Silencing PD-1 using PH-762 (PD-1 targeting INTASYL compound) to improve Iovance TIL effector function using Gen 2 manufacturing method

Inbar Azoulay Alfaguter[1], Michelle Simpson-Abelson[1], Krit Ritthipichai[1], Kenneth D'Arigo[1], Florangel Hilton[1], Marcus Machin[1], Dingxue Yan[2], James Cardia[2], Maria Fardis[1], and Cécile Chartier[1]

[1]Iovance Biotherapeutics, Inc. Tampa, FL and San Carlos, CA, USA; [2]Phio Pharmaceuticals Marlborough, MA, USA

IOVANCE BIOTHERAPEUTICS

ADVANCING IMMUNO-ONCOLOGY

999 Skyway Road, STE 150, San Carlos, CA 94070

For more information, please contact kellyditrapani@iovance.com

BACKGROUND

- Adoptive T-cell transfer with tumor infiltrating lymphocytes (TIL) is an investigational immunotherapy for advanced solid cancers. Ongoing Phase II clinical trials of Iovance's lifileucel and LN-145 TIL products have demonstrated efficacy with ORRs of 36.4% and 44% in patients with melanoma and cervical cancer, respectively.[1,2]
- Anti-PD-1 therapy has been widely used as a first-line therapy in several types of cancer. TIL infusion products from the patients previously treated with anti-PD-1 therapy still sustain PD-1 expression, especially the subset of tumor antigen-specific TIL.[3]
- Building on the therapeutic efficacy of PD-1 blockade, we reasoned that intrinsic silencing of PD-1 in our TIL products, may provide similar benefits to systemic administration of anti-PD-1 therapy, while decreasing the side effects associated with systemic anti-PD-1.[3]
- INTASYL compounds are self-delivering chemically modified siRNAs, which have the ability to penetrate cells and silence specific target genes with high efficiency.[4] Furthermore, a silencing approach yielding a transient transcript knockdown, may prove favorable when compared with permanent genetic modification in the context of T cell regulation.
- Here, we tested the silencing efficiency of a PD-1-targeted INTASYL compound, termed PH-762, in TIL and its effect on TIL phenotype and function.

[1] Sarnaik A, et al. Safety and efficacy of cryopreserved autologous tumor infiltrating lymphocyte therapy (LN-144, lifileucel) in advanced metastatic melanoma patients who progressed on multiple prior therapies including anti-PD-1. *J Clin Oncol*. 2019;37:2518-2518.

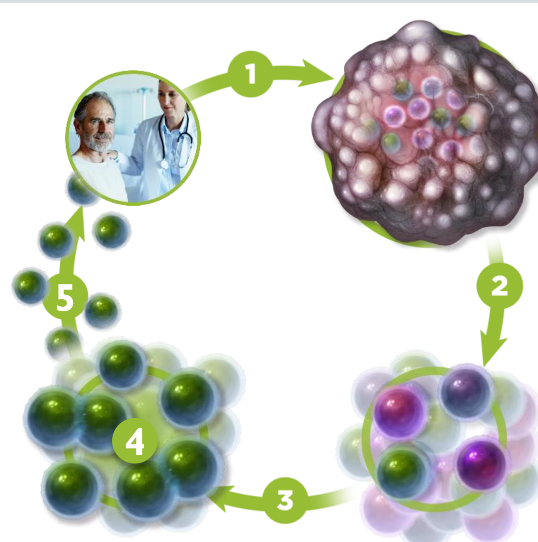
[2] Jazaeri A , et al. Safety and efficacy of adoptive cell transfer using autologous tumor infiltrating lymphocytes (LN-145) for treatment of recurrent, metastatic, or persistent cervical carcinoma. *J ClinOncol*. 2019;37:2538-2538.

[3] Gros A, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *J Clin Invest*. 2014;124:2246-2259.

[4] Ligtenberg M A, et al. Self-Delivering RNAi Targeting PD-1 Improves Tumor-Specific T Cell Functionality for Adoptive Cell Therapy of Malignant Melanoma. *Mol Ther*. 2018;26:1482-1493.

