**SUMMARY**

Tumor-reactive human CD8+ CD39+ CD103+ (Double Positive, DP) T cells are predominantly found in the tumor microenvironment with an exhausted phenotype (significantly high levels of CD39, PD-1, CTLA-4, and TIM-3). Our unique expansion method allows these DP cells to grow from thousands into billions, traffic to the tumor site, recognize autologous tumor, and facilitate tumor regression. We tested the CD8 DP TIL in vivo using a xenograft model with immune-compromised mice that constitutively secrete human IL-2 (NOD:Rt2), which was necessary for the long-term survival of TIL in the periphery and for tumor regression. These preclinical data were the basis for our Phase 1 human clinical trial design for the adoptive transfer of CD8 DP TIL (AGX148). The trial is a first-in-human protocol for adults with solid tumors (NCT05095220) consisting of three cohorts: 2 weeks, 3 weeks, or 4 weeks of IL-2 administration after adoptive TIL transfer. Each cohort contains six patients; 3 receiving DP TIL alone and 3 receiving DP TIL with PD-1 siRNA knockdown using INTASYL™ compound PH-762. The first 3 patients have been treated, all previously failed standard therapy, including checkpoint blockade. No serious adverse events were observed. Two of the three patients had partial responses previously failed standard therapy, including checkpoint blockade. No serious adverse events were observed. Two of the three patients had partial responses.

**PRECLINICAL PDX DATA**

**Based on the image, there is a diagram illustrating the survival experiment in NOD or IL-2 NOD mice, comparing the effect of blood vs. tumor injection. The diagram shows a comparison of different treatment groups over time, with axes indicating survival and time.**

**PRELIMINARY CLINICAL TRIAL RESULTS**

**Based on the image, there is a table summarizing the preliminary clinical trial results for 3 patients treated with INTASYL™ PH-762. The table includes information on patient ID, cancer type, % of DP cells sorted, % viability, # DP cells sorted, and expansion fold.**

**CONCLUSIONS**

- CD8+ CD39+ CD103+ DP TIL-reactive TIL are found in a variety of solid tumor types at varying frequencies and have a significant "exhausted" phenotype.
- In preclinical PDX models, adoptively transferred DP TIL persisted long-term, trafficked to and cleared autologous tumors in the presence of IL-2.
- TCR-seq showed distinct TCR repertoire in the DP vs DN TIL.
- Single-cell TCR-seq and gene-seq demonstrated diversity of phenotypes within the DP TIL product.
- Using AgonOx’s AGX148 selection and expansion method, DP TIL can be grown in vitro to billions of highly functional cells.
- AgonOx's AGX148 selected-TIL product minimizes bystander expansion and is highly enriched with tumor-reactive T cells.
- INTASYL™ PH-762 treatment reduced PD-1 protein expression on AGX148 TIL Product.
- Phenotype of blood and tumor biopsies revealed distinct phenotypic changes after TIL treatment in both humans and mice.
- Preliminary clinical trial results from 3 patients showed evidence of tumor regression at day +29 (2 PR (melanoma) and 1 SD (thyroid cancer) with 1 PR (endometrial cancer)).