

SNUH Harvard collaboration proposal

Dennis L. Kasper, MD -Harvard Medical School

Specific Aims

Since only a subset of cancer patients respond to ICI therapy, research into possible approaches to enhance immunological therapy of cancer has received a lot of attention. In a series of seminal studies, gut microbiome composition was highlighted as a strong predictor of response to ICI therapy. Treatment with single strains of commensal bacteria have not been reported to be successful. Moreover, commensal bacteria are known to induce multiple diverse immunomodulatory effects in their host, which could result in unwanted side-effects that are independent of ICI therapy. Since organism growth cannot be controlled, the use of a live organism risks over-growth or niche competition in the recipient's microbiome, which can disrupt the delicate equilibrium between bacteria in the gut. However, it has been demonstrated that fecal microbiome transplants (FMTs) from ICI-responder patients to non-responder patients could increase response of non-responders to ICI treatment. FMT is a treatment that lacks a mechanistic understanding and very likely, even in successfully treated patients, works by different mechanisms in many or most patients. There had been little mechanistic insight into how gut microbes modulate efficacy of check-point blocking therapy until our recent publication (**Park, et al Nature 2023**). By carefully sorting through a complex human microbiota that in mice facilitated check point blocking therapy, we found that colonization of GF mice with a single strain, *Coprobacillus cateniformis*, was sufficient to promote anti-tumor immunity in non-responders to anti-PD-L1 or PD-1 therapy and that mechanistically *C. cateniformis* colonization suppresses PD-L2 on dendritic cells in both the MLNs (mesenteric lymph nodes) and tumor dLNs (draining lymph nodes). Our data suggested that *C. cateniformis* promotes response to anti-PD-L1 by suppressing PD-L2 and that this signal may originate in the MLNs and travel to the tumor dLNs.

Our preliminary immunochemical studies indicate the molecule from *C. cateniformis* that is responsible for the immune modulation of the PD-L2/RGMB pathway is a lipid. We have now found that this lipid from a gram- positive bacteria modulates the expression of PD-L2 through innate immune signaling mediated by TLR4. Colonization of previously germ-free mice with *C. cateniformis* significantly reduced PD-L2 expression in CD11b+ cells and CD11c+ cells in the tumor dLNs and reduced PD-L2 expression in CD11c+ cells in the MLNs. Additionally, in vitro treatment of bone-marrow-derived dendritic cells (BMDCs) with soluble surface extracts of *C. cateniformis* downregulated PD-L2 expression, and this suppression of PD-L2 was reduced in MYD88 and TLR4 knockout DCs. Using the insight that lower PD-L2 levels facilitated PD-L1 or PD-1 therapy in mice and that this was impacting signaling thru RGMB on T cells, we determined that monoclonal blocking antibodies to RGMB or PD-L2 in conjunction with PD-L1 or PD-1 therapy were sufficient to induce tumor regression in mice previously resistant to PD-1 or PD-L1. In this proposal we will define in detail the bacterial molecule responsible for initiating this event, the innate receptor through which this molecule is signaling, and the initiating signaling mechanisms responsible for decreasing PD-L2 expression on DCs.

Specific Aim 1, Determine the lipid that promotes the efficacy of ICI therapy

- 1a. Scale up the HPLC separation of the *C. cateniformis* lipid extract
- 1b. Determine the structure of the lipid down-regulating PD-L2 in BMDCs
- 1c. Chemically synthesize the lipid
- 1d. Validation of lipid efficacy in tumor models

Specific aim 2: Characterize the TLR4 recognition and signaling of the lipid down-regulating PD-L2.

- 2a. Pull-down of FLAG-tagged TLR4 complexed with the lipid and other lipid binding proteins
- 2b. Immunoprecipitation of native TLR complexed with the lipid and other lipid-binding proteins
- 2c. Pull-down of the biotinylated lipid complexed with TLR4 and other lipid-binding proteins
- 2d. CRISPR-Cas9 KO of the target proteins in BMDCs and confirm the KO cells no longer respond to the lipid