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, naïve 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 Crown-Bio (Taicang).

**REFERENCES**


247 ASSESSMENT OF SENSITIVITY TO A PD-1 CHECK POINT INHIBITOR AND CISPLATIN IN BLADDER CANCER PATIENT-DERIVED XENOGRAFTS 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 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.

246 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-(L)-1 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
TMB performs as a predictive biomarker to ICIs in different PD-L1 expression subgroups is not well characterized.

**Methods**
We collected clinicopathologic and genomic data from NSCLCs which underwent targeted NGS and TMB assessment at DFCI. An unbiased recursive partitioning (URP) algorithm was used to investigate an optimal TMB cut-off with respect to objective response rate (ORR) in the subset of pts treated with ICIs. This TMB cut-off was then validated in the prospective POPLAR/OAK cohort.

**Results**
Among 3560 NSCLCs identified, median TMB was significantly higher among current smokers compared to former (P<0.0001) and never smokers (P<0.0001), and there was a significant correlation between TMB and pack-years (figure 1A-B). Pts with BRAF or KRAS mutations had the highest median TMB (10.9 and 9.8 mutations/Megabase [mut/Mb], respectively), while tumors with RET and ALK alterations had the lowest median TMB of 5.3 mut/Mb (figure 2A-B). Tumors with PD-L1 expression of ≥50% had significantly higher median TMB compared to those with a PD-L1 expression of 1–49% (P=0.002) and <1% (P<0.0001). Among pts treated with ICIs (N=690), URP identified an optimal grouping TMB cut-off for ORR of 19.0 mut/Mb, which corresponded to the 90th percentile. Pts with a TMB of ≥19.0 mut/Mb had a significantly higher ORR (45.2% vs 20.1%, P<0.0001) and longer median PFS (11.0 vs. 2.9 months, HR:0.49, P<0.0001) and OS (20.8 vs. 11.2 months, HR:0.59, P=0.001) compared to those with a TMB of <19.0 mut/Mb (figure 3A-C). A TMB of ≥19.0 mut/Mb was an independent predictor of improved PFS and OS at multivariable analysis (table 1). A TMB within the top 10th percentile was confirmed to correlate with improved ORR and PFS in atezolizumab arm but not in the docetaxel arm of the POPLAR/OAK trials (figure 4A-B). When TMB and PD-L1 where integrated in the URP, we identified an optimal cut-off of 19 mut/Mb among cases with a PD-L1 expression of ≥25%, and of 8.4 mut/Mb among those with a PD-L1 expression of >25%, suggesting that TMB differentially impacts response to immunotherapy among PD-L1 high versus low NSCLCs (figure 5).

**Abstract 246 Figure 1**
(A) Correlation between TMB and smoking status. (B) Linear correlation between TMB and pack-years on a continuous scale.

**Abstract 246 Figure 2**
(A) TMB distribution across genomically defined subsets of NSCLC. (B) Pairwise comparison in TMB distribution among genomically defined subsets of NSCLC. Reported in each box are the Q-values for each comparison (FDR method of Benjamini and Hochberg).

**Abstract 246 Figure 3**
(A) Overall response rate, (B) progression-free survival and (C) overall survival to PD-(L)1 inhibition in patients with NSCLC and a TMB of ≥19.0 vs <19.0 mut/Mb, as determined by unbiased recursive partitioning.

**Abstract 246 Figure 4**
(A) Overall response rate, progression-free survival and overall survival to atezolizumab in patients enrolled in the POPLAR/OAK trials and a bTMB of ≥25 (top 10th percentile) vs <25.0 (lower 90th percentile) mut/Mb. (B) Overall response rate, progression-free survival and overall survival to docetaxel in patients enrolled in the POPLAR/OAK trials and a bTMB of ≥25 (top 10th percentile) vs <25.0 (lower 90th percentile) mut/Mb. bTMB, blood tumor mutational burden.

**Abstract 246 Figure 5**
Unbiased regression tree algorithm recursively identifies that different TMB cut-offs impact response to immunotherapy in PD-L1 TPS high (>25) vs low (<25%) NSCLCs. TPS, tumor proportion score.
Conclusions The impact of TMB may vary across PD-L1 expression subgroups. Rational integration of TMB and PD-L1 expression may identify NSCLCs with the greatest likelihood of response or resistance to ICIs.

