Ex vivo ATLAS-identified inhibitory neoantigens promote mouse melanoma tumor progression

Hanna Starobinets1, Kyle Ferber1, Jason R. Dobson1, Peri Matatia1, Erik Carter1, Adrienne Li1, Michael O’Keeffe1, Crystal Cabral1, Matthew Lanchantin1, Erick Donis1, James Loizeaux2, James Foti1, Abba Dhaneshwar2,3, Wendy Broom1, Pamela Carroll1, Paul Kirschmeier2,3, Jessica B. Flechtn1, Hubert Lam1

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1 Genocea Biosciences Inc., Cambridge MA USA;
2 Belfer Center for Applied Cancer Science, Boston MA USA; 3 Dana-Farber Cancer Institute, Boston MA USA

Background
Neoadaptogens are attractive targets for personalized cancer immunotherapy due to their recognition as foreign antigens not subject to central tolerance. Personalized cancer vaccines leverage neoantigens to direct the immune system to specifically recognize tumor cells for their destruction. Although not well understood, published data also suggest that some immunotherapies result in hyperprogression1. One hypothesis for this phenomenon is antigen-specific immune modulation by T cells. ATLAS® is a T cell profiling platform whereby putative antigens can be screened ex vivo using autologous antigen presenting cells (APCs) and T cells2. Antigens are differentially characterized as stimulatory or inhibitory by significant up- or downregulation of T cell cytokine secretion relative to control responses; thus, the ATLAS bioassay allows for identification and characterization of desired as well as potentially unwanted antigen-specific T cell responses. A melanoma model was employed to identify murine stimulatory and inhibitory neoantigens using ATLAS®. Candidate neoantigens were manufactured as synthetic long peptides and delivered subcutaneously to C57BL/6 mice with or without adjuvant to elucidate the ability of stimulatory or inhibitory vaccines to impact tumor growth.

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mATLAS-defined inhibitory antigens promoted tumor hyper-progression

A) C57BL/6 mice were immunized with 1x10⁷ B16F10 cells on day 0 and subcutaneous immunization was performed on day 3, 10, and 17 with PBS, a pool of 4 inhibitory antigens, or a pool of 4 stimulatory antigens. C) Kaplan-Meier analysis of mice survival after inhibitory and stimulatory peptide immunization. Shaded area indicates 95% confidence intervals. D) Individual tumor growth kinetics in inhibitory or combined PBS and stimulatory groups. Boxed region indicate areas of accelerated tumor kinetics in inhibitory group. E) H&C analysis of two representative PBS and hyperprogressing tumors. Quadrant of CD8+ T cell infiltration into tumor.

Conclusions
ATLAS screening in mouse melanoma identified inhibitory and stimulatory neoantigens with parallels to human ATLAS neoantigen screening data. MHC-binding prediction algorithms failed to identify most ATLAS-defined stimulatory neoantigens and may mischaracterize inhibitory neoantigens. Immunization with inhibitory peptide antigens caused tumor hyper-progression in mice. The phenomenon of tumor hyper-progression has been observed in a subset of patients treated with checkpoint inhibitors1,2. Immunization with stimulatory peptide antigens with adjuvant were immunogenic and promoted anti-tumor efficacy. Stimulatory vaccine responses may be enhanced with engagement of neoantigen-specific CD8+ T cell responses and/or combination with checkpoint inhibitor therapy. Additional studies will continue to explore efficacy of mATLAS®-defined neoantigen vaccines and the mechanism of mATLAS-defined inhibitory antigens.

References
2. J. Flaherty and J. C. Thompson, mATLAS Screening and mATLAS Immunotherapy in Patients with Necrotic Solid Tumors, Cancer Immunol Res 2016, 4(10):862-864