A window-of-opportunity study with atezolizumab and the oncolytic virus pelareorep in early Breast Cancer (AWARE-1)

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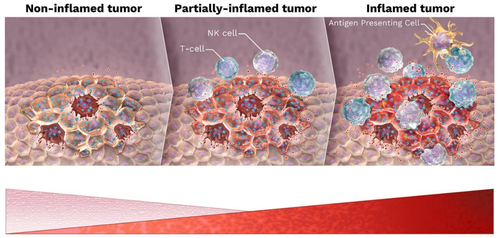
BACKGROUND

- Pelareorep (pela) is an intravenously (IV) delivered and systemically available unmodified oncolytic reovirus that can replicate in tumor tissue and induce a T cell inflamed phenotype[1].



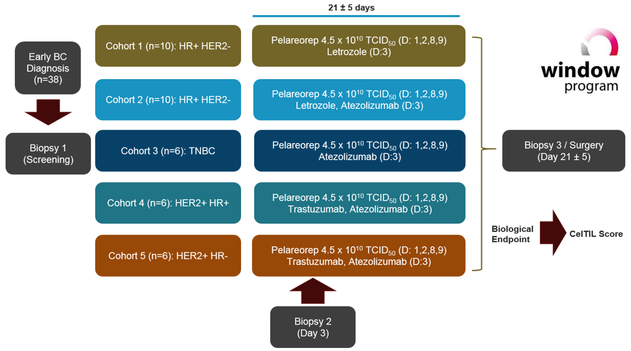
Pelareorep mechanism of action. Pelareorep selectively infects cancer cells leading to tumor cell lysis. The virus also mediates anti-tumor immunity by activating both innate and adaptive immune response. We hypothesize that pelareorep mediated immune responses will boost anti-PD-L1 response.

- A previous phase 2 study in metastatic breast cancer demonstrated a statistically significant improvement in overall survival (OS) in patients treated with pela given in combination with paclitaxel (PTX) versus PTX alone[2]. We hypothesized that the OS benefit from pela + PTX may be attributed 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, we and SOLTI research group are conducting the AWARE-1 study (NCT04102618) in patients with early breast cancer.

- The **primary endpoint of the study is CelTIL score[3]**, a metric for quantifying the changes in tumor cellularity and tumor infiltrated lymphocytes (TILs), where an increase in CelTIL is associated with a favorable response to treatment.

STUDY DESIGN & METHODS

- AWARE-1 is a window-of-opportunity study to evaluate the safety and effect of pela ± atezolizumab on the tumor microenvironment (TME) in 38 women with early breast cancer.



STUDY OBJECTIVES

- Primary objective: to evaluate CelTIL score 3 weeks following initiation of treatment in each cohort.
- Secondary objective: to evaluate immunological changes within the tumor and peripheral blood.

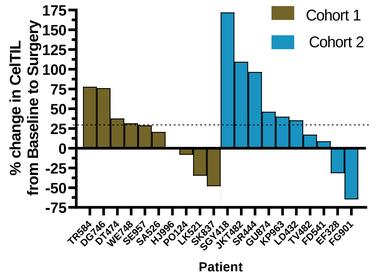
Here we present the identified differences between HR+HER2- patients receiving pela in the absence or presence of atezolizumab (Cohorts 1 and 2, respectively).

RESULTS

CelTIL score - Cohort 2 achieved the study's primary endpoint

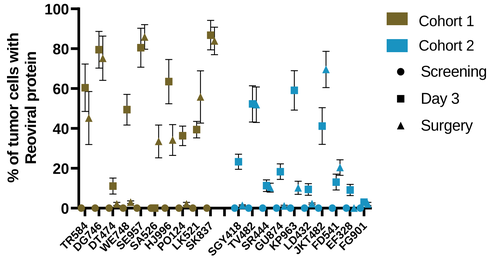
Primary endpoint: a ≥30% increase in CelTIL score from baseline in at least 50% of the patients

- **Cohort 2 has achieved the study's primary endpoint with 60% of patients showing an increase in CelTIL ≥30%**
- Cohort 1 did not meet the study's primary endpoint with 40% of patients showing an increase in CelTIL ≥30%, although a promising trend toward increased CelTIL ≥30% was observed in this cohort, as was efficient pela replication in the tumor biopsies of Cohort 1 (see Figure below)
- CelTIL score = −0.8 × tumor cellularity (in %) + 1.3 × TILs (in %). The minimum and maximum unscaled CelTIL scores will be −80 and 130. This unscaled CelTIL score is then scaled to reflect a range from 0 to 100 points.
- **An increase in CelTIL score is associated with better treatment outcomes[1]**



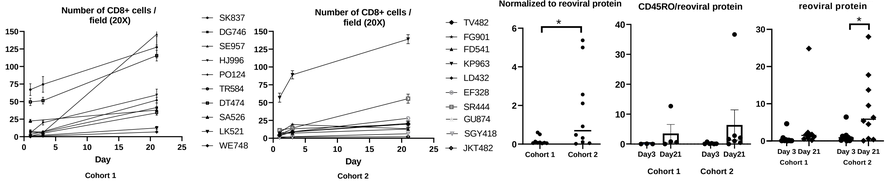
Pelareorep replication is seen in all tumor biopsies and promotes CD8+ T cell infiltration

Productive reoviral/pelareorep infection is seen in all post-treatment tumor biopsies, averaging 52% of tumor cells at day 3 and 42% at surgery (day ~21) assessed by immunohistochemistry. Viral replication was not observed in adjacent normal tissue.



