INTASYL™ PH-762 self-delivering RNAi targeting PD-1 enhances the therapeutic efficacy of systemically administered HER2-targeted CAR-T cells in a SKOV3 model of human ovarian adenocarcinoma in NCG mice

Benjamin Cuiffo, Melissa Maxwell, Dingxue Yan, Brianna Rivest, James Cardia and Simon P. Fricker

Abstract: 431

Phio PHARMACEUTICALS

ASGCT 2021 Virtual ANNUAL MEETING

Abstract

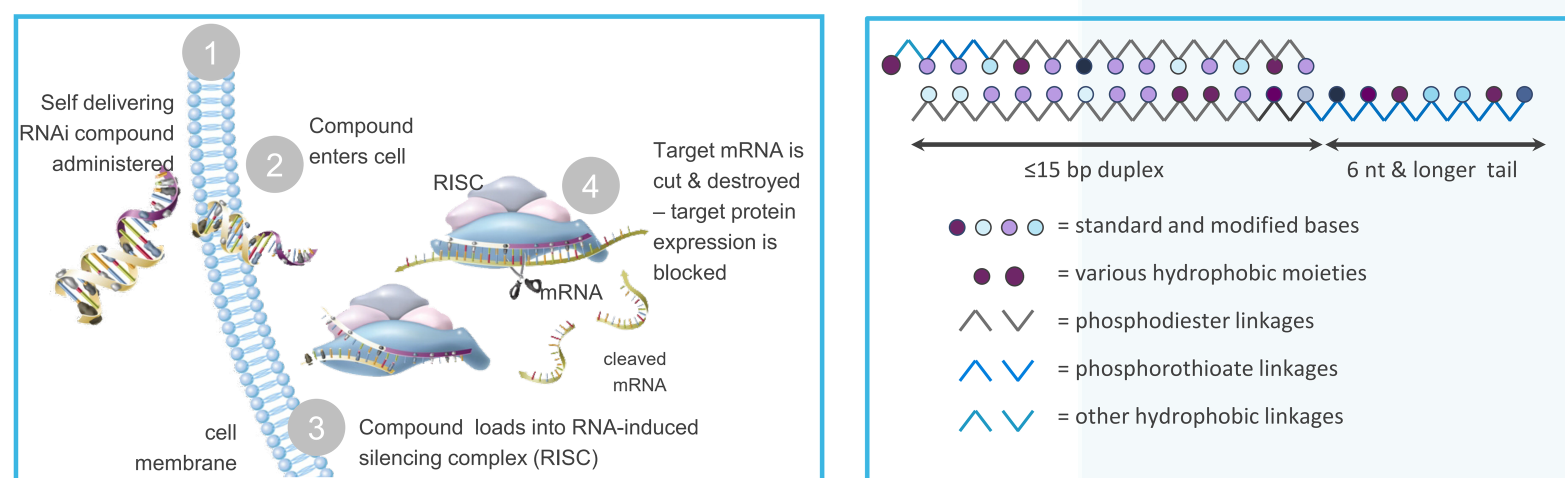
Targeted chimeric antigen receptor engineered T cells (CAR-T) therapies have proven clinically successful in treating hematological malignancies. However, the therapeutic potential of CAR-T in treating solid tumors remains limited, due in part to intrinsic mechanisms of T cell exhaustion triggered by the immunosuppressive tumor microenvironment of solid tumors, including tumor-mediated upregulation of T cell inhibitory receptors such as PD-1. To overcome this hurdle, strategies to functionally silence PD-1 and/or other mediators of T cell exhaustion are under development. Gene editing strategies to permanently delete PD-1 have been demonstrated to enhance CAR-T efficacy against solid tumors in preclinical models. However, an early clinical study showed a very low editing frequency of 5.8% and in edited T cells the median PD-1 expression disruption was <50%, raising doubts about the adequacy of CRISPR-Cas9-mediated PD-1 disruption in T cells[1,2]. In addition, the outlook for gene edited CAR-T therapies remains uncertain due to safety considerations (permanent deletion of T cell suppressive mechanisms and off-target impacts of gene editing to the CAR-T product), regulatory hurdles, manufacturing complexity, and cost.

