Self-Delivering RNAi Targeting BRD4 (PH-894) Improves the Phenotype of HER2-CAR-T Cells During Expansion

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Abstract# 222

Abstract

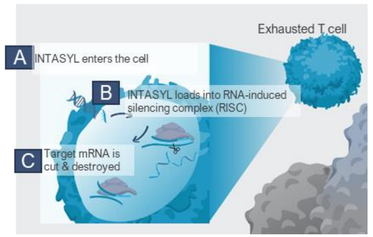
Chimeric antigen receptor (CAR) T cell therapies can elicit remarkable responses toward treating hematological malignancies. However, the full therapeutic potential of CAR-T in treating solid tumors remains unrealized. Strategies under investigation to improve CAR-T therapeutic efficacy include *ex vivo* intervention during manufacture to improve the quality and potency of the CAR-T product. The activation and expansion phases of a typical CAR-T manufacturing process with anti-CD3/CD28 antibody coated beads or similar are known to induce exhaustion and differentiation phenotypes, which have a negative impact on the potency of the final therapeutic product. Gene therapy approaches have shown promise in this area; however, obstacles remain including safety considerations deriving from the permanent deletion of T cell suppressive mechanisms, potential off-target impacts of gene editing the cell product, and technical challenges of low editing efficiencies and process integration.

The INTASYL™ platform is a self-delivering RNAi technology that imparts small molecule-like properties to siRNAs, providing efficient delivery to target cells without need for specialized formulations or drug delivery systems, allowing seamless incorporation 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 T cell suppressor mechanisms.

Here we assessed the potential of the BRD4-targeted INTASYL PH-894 to improve the quality and potency of HER2-targeted CAR-T cells (HER2-CAR-T) in a 19-day model of CAR-T expansion. HER2-CAR-T cells were activated with plate-bound OKT3 antibody (10 ng) and anti-CD3/CD28 beads on Day 0 and Day 6 respectively and treated with PH-894 (2 µM), a non-targeting control INTASYL (NTC; 2 µM), or vehicle (PBS) on Day 0 and Day 5. PH-894 reduced the expansion-associated induction of BRD4 and BRD4-regulated MYC. Additionally, PH-894 mitigated the induction of inhibitory receptors and markers of T cell exhaustion, TIGIT and TIM3, on $CD8^+$ HER2-CAR-T cells and secretion of expansion-induced immunosuppressive IL-10. PH-894 elicited durable repression of PD-1 on $CD4^+$ HER2-CAR-T cells (through Day 19). Finally, PH-894 preserved putative T cell stem-cell memory (T_{scm} defined by $CCR7^+CD62L^+CD95^+CD45RA^+$) and central memory ($T_{cm}$ defined by $CCR7^+CD62L^+CD95^+CD45RA^-$) $CD8^+$, phenotypes associated with cell persistence, on HER2-CAR-T populations at Day 19 that were otherwise depleted by cell expansion without PH-894 use.

These data provide proof-of-concept to suggest that silencing BRD4 with INTASYL PH-894 can be used to improve the phenotype of CAR-T during activation and expansion phases of their manufacturing process by reducing exhaustion, immunosuppression, and conferring a phenotype associated with cell persistence, thus serving to improve the quality of a final CAR-T cell product. Follow-up studies are ongoing on the use of PH-894 in T cell adoptive cell therapy.

PH-894 is a self-delivering RNAi therapy (INTASYL™) designed to specifically target BRD4



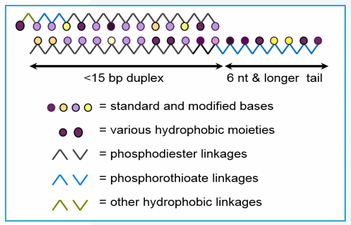


Figure 1. INTASYL™ mechanism of silencing and structure

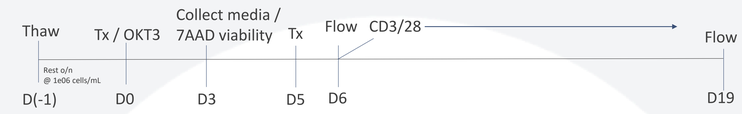


Figure 2. Experiment Design

Schematic of the CAR-T expansion model with INTASYL™ treatment (Tx; 2 µM) and readout timepoints indicated.

