Increasing activation of human tumor-reactive T cells (CD39+CD103+CD8+) by gene silencing of PD1 with self-delivering RNAi INTASYL™

Colin J. Thalhofer[1], Ryan Montler[1], Melissa Maxwell[3], Dingxue Yan[3], James Cardia[3], Simon P. Fricker[3], Nicholas Morris[1], Jacob Moses[1], Joshua F. Rios[1], Tarsem Moudgil[2], Bernard A. Fox[2], Richard B. Bell[2], Andrew D. Weinberg[1,2]

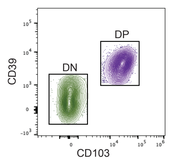
[1]AgonOx, Inc., Portland, OR, USA; [2]EACRI, Providence Cancer Center, Portland, OR, USA; [3]Phio Pharmaceuticals, Marlborough, MA, USA

Introduction

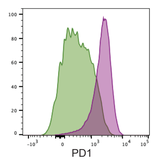
- Tumor Infiltrating Lymphocyte (TIL) therapy has proven effective for patients with stage IV melanoma, however there are critical issues that can limit the efficacy of standard TIL therapy across a wide range of different malignancies.
- We and others have shown that some tumor types contain a low percentage of tumor-specific T cells. We hypothesize that most of the patients that do not respond to TIL therapy are likely receiving a low percentage of tumor-reactive T cells and therefore a high percentage of non-therapeutic bystander TIL.
- We have developed a streamlined method that expands a highly enriched fraction of tumor-reactive T cells contained within the CD39+CD103+CD8+ TIL in greater than 90% of patient samples from a wide variety of malignancies (melanoma, colon cancer, head and neck cancer, etc.). This TIL product displays a broad repertoire of tumor-specific TCRs. The expanded CD39/CD103 TIL can kill autologous tumors in vitro, but the possibility remains that they could revert to a suppressed or exhausted state when they reach the tumor microenvironment upon transfer back into patients.
- To mitigate the suppressive effects of the tumor microenvironment we have evaluated Phio Pharmaceuticals' self-delivering RNAi INTASYL™ platform to silence PD1 in the expanded TIL product with PH-762.
- The TIL product was treated during the rapid expansion phase of the protocol with either nontargeting control compounds (**NTC**) or PD1 targeting INTASYL™ compounds. PD1 protein levels and TIL functionality were assessed via flow cytometry and cytokine bead array.

Background

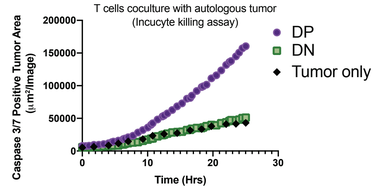
CD8+ human TIL subsets



High expression of PD1 in DP TIL



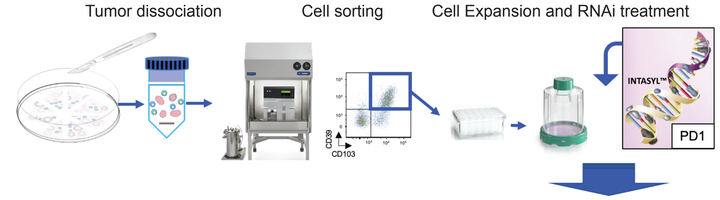
Expanded DP (CD39+CD103+) T cells are tumor-reactive



Hypothesis

We hypothesized that treating our tumor-reactive DP T cells with self-delivering RNAi INTASYL™ compounds targeting *PDCD1* mRNA (PH-762) during rapid expansion would result in reduced PD1 expression, an enhanced functional phenotype, and a more potent adoptive cell therapy product.

Experimental Methods



1 PD1 modulation with RNAi

Dose response in DP T cells to *PDCD1*-targeting INTASYL™

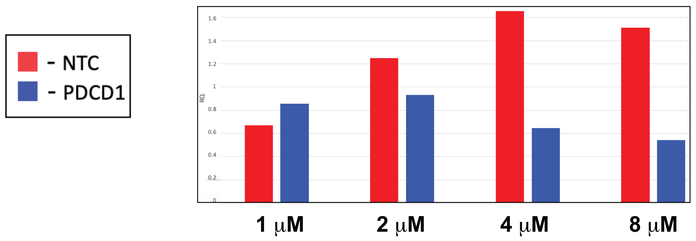
- **A** *PDCD1* mRNA expression by PCR
- **B** PD1 protein expression by flow cytometry
  - 24 hours post-restimulation with aCD3/CD28/CD2
  - PD1 expression time-course post-1st and 2nd stim.