The INTASYL™ platform is a self-delivering RNAi technology that imparts small molecule-like properties to interfering RNAs, providing efficient delivery (close to 100%) to target cells without need for specialized formulations or drug delivery systems. As such INTASYL can be easily incorporated into CAR-T manufacturing protocols. INTASYL provides robust and highly specific on-target gene silencing. While durable, the effects of INTASYL-mediated silencing are transient, potentially mitigating safety considerations arising from permanent deletion of either T cell suppressors such as PD-1 or off target genes.

Here we assessed the potential of the PD-1 targeted INTASYL PH-762 to enhance the therapeutic efficacy of HER2-targeted CAR-T cells (HER2CART; ProMab) in treatment of a subcutaneous HER2-expressing SKOV3 model of human ovarian cancer in NCG mice. On-target silencing of PD-1 was demonstrated in HER2CART cells *in vitro*. For the *in vivo* study, 5 µM PH-762 was administered to the HER2CART cell product under simultaneous activation with human CD3/CD28-coated beads (simulating a CAR-T manufacturing expansion phase); control HER2CART cells were similarly bead-activated but remained untreated. Twenty-four hours-post INTASYL treatment / activation, a subtherapeutic dose (5e05 cells) of viable INTASYL-treated or untreated CAR-T cells were administered intravenously to mice bearing SKOV3 tumors of ~150 mm³ (N = 4 / group); a control group was administered vehicle only. Tumor volumes and body weights were recorded. Compared to vehicle, HER2CART therapy initially inhibited mean tumor volume growth over the first half of the study, however, tumor volumes of untreated HER2CART therapy groups caught up to those of the vehicle control group by study conclusion (Day 15). In contrast, INTASYL PH-762-treated inoculum of HER2CART cells (PH-762 HER2CART) exerted a statistically significant, robust and durable inhibition of tumor growth. Tumors were collected at endpoint, dissociated, and stained with human-specific antibody panels to uncover PH-762 mediated phenotypic changes to the tumor resident CAR-T cells.

Taken together, these data using a HER2-targeted CAR-T cell product against a HER2-expressing ovarian cancer xenograft provide proof of concept for the application of PD-1 checkpoint inhibition with INTASYL in CAR-T cells prior to adoptive cell transfer to enhance the therapeutic efficacy of CAR-T cell therapy.

Figure 1. INTASYL™ mechanism of silencing and structure



INTASYL compound

PH-762, an INTASYL (self delivering RNAi) compound targeting PD-1 mRNA

USAGE

01 Enhancement of adoptive cell therapies (ACT)

02 Direct injection into tumor microenvironment (TME)[3,4]

[1] Lu *et. al.* Nature Medicine May 2020: 732-40
[2] Rosell *et. al.* Front. In Oncology May 2020: 1726
[3] Cuiffo *et. al.* SITC Annual Meeting 2020, virtual
[4] Cuiffo *et. al.* AACR Annual Meeting 2021, virtual

