

model. Both heterozygous and homozygous hTLR8 KI mice are viable with inflammatory phenotypes, i.e. enlarged spleens and livers, and significantly higher IL-12 p40 levels under TLR8 agonist treatment. In this study, we evaluated the potential use of hTLR8 mice for cancer immunotherapy studies.

Methods hTLR8 mice, together with naïve C57BL/6 mice, were inoculated with MC38 syngeneic tumor cells. Tumor bearing mice were grouped at a mean tumor volume of approximately 100 mm³ for treatment with PBS or 10 mg/kg anti-PD-1 (RMP1-14) antibody. At the efficacy endpoint, spleens and tumors were collected for flow cytometry profiling.

Results Anti-PD-1 treatment of MC38 tumors in naïve C57BL/6 led to moderate tumor growth inhibition (TGI = 54%). Interestingly, anti-PD-1 treatment showed improved efficacy in hTLR8 mice (TGI = 79%), including 2/10 tumors with complete tumor regression. In comparison, non-treated MC38 tumor growth rate was slower in hTLR8 mice than in naïve mice. Anti-PD-1 treated hTLR8 mice also had significantly increased IFN- γ and TNF- α positive CD4+ T cells in the spleen, along with higher numbers of differentiated effector T cells. In addition, hTLR8 mice have activated dendritic cells and macrophages, acting as critical steps in initiation of the inflammatory process, with higher levels of pro-inflammatory cytokines, such as IL-6, IFN- γ , TNF- α , and IL-1 β , which may promote Th1 priming and differentiation of T cells into IFN- γ or TNF- α producing cells.

Conclusions hTLR8 mice offer a great tool to model cancer immunotherapy in an inflammatory/autoimmunity prone background. Moreover, hTLR8 mice can be effectively used to shift a 'cold' tumor phenotype to 'hot' tumors in a syngeneic setting.

Ethics Approval Animal experiments were conducted in accordance with animal welfare law, approved by local authorities, and in accordance with the ethical guidelines of Crown-Bio (Taicang).

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ASSESSMENT OF SENSITIVITY TO A PD-1 CHECK POINT INHIBITOR AND CISPLATIN IN BLADDER CANCER PATIENT-DERIVED XENOGRAPTS WITH VARIOUS LEVELS OF PD-L1 EXPRESSION IN HU3D4NCG MICE

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Background Bladder cancer is the fifth most common cancer in the US, and the ninth most common cancer worldwide. Treatment of bladder cancer has evolved over time to encompass traditional modalities of chemotherapy and surgery, but has been particularly impacted by the recent use of immunotherapy. Modern immunotherapy has focused on checkpoint protein inhibitors that impede immune function. The inhibitors for several checkpoint targets (programmed death-ligand 1 [PD-L1], programmed cell death protein1 [PD-1], and cytotoxic T-lymphocyte-associated protein 4 [CTLA4]) were either

approved or in late-stage development. In this study we examined the effect of PD-1 inhibitor pembrolizumab and cisplatin in a panel of bladder patient-derived xenografts (PDX) with distinct patterns of PD-L1 expression in CD34+ stem cell humanized NCG (HuCD34NCG) mice.

Methods Three bladder PDX models PNX0428, PNX0434 and PNX1028 have been established under informed consent from the patients at the Fox Chase Cancer Center, Philadelphia. These models have been profiled for the levels of PD-L1 protein using immunohistochemical staining with SP263 antibody (Ventana) and used to establish the growth kinetics and sensitivity to the PD-1 check point inhibitor pembrolizumab and standard of care chemotherapeutic agent cisplatin in female HuCD34NCG and standard NCG mice from Charles River Laboratories.

Results We have established the ability of three bladder PDX models to grow in both the HuCD34NCG and standard NCG mice. The tumor growth kinetics of these models was slightly delayed in HuCD34NCG animals compared to NCG. We observed variable responses to cisplatin and pembrolizumab treatments among the PDX models that did not correlate with the level of PD-L1 expression in these tumors. Despite the presence of ~70% PD-L1 positive cells in the PNX0428 model, these tumors produced minor responses to pembrolizumab in HuCD34NCG mice that correspond to progressive disease in patients. Interestingly, pembrolizumab treatment in the PNX1028 model and even more significantly in the PNX0434 model in HuCD34NCG mice produced strong statistically significant tumor growth inhibition that correlates with stable disease in patients despite negative staining for PD-L1 protein in these tumors. The standard of care treatment cisplatin produced significant tumor growth inhibition in all three PDX models in both HuCD34NCG and standard NCG mice.

Conclusions Our data indicates that abundant expression of PD-L1 protein in tumors should not be used as the only biomarker for patient stratification for the treatment with PD-1/PD-L1 check point inhibitors. The HuCD34NCG mouse model is an effective tool for supporting tumor growth and evaluating immunotherapies.

Ethics Approval Animal studies were approved by Nexus Pharma, IACUC number 08-22. Three bladder PDX models PNX0428, PNX0434 and PNX1028 have been established under informed consent from the patients at the Fox Chase Cancer Center, Philadelphia, IRB protocol 11-866.

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CLINICOPATHOLOGIC AND GENOMIC CORRELATES OF TUMOR MUTATIONAL BURDEN AND ITS IMPACT ON PD-(L)1 INHIBITION EFFICACY IN NON-SMALL CELL LUNG CANCER ACCORDING TO DIFFERENT PD-L1 EXPRESSION SUBGROUPS

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Background High tumor mutational burden (TMB) and PD-L1 expression are associated with improved clinical outcomes in patients (pts) with non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs). However, how