OVERVIEW OF TIL THERAPY PROCESS

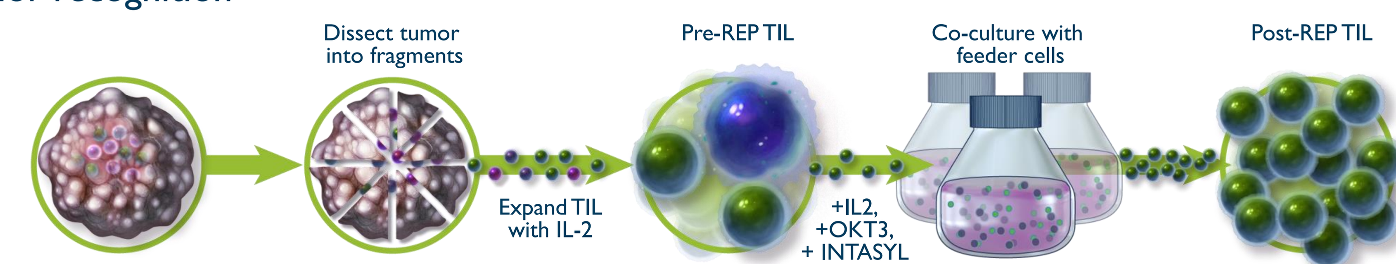
1. The tumor is excised from the patient and transported to the GMP manufacturing facility.
2. Upon arrival, the tumor is fragmented and placed in flasks with IL-2 for a pre-Rapid Expansion protocol (pre-REP).
3. Pre-REP TIL are further propagated in a REP protocol in the presence of irradiated PBMCs, anti-CD3 antibody, and IL-2.
4. TIL products are assessed for phenotype and effector function.
5. Prior to infusion of expanded TIL, patients receive a non-myeloablative lymphodepletion regimen consisting of cyclophosphamide and fludarabine. Following infusion of TIL, patients receive a short duration of high-dose IL-2 to support growth and engraftment of transferred TIL.



Experimental Design

Tumor samples derived from melanoma, head and neck, sarcoma, lung, and breast cancers were subjected to research-scale pre-REP. Pre-REP TIL were subsequently expanded in the presence or absence of either non-targeting control (NTC) or PH-762 (PD-1 targeting) INTASYL compounds. Post-REP TIL were analyzed for:

- Knockdown (KD) efficiency
- Comprehensive phenotypic and functional characterization
- Tumor recognition



RESULTS

Figure 1. High knockdown efficiency of PD-1 was observed in TIL products from multiple solid tumor histologies

Evaluation of PD-1 knockdown in TILs. TILs expanded with non-targeting control (NTC) or PH-762 (PD-1 targeting) INTASYL compounds, were stained for PD-1 and analyzed by flow cytometry. **(A)** Representative pseudo color plots depicting PD-1 reduction in PH-762-treated melanoma TIL. **(B)** Nineteen tumor samples from 5 histologies (melanoma n=8, breast cancer n=6, lung n=3, head and neck n=1, sarcoma n=1) were used to derive PH-762 TIL. Percentage of PD-1 KD was calculated by the formula: (1-(% of CD3+PD-1+ in PH-762 TIL / % of CD3+PD-1+ in NTC TIL)) x100. Average percentages of KD efficiency ± SEM are plotted for each histology.

Highly efficient PD-1 KD ranging from 68 to 95 % (with an average of 85%) was obtained in TIL treated with PH-762.

