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

Simon Tarpinian[1], Jenny Rowe[3], Ruziboy Husanov[1], Uma Saha[1], Prabal Banerjee[1], Rukiye Eraslan[1], Beverly Jones[1], Stephen Festin[3], Vladimir Khazak [1, 2]
[1]Invivotek, Hamilton, NJ, [2]Nexus Pharma, Philadelphia, PA, [3]Charles River Laboratories Research Models and Services (RMS), Wilmington, MA

P247
Presented at SITC 2020

1 INTRODUCTION

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 (HuCD34 NCG) mice.

2 METHODS

Generation and Maintenance of Bladder PDX tumor models

Three bladder PDX models PNX0428, PNX0434 and PNX1028 have been established under informed consent from the patients at the Fox Chase Cancer Center, Philadelphia. The impersonalized patient information is presented in **Table 1**.

Table 1. Impersonalized patient information

#	Model	Tumor Status	Cancer Type	Histology	Harvest Site	Disease Stage	Tumor Grade	Neoadjuvant Treatment	Adjuvant Treatment	Age	Gender	Ethnicity
1	PNX0428	Primary	Bladder	Urothelial Carcinoma	Bladder	IIIA	High Grade	Gemcitabine/ Cisplatin, PR	NA	75	Female	Caucasian
2	PNX0434	Metastatic	Bladder	Urothelial Carcinoma	Bladder	IIIA	High Grade	ddMVAC: Doxorubicin/ Cisplatin/ Methotrexate/ Vinblastin, PR	NA	70	Male	Caucasian
3	PNX1028	Primary	Bladder	Sarcomatoid Carcinoma	Bladder	II	Unknown	Naïve	NA	66	Male	Caucasian

To create PDX models bladder tumor tissues were collected from the human pathology facility in ice cold transport media. Tissues were minced on ice, mixed in RPMI/Matrigel and implanted subcutaneously in both flanks of NSG mice. Additional fragments of tumor tissue were snap-frozen for future RNA and/or DNA extraction and WES and RNA Seq analysis, and fixed and processed into paraffin blocks for histological evaluation. The third passage (P3) tumors were applied for further xenograft studies. The genomic characterization of PDX models is presented in **Table 2**.

Table 2. PDX Model Characterization

#	Model	Cancer Type	Histology	WES Mutations	RNA seq: Gene Expression	Biomarkers	Time to 100 mm³
1	PNX0428	Bladder	Urothelial Carcinoma	1042 mutated genes; available upon request	RNA seq; available upon request	NA	35 days
2	PNX0434	Bladder	Urothelial Carcinoma	Ongoing	Ongoing	NA	35 days
3	PNX1028	Bladder	Sarcomatoid Carcinoma	1001 mutated genes; available upon request	RNA seq; available upon request	vimentin positive; cytokeratin (AE1/AE3, CAM5.2, CK5/6, CK903) negative; A103, S100, HMB45, MITF, actin, desmin, CD117 and p63 negative	21 days

Tumor Implantation

Female HuCD34 NCG and NCG mice (provided by Charles River Laboratories) were implanted subcutaneously on the right flank according to the procedure described above with n=5 mice per treatment group for each bladder PDX model.

Checkpoint Inhibitor and Chemotherapy Dosing Regimens

Pembrolizumab (Keytruda, Merck) was given at 10mg/kg, IV, Q1Wx3. Cisplatin (DDP, Mylan) was given at 5 mg/kg, IP, Q1Wx3. The study treatment protocol is presented in **Table 3.**

Immunohistochemistry

Tumor tissues have been profiled for the levels of PD-L1 protein expression using immunohistochemical staining with rabbit monoclonal antibody SP263 (Ventana) according to manufacturer's instructions.

