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Evaluation of Autologous and Allogeneic T Cell-Based Therapies in *Ex-vivo* and *In-Vivo* Xenograft Tumor Models

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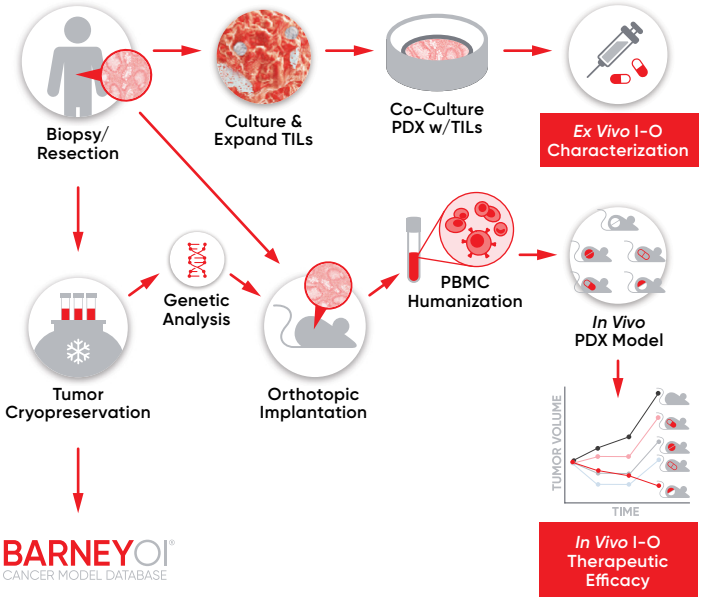
The field of adoptive T cell-based immunotherapy, including tumor-infiltrating lymphocytes (TILs), T cell receptor (TCR) & Chimeric antigen receptor (CAR) – T cells, has undergone unprecedented growth, specifically later in delivering an effective and durable clinical response. Notable success with CAR-T cells is limited to hematological malignancies while clinical gains with solid tumors is still a challenge. Solid tumors' heterogeneous target expression and loss, T cell status and metabolic fitness, T cell trafficking into the tumor, and the hostile immunosuppressive tumor microenvironment (TME) have hampered T cell efficacy in patients. There is a lack of clinically relevant solid tumor models for the translation of T cell-based therapies into the clinic. Here, We present orthotopic patient-derived xenografts (PDX) as an essential translational platform for the pre-clinical evaluation of T cell-based immunotherapies.

SUMMARY

The tumor implantation site determines the outcome of different therapeutic responses validated with anti-mouse VEGFR2 antibody (Ab), focal irradiation, and allogeneic 3rd generation CAR-T cell therapy (scFv(HER2)-CD28-4-1BB-CD3zeta CAR-T). For anti-angiogenic therapy, differences in response are driven by differential in vivo therapeutic efficacy and immune cell composition. For cell therapy, a dose-dependent killing of HER-2 expressing CDX (A549) and PDX (NSCLC adenocarcinoma) was observed with HER-2 targeting CAR-T cells in an ex-vivo co-culture assay. This was in concordance with the activation, effector function, and differentiation status of the CAR-T cells. Further, to imitate the patient population and the effect of solid TME, CAR-T cells were assessed for their efficacy and immune response differences in the subcutaneous and orthotopically implanted HER-2 expressing PDX in immunodeficient NOG mice. Our results indicate discordant tumor infiltration and immune cell functional status under different implantation sites, favoring orthotopic implantation as a superior translational model for T-cell immunotherapy evaluation. Our findings highlight the importance of testing T cell-based immune therapies in the appropriate translational tumor models with better certainty of clinical outcome.