2 Testing T cell Functionality

- **C**
  - T cell proliferation fold expansion
  - Activation/Expression - TCR stimulation anti-CD3
- **D**
  - Coculture with autologous tumor cells
  - Autologous tumor killing by Incucyte
  - Activation markers by flow cytometry
  - Cytokine production by bead array (CBA)

1 PD1 knock-down in expanded CD39+CD103+CD8+ (DP) T cells

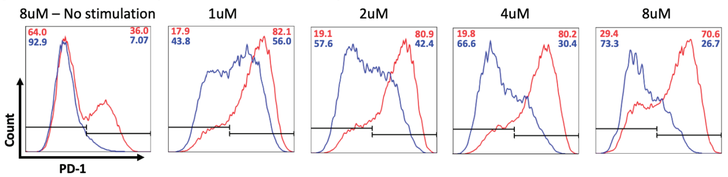
A *PDCD1*-targeted INTASYL™ mRNA silencing is dose-dependent



- DP T cells were expanded for and treated with either Non-targeting control oligos (NTC) or *PDCD1*-targeting RNAi oligos for the last 7 days and *PDCD1* mRNA was evaluted by qPCR
- Relative *PDCD1* gene expression was determined by qPCR after a 14 day REP

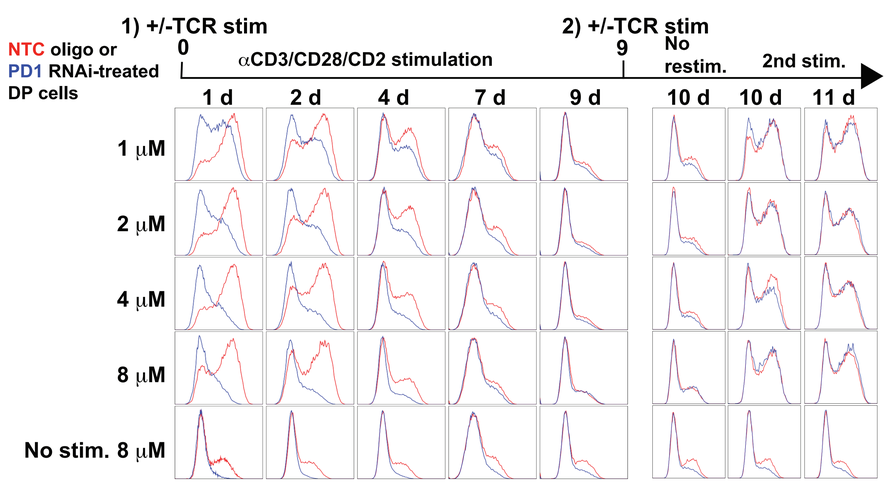
B PD1 protein knock-down is dose-dependent

- Sorted, expanded, and RNAi-treated DP T cells from a colon cancer patient were analyzed for PD1 expression 24 hours post-restimulation with αCD3/CD28/CD2



- PD1 protein expression was analyzed by flow cytometry the frequency of DP T cells that are PD1**(+)** upper right or PD1**(-)** upper corners left (NTC or *PDCD1*)

- PD1 protein knock-down is sustained for up to seven days following 1st stim.



- PD1 protein dose-dependent protein knock-down was observed for up to 7 days and returned to normal levels upon 2nd restim.

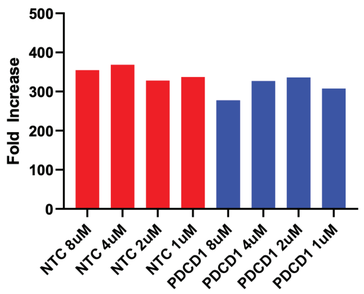
2 DP T cell Functionality after Treatment with *PDCD1*-targeting RNAi INTASYL™

C *PDCD1*-targeted INTASYL™ mRNA silencing does not inhibit T cell proliferation during REP

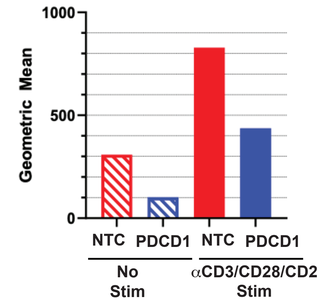
Two Week Rapid Expansion Protocol in Grex Plates