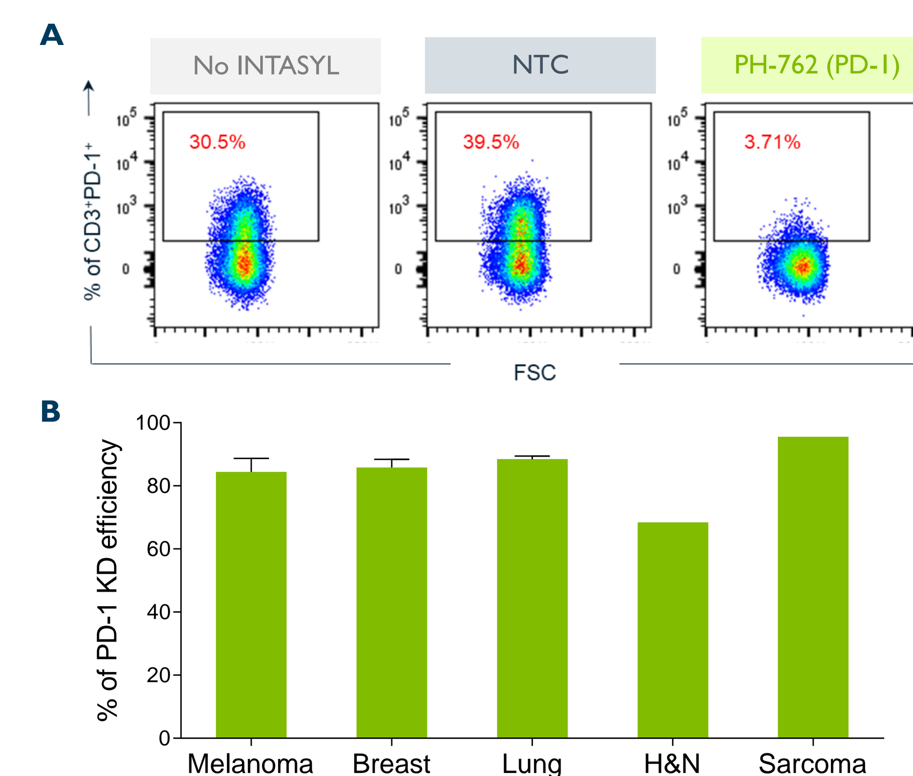


Figure 2. Knockdown efficiency of PD-1 mediated by PH-762 was sustained for seven days after TIL harvest

Assessment of PD-1 KD efficiency in TIL after harvest. TIL expanded with non-targeting control (NTC) or PH-762 (targeting PD-1). **(A)** PD-1 levels were monitored by flow cytometry for 7 days in post-REP TIL samples derived from melanoma, lung, and breast cancers (n=3). Results are shown as percentage of PD-1 KD efficiency calculated by the formula described in Figure 1B. Error bars are shown as SEM. Unpaired t-test revealed no statistically significant differences between the time points.

Subtle decrease in PD-1 KD efficiency was observed on day 7 after harvest.

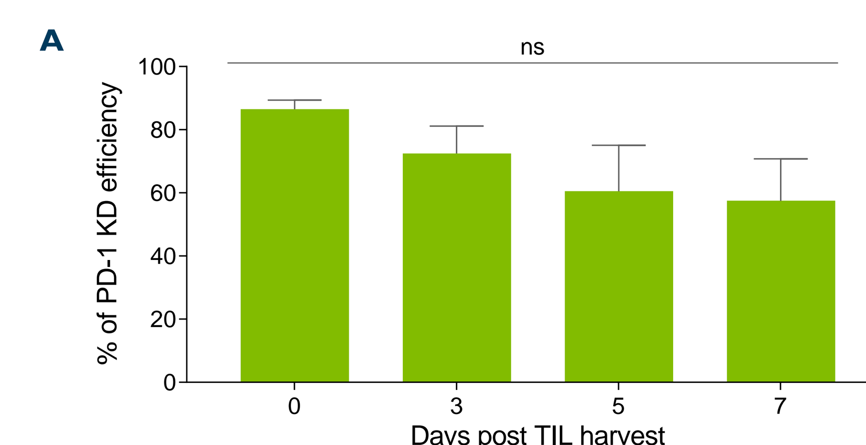


Figure 3. TIL expanded with PH-762 exhibited similar phenotype relative to controls

Phenotype analysis of TIL expanded with PH-762. TIL expanded with non-targeting control (NTC) or PH-762 (PD-1 targeting) INTASYL compounds were stained for T-cell lineage (CD3, CD4, and CD8) and memory markers (CD45RA and CCR7) and analyzed by flow cytometry. **(A)** Percentages of $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cell subsets generated from four different histologies including melanoma (n=3), breast (n=4), lung (n=3), and sarcoma (n=1) are shown in bars with vertical lines indicating standard errors (SEM). ns indicates not significant. **(B)** Average percentages of memory T-cell subsets (Effector memory; TEM, Central memory; TCM, and Effector memory RA; TEMRA) are shown in bars with vertical lines indicating standard errors(SEM).