IMMUNO-ONCOLOGY (IO) TRANSLATIONAL WORKFLOW



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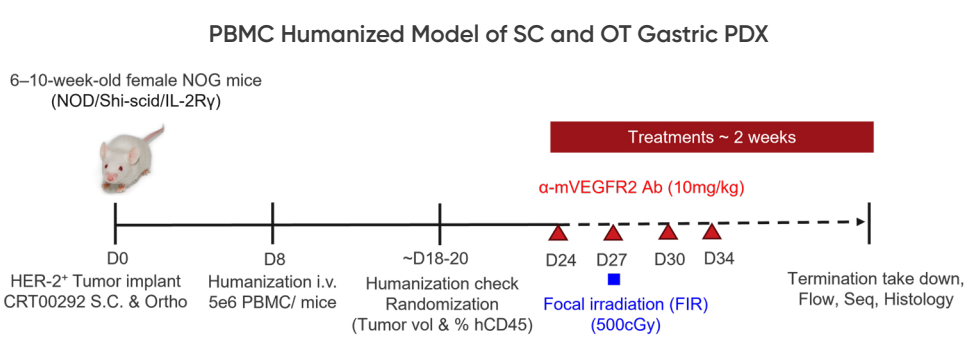
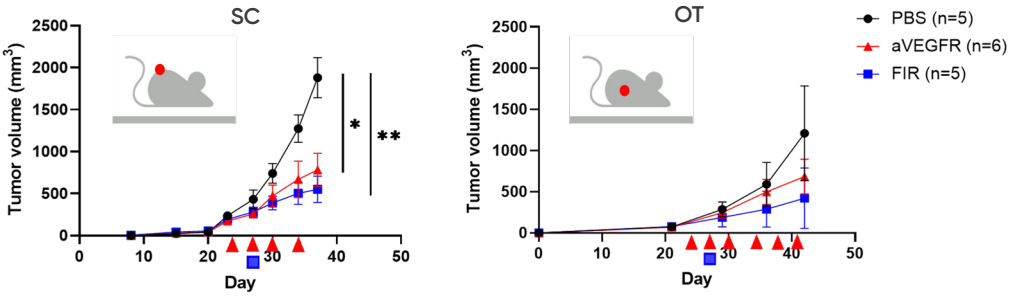
RESULTS

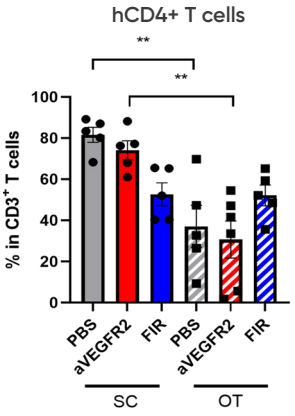
Orthotopic (OT) implanted *in vivo* models show decreased therapeutic efficacy and lower CD4+ T cell recruitment compared to subcutaneous (SC) implanted PDX

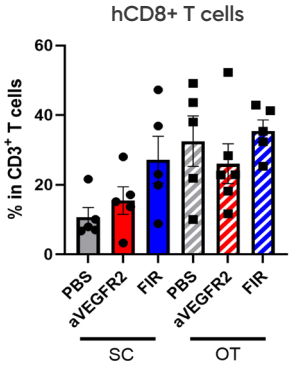


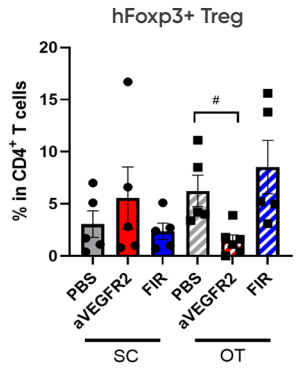
Gastric PDX was implanted subcutaneously and orthotopically in female NOG mice, 6-10 weeks of age. After one week of tumor implantation, both groups were humanized with 5x106 donor PBMCs. Mice were randomized depending on blood check of humanization and tumor volume (40–100mm3). Both groups received anti-mVEGFR2 antibody (Ab) treatment for 2 weeks or one dose of focal irradiation (FIR) (500cGy).



The tumor growth curve of the SC group was measured via caliper twice a week, and the OT group was measured via the Aspect M3 Compact MRI system once a week. *P<0.05, **P<0.01 by the two-way ANOVA.







TILs were further evaluated by % of hCD4, hCD8, and hTreg cells using the Cytek Aurora spectral flow cytometer and FlowJo analysis. SC group, PBS n=5, aVEGFR2 n=5, FIR n=5; OT group, PBS n=5, aVEGFR2 n=6, FIR n=5. *P<0.05, **P<0.01 by the two-way ANOVA. #P<0.05 by the Student's t-test.

