In this preliminary, proof-of-principal study, two dogs presenting to their veterinarians with histologically confirmed metastatic lung tumors via immune checkpoint inhibition. While immune checkpoints expressed on lung tumors are not amenable to RASON inhibition, immune cells resident in the bronchial epithelium and BALC represent good targets for the RASON approach to checkpoint inhibition. E.g., SIRP-alpha is a receptor expressed by myeloid lineage cells such as dendritic cells (DCs), tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). When CD47, found on the surface of tumor cells, binds to SIRP-alpha on immune effector cells, the anti-tumor action of such immune effector cells becomes significantly diminished. We hypothesized that RASONs targeting mRNA of immune checkpoint proteins found on immune effector cells would eliminate checkpoint proteins from their surface, such that when they were signaled to home in on lung tumors, they would arrive in the tumor-associated microenvironment in a state impervious to checkpoint ligands expressed on the surface of tumor cells. To test this hypothesis, we applied a RASON protocol to dogs with spontaneous lung tumors presenting to their veterinarians.

Methods
In this preliminary, proof-of-principal study, two dogs with histologically confirmed metastatic lung tumors were administered RASONs targeting PD1, CTLA-4 and SIRP-alpha, by inhalation, twice weekly for eight weeks.

Results
X-ray analysis performed two weeks after the conclusion of RASON treatment showed dramatic results. One dog showed complete tumor dissolution (figure 1), and the second dog showed near total tumor dissolution, with faint shadows remaining (figure 2).

Conclusions
While these are preliminary results, and need to be dramatically expanded, they provide an initial indication that the RASON approach might prove to be an effective addition to immune checkpoint inhibition. It possesses certain advantages over small molecule or antibody approaches to checkpoint inhibition. For example, rather than being delivered systemically, RASONs are delivered by inhalation directly to the target tissues— the bronchial epithelium and BALC. Furthermore, it may be possible to reduce the toxicity of systemic treatments targeting checkpoint proteins on tumor cells, by reducing or eliminating their ligands on immune effector cells. In as much as the RASON approach to the treatment of human asthma failed in clinical trials as a result of its induction of an influx of macrophages into the lung, the ability to render TAMs impervious to the presence of tumor-associated immune checkpoints suggests that the RASON approach may hold considerable promise for the treatment of lung tumors.

Ethics Approval
All research reported here involved informed consent by owners of dogs with spontaneous lung neoplasms, for which no satisfactory alternative treatment was available, and was performed in strict compliance with both the Basle Declaration, to which the laboratory is a signatory member, as well as guidelines published by the International Council for Laboratory Animal Science (ICLAS).

Consent
N/A

REFERENCES

Abstract 244 Figure 1
Canine 2 presented with a 3-cm spherical tumor (circled, left). After RASON treatment (right), tumor underwent complete regression

Abstract 244 Figure 2
Canine 1 presented with one 9-cm tumor and four smaller tumors ranging from 1–2 cm (tumors are marked with dot marks).
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-1β, 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 CrownBio (Taicang).

**REFERENCES**

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**ASSESSMENT OF SENSITIVITY TO A PD-1 CHECK POINT INHIBITOR AND CISPLATIN IN BLADDER CANCER PATIENT-DERIVED XENOGRAPHS WITH VARIOUS LEVELS OF PD-L1 EXPRESSION IN HUCD34NCG MICE**


**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 protein 1 [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**


**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...