A Bispecific PD-L1:CD3 T-cell Engager Potently Kills Intractable Primary And Metastatic Intracranial Tumors

Emily J Girard, Shelli M Morris, Kristina Pilat, Alison M Williams, Kenneth Brasel, Heather Conti, Parvathi Muthuraman, Raymond Ruff, Hailey Hentschel, Steven Chen, Chunfeng Yin, Zachary R Crook, Jason P Price, Andrew J Mhyre, James M Olson

Ben Towne Center for Childhood Cancer and Blood Disorders Research, Seattle Children's Research Institute

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INTRODUCTION Central nervous system (CNS) tumors represent a large unmet medical need. Many pediatric and adult brain cancers and other cancers (e.g., lung, colon, breast) with CNS involvement remain largely intractable despite standard of care and advanced treatment strategies. We engineered a bispecific PD-L1:CD3 T-cell engager (TCE) and discovered promising efficacy in a wide range of pre-clinical intracranial tumor models, including some models with intact blood brain barriers.

STUDY DESIGN The purpose of this work was to explore the utility of a PD-L1:CD3 TCE for the treatment of intracranial tumors. Experiments were performed to determine target expression level and T-cell killing sensitivity *in vitro*. *In vivo* murine experiments evaluated anti-tumor efficacy, pharmacodynamic response, and tolerability. Pharmacokinetics and pharmacodynamics were evaluated in a dose range finding study in non-human primates. *In vivo* efficacy studies were performed as single experiments with replicate experiments run for models PBT-29FH, PBT-27FH, and NCI-H1975. Experiments were performed in adult male and female NOD.Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/SzJ (NSG) mice from Jackson Laboratories, with 6-10 mice per study arm. Mice were enrolled in treatment groups normalizing for bioluminescent tumor signal or body weight at a time point determined from historic tumor latency. Pharmacodynamic studies were performed twice in PBT-29FH xenografts and enrolled 4-5 mice per treatment arm. Mice in efficacy and pharmacodynamic studies received 0.5-1 nmol (1.5-3 mg/kg) TCE by intravenous injection twice weekly and 7.5x10^6 activated T cells (ATC) once weekly. TCE tolerability was performed twice in humanized PD-1/PD-L1 mice purchased for GenOway (genO-hPD-1/PD-L1) with 3-5 mice per dose level. One NHP dose range finding study was conducted with 3 animals at each of 3 dosing levels.

RESULTS PD-L1 was expressed and up-regulated by exposure to IFNγ in all 36 cell lines evaluated, representing diverse tumor models (Fig. 1). TCE anti-tumor activity was evaluated in 8 orthotopic models of primary brain tumor: 2-diffuse midline glioma (DMG), 1-medulloblastoma, 2-high-grade glioma, and 3-glioblastoma. Overall and median survival were significantly extended in 7 of 8 models (Fig. 2A,B,C). Additionally, TCE treatment elicited pharmacodynamic effects (PD-L1 up-regulation and T-cell recruitment to the tumor) in the presence of an intact blood brain barrier, as indicated by albumin staining (Fig. 2B). In addition to activity in primary brain tumor models, TCE showed robust anti-tumor responses against models of CNS metastases. All 6 models (3-lung adenocarcinoma, 1-colorectal carcinoma, and 2- triple negative breast cancer) had 100% survival of treated mice after 60-days. Bioluminescent tracking revealed that 53% of tumors across model types were eliminated by TCE treatment, 39% showed static tumor presence, and 8% of tumors progressed (Fig.3A). Further studies in the intracranial NCI-H1975 lung metastases model demonstrated efficacy at doses as low as 0.03 mg/kg and an additive response when combined with standard of care agent osimertinib (Fig. 3B,C). Dose range tolerability in PD-1/PD-L1 humanized mice showed no overt toxicity with transient cytokine stimulation, and no change in CD3$^+$ cell distribution, or loss of non-neoplastic PD-L1$^+$ cells (Fig. 4). A dose range finding study in non-human primates revealed pharmacodynamic responses at 0.03 mg/kg with toxicities limited to grade 1. At 0.3 mg/kg grade 2-4 toxicities were observed (Table 1).

PD-L1 is Expressed and Upregulated by All Tumor Models Evaluated

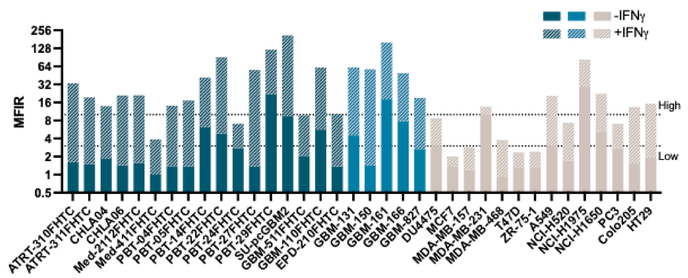

Figure 1: PD-L1 expression by flow cytometry. Baseline (solid) and induced (dashed-50 ng/ml IFNγ, 48hr) PD-L1 expression in cell culture lines of **pediatric brain tumors**, **adult glioblastoma**, and **adult peripheral tumors**.