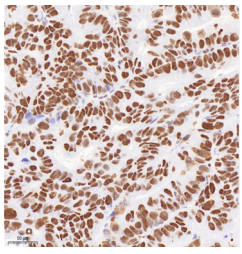
MRI

OT, PBS, Day 29

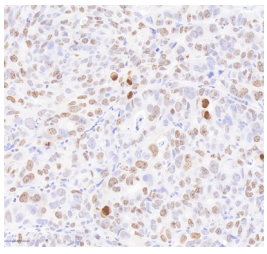
Repetitive MRI image of gastric PDX model at D29. Yellow arrowhead.

QC

Human Nuclei

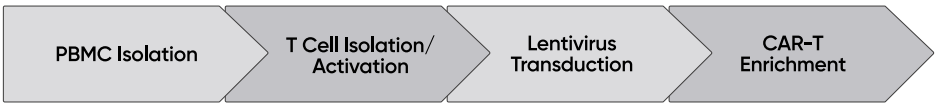


Ki67

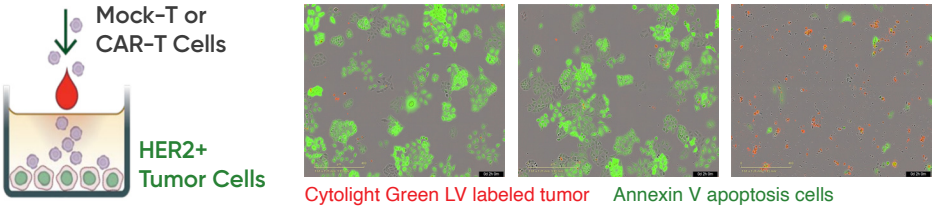


Immunohistochemistry (IHC) QC with anti-Human nuclei and human Ki67. Scale bar, 50µm

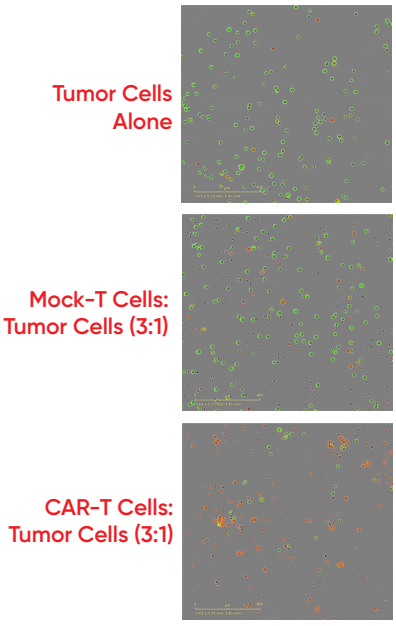
HER2-targeting CAR-T cells significantly drives apoptosis in HER2+ tumors (CDX/PDX)

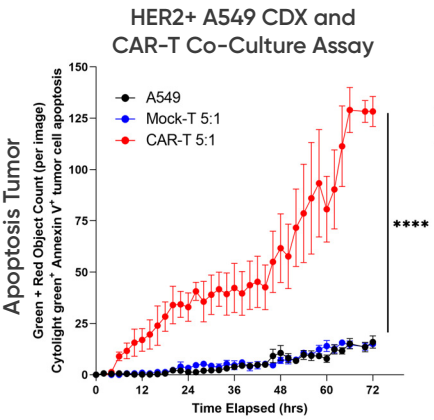


The workflow of the 3rd generation CAR-T generation (scFv(HER2)-CD28-4-1BB-CD3zeta).

