Overcoming Resistance to PD-1 Checkpoint Blockade for Cancer Immunotherapy

Arlene Sharpe

The PD-1 pathway delivers inhibitory signals that function as a brake for immune responses and has wide-ranging immunoregulatory functions. This pathway regulates the critical balance between stimulatory and inhibitory signals needed for effective immune responses to microbes and tumors, as well self-tolerance. The critical role of PD-1 in limiting anti-tumor immunity is demonstrated by the transformative effects of PD-1 cancer immunotherapy. Although PD-1 pathway inhibitors are revolutionizing cancer treatment, mechanistic insights are needed to understand why PD-1 pathway blockade works or fails in order to identify responders to PD-1 cancer immunotherapy and to develop rational combination therapies. This talk will discuss studies in mouse models that have identified mechanisms by which the PD-1 pathway regulates anti-tumor T cell responses, and gene perturbation approaches to discover new cancer immunotherapy targets and their mechanisms of action.

Arlene Sharpe

Kolokotrones University Professor

Chair, Dept. of Immunology

Vice Director, Gene Lay institute

Harvard Medical School

NAME: **Arlene H. Sharpe**

POSITION TITLE: Professor of Kolokotrones University

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Radcliffe College, Cambridge, MA	A.B.	09/1971	05/1975	Biochemistry
Harvard University, Cambridge, MA	Ph.D.	09/1975	05/1981	Microbiology
Harvard Medical School, Boston, MA	M.D.	09/1977	05/1982	Medicine

A. Positions, Scientific Appointments and Honors

1. Positions, Scientific Appointments

2023-Present. Kolokotrones University Professor, Harvard University
2023-Present. Vice-Director, Gene Lay Institute of Immunology & Inflammation, BWH, MGH, HMS
2019-Present. Member, Broad Institute
2018-Present. Chair, Department of Immunology, HMS
2015-Present. Leader, Cancer Immunology Program, DF/HCC
2003-Present. Professor of Pathology, BWH

2. Awards

2024. Academy Member, American Academy of Arts & Sciences

2024. Harrington Prize for Innovation in Medicine

2023. Switzer Prize

2022. FASEB Excellence in Science Lifetime Achievement Award

2022. American Association of Immunologists, Lifetime Achievement Award

2022. American Society for Investigative Pathology Rous-Whipple Award

2022. Fellow, American Association of Immunologists

2021. Fellow, SITC Academy of Immuno-Oncology

2020. Fellow, National Academy of Inventors

2020. Richard V. Smalley Memorial Award, Society for Immunotherapy of Cancer

2019. Fellow, American Association for Cancer Research

2018. Member, National Academy of Science

2018. Member, National Academy of Medicine

2017. Recipient, Warren Alpert Foundation Prize

2017-2024. Highly Cited Researcher (top 1%), Thomson Reuters/Clavarite

2016. Citation Laureate, Thomson Reuters

2014-2015. Highly Cited Researcher (top 1%), Thomson Reuters

2014. William B. Coley Award, Cancer Research Institute

B. Contributions to Science including representative, relevant publications

1. Defining the functions of the B7-1 and B7-2 pathway in T cell activation and tolerance.
2. Our studies of B7-1 deficient mice provided the first *in vivo* evidence for the existence of alternative CTLA4 counter-receptors. As a result, a second CTLA4 counter-receptor, B7-2, was cloned. Our studies revealed that B7-2 is the major early activating costimulator for initiating immune responses. The discovery of B7-2 led us to compare B7-1 and B7-2 functions. We found that B7-1 and B7-2 have critical, overlapping roles in germinal center formation and Ig class switching *in vivo*, and both contribute to T helper cell differentiation. In the mouse model of Multiple Sclerosis, experimental autoimmune encephalomyelitis (EAE), we found that B7-1 and B7-2 have critical overlapping roles not only in the initial activation and expansion of self-reactive T cells, but also in the effector phase of encephalitogenic T cell activation within the central nervous system. The role for B7-1/B7-2 costimulation during the effector phase of autoimmune disease had not been appreciated previously. These findings inspired development of pathway antagonists to block pathogenic T cell responses.
3. Defining the critical inhibitory functions of CTLA-4 in vivo.
   Our studies with CTLA-4 deficient mice revealed the critical function inhibitory function for CTLA-4, and a previously unsuspected means by which costimulation can regulate responses, showing that costimulation can have both positive and negative regulatory roles. The phenotype of the CTLA-4 deficient mouse strain suggested a critical role for CTLA-4 in regulating T cell tolerance. We demonstrated an essential role for CTLA-4 in regulating the induction of anergy *in vivo.* More recently, we generated CTLA-4 conditionally deficient mice and used them to dissect CTLA-4 function CD4+ FoxP3- T cells and regulatory cells (Tfr).
4. Defining the role of PD-1 and its ligands in regulating T cell activation, tolerance and exhaustion.
   Our studies first demonstrated that PD-L1 and PD-L2 can inhibit T cell proliferation and cytokine production *in vitro*. We determined that the PD-1:PD-L pathway exerts critical inhibitory functions in T cell activation, tolerance, chronic viral infections and tumors. We also showed that PD-L1 is expressed on tumors. We demonstrated that this pathway controls multiple tolerance checkpoints that prevent autoimmunity. We also identified a novel role for PD-L1 on non-hematopoietic cells in regulating self-reactive T cells. In collaboration with the laboratories of Drs. Rafi Ahmed and Gordon Freeman, we discovered that the PD-1:PD-L1 pathway contributes directly to T cell exhaustion and lack of viral control during chronic LCMV infection. These studies revealed the therapeutic potential for this pathway for treating T cell exhaustion and have translated into clinical trials and cancer immunotherapy. We are currently investigating other coinhibitory pathways and their interplay with the PD-1 pathway in tolerance, infection and cancer.
5. Defining the functions of T follicular regulatory cells (Tfr).
   Tfr cells are a recently discovered Treg subset that inhibits humoral immunity. We have developed methods to determine mechanisms of Tfr cell suppression, and found that Tfr cells can prevent activation

of both Tfh and B cells. By separating analyzing Tfh and Tfr cells, we determined that Tfr cell differentiation is restrained by PD-1 and CTLA-4. We also found that PD-1 inhibits Tfr suppressive function, while CTLA-4 is a mediator of Tfr suppressive capacity.

