3. Tsukita Y, Fujino N, Miyauchi E, *et al*. Axl Kinase drives immune checkpoint and chemokine signalling pathways in lung adenocarcinomas. *Molecular Cancer* 2019;**18**:24.
4. Terry S, Abdou A, Engelsen AST, *et al*. AXL targeting overcomes human lung cancer cell resistance to NK- and CTL-Mediated Cytotoxicity. *Cancer Immunol Res* 2019;**7**(11):1789–1802.
5. Scutera S, Fraone T, Musso T, *et al*. Survival and migration of human dendritic cells are regulated by and IFN-alpha-Inducible Axl/Gas6 Pathway. *J Immunol* 2009; **183**:3004–3013.
6. Wilson C, Ye X, Pham T, *et al*. AXL Inhibition sensitizes mesenchymal cancer cells to antimitotic drugs. *Cancer Res* 2014;**74**(20):5878–5890.
7. Ludwig KF, Du W, Sorrelle NB, *et al*. Small-Molecule Inhibition of Axl targets tumor immune suppression and enhances chemotherapy in pancreatic cancer. *Cancer Res* 2018; **78**(1):246–255.
8. Brand TM, Iida M, Stein AP, *et al*. AXL mediates resistance to cetuximab therapy. *Cancer Res* 2015;**74**(18):5152–5164.
9. Guo Z, Li Y, Zhang D, Ma J. Axl Inhibition induces the antitumor response which can be further potentiated by PD-1 blockade in the mouse cancer models. *Oncotarget* 2017; **8**(52):89761–89774

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0509

510 HELPING THE KILLERS: INNOVATIVE CANCER IMMUNOTHERAPY HARNESSING QUASI-UNIVERSAL TUMOR ANTIGEN-SPECIFIC CD4 T CELLS

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Background While cancer immunotherapy has mainly focused on exploiting CD8 T cells given their role in the direct elimination of tumor cells, increasing evidence highlights the crucial roles played by CD4 T cells in anti-tumor immunity. However, their very low frequency, the lack of robust algorithms to predict peptide binding to MHC class II molecules and the high polymorphism of MHC class II molecules render the study and use of circulating tumor antigen-specific CD4 T cells challenging. In this regard, the HLA-DRB3*02:02 gene encoding an HLA allele that is expressed by half of the Caucasian population, offers a way to identify CD4 T cell-defined tumor antigens with broad cancer patient coverage.
Methods Here, we aim to identify, isolate and functionally characterize 'quasi-universal' human tumor antigen-specific HLA-DRB3*02:02-restricted CD4 T cells in cancer patients. Using an algorithm we recently developed in house,[1] tumor-associated antigenic peptides binding to this allele are identified. We have generated a large collection of HLA-DRB3*02:02-restricted CD4 T cell clones of different tumor-antigen specificities. We will perform in vitro co-cultures of CD4 T cell clones with tumor cells to measure cytokine secretion, their tumor cell killing and their phenotypic profile (PD-1, TIM3, TIGIT, 4-1BB, CD40L, LAG3, VISTA, OX40). We will sequence and clone the TCR of the most promising candidates for adoptive cell transfer therapy. Lastly, we will directly evaluate the presence of these cells ex-vivo and longitudinally monitor them in patients.
Results N/A
Conclusions Together, these results should contribute valuable targets for coordinated CD4 and CD8 T cell-based immunotherapy of cancer.

REFERENCE

1. Racle, J., *et al*., Robust prediction of HLA class II epitopes by deep motif deconvolution of immunopeptidomes. *Nat Biotechnol* 2019. **37**(11): p. 1283–1286.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0510

511 TUMOR IMMUNE MICROENVIRONMENT IN ADULT MICE ASYNCHRONOUSLY CROSS-FOSTERED AS PUPS

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Background Immune responses to cancer are highly variable and influenced by genetic and environmental factors.[1] Syngeneic tumor models in mice with intact immune systems are required to study anti-tumor immune responses but are unable to adequately model varied immune responses. Classically, different mouse strain backgrounds have been used to compare different immune responses to cancer immunotherapy, but this approach is limited by the inability to administer identical tumor cell lines, keeping constant the tumor while experimentally varying the immune response. Proper establishment of the immune system begins in early life and is regulated by environmental cues from maternal breast milk and the developing microbiota. To disrupt these cues prior to weaning, newborn pups can be cross-fostered to dams that delivered their litters asynchronously, either 2 weeks earlier or later, a model referred to as asynchronous cross-foster (ACF).[2] We previously demonstrated that ACF can profoundly skew the immune profile of genetically identical offspring.[2] Young ACF mice exhibited enhanced Th2 immunologic skewing and reduced peripheral tolerance in response to antigen, which resulted from impaired development of peripherally-induced regulatory T cells (pTreg). Adult mice that underwent ACF also exhibited altered systemic cytokine expression even in the absence of immunologic stimuli, suggesting that ACF has lasting impact on the immune system. Because peripheral tolerance and immune skewing directly impact anti-tumor immunity,[3] we hypothesized that ACF would also impact the immune response to tumor growth.
Methods To measure impact of ACF on tumor growth and tumor infiltration, we introduced EL4 lymphoma cells into 7-week-old mice with the following foster schemes: conventionally reared mice, 1-day-old pups cross-fostered with 10-day post-partum dam (ACF1 to ppd10), and 13-day-old pups cross-fostered with 1-day post-partum dams (ACF13 to ppd1). Immune infiltration at tumor endpoint was measured using flow cytometry.
Results EL4 tumor growth was increased in ACF mice compared to conventionally-reared controls. Further, the immune infiltrate at endpoint was altered, with ACF mice having fewer natural killer (NK) cells, dendritic cells, and activated cytotoxic CD8+ T cells in the tumor microenvironment.
Conclusions Our observations support the hypothesis that ACF impacts tumor growth and immune infiltration. Future directions include phenotyping the immune infiltrate with finer resolution, the study of additional tumor models, and investigation of the effects of ACF on spontaneous tumor incidence and immunotherapy efficacy. Development of this novel model could provide valuable insight into early life factors that influence anti-tumor immunity.
Ethics Approval The study was approved by Mayo Clinic's IACUC approved all uses in this study, approval number A00004845.

REFERENCE

1. Radiloff DR, Rinella ES, and Threadgill DW. Modeling cancer patient populations in mice: complex genetic and environmental factors. *Drug Discov Today Dis Models* 2008; **4**:83–88.