Property of Phio Pharmaceuticals

PH-762 silences PD-1 and enriches CD8⁺ and CD25⁺ in activated HER2CART cells

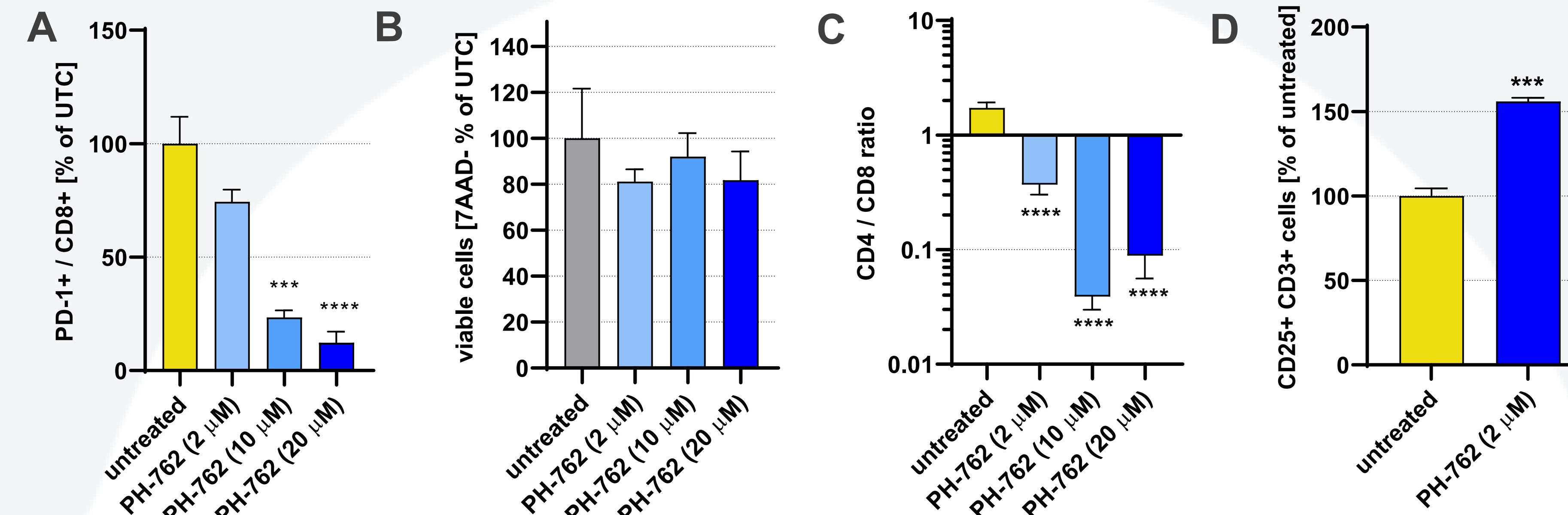


Figure 2. PH-762 silences PD-1 and enriches CD8⁺ and CD25⁺ populations in activated HER2CART cells *in vitro*

A. On-target PD-1 silencing as percentage (%) of untreated CD8 HER2CART cells seven days post-activation by CD3/CD28 beads. PH-762 provided dose associated silencing of it's target PD-1. **B.** Cell viability (number of 7AAD-negative intact (SSC vs FSC) cells) as % of untreated cells seven days post-activation by CD3/CD28 beads. PH-762 did not significantly impact cell viability. **C.** Ratio of CD4⁺ to CD8⁺ cells normalized to untreated cells seven days post-activation by CD3/CD28 beads. PH-762 provided dose associated enrichment of CD8 vs CD4 T cells. **D.** CD25⁺ CD3⁺ cells as % of untreated cells four days post treatment with PH-762 and two days post co-culture with MCF7 cells (1:1). PH-762 enriched for activated cells. Statistical significance was assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests or unpaired two-tailed students t test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

PH-762 enhances the anti-tumor efficacy of HER2CART adoptive cell therapy without impact on tolerability in the SKOV3 model of human ovarian cancer in NCG mice