6. Defining novel regulators of immunity using in vivo CRISPR screens.
   We have developed a system for perturbation of genes in immune cells *in vivo* using CRISPR-Cas9. This system expands the breadth of immune lineages that can be edited including naïve T and B cells, macrophages and dendritic cells, and enables in vivo pooled screens to discover immunoregulatory genes and knockout individual genes to characterize their function.

C. Research Support (ongoing and completed in the last 3 years)

1. 2024-2029. Oncolytic virus therapeutic responses occur from changes in the glioblastoma immune microenvironment (USD $17,295)
2. 2023-2028. Elucidating how TIGIT agonists regulate CNS autoimmunity exacerbated by PD-1 blockade (USD $3,156,940)
3. 2023-2028. Costimulatory Mechanisms of Autoimmunity (Core B) (USD $741,565)
4. 2023-2027. Defining mechanisms to promote antitumor immunity by modulating one-carbon metabolism (USD $2,831,180)
5. 2023-2027. Calico Life Science Research Project (USD $10,000,000)
6. 2023-2026. PhiBio Project (USD $750,000)
7. 2023-2024. Progenitor cell states contributing to aging and lung cancer (USD $67,800)
8. 2022-2025. DoD Improving the durability of immunological memory during anti-PD-1 immunotherapy (USD $825,247)
9. 2022-2025. MRA Improving the durability of immunological memory during anti-PD-1 immunotherapy (USD $247,425)
10. 2021-2026. Establishing the Liposarcoma Research Initiative for the Benefit of Patients Worldwide (USD $717,395)
11. 2020-2025. Synergies among inhibitory receptors in tolerance, cancer and antiviral immunity (USD $2,281,510)
12. 2020-2025. Understanding and Overcoming T cell Immunosuppression in Glioblastoma (USD $696,568)
13. 2020-2025. AbbVie : Research Alliance Agreement (USD $1,558,440)
14. 2021-2026. Cancer Center Support Grant – HMS Program Leaders/Mouse Engineering Core (USD $823,760)
15. 2019-2024. T Cell Costimulatory Pathways: Functions and Interactions (USD $3,974,775)
16. 2019-2023. DF/HCC Kidney Cancer SPORE (USD $312,668)
17. 2016-2021. Dana-Farber/Harvard Cancer Center Support Grant (USD $23,075)
18. 2018-2021. Alliance Agreement - Targeting metabolism to modulate T cell activation and effector function (USD $1,000,000)
19. 2018-2022. Abbreviated Targeted Therapy to Improve Anti-PD-1 Inhibitor Efficacy in Melanoma (USD $809,227)
20. 2018-2023. Systems Pharmacology of Therapeutic and Adverse Responses to Immune Checkpoint and Small Molecule Drugs (USD $949,200)
21. 2017-2023. Costimulatory Mechanisms of Autoimmunity (USD $2,692,002)
22. 2022-2023. A Platform to Systematically Identify Free Fatty Acid Regulation of anti-Tumor Immunity in Obesity (USD $19,999)
23. 2022-2023. Characterization of the immunomodulatory effects of ERAS-601 and ERAS-007 alone and in combination with an anti-PD-1 agent (USD $223,378)
24. 2021-2023. Moderna Project (USD $322,414)

References

1. Freeman GJ, et al. Uncovering of functional alternative CTLA-4 counter-receptor in B7-deficient mice. Science 262, 907-9 (1993).

2. Tivol EA, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3, 541-7 (1995).
3. Latchman Y, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2, 261-8 (2001).
4. Keir ME, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med 203, 883-95 (2006).
5. Sage PT, et al. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. Nat Immunol 14, 152-61 (2013).
6. Sage PT, et al. Defective TFH Cell Function and Increased TFR Cells Contribute to Defective Antibody Production in Aging. Cell Rep 12, 163-71 (2015).
7. LaFleur MW, et al. A CRISPR-Cas9 delivery system for in vivo screening of genes in the immune system. Nat Commun 10, 1668 (2019).
8. LaFleur MW, et al. PTPN2 regulates the generation of exhausted CD8$^+$ T cell subpopulations and restrains tumor immunity. Nat Immunol 20, 1335-1347 (2019).
9. Ringel, et al. Obesity Shapes Metabolism in the Tumor Microenvironment to Suppress Anti-Tumor Immunity. Cell 183, 1848-1866.e26 (2020).
10. Pauken KE, et al. Single-cell analyses identify circulating anti-tumor CD8 T cells and markers for their enrichment. J Exp Med 218, e20200920 (2021).
11. Rowe JH, et al. Formate supplementation enhances anti-tumor CD8+ T cell fitness and efficacy of PD-1 blockade. Cancer Discovery 13, 2566-2583 (2023).
12. LaFleur MW, et al. X-CHIME enables combinatorial, inducible, lineage-specific and sequential knockout of genes in the immune system. Nat Immunol 25, 178-188 (2024).