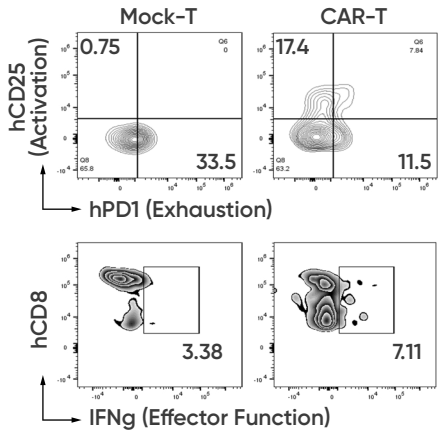


HER2-expressing A549 cells were labeled with Cytolight green using LV. 1,000 tumor cells were co-cultured with 5,000 Mock-T or CAR-T cells in the presence of Incucyte Annexin V Red Dye with 300U hIL2 media. The final image of the tumor and Mock-T or CAR-T cells co-culture after 3 days of incubation. Green, Cytolight green labeled A549 tumor; Red, apoptosis cells.

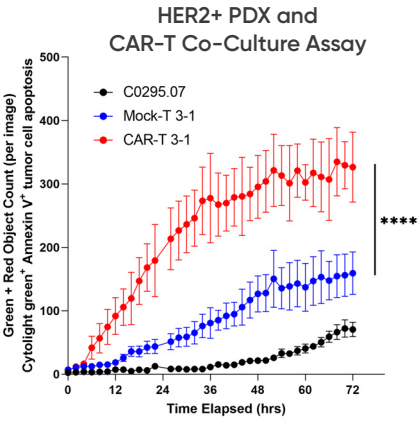




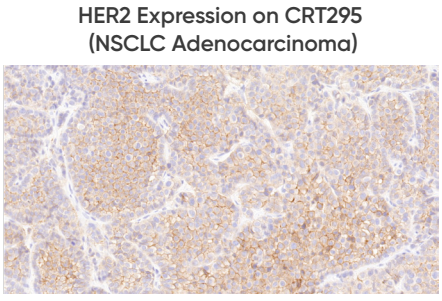
The apoptotic tumor cell count was quantified over time using the Satorius Incucyte S3 Imaging system.
****P<0.001 by the two-way ANOVA.

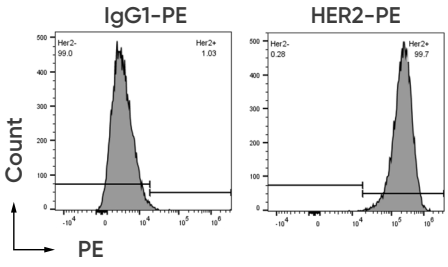


Immune phenotyping of CAR-T cells was characterized by spectral flow cytometry.



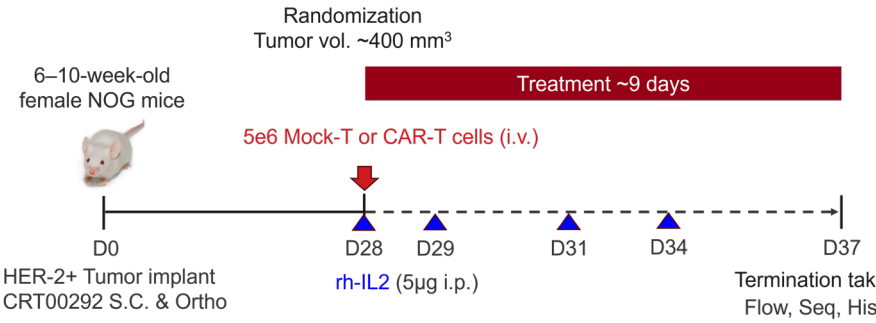
Co-culture killing assay of CAR-T cells and selected PDX tumor cells. The apoptotic tumor cell count was quantified and graphed over time using the Satorius Incucyte S3 Imaging system.
****P<0.0001 by the two-way ANOVA.



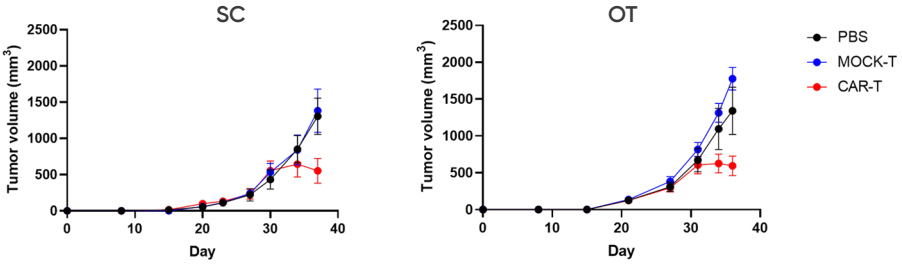


High HER2-expressing PDX was selected from the Certis BARNEY database. The validation of HER2 antigen expression in CRT00295 PDX was confirmed by IHC and Flow cytometry.