PH-762 (PD-1 targeting INTASYL compound) treatment had no impact on T-cell lineage and memory T-cell subsets.

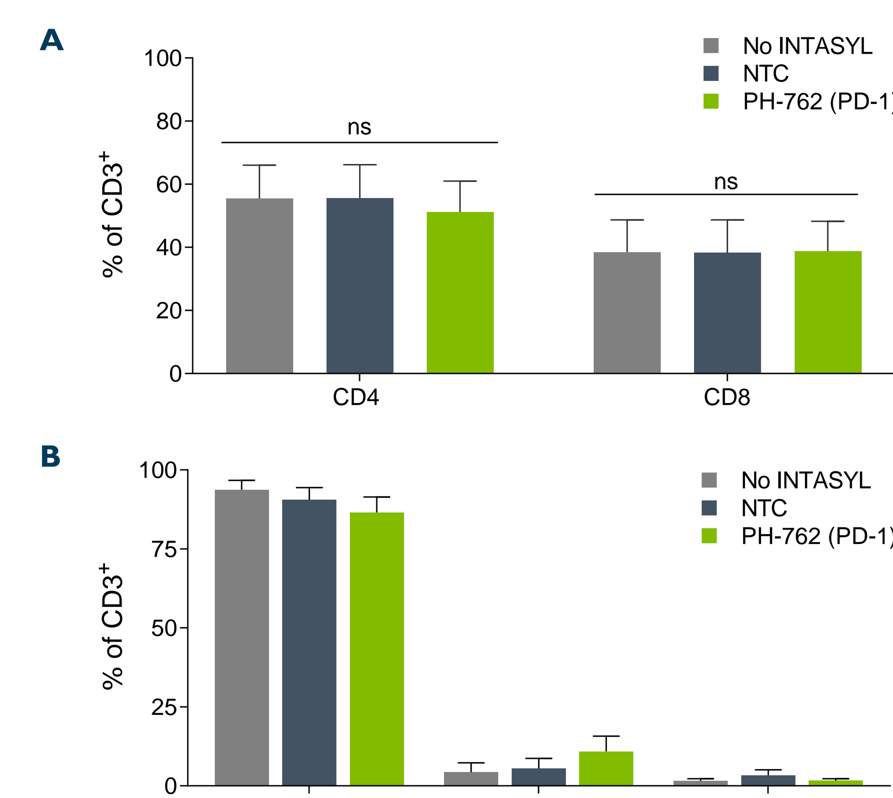


Figure 4. PD-1 knockdown TIL displayed activated T-cell phenotype while co-inhibitory/exhaustion markers remained unchanged

Differentiation, activation, and exhaustion status of PD-1silenced TIL. TIL from 5 histologies including melanoma, breast, sarcoma head and neck and lung were expanded with PH-762 (PD-1 targeting) or non-targeting control (NTC) INTASYL compounds (n≥5). Post-REP TIL were stained for differentiation markers (CD27, CD28, and CD56), activation markers (CD25, 4-1BB, and OX40), and exhaustion markers (TIM-3, LAG-3, TIGIT, and DNAM) and analyzed by flow cytometry. The fold changes of differentiation markers **(A)**, activation markers **(B)**, and exhaustion markers **(C)** are depicted in bars with vertical lines indicating standard errors (SEM). Fold changes were calculated as the ratio of protein expression in TIL expanded with PH-762 (PD-1 targeting) and non targeting control (NTC) INTASYL compounds.

** indicates p-value<0.01. Statistical significance was determined by unpaired t-test.

An increased expression of 4-1BB and OX40 in TIL expanded with PH-762 (PD-1 targeting INTASYL compound) showed activated T-cell phenotype while differentiation and exhaustion status was unaffected.