- DP T cells from a melanoma cancer patient were sorted and expanded in Grex plates and treated with NTC oligos or *PDCD1*-targeting RNAi INTASYL™
- *PDCD1*-targeting RNAi INTASYL™ treated DP cells expanded to equivalent numbers during the 14 day REP
- As observed in the DP from a colon cancer patient in (B) we also observed that *PDCD1*-targeting RNAi INTASYL™ treated DP cells from a melanoma patient was able to knock down PD1 protein levels

- Fold Expansion of DP T cells

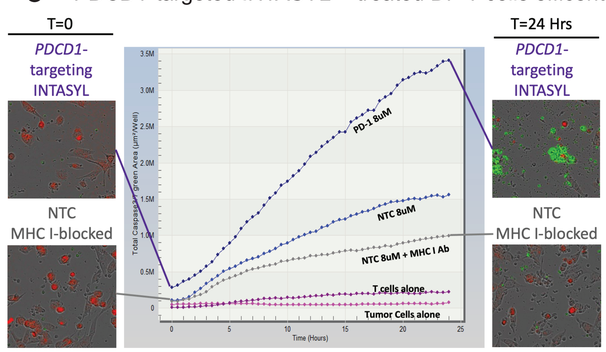


- PD1 expression Post-REP 48hr after anti-CD3/CD28 Restimulation



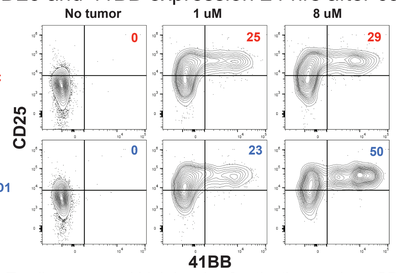
D DP T cells treated with *PDCD1*-targeted INTASYL™ are highly functional when cocultured with autologous tumor cells

- *PDCD1*-targeted INTASYL™ treated DP T cells efficently kill autologous tumor cells



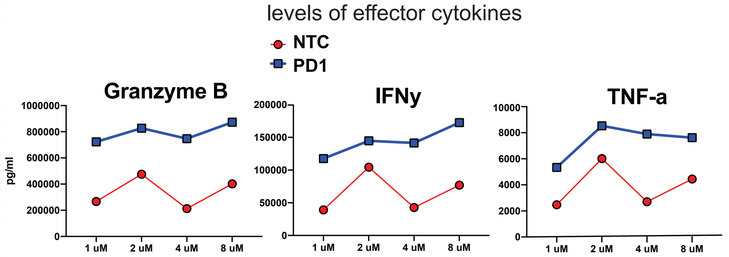
- Melanoma patient-derived DP T cells treated with NTC or *PDCD1*-targeted RNAi compounds during REP as in (C) and then cocultured with autologous tumor cells
- Autologous tumor cell killing was analyzed by labeling patient-derived autologous tumor cells with Cytolight Rapid Red and killing was observed using Caspase 3/7 green

- CD25 and 41BB expression 24 hrs after coculture



- DP T cells expressed high levels of activation markers CD25 and 41BB in the presence of autologous tumor

- DP T cells treated with *PDCD1*-INTASYL™ produced high levels of effector cytokines



- Cytokine production was analyzed 24 hrs after coculture by cytokine bead array
- DP T cells treated with NTC oligos or *PDCD1*-targeting RNAi INTASYL™ were both able to become activated and produce effector cytokines in response to being cocultured with autologous tumor cells.

Conclusions

- We have found that CD39+CD103+CD8+ (DP) patient-derived TIL are highly enriched for tumor-reactivity, can be efficently isolated and expanded in vitro.
- Silencing of PD1 expression in the expanded TIL product was obtained by adding the self-delivering RNAi compounds to the cell culture media, without needing transfection media, delivery formulations or electroporation.
- The *PDCD1*-targeted INTASYL™ (PH-762) treatment yielded a TIL product that responded to autologous tumor with increased production of IFN-γ, TNF-α and Granzyme B, activation marker expression (CD25 and 41BB), and efficent killing relative the the NTC-treated cells.

Future Directions

These data highlight a promising combination to improve the activity of tumor-reactive TIL in future human clinical trials.

Acknowledgements

We would like to thank the members of the EACRI community, especially the Flow Core, Clinical Trial Team, and Cancer Research Animal Division (CRAD) with special thanks to Mark Schmidt, Stacy (Christine) Spencer, and Miranda Gilchrist.