Combination Immune Checkpoint Inhibitors for the Treatment of Human Colon Carcinoma in NSG Mice Engrafted with Human PBMC.

Martin R. Graf; Jason M. Davis; Anya Avrutskaya; Lynnell Thorne; Mark Ellison; Vivek Mahajan; Thi Bui; Aidan Synnot; Robert J. Mullin and Paula L. Milliani de Marval.

Charles River Discovery Services, Morrisville, NC, USA

**ABSTRACT**

Over the past decade there has been an increasing interest for preclinical models useful for evaluating the efficacy of checkpoint inhibition-based cancer immunotherapeutics. However, the recently developed humanized mouse models require the engraftment of human tumors into immunodeficient mice to develop a tumor load large enough to study the tumor response to checkpoint inhibitors. Here we report on the development of a xenogeneic humanized NSG mouse model, which provides a minimally invasive and highly dynamic system to study the efficacy of checkpoint inhibition-based strategies. In this model, human primary peripheral blood mononuclear cells (PBMC) are engrafted into NSG mice to establish a host immune system. We report here that this model provides an effective and efficient system to test the efficacy of checkpoint inhibition-based strategies. In this study, humanized NSG mice were engrafted with human peripheral blood mononuclear cells (PBMC) at 3x10^7 cell/animal (Hemacare; PBMC-HLA-A*01/01, PBMC-HLA-A*01/02; PBMC- HLA- A*03/24). Seven days post engraftment, animals were implanted with 5x10^6 RKO tumor cells in 50% Matrigel, subcutaneously in the lower hind limb. Peripheral blood was collected in tubes containing K2EDTA and immune cell populations were analyzed by flow cytometry for the expression of PD-L1 and PD-L2. CD8 T expansion. In addition, this study demonstrates that there is a therapeutic window to evaluate cancer treatments before the onset of xenogeneic GvHD in this model.

**RESULTS**

Here we present the response to the checkpoint inhibitors pembrolizumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in the human RKO colorectal carcinoma model. This high-grade adenocarcinoma cell line was originally established from the primary resected tumor of a patient with metastatic colorectal cancer and has been extensively described in the literature. PD-L1 and PD-L2 expression was assessed in a panel of 9 human tumor cell lines before xenografting into the NSG mice. Four cell lines, HT-1080, Colo205, RKO, HCT116, MDA-MB-231, Calu6, HCC827, SKOV3, were analyzed by flow cytometry for the expression of PD-L1 and PD-L2. A 5% flow cytometry gate on the CD45 population was used to identify leukocytes. By day 22 most of the hCD45 cells were CD3+ distributed between CD4 and CD8 subsets. There is a limited presence of naive T cells, central memory and effector memory T cells. The hPBMC-NSG mouse model is responsive to pembrolizumab alone and in combination with ipilimumab. Ipilimumab produced a modest, yet significant tumor growth inhibition in the RKO humanized NSG model. The RKO colorectal carcinoma model is responsive to pembrolizumab alone and in combination with ipilimumab, pembrolizumab produced a modest yet significant tumor growth inhibition in the NSG mice model.