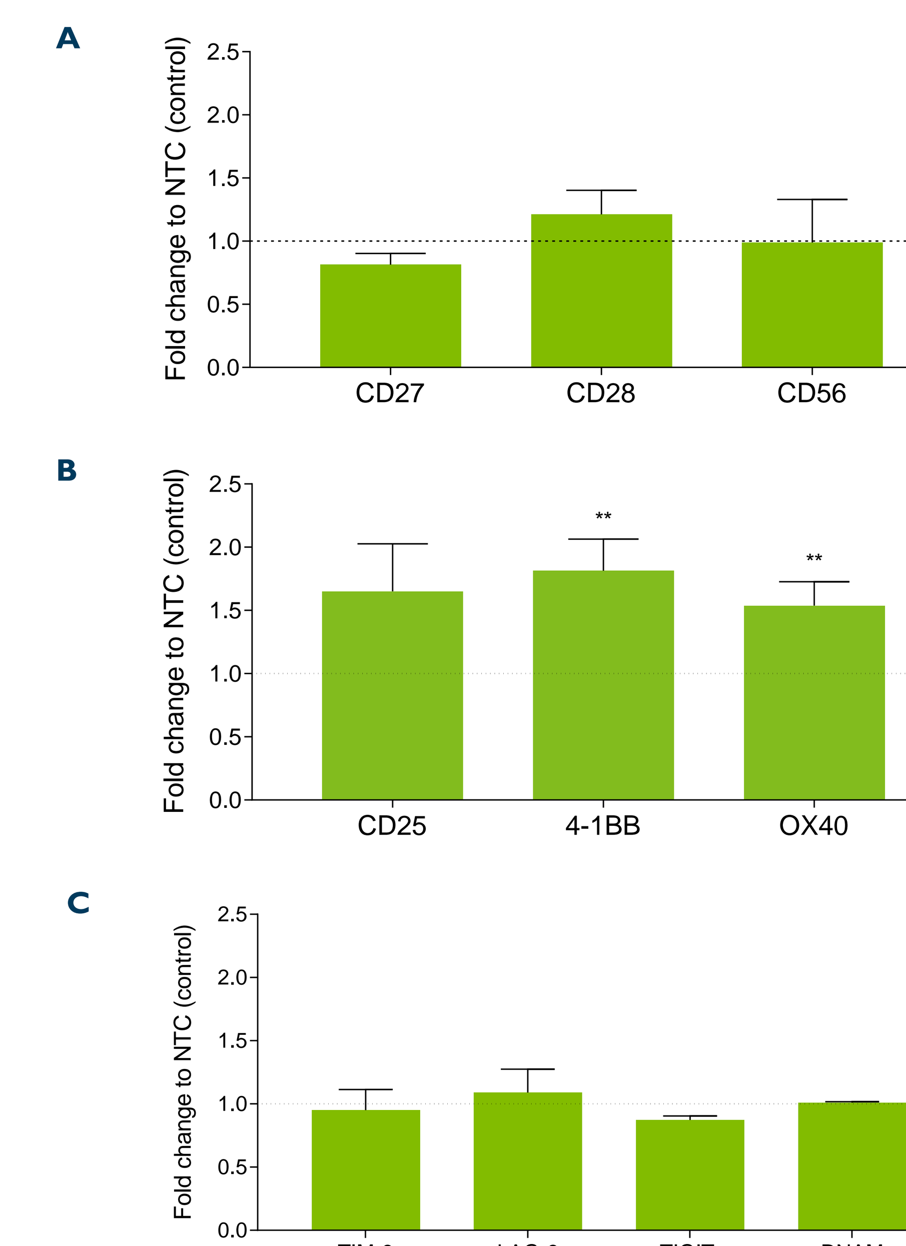
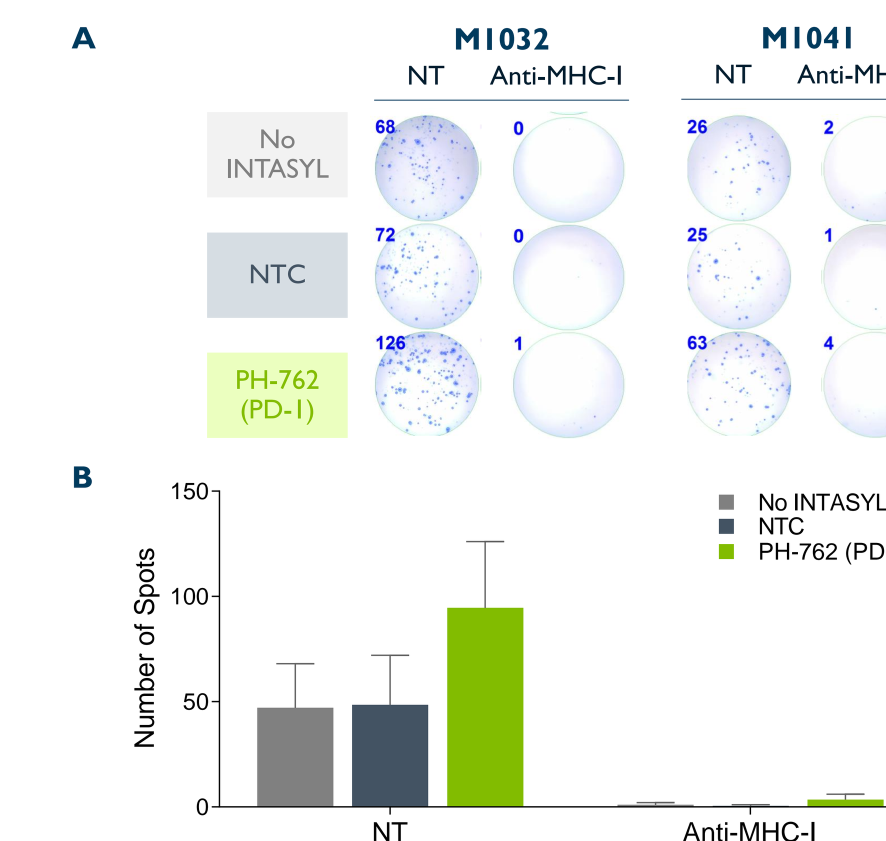


