CERTIS™ ONCOLOGY INTELLIGENCE

# *In-vivo* Tumor Implantation Site Exhibits Differential Immune Response in Solid Tumors

Rajeev Shrimali, PhD[1]; Jasmine Borroel[1]; Carmen Sunico, PhD[1]; Kristen Buck[1]; Javier Rodriguez[1]; Long Do, PhD[1]; Warren Andrews, PhD[1]; Yuan-Hung Chien, PhD[1]; Christophe Pedros, PhD[1]; Bianca Carapia[1]; Rowan Prendergast[1]; Bridget Corcoran[1]; Jantzen Sperry, PhD[1]; Jonathan Nakashima, PhD[1].

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Successful discovery and development of cancer therapeutics depend on testing agents in the most clinically relevant translational models. Emerging evidence highlights the importance of the local tumor microenvironment (TME) in evaluating the efficacy of new therapeutics, especially for immunotherapies. Historically, patient-derived xenograft (PDX) modeling involves subcutaneous implantation, which has been shown to minimally represent the actual human tumor site[1-8]. Here, Certis uses peripheral blood mononuclear cell (PBMC) humanized PDX models to demonstrate differences in the TME, overall immune response, and the pharmacological outcome, between subcutaneous (SC) and orthotopic (Ortho / OT) PDX models. These findings highlight the importance of testing new therapies in the most clinically relevant setting for greater translation into clinical success.

## METHODS

Patient biopsies were surgically implanted subcutaneously into the right rear flanks or into their respective orthotopic location (e.g., into the stomach for gastric PDX CRT00292 BarneyOI™ model) of the female NOG mice. To monitor tumor growth, SC tumors were measured via caliper twice weekly, and Ortho tumors were imaged with the M3™ compact MRI from Aspect Imaging. For the humanized study, $1x10^6$ - $5x10^6$ donor PBMCs were injected intravenously via tail vein before or after tumor implantation depending on respective tumor growth. Therapeutics were formulated and administered per manufacturer's instructions or past publications. To determine % hCD45 chimerism, weekly in-life blood samples were collected, and RBC lysed per manufacturer's protocol (Thermo Fisher Scientific) and further processed by standard flow staining protocol. For RNA-Seq analysis, mouse contamination was removed (Xenome) and aligned to Human GRCh38 genome using STAR/RSEM, and differential gene expression was performed using edgeR against matched normal tissue from the Genotype-Tissue Expression (GTEx) project. Gene set enrichment analysis (GSEA) was performed to find enriched pathways (KEGG). For tumor-infiltrating lymphocyte (TIL) analysis, tumors were surgically removed and dissociated into single cell suspension using the Miltenyi Biotec gentleMACS™ Dissociator. Immune populations were analyzed using the Cytek™ Biosciences Aurora 3 spectral flow cytometer.

## CONCLUSIONS

Tumor implantation site determines the outcome of therapeutic response including immune check point inhibitors. Differences in response is driven by differential gene signature, T cell recruitment, infiltration and functional status. Orthotopic PDX models provide a clinically relevant and translative platform for advancing various cancer therapeutics, including immunotherapies.

## CITATIONS & ACKNOWLEDGEMENTS

[1]Certis Oncology Solutions, San Diego, CA.

aspectimaging TACONIC Models For Life.

ACCESS BARNEYOI™ CANCER MODEL DATABASE certisoncology.com/tumor-bank

## RESULTS

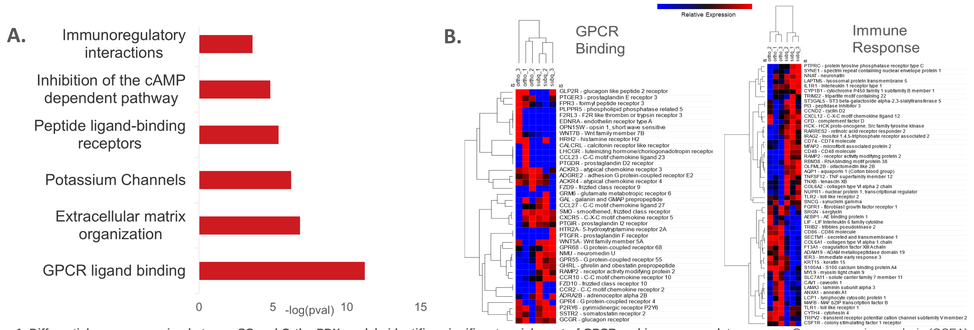


**Figure 1. Differential gene expression between SC and Ortho PDX models identifies significant enrichment of GPCR and immune regulatory genes.** Gene expression analysis (GSEA) against matched normal tissue from the GTEx project. **A**. Pathway enrichment analysis of top differentially expressed genes between SC and Ortho tissue of PDX models show pathways that may functionally contribute to differences in therapy response. **B**. Upregulated (red) and downregulated (blue) genes compared to matched normal (GTEx) highlight gene expression dysregulation between SC and Ortho PDX models in GPCR binding and immune response.