Ethics Approval Clinicopathologic data were collected from patients with advanced NSCLC who had consented to a correlational research study (DF/HCC protocol #02-180).

Abstract 246 Table 1 Multivariable Cox regression for PFS and OS among patients with NSCLC treated with PD-(L)1 blockade.

<table>
<thead>
<tr>
<th>Progression-free survival</th>
<th>Univariate Hazard Ratio (95%CI)</th>
<th>P-value</th>
<th>Multivariable Hazard Ratio (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.59 (0.43-0.81)</td>
<td>0.03</td>
<td>0.64 (0.49-0.87)</td>
<td>0.03</td>
</tr>
<tr>
<td>PD-L1 expression</td>
<td>0.592 (0.48-0.75)</td>
<td>&lt;0.0001</td>
<td>0.594 (0.49-0.77)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (75 vs 90)</td>
<td>0.91 (0.75-1.12)</td>
<td>0.32</td>
<td>0.72 (0.63-0.86)</td>
<td>0.04</td>
</tr>
<tr>
<td>Male (male vs female)</td>
<td>0.81 (0.69-0.98)</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ECOG - 0 (0 vs 1)</td>
<td>0.35 (0.28-0.43)</td>
<td>&lt;0.0001</td>
<td>0.36 (0.28-0.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smoking history (ever vs never)</td>
<td>0.78 (0.50-0.92)</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ERL lays metastasis (yes vs no)</td>
<td>0.83 (0.68-1.05)</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Line of therapy (1 vs 3)</td>
<td>0.54 (0.42-0.79)</td>
<td>&lt;0.0001</td>
<td>0.72 (0.55-0.93)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abstract 248 Figure 1 Bulk and single-cell (sc) RNA-sequencing (RNA-seq) of MDOTS identifies an anti-PD-1 (αPD-1) resistant subpopulation of persister cells. IgG= isotype control.

DOTS. In vitro culture studies were conducted with or without cytokines (100 ng/ml) or drugs (500 nM). In vivo studies in mice bearing MC38 or CT26 tumors evaluated the combinatorial strategy with PD-1 blockade. We further evaluated our findings in scRNA-seq of an αPD-1 refractory colorectal cancer (CRC) patient tumor.

Results Bulk RNA-seq of αPD-1 treated DOTS revealed a mesenchymal resistant phenotype with upregulated TNF-α/NFκB signaling (figure 1). scRNA-seq further identified a discrete sub-population of immunotherapy persister cells (IPCs). These cells expressed a stem-like phenotype including down-regulation of E2F targets indicative of quiescence, suppression of interferon-γ response genes, induction of hybrid epithelial-to-mesenchymal state, and active IL-6 signaling (figure 1). Ly6a/stem cell antigen-1 (Sca-1) and Snai1 were found to be differentially upregulated in IPCs resistant to PD-1 blockade (not shown). Sca-1 positivity was confirmed in pre-existing tumor populations in vitro (figure 2). When enriched via sorting, these cells remained more persistently Sca-1+ at 96 hours in culture of CT26 compared to MC38 cells, related to increased autocrine IL-6 production by CT26 Sca-1+ cells. Indeed, IL-6 supplementation was capable of expanding Sca-1+ cells in culture (figure 2). Sca-1+ cells expressing ovalbumin peptide were refractory to OT-1 T cell mediated killing and failed to upregulate MHC class-1 antigen presentation (H-2Kb) in response to IL-6, in contrast to interferon-γ (not shown). Analysis of RNA-seq data further identified Birc2/3 as potential targets limiting TNF-mediated apoptosis of these cells (not shown). Notably, Birc2/3 antagonism depleted Sca-1+ IPCs in vitro and significantly potentiated the impact of PD-1 blockade in vivo in MC38, and less robustly in CT26 (figure 3). Evaluation in a microsatellite-instability high CRC patient identified a pre-existent IPC subpopulation within the αPD-1 refractory pre-treatment tumor, with high SNAI1 expression compared to CRC samples in TCGA (figure 4).