TCE is Efficacious Against Pediatric and Adult Brain Tumor Orthotopic Xenografts

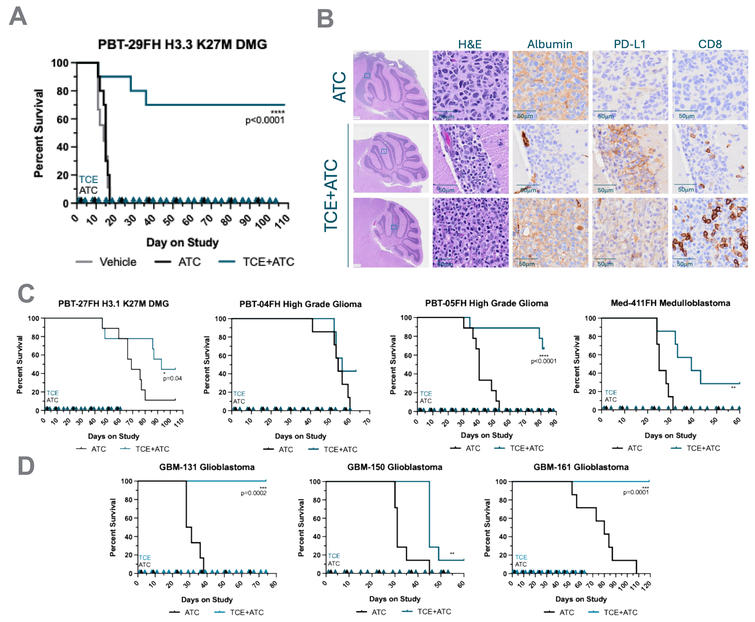

Figure 2: Efficacy of systemically administered TCE against intracranial xenografts. **(A)** Kaplan-Meyer curve for mice bearing PBT-29FH DMG xenografts treated with vehicle, ATC, or TCE+ATC. **(B)** TCE pharmacodynamic effect after 10-day treatment of PBT-29FH xenografts. **(C-D)** Kaplan-Meyer curves for mice with intracranial xenografts of pediatric (C) or adult (D) brain tumors.

TCE is Efficacious Against Intracranial Metastases Xenograft Models

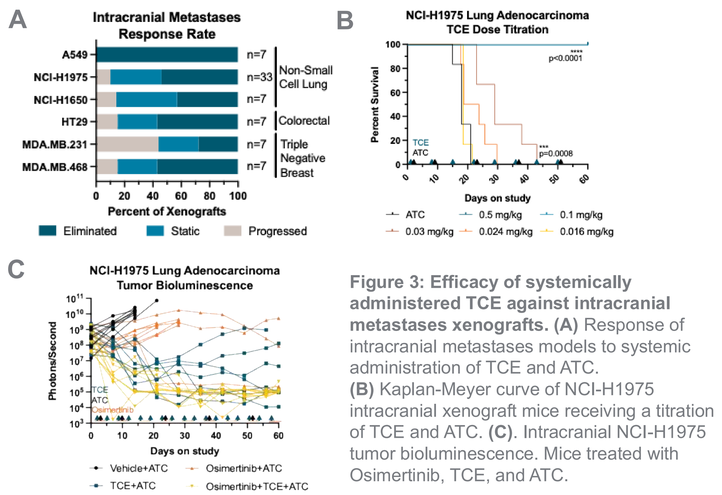

Figure 3: Efficacy of systemically administered TCE against intracranial metastases xenografts. (A) Response of intracranial metastases models to systemic administration of TCE and ATC. **(B)** Kaplan-Meyer curve of NCI-H1975 intracranial xenograft mice receiving a titration of TCE and ATC. **(C)**. Intracranial NCI-H1975 tumor bioluminescence. Mice treated with Osimertinib, TCE, and ATC.

Cytokine Release is Transient and PD-L1$^+$ Cells are Spared in Humanized PD-L1 Mice

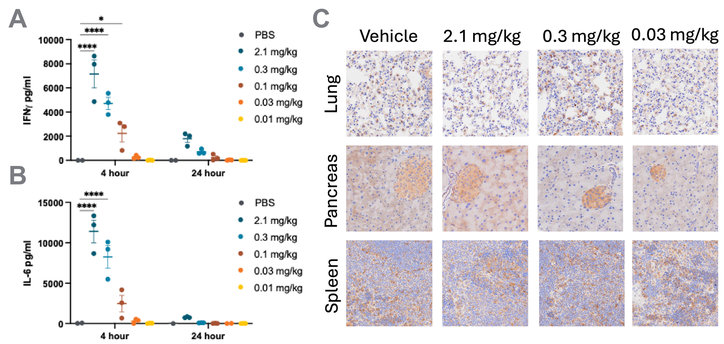

Figure 4: TCE is well tolerated in humanized PD-1/PD-L1 mice. (A-B) serum concentration of IFNγ (A) or IL-6 (B) 4 and 24 hours after TCE administration. **(C)** Immunohistochemistry staining for PD-L1.

Pharmacodynamic Responses Observed in Non-human Primates

Cohort 1: 0.01 mg/kg Dose Level	Cohort 3: 0.3 mg/kg Dose Level
• 0.001mg/kg step dose used in dose 1 • No signs of toxicity observed	• One grade 4 toxicity • Two subjects had transient grade 1-2 toxicity • No pancreatic insufficiency (glucose, lipase, amylase stable) • No pancreatic islet damage by IHC • No respiratory insufficiency • No lung damage by IHC
Cohort 2: 0.03 mg/kg Dose Level	
• Transient elevation of body temperature (<2°C) • Transient lymphopenia • No other signs of toxicity	

Table 1: NHP study results

Conclusions and Future Directions

- PD-L1:CD3 TCE had potent efficacy against intracranial xenograft models representing pediatric and adult primary brain tumors and peripheral tumors with central nervous system metastases.
- TCE was administered to humanized PD-L1 mice without significant toxicity or loss of PD-L1$^+$ cells at doses that eliminated intracranial xenografts.
- TCE administration to non-human primates was tolerated at doses effective in xenograft models.
- Preclinical results from this TCE recommend continued development of a clinical candidate.

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