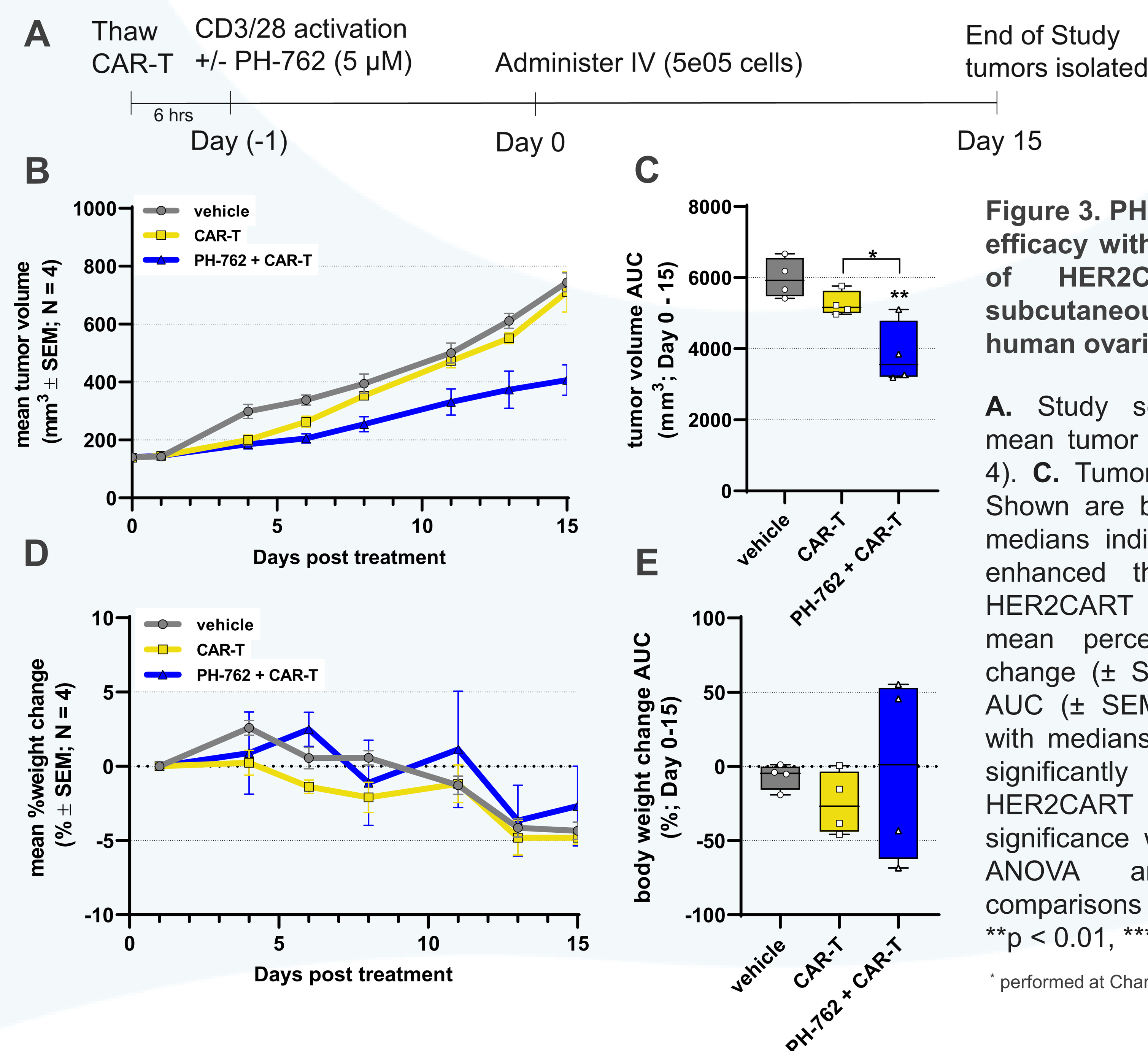


Figure 3. PH-762 improves therapeutic efficacy without impacting tolerability of HER2CART ACT in a subcutaneous SKOV3 model of human ovarian cancer.

A. Study schematic. **B.** Longitudinal mean tumor volume (mm³ ± SEM; N = 4). **C.** Tumor volume AUC (Day 0-15). Shown are box and whisker plots with medians indicated. PH-762 significantly enhanced the anti-tumor efficacy of HER2CART therapy. **D.** Longitudinal mean percentage (%) body weight change (± SEM). **E.** % weight change AUC (± SEM). Box and whisker plots with medians indicated. PH-762 did not significantly impact the tolerability of HER2CART therapy. Statistical significance was assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

* performed at Charles River Laboratories, Worcester, MA

Silencing of PD-1 in HER2CART cells by PH-762 results in a more active cell phenotype in the tumor microenvironment

