High Dimensional Profiling Analysis of Successful Cancer Immunotherapy

James P. Allison and Robert D. Schreiber

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Balzan GPC Adviser: Jules Hoffmann
Principal Investigator: Robert D. Schreiber
Deputy Principal Investigator: Maxim Artyomov
Additional Investigators: Matthew M. Gubin, Daniele Runci, Ekaterina Esaulova
Affiliated Institutions: Department of Pathology and Immunology, Washington University School of Medicine
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James P. Allison is Chairman of the Department of Immunology, Executive Director of the Immunotherapy Platform, and Director of the Parker Institute for Cancer Immunotherapy, University of Texas MD Anderson Cancer Center in Houston, Texas. Robert D. Schreiber currently holds several positions at the Washington University School of Medicine in St. Louis: the Andrew M. and Jane M. Bursky Distinguished Professor at the Department of Pathology and Immunology, Professor of Molecular Microbiology, Director at the Andrew M. and Jane M. Bursky Center for Human Immunology and Immunotherapy Programs and Program Co-Leader in Tumor Immunology at the Alvin J. Siteman Cancer Center.

Research Project Summary – Robert Schreiber

Although immune checkpoint blockade (ICB) therapy can induce durable clinical responses in a subset of cancer patients, the molecular and cellular changes associated with successful ICB therapy remain incompletely defined. The research in this proposal will leverage a well-characterized mouse tumor model developed in the Schreiber Lab with state-of-the-art high dimensional profiling approaches [i.e., single cell RNAseq (scRNAseq) and Time of Flight Mass Cytometry (CyTOF)] that are now established in the lab to better understand successful versus unsuccessful outcomes of
cancer immunotherapy. The project also seeks to identify new predictive biomarkers to not only help stratify patients with respect to the types of immunotherapies they receive but also provide early feedback on their responses to immunotherapy.

Previous work in the Schreiber Lab led to the generation and characterization of the T3 methylcholanthrene (MCA)-induced mouse sarcoma line that has proven highly valuable in studying natural and therapeutic immune responses to cancer. T3 sarcoma cells form progressively growing tumors when injected into naïve wild type mice but these tumors are rejected when tumor-bearing mice are treated with either (a) ICB monoclonal antibodies (mAb) such as anti-PD-1 and/or anti-CTLA-4, (b) other immunomodulatory mAbs, or (c) tumor-specific neoantigen vaccines. As a consequence of these efforts, much is now known about the immune response against T3 tumors, including their mutational landscape, the identities of the dominant and subdominant T3 neoantigens, and the optimal timing for administration of the different immunotherapies to achieve durable responses to established T3 tumors in mice.

Recently, the team used complementary scRNAseq and CyTOF approaches to identify tumor-infiltrating immune cells from either progressively growing T3 tumors in mice treated with control mAb or tumors undergoing rejection in mice treated with anti-PD-1 and/or anti-CTLA-4. Unbiased assessment of the transcriptional status of tumor infiltrating immune cells by scRNAseq identified common and distinct alterations induced by the different ICB treatments in both lymphoid and myeloid cell populations. Specifically, multiple subpopulations of effector CD4+ and CD8+ T cells, Tregs and NK cells were identified along with five distinct macrophage subpopulations. The latter were distinguishable by the combinatorial presence or absence of mrc1 (CD206), CX3CR1, CD1d1 and/or Nos2 (iNOS). The macrophage subpopulations spanned the spectrum of macrophage activation states and changed dynamically during ICB therapy. These findings were confirmed and extended at the protein level using CyTOF and conventional flow cytometry.

This proposal will use scRNAseq and CyTOF together with conventional immunologic approaches in longitudinal studies to define the temporal relationships that result in generation of the different lymphocyte and macrophage subpopulations. Experiments will be conducted to explore whether individual subpopulations arise as a consequence of reprogramming of cells from other subpopulations or from new cell infiltration into the tumor. The effects of different immunotherapies, either alone or in combination, on the development of cellular subpopulations will be assessed. The generality of the
observations will be explored by performing comparable experiments on, first, other MCA-sarcomas and then, on other types of mouse tumors. Various cellular subpopulations will be isolated by cell sorting and their anti-tumor versus pro-tumor functional activities assessed. Finally, using the data obtained in the mouse studies as a guide, samples will be taken from human cancer patients before, during, and after immunotherapy and analysed and the results linked to clinical outcome.

The research team, consisting of one senior and four young investigators at different levels, is both international and multigenerational. The project will be conducted in the Department of Pathology and Immunology at Washington University School of Medicine in St. Louis, and is scheduled for 2018-2020. Results of the studies will be published in peer reviewed scientific journals with acknowledgement of the second half of the 2017 Balzan Prize to Robert Schreiber for Immunological Approaches in Cancer Therapy. Towards the end of the research project, a Symposium on Immunotherapy of Cancer highlighting the results of this study and studies by other laboratories working in the immuno-oncology area may be organized in conjunction with the Balzan Foundation.