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The Manguso lab is working to improve the efficacy of cancer immunotherapy. We use a range of approaches including mouse models, functional genomics, cellular immunology, and single-cell profiling to understand how cancers evade the immune system. Our lab has pioneered the use of *in vivo* genetic screens with CRISPR to identify new immunotherapy targets and resistance mechanisms. Using these approaches, we identified the tyrosine phosphatase PTPN2, a critical regulator of immunotherapy sensitivity in tumor cells. We also identified the dsRNA-editing enzyme ADAR1 as a checkpoint that regulates the sensing of self-dsRNA by tumor cells. Our results indicate that there are dozens of ways that cancers can be targeted by the immune system, and we are working to understand the new mechanisms revealed by our studies. In the long term, these approaches will enable a new understanding of how the immune system interacts with cancerous tissue and how the interaction can be manipulated to destroy tumors.

Over the last decade, critical discoveries in immunology and cancer biology have revealed how tumors are shaped by the immune system and how they evolve to evade it. We now know that disrupting immune checkpoints such as CTLA-4 and PD-1/PD-L1 can lead to T cell-mediated elimination of tumors. However, there is still a critical unmet need, as the vast majority of patients with cancer do not benefit from current immunotherapies. Our most pressing challenge is to discover the next generation of immunotherapies that can bring clinical benefit to the majority of patients.

To discover immunotherapy targets and resistance mechanisms in high throughput, we have developed an *in vivo*, CRISPR-based genetic screening system to identify genes that regulate tumor cell sensitivity to immunotherapy (Manguso et al, *Nature* 2017). We genetically modify mouse cancer cell lines that can be transplanted into animals and used as immunotherapy models. After delivery of Cas9 and libraries of single guide RNAs (sgRNAs), we implant pools of modified tumor cells into animals that are treated with immunotherapy. In a single experiment we can determine genes that, when deleted, increase or decrease sensitivity to immunotherapy (Figure 1). This strategy has enabled the rapid and simultaneous identification of new targets and resistance mechanisms that are potent regulators of anti-tumor immunity.

This powerful, unbiased discovery system allows us to identify targets and resistance mechanisms with no previously identified roles in immunotherapy. Three examples illustrate the power of this system for discovery: 1) we found that deletion of the phosphatase PTPN2 enhanced tumor cell sensitivity to immunotherapy. While PTPN2 was known to negatively regulate T cell receptor activation, our screens determined that it is also the most potent suppressor of interferon-gamma sensing in tumor cells; 2) we discovered that the non-classical MHC-I gene HT-T23/Qa-1 (HLA-E) is a

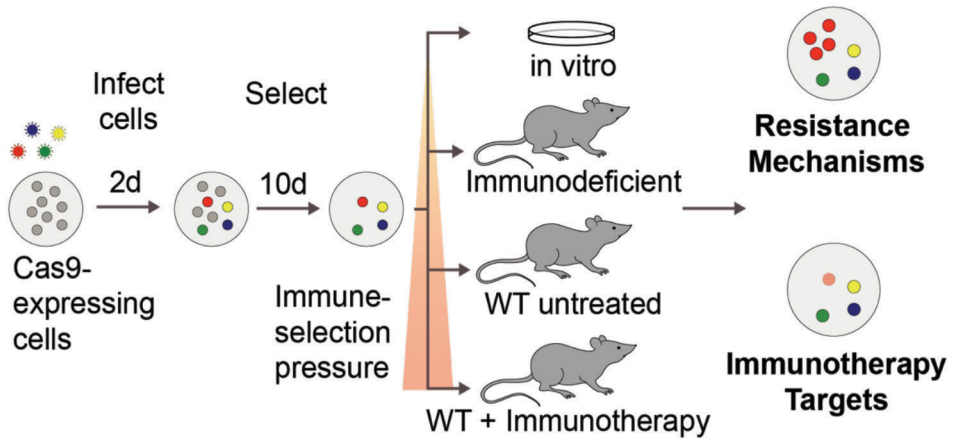

Diagram of in vivo CRISPR screening system. Pools of Cas9-expressing, sgRNA library-transduced tumor cells are implanted into either wild-type or immunocompromised mice. After 2 weeks, tumors are harvested and genomic DNA is extracted from tumor tissue. Next generation sequencing of the sgRNA library is used to identify resistance mechanisms or immunotherapy targets.

major immune checkpoint that limits anti-tumor immunity by T cells and NK cells; 3) our screens identified that deletion of ADAR1, an adenosine deaminase acting on RNA unmasks endogenous dsRNA that can be recognized by the cytosolic pattern recognition receptors PKR and MDA5, and can overcome resistance to immunotherapy caused by loss of antigen presentation (Ishizuka & Manguso et al, *Nature* 2019). Previously, these genes were not known or prioritized targets in immuno-oncology, but our unbiased approach enables discoveries that would have otherwise been unlikely.

We have demonstrated that *in vivo* CRISPR screens are a powerful way to discover new targets and probe the interaction of tumor cells with the host immune system. We can now broadly apply these genetic tools to advance our understanding of how immunotherapy works, why it may fail, and how we can improve it. Ongoing projects in the lab include:

1. Discover novel immunotherapy targets and mechanisms of resistance across several well-characterized mouse cancer models
2. Identify pathways that can overcome acquired resistance to immunotherapy
3. Understand how we can manipulate antigen presentation to enhance immunotherapy

These projects will define new ways to generate anti-tumor immune responses, reveal pathways that can be targeted to enhance these responses across cancer types, and anticipate and overcome the mechanisms by which tumors will become resistant. More broadly, these studies will improve our understanding of how tumors evolve under the selective pressure of immune surveillance and enable the development of more effective therapeutics.

Selected Publications:

Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, Yates KB, Lako A, Felt K, Naik GS, Manos M, Gjini E, Kuchroo JR, Ishizuka JJ, Collier JL, Griffin GK, Maleri S, Comstock DE, Weiss SA, Brown FD, Panda A, Zimmer MD, **Manguso RT**, Hodi FS, Rodig SJ, Sharpe AH, Haining WN. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol.* 2019 Mar;20(3): 326-336.

Ishizuka JJ*, **Manguso RT***, Cheruiyot C, Bi K, Panda A, Iracheta-Vellve A, Miller BC, Yates KB, Dubrot J, Du P, Buchumenski I, Ayer A, Comstock DE, Griffin GK, Brown FD, Ahmad S, Collins NB, Long AH, Pope HW, Zimmer MD, Kohnle I, Sen DR, Doench JG, Kozono D, Hur S, Levanon EY, Haining WN. Loss of the RNA editing enzyme ADAR1 in tumors improves response to immunotherapy and overcomes therapeutic resistance. *Nature*. 2019 Jan;565(7737):43-48.

Manguso RT, Pope HW, Zimmer MD, Brown FD, Yates KB, Miller BC, Collins NB, Bi K, LaFleur MW, Juneja VR, Weiss SA, Lo J, Fisher DE, Miao D, Van Allen E, Root DE, Sharpe AH, Doench JG, Haining WN. In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature*. 2017 Jul 27;547(7664): 413-418.

* Denotes equal contribution