development", ICGEB, New Delhi, India.

12/17 "Highly Thermostable Single-Dose Vaccines", Hilleman Laboratories of the Wellcome Trust, New Delhi, India.

3/18 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Harvard Medical School Program in Virology, Boston, MA.

6/18 "Imaging Polyomavirus Nuclear Assembly Factories", 5th Workshop on Emerging Issues in Oncogenic Virus Research, Puglia, IT.

7/18 "Nuclear Virus Factories in Polyomavirus-Infected Cells", FASEB Conference on Virus Structure and Assembly, Steamboat Springs, CO.

11/18 "Nuclear Virus Factories in Polyomavirus-Infected Cells", Biochemistry Department, Emory School of Medicine, Atlanta, GA

07/20 "Single shot, Thermostable Human Papillomavirus Vaccines Prepared with Atomic Layer Deposition Technology," 36th International Papillomavirus Meeting, Barcelona (virtual).

11/21 "Single shot, Thermostable Human Papillomavirus Vaccines Prepared with Atomic Layer Deposition Technology," 37th International Papillomavirus Meeting, Toronto, Canada (virtual, invited speaker).

6/22 "Proteomic Profiles of Murine Polyomavirus (MuPyV) Replication Centers Using iPOND Coupled with Mass Spectrometry", 6th Workshop on Emerging Issues in Oncogenic Virus Research, Puglia, IT.

7/22 “Proteomic Profiles of Murine Polyomavirus (MuPyV) Replication Centers Using iPOND Coupled with Mass Spectrometry”, DNA Tumour Virus Meeting, Cambridge UK.

CURRENT SUPPORT

9 R01 AI151636-35A1 (previously RO1 CA37667-34) 1/1/20-12/31/25
NIH/NIAID (3.0 Summer, 0.9 academic) $247,500 (direct, annual)
“Mechanisms in Polyomavirus Assembly”
PI: R.L. Garcea

The major goals of this project are to define the assembly of murine polyomavirus in the cell nucleus using genetic, biochemical, and high-resolution imaging approaches.

2 P50 CA 098252-12 9/1/19-8/31/24
NIH/NCI SPORE in Cervical Cancer (0.45 academic) $77,530 (direct annual)
P.I. T.C. Wu, Johns Hopkins University School of Medicine
Project 1: “HPV16 L1 Capsomeres as a Second Generation Preventive HPV Vaccine”
P.I. Robert L. Garcea
The goal of this project is to support the preclinical and phase I study of a capsomeric form of an HPV vaccine.

Bill and Melinda Gates Foundation
INV-049917 11/15/22-11/14/24 (2.0 calendar months) $723,385 (total direct)
“Atomic layer deposition-based coatings for HIV vaccine applications”
P.I.: Robert L. Garcea; co-PI: Theodore Randolph
The goal of this multi-institute project is to apply ALD vaccine technology to new HIV gp120 vaccine formulations.

CHAVD 5UM AI144462-05 Administrative Supplement:
7/1/23- 6/30/24 $100,000 (total direct)
Consortium for HIV/AIDS Vaccine Development: Evaluating slow-release technologies for enhanced humoral immunity from single-administration vaccines.
P.I.s Robert Garcea and Theodore Randolph, University of Colorado, Boulder.

NIMBL: Global Health Fund Pilot Project to Assess Stabilization Technologies for mRNA-LNP Vaccines.
1/1/24-9/30/24 $109,623 (total direct)
P.I.s: Theodore Randolph and Robert Garcea, University of Colorado, Boulder.

OVERLAP

None.

RECENT SUPPORT

Bill and Melinda Gates Foundation: Innovations in Vaccine Manufacturing for Global Markets
INV- 042180 2/16/22- 6/30/23 (2.0 calendar months) $403,128 (total direct)
"A Single Administration Prime-Boost Rabies Vaccination Platform"
P.I.: Robert L. Garcea; co-PI: Theodore Randolph
The goal of this project is to apply the recently developed nanoparticle-based vaccine platform to rabies vaccines.

Bill and Melinda Gates Foundation: Innovations in Vaccine Manufacturing for Global Markets
INV-002149 7/25/19-6/30/21 (2.0 calendar months; no cost extension) $1,146,744 (total direct)
"**Pulsatile Release and Platform Upgrade"**
P.I.: Robert L. Garcea; co P.I.: Theodore Randolph
The goal of this project is to develop a nanoparticle based vaccine platform capable of delivering both prime and boost immunizations with a single shot.

ALSAM Foundation Therapeutic Innovation Grant
1/1/19-12/31/21 ($200,000 total direct)
"Development and formulation of a phage nanoparticle vaccine system"
P.I.s: Robert L Garcea, Theodore Randolph, Carlos Catalano
The goal of this project is to use lambda-phage particles to present epitopes in immunization.

State of Colorado Office of Economic Development and International Trade: Advanced Industries Accelerator Program 7/1/17-5/30/20 ($183,487 total direct)
"Development of a Next Generation, Highly Thermostable Human Papillomavirus Vaccine"
P.I.: Robert L. Garcea; co-P.I.: Theodore Randolph
The goal of this project is to complete pre-clinical studies on a thermostable, tetravalent HPV vaccine in preparation for GLP.

Bill and Melinda Gates Foundation: Innovations in Vaccine Manufacturing for Global Markets
OPP1153439 10/14/16-6/30/19 (2.5 calendar months) $578,668 (annual)
"A Single Administration Prime-Boost Vaccination Platform"
P.I.: Robert L. Garcea; co P.I.: Theodore Randolph
The goal of this project is to develop a nanoparticle based vaccine platform capable of delivering

both prime and boost immunizations with a single shot.