Figure 5. PD-1 silenced TIL heightened IFN-γ secretion in response to re-stimulation with autologous tumor cells

Assessment of IFN-γ secretion following T-cell re-stimulation with autologous tumor cells. Two melanoma TILs (M1032 and M1041) expanded with PH-762 (PD-1 targeting) or non-targeting control (NTC) INTASYL compounds were co-cultured with autologous tumor cells at the ratio of 1 to 2 and assessed for IFN-g secretion by ELISpot. Anti-MHC- class I blocking antibody was used as a control. Each condition was done in duplicates. **(A)** Representative ELISpot images show the spot counts of each condition. A marked increase in IFN-g spot counts was observed in TIL expanded with PH-762 (PD-1 targeting INTASYL compound). **(B)** Average of ELISpot counts from both M1032 and M1041 TILs are shown in bars with vertical lines indicating standard errors (SEM).

PD-1 silencing mediated by PH-762 (PD-1 targeting INTASYL compound) enhanced TIL effector function.



CONCLUSIONS

- INTASYL-mediated silencing of PD-1 with PH-762 in TIL was highly efficient with average of 85% knockdown efficiency in different histologies.
- TIL expanded with PH-762 exhibited activated T-cell phenotype indicated by significant increase in 4-1BB and OX40.
- Inhibitory and exhaustion molecules remained unaffected, suggesting that compensatory mechanisms were not triggered by PD-1 silencing.
- PD-1 knockdown TIL displayed elevated IFN-γ secretion when co-cultured with autologous tumor cells, indicating improved effector function upon specific T-cell re-stimulation.
- These data elucidate the impact of PH-762 in TILs and support evaluating these new PD-1-silenced TIL in the clinic.

DISCLOSURE

- This study and poster are sponsored by Iovance Biotherapeutics, Inc.
- All authors are employees of the indicated biotechnology companies, and may have stock options.

ACKNOWLEDGMENT

- All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors.
- The authors would like to thank the participating patients and their families for donation of material used in this study.

© Iovance Biotherapeutics, Inc. 2019