PH-894 suppresses induction of its target BRD4 along with downstream MYC and suppressive checkpoint molecules TIGIT and TIM3 in $CD8^+$ HER2-CAR-T cells during CAR-T expansion

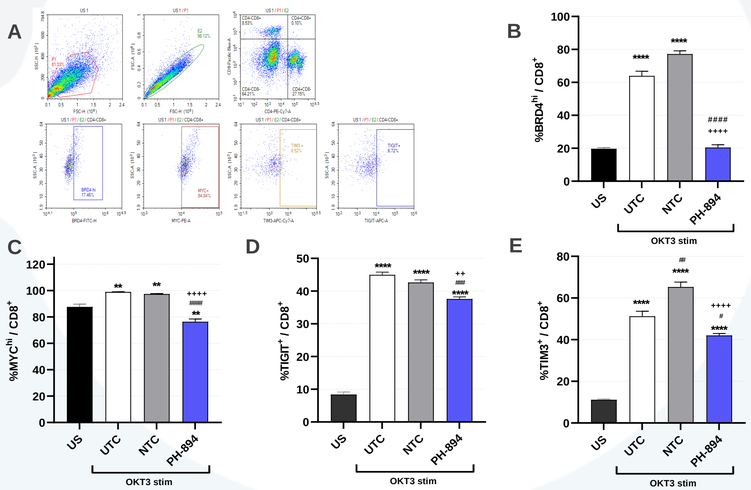


Figure 3. PH-894 suppressed induction of its target BRD4 along with downstream MYC and immune suppressive checkpoint molecules TIGIT and TIM3 in $CD8^+$ HER2-CAR-T cells by flow cytometry on Day 6 post Tx / stimulation in the expansion.

Flow cytometry from Day 6 (D6) post Tx with 2 µM PH-894 and stimulation with 10 ng CD3-agonist antibody (OKT3). **A.** Representative flow gating. **B.-E.** D6 group means ± SEM (n = 3) are shown. **B.** $BRD4^{hi}$ $CD8^+$ HER2-CAR-T cells. **C.** MYC^{hi} $CD8^+$ HER2-CAR-T cells. **D.** $TIGIT^+$ $CD8^+$ HER2-CAR-T cells. **E.** $TIM3^+$ $CD8^+$ HER2-CAR-T cells. PH-894 Tx resulted in statistically significant suppression of OKT3-induced BRD4 levels along with downstream MYC and immune checkpoints TIGIT and TIM3 compared to OKT3-stimulated but untreated control (UTC) or compared to Tx with a chemically-identical but nontargeting control INTASYL (NTC). Statistical significance of differences in means were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001; ***p <0.001; **p <0.01; *p <0.05; * = vs US; # = vs UTC; + = vs NTC.

PH-894 suppresses expansion-induced secretion of immune-suppressive IL-10 from HER2-CAR-T cells

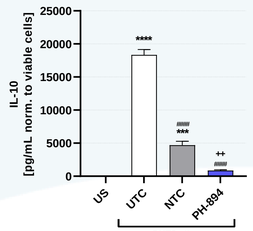


Figure 4. Secretion of IL-10 by HER2-CAR-T cells was assessed by Cytometric Bead Array (CBA) assay.

Cytometric bead array (CBA) assay normalized for equivalent numbers of viable cells detected IL-10 secreted by HER2-CAR-T cells in the conditioned media on Day 3 post Tx/OKT3-stimulation. PH-894 Tx resulted in statistically significant suppression of secretion of stimulation-induced IL-10 levels compared to UTC or compared to NTC. Group means ± SEM (n = 3) are shown. Statistical significance of differences in means were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001; ***p <0.001; **p <0.01; *p <0.05; * = vs US; # = vs UTC; + = vs NTC.

