The oncolytic virus pelareorep in combination with immune checkpoint inhibitor activates T-cell functioning in early breast cancer patients – immunophenotype results from AWARE-1 study

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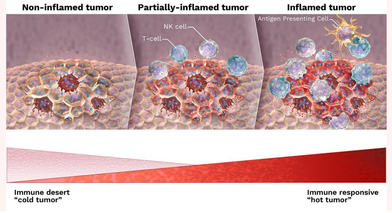
BACKGROUND

- Pelareorep (pela) is an intravenously delivered unmodified oncolytic reovirus that can selectively replicate in tumor cells and induce a T cell inflamed phenotype[1] (Fig 1).
- A previous phase 2 study in metastatic breast cancer (BC) demonstrated statistically significant improvement in overall survival (OS) in patients (pts) treated with pela combined with paclitaxel (PTX) versus PTX alone[2]. We hypothesized that the OS benefit from pela + PTX is due to an adaptive T cell response triggered by pela. To examine if pela can mediate the priming of an anti-tumor immune response, and to assess the impact of checkpoint blockade therapy on this response, Oncolytics biotech Inc. and the SOLTI research group conducted the AWARE-1 window of opportunity study (NCT04102618) in pts with early BC.

Figure 1: Pelareorep's mechanism of action.

Pelareorep selectively infects cancer cells leading to tumor cell lysis. The virus also mediates anti-tumor immunity by activating both innate and adaptive immune responses. We hypothesize that pelareorep-mediated immune responses and its effect on the tumor microenvironment will boost responses to anti-PD-L1 therapy.



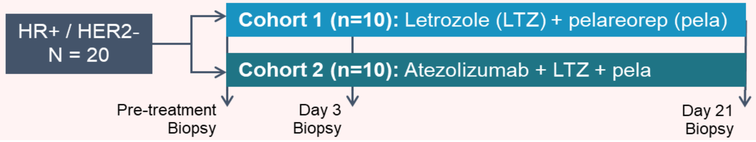
- The primary endpoint is CelTIL score, a metric for quantitating changes in tumor cellularity and tumor infiltrating lymphocytes (TILs)[3]. An increased CelTIL score is associated with favorable responses to treatment (CelTIL score = −0.8 × tumor cellularity (%) + 1.3 × TILs (%).
- Previously reported AWARE-1 data showed that pela combined with atezolizumab (atezo) resulted in CelTIL score increases of >30% in 60% of HR+/HER2- newly diagnosed, early BC patients, thereby meeting the study's primary endpoint. Patients who received pela without atezo showed increases of >30% in CelTIL score in 40% of patients[4].
- Increased CelTIL scores were accompanied by a favorable immunologic response in both the tumor and the blood as demonstrated by[4,5]:
  - Upregulation of PD-L1 and caspase 3 expression in tumor tissue
  - Increased CD8+ and memory T cells in tumor tissue
  - A more favorable CD8:Treg ratio, indicating a less immunosuppressive tumor microenvironment (TME) along with an increase in markers of T cell activation and no significant change in markers of T cell exhaustion
  - Changes in the T cell populations including decreases in clonal T cell diversity, which was associated with increased CelTIL scores and TILs at surgery
- Here we present additional flow cytometry results from AWARE-1 for HR+/HER2- patients who received letrozole + pela in the absence or presence of atezo (Cohorts 1 and 2, respectively).

STUDY DESIGN & METHODS

- AWARE-1 is a window-of-opportunity study designed to evaluate the safety and effect of neoadjuvant letrozole + pela ± atezo on the TME and peripheral blood cell populations (Fig 2). Treatment naïve HR+/HER2- early BC patients were enrolled in two cohorts: Cohort 1 (C1) – pela + letrozole (n=10); and Cohort 2 (C2) – pela + letrozole + atezo (n=10). Pela was administered on days 1, 2 and 8, 9, while atezo was administered on day 3. Blood and tumor samples were collected pre-treatment and on days 3 and 21. We investigated the immune cell composition of blood using a multicolor flow cytometry to identify different subsets of immune cells.