Flow Cytometry

QC screening performed on peripheral blood of HuCD34 NCG mice at ~14 weeks post-injection of human cord blood derived CD34⁺ cells. Samples acquired using the Attune™ NxT Flow Cytometer 4-laser system (ThermoFisher). Post-acquisition analyses performed using FlowJo v10.4 (FlowJo) for gating and GraphPad prism (GraphPad Software) for data representation.

3a RESULTS: PD-L1 Staining

Figure 1

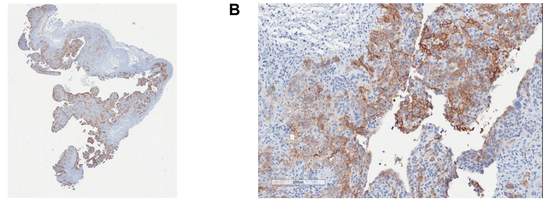

Figure 1. IHC staining of PNX0428 tumor tissue with PD-L1 antibody SP263 (Ventana). **A** -1x and **B** -20x magnification. An immunostaining with anti-PD-L1 rabbit monoclonal antibody determined islands of PD-L1 positive cells in PNX0428 bladder PDX tissues. The scanning estimated 70% of the cells were positive for PD-L1 protein. IHC staining of PNX0434 and PNX1028 tumor tissues with anti-PD-L1 antibody was considered negative (data not shown).

3b RESULTS: Humanization Levels of HuCD34 NCG mice

Figure 2

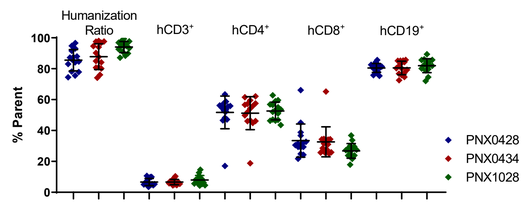

Figure 2. Humanization levels in peripheral blood. QC screening at ~14 weeks post-injection of human cord blood derived CD34+ cells. HuCD34 NCGs were randomized into three tumor implantation groups based on levels of human cell populations. Data are shown as Mean ± SD. Humanization Ratio: Percent of hCD45⁺ cells of total lymphocytes; hCD3⁺: Frequency of parent hCD45⁺; hCD4⁺ and hCD8⁺: Frequency of parent hCD45⁺hCD3⁺; hCD19⁺ Frequency of parent hCD45⁺hCD3⁻.

3c Response Summary: Tumor Growth Inhibition Analysis

Table 3

Group	PDX Model	NCG	HuCD34 NCG	Treatment Regimen Agent	MTV	%TGI	Statistical Significance (vs G1)	Median TTE	Mean BW Nadir
1	PNX0428	5		Vehicle/Saline	1063		....	19	1.0%
2	PNX0428	5		Cisplatin	602	43	ns	19	-3.7%
3	PNX0428	5		Pembrolizumab	1505	-42	ns	19	0.9%
1	PNX0428		5	Vehicle/Saline	500		....	19	-2.0%
2	PNX0428		4	Cisplatin	116	77	*	19	-15.0%
3	PNX0428		5	Pembrolizumab	359	28	ns	19	-1.6%
1	PNX0434	5		Vehicle/Saline	708		....	19	3.2%
2	PNX0434	5		Cisplatin	214	70	ns	19	-20.6%
3	PNX0434	5		Pembrolizumab	934	-32	ns	19	6.6%
1	PNX0434		4	Vehicle/Saline	1271		....	19	5.0%
2	PNX0434		4	Cisplatin	225	82	*	19	-21.9%
3	PNX0434		5	Pembrolizumab	472	63	*	19	-2.6%
1	PNX1028	5		Vehicle/Saline	2314		....	6	-4.9%
2	PNX1028	5		Cisplatin	1060	54	ns	22	-12.3%
3	PNX1028	5		Pembrolizumab	2646	-14	ns	6	-8.3%
1	PNX1028		3	Vehicle/Saline	1698		....	15	7.3%
2	PNX1028		5	Cisplatin	58	97	**	21	-27.0%
3	PNX1028		4	Pembrolizumab	922	46	*	21	-8.9%

Table 3. Analysis of treatment efficacy in HuCD34 NCG and NCG mice. Treatment outcome was based on percent tumor growth inhibition (%TGI). Percent TGI was calculated using the formula: **%TGI = [1-(MTVdrug treated/ MTVcontrol)] x 100**.
Statistical significance was determined using One way Anova (Tukey's Multiple Comparison Test). *p < 0.05, **p < 0.001
MTV = maximum tumor volume.
TTE = time to end point.