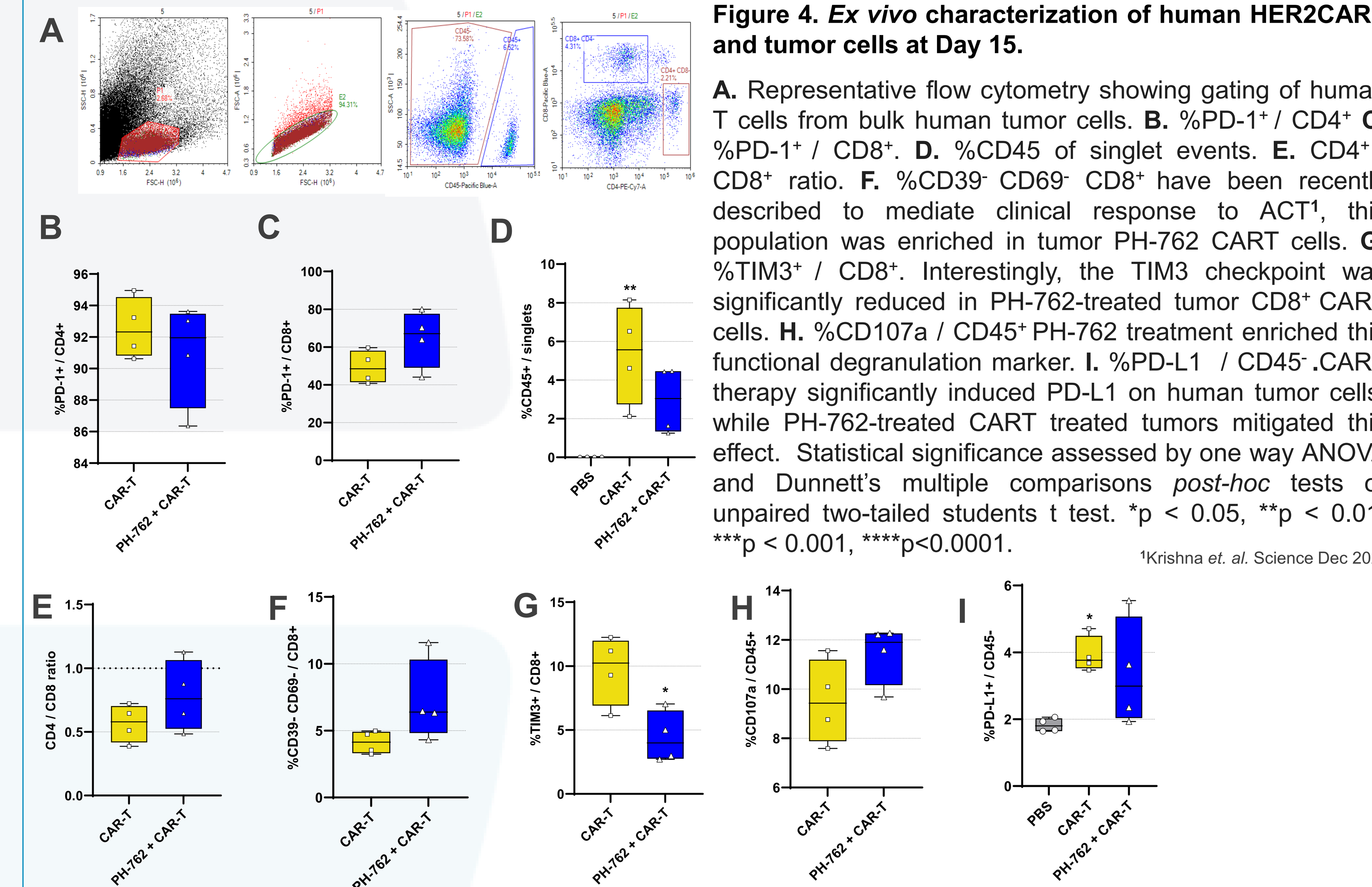


Figure 4. *Ex vivo* characterization of human HER2CART and tumor cells at Day 15.