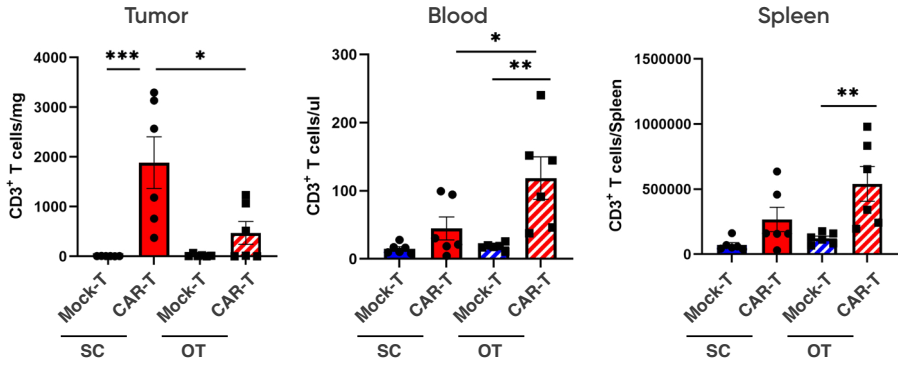
HER2-targeting CAR-T cells show a similar *in vivo* efficacy in both SC and OT models, but lower ability of tumor infiltration with higher activity and lower central memory T (T_{CM}) cell subset in the OT group



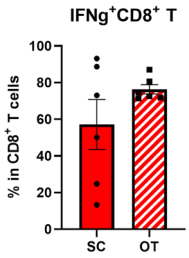
Gastric PDX (CRT00292) was implanted subcutaneously and orthotopically in female NOG mice, 6-10 weeks of age. When the tumor range both in SC and Ortho groups reaches approximately 400mm3 – 500mm3, mice were randomized into different groups with n = 6 mice/group and received 5e6 control Mock-T or CAR-T cells (HLA match between T cells donor and PDX tumor) followed by four doses of 5µg rh-IL2.

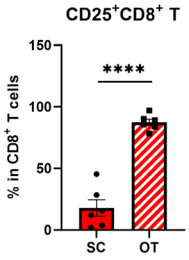


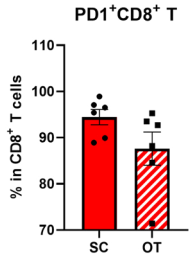
The tumor growth curve of the SC group was measured via caliper twice a week, and the OT group was measured via the Aspect M3 Compact MRI system once a week.
***P<0.001; ****P<0.0001 by the two-way ANOVA.

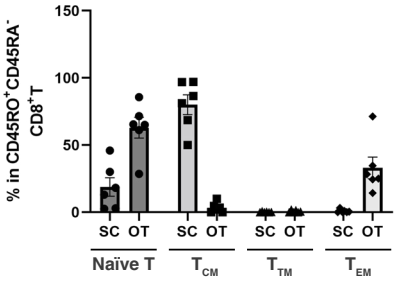


Absolute cell count of hCD3+ T cells in tumor, blood, and spleen collected from both SC and OT groups. *P<0.05; **P<0.01; ***P<0.001 by the two-way ANOVA.









Functional characterization and differentiation status of tumor-infiltrating CD8+ CAR-T using the Cytek Aurora spectral flow cytometer and FlowJo analysis.
****P<0.0001 by the Student's t-test.

OBSERVATIONS & RESULTS

- **PDX tumor implantation site drives differential immune cell composition under different treatments**
- **HER2-targeting CAR-T cells induce apoptosis in both HER2+ CDX and PDX models**
- **HER2-targeting CAR-T cells show different rates of tumor infiltration, and effector status in different implantation sites**

ACKNOWLEDGEMENTS

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