3d RESULTS: Tumor Study

Figure 3

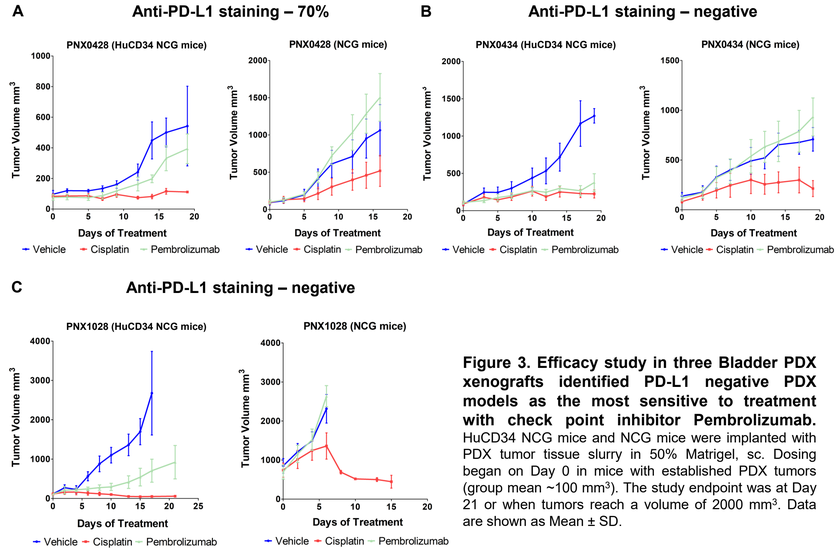

Figure 3. Efficacy study in three Bladder PDX xenografts identified PD-L1 negative PDX models as the most sensitive to treatment with check point inhibitor Pembrolizumab. HuCD34 NCG mice and NCG mice were implanted with PDX tumor tissue slurry in 50% Matrigel, sc. Dosing began on Day 0 in mice with established PDX tumors (group mean ~100 mm³). The study endpoint was at Day 21 or when tumors reach a volume of 2000 mm³. Data are shown as Mean ± SD.

4 CONCLUSION

- Confirmed ability of three proprietary bladder PDX models to form tumors in HuCD34 NCG mouse model.
- Evaluated immuno-oncology therapy Pembrolizumab and Standard of Care chemo regimen, Cisplatin, in three bladder PDX models with various levels of PD-L1 expression in HuCD34 NCG and standard NCG mice.
- Mice treated with Pembrolizumab developed no adverse effects during the study. Treatment with Cisplatin was associated with significant weight loss in both HuCD34 NCG and standard NCG mice.
- Treatment with Cisplatin produced significant tumor growth inhibition in all three PDX models and the effect was not associated with the type of NCG mice.
- Treatment of PNX0434 and PNX1028 models with Pembrolizumab in HuCD34 NCG mice produced statistically significant tumor growth inhibition that is reminiscent of a stable disease phenotype in patients.
- **Data indicate 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 HuCD34 NCG mouse model is an effective tool for supporting tumor growth and evaluating immunotherapies.

Acknowledgements: The authors thank Dr. Qi Cai from the animal pathology facility at Fox Chase Cancer Center for her time and efforts in anti-PD-L1 IHC analysis and Dr. Christoph Eberle from Charles River Discovery for performing flow cytometry.