PH-894 treatment reduced PD-1 on $CD4^+$ and $CD8^+$ HER2-CAR-T cells and increased $CD69^-$ $CD39^-$ stem-like $CD8^+$ HER2-CAR-T cells in the expansion product (Day 19)

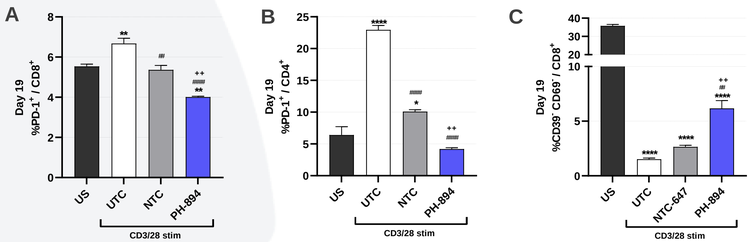


Figure 5. Effect of PH-894 treatment on HER2-CAR-T expansion product phenotype at Day 19.

Flow cytometry data shows D19 post-REP group means ± SEM (n = 3). **A.** $PD\text{-}1^+$ $CD8^+$ HER2-CAR-T cells. **B.** $PD\text{-}1^+$ $CD4^+$ HER2-CAR-T cells. **C.** $CD39^-$ $CD69^-$ $CD8^+$ HER2-CAR-T cells. PH-894 Tx on D0 and D5 resulted in statistically significant suppression of REP-induced PD-1 on both $CD4^+$ and $CD8^+$ HER2-CAR-T cells and enhanced stem-like $CD39^-$ $CD69^-$ $CD8^+$ HER2-CAR-T cell product post-REP (Day 19) compared to UTC or compared to NTC. Statistical significance of differences in mean were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001; ***p <0.001; **p <0.01; *p <0.05; * = vs US; # = vs UTC; + = vs NTC.

PH-894 preserved putative T_{scm} and T_{cm} $CD8^+$ HER2-CAR-T cell populations in the expansion product (Day 19)

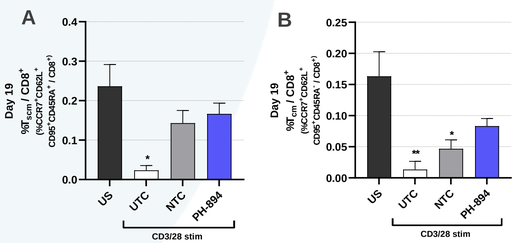


Figure 6. Effect of PH-894 treatment on $CD8^+$ memory T cell populations in the HER2-CAR-T cell expansion product (Day 19).

Flow cytometry data shows D19 group means ± SEM (n = 3). **A.** Putative T stem cell memory (T_{scm}; $CCR7^+$ $CD62L^+$ $CD95^+$ $CD45RA^+$) $CD8^+$ HER2-CAR-T cells. **B.** Putative T central memory (T_{cm}; $CCR7^+CD62L^+CD95^+CD45RA^-$) $CD8^+$ HER2-CAR-T cells. While activation/expansion significantly depleted these populations in the final cell product, PH-894 Tx on D0 and D5 preserved these populations in the Day 19 expansion product. Statistical significance of differences in means were assessed by one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ****p<0.0001; ***p <0.001; **p <0.01; *p <0.05; * = vs US.

Conclusions

- PH-894 treatment during expansion of HER2-CAR-T cells with OKT3 and CD3/28 activator beads demonstrated improved phenotype of the final cell product.
- PH-894 suppressed induction of its target BRD4 along with downstream MYC and suppressive checkpoint molecules TIGIT and TIM3 in $CD8^+$ HER2-CAR-T cells.
- PH-894 suppressed activation-induced secretion of immune-suppressive IL-10 from HER2-CAR-T cells.
- PH-894 reduced PD-1 on $CD4^+$ and $CD8^+$ HER2-CAR-T cells and increased $CD69^-$ $CD39^-$ stem-like $CD8^+$ in the HER2-CAR-T cell expansion product.
- PH-894 preserved putative T_{scm} and T_{cm} $CD8^+$ HER2-CAR-T cell populations in the expansion product.
- These data suggest that silencing BRD4 with INTASYL PH-894 can be used to improve the phenotype of CAR-T cell products during activation/expansion phases of the manufacturing process.