Figure 2: AWARE-1 study design and objectives

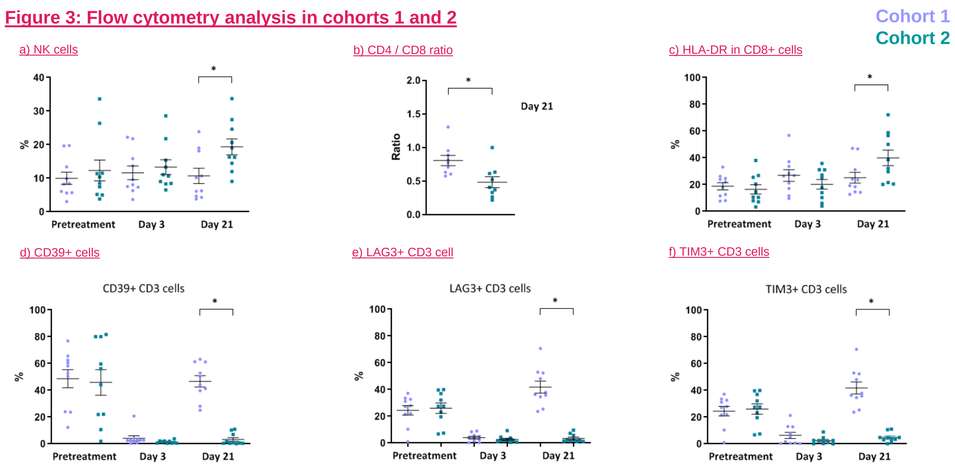
- **Primary objective:**
  To evaluate CelTIL score 3 weeks following initiation of treatment
- **Secondary objective:**
  To evaluate immunological changes within the tumor and peripheral blood



RESULTS - FLOW CYTOMETRY

- Flow cytometry analysis showed a significant increase in natural killer (NK) cells on day 21 in C2 compared to C1 (≈2-fold, p-value=0.0166) (**Fig 3a**).
- A statistically significant decrease in CD4/CD8 ratio was observed when C2 was compared to C1 on day 21 when normalized to the day 3 values (≈1.5-fold, p-value=0.0142) (**Fig 3b**). Moreover, an increase in HLA-DR expression in the CD8 population was detected in C2 vs C1 on day 21 (≈1.5-fold, p-value =0.0632) (**Fig 3c**).
- Assessment of exhaustion markers showed that pela administration decreased CD39, LAG3 and TIM3 markers on day 3. However, low levels of these markers were only maintained at day 21 in C2 patients who had received atezo on day 3 (**Fig 3d, e, f**). No differences were observed in BTLA, CTLA-4 and PD1 markers.

Figure 3: Flow cytometry analysis in cohorts 1 and 2



CONCLUSION

Here we show flow cytometry data from the blood samples of AWARE-1 patients (Cohorts 1 and 2) demonstrating further the pela-mediated activation of immune cells, which becomes even more pronounced with the addition of atezo (Cohort 2). These data, together with previously reported AWARE-1 results, illustrate pela's ability to induce an inflamed tumor phenotype and an activated cytotoxic blood immune cell repertoire. Additionally, they show that atezolizumab acts synergistically with pela to enhance these effects. In summary, the AWARE-1 results support pela's immune-based mechanism of action and suggest that combining pela with atezo may improve outcomes in BC patients.

References
[1] Samson et al. Sci Transl Med 2018;10. [2] Bernstein et al. Breast Cancer Res Treat (2018);167:485-93. [3] Nuciforo et al. Ann Oncol (2018), 29: 170-77.[4] Manso et al. AACR Virtual Annual Meeting (2021). [5] J Gavila et al. ESMO BC (2022)