A. Representative flow cytometry showing gating of human T cells from bulk human tumor cells. **B.** %PD-1⁺ / CD4⁺ **C.** %PD-1⁺ / CD8⁺. **D.** %CD45 of singlet events. **E.** CD4⁺ / CD8⁺ ratio. **F.** %CD39⁻ CD69⁻ CD8⁺ have been recently described to mediate clinical response to ACT[1], this population was enriched in tumor PH-762 CART cells. **G.** %TIM3⁺ / CD8⁺. Interestingly, the TIM3 checkpoint was significantly reduced in PH-762-treated tumor CD8⁺ CART cells. **H.** %CD107a / CD45⁺ PH-762 treatment enriched this functional degranulation marker. **I.** %PD-L1 / CD45⁻ .CART therapy significantly induced PD-L1 on human tumor cells, while PH-762-treated CART treated tumors mitigated this effect. Statistical significance assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests or unpaired two-tailed students t test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

[1] Krishna *et. al.* Science Dec 2020.

PH-762 enriches antigen-exposed HER2CART cells for memory / stem markers

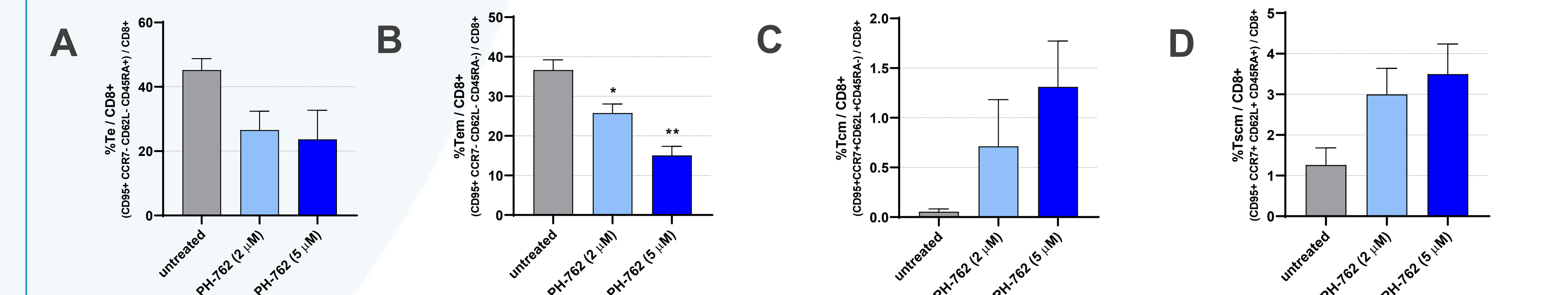


Figure 5. PH-762 promotes CD8 memory / stem phenotype in HER2CART co-cultured with MCF7 tumor cells.

HER2CART cells were cultured with MCF7 cells for 13 days. T cell differentiation phenotypic markers were assessed by immunostaining / flow cytometry. **A.** %CD95⁺CD45RA⁺CCR7⁻CD62L⁻ / CD8⁺ effector T (Te) cells **B.** %CD95⁺CD45RA⁻CCR7⁻CD62L⁻ / CD8⁺ effector memory T (Tem) cells **C.** %CD95⁺CD45RA⁻CCR7⁺CD62L⁺ / CD8⁺ central memory T (Tcm) cells **D.** %CD95⁺CD45RA⁺CCR7⁺CD62L⁺ / CD8⁺ memory stem T (Tscm) cells Statistical significance assessed by one way ANOVA and Dunnett's multiple comparisons *post-hoc* tests *$p < 0.05$, **$p < 0.01$.

Conclusions

- **PH-762 significantly enhanced the antitumor efficacy of HER2CART cells *in vivo*, with no impact on tolerability of the therapy.**
- **PH-762 silenced PD-1 in a dose associated manner in activated HERCART cells up to ~90%, without significant impact on viability, while enriching for CD8⁺ and CD25⁺ cells.**
- ***Ex vivo* analyses of PH-762-treated HER2CART cells isolated from tumors suggest PH-762 may enhance CAR-T function through multiple mechanisms including enhanced efficiency, function (degranulation), decreased suppressive potential, and promotion of memory / stem populations.**
- **T cell memory / stem differentiation markers were promoted, and terminal differentiation markers reduced on PH-762-treated HER2CART in an *in vitro* coculture assay with MCF7 tumor cells.**