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

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BACKGROUND

- Adoptive T-cell transfer with tumor infiltrating lymphocytes (TIL) is an investigational immunotherapy for advanced solid malignancies. Early-phase trials of OncoGenex Phase 1/2 trials of Iovance’s Melon and LU-1 INTASYL products have demonstrated efficacy of ORRs of 34.6% and 44% in patients with melanomas and cervical cancer respectively.1
- Anti-PD-1 therapy has been widely used as a first-line therapy in several types of cancer TIL infusion is a key component of this therapy. Several studies have shown that silencing PD-1 within TILs, especially the subset of tumor antigen-specific TILs, may prove favorable when compared with non-specific PD-1 silencing. Furthermore, a silencing approach yielding a transient transcript knockdown, may prove favorable when compared with permanent genetic modification in the context of TILs.
- Here, we tested the silencing efficiency of a PD-1-targeting INTASYL compound, termed PH-762, in TIL and in effect on TIL phenotype and function.

RESULTS

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 media with IL-2 for a pre-Transplant Expansion protocol (pre-REP).
3. Pre-REP TILs are further propagated in a REP protocol in the presence of stimulated RPMI media and IL-2.
4. TIL products are assessed for phenotypic and effector function.
5. Prior to infusion of expanded TILs, patients receive a non-replicable lymphodepletion regimen consisting of cyclophosphamide and fluorouracil.

Experimental Design

Tumor samples derived from melanoma, head and neck, sarcoma, lung, and breast cancers were sourced to research pre-REP. Pre-REP TILs were subsequently expanded in the presence of absence of either non-targeting control (NTC) or PH-762 (PD-1 targeting) INTASYL compounds. Pre-REP TILs were analyzed for:
- Knockdown (KD) efficiency
- Comprehensive phenotypic and functional characterization

Tumor recognition

Evaluation of PD-1 knockdown efficiency was performed using TILs expanded with PH-762 targeting INTASYL compound in the presence or absence of NTC. Expression levels were measured using flow cytometry. CD3+PD-1+TILs were stained for PD-1 protein expression. Pre-REP TILs from PH-762 targeting INTASYL compound were incubated with either 1 (negative control), 1 (PD-1 KD efficiency) or 1 targeting control (NTC) compound. Flow cytometry was performed to determine the percentage of PD-1 knockdown efficiency. The ratio of protein expression was used to calculate the percent knockdown efficiency in different TIL products. A fold change of 1 indicates a significant increase in PD-1 expression. An increased expression of 4-1BB and OX40 in TIL expanded with PH-762 targeting INTASYL compound was observed, improving TIL phenotype and effector function upon specific TIL recognition.

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

Assessment of PD-1 KD efficiency in TIL after harvest. TIL expanded with targeting control (NTC) or PH-762 targeting (1) were expanded for seven days post-harvest. Effector memory (TEMRA) and Central memory (TCM) TILs were expanded with NTC or PH-762 targeting INTASYL compound, and stained for PD-1 protein expression.

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

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

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CONCLUSIONS

- INTASYL-mediated silencing of PD-1 on PH-762-TIL was highly efficient with an average of 85% knockdown efficiency in different tumor types. TIL expanded with PH-762-NCT exhibited activated T-cell phenotype with increased expression of 4-1BB and OX40. Inhibitory and exhaustion molecules remained unaltered suggesting that compensatory mechanisms were not required in PH-762 TILs. PD-1 knockdown TILs exhibited silenced IFNγ secretion when co-cultured with targetable tumor cells, indicating improved effector function upon specific TIL recognition. These data elucidate the impact of PH-762 TILs and support evaluating these new PD-1 targeting TILs in the clinic.