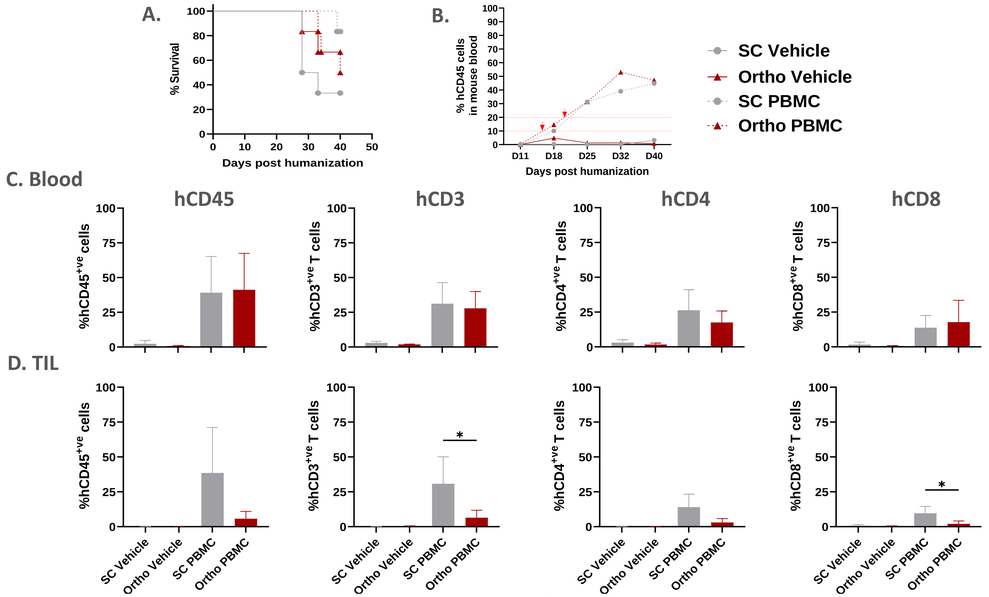


**Figure 2. PBMC humanized model of SC and Ortho gastric PDX.** Gastric PDX (CRT00292 BarneyOI™ Model) was implanted subcutaneously and orthotopically in female NOG mice, 8-10 weeks of age. When tumors reached 60-100mm$^3$ both groups were humanized with $5x10^6$ donor PBMCs and further evaluated for **A.** % Survival, **B.** % hCD45 chimerism, **C.** % hCD45, % hCD3, % hCD4 and % hCD8 cells of peripheral live leucocytes and **D.** % hCD45, % hCD3, % hCD4 and % hCD8 cells of live TIL. B, C & D are multi-color flow cytometer studies carried out on Cytek Biosciences Aurora 3 spectral flow cytometer. *Unpaired student's T test $p \leq 0.05$.

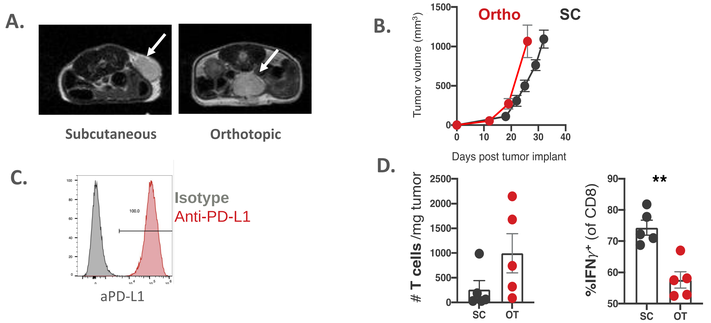


**Figure 3. SC and Ortho implantation effect on *in vivo* PBMC recruitment.** **A.** M3 compact MRI from Aspect imaging **B. G**rowth curves of a liposarcoma PDX model (CRT00395 BarneyOI™ Model ) implanted in the rear flank or abdomen of PBMC-humanized animals. **C.** Confirmation of PD-L1 status by spectral flow cytometry. **D.** Differential TIL recruitment and immunophenotypes of untreated tumors in locations (n=8).

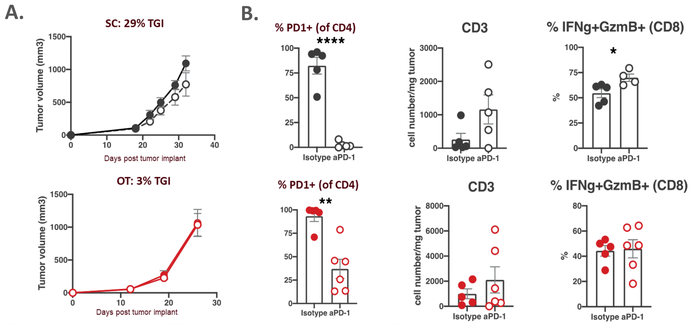


**Figure 4. SC and Ortho (OT) implantation effect on *in vivo* efficacy and immunophenotypes when treated with pembrolizumab. A.** Growth curves of SC and Ortho (OT) liposarcoma PDX model (CRT00395 BarneyOI™ Model) under anti-PD1 treatment show enhanced efficacy in the subcutaneous setting. **B.** Differential PD1 expression, recruitment and effector status of TIL from subcutaneously and orthotopically located tumors (n=6).

## OBSERVATIONS & RESULTS

PDX tumor implantation site drives differential:

- Immune gene signature
- Immune cell recruitment, infiltration and effector status
- Overall immune response to immune check point inhibitors

## REFERENCES

**1** Day CP et al. *Cell*. (2015). **2** Nakano K et al. *J Toxicol Pathol*. (2018). **3** Akter F et al. *BBA Rev Cancer*. (2021). **4** Julien S et al. *Clin Cancer Res*. (2012). **5** Walters DM et al. *PLoS One*. (2013). **6** DeRose YS et al. *Nat Med*. (2011). **7** Patrizii M et al. *Front Oncol*. (2018). **8** Tran Chau V et al. *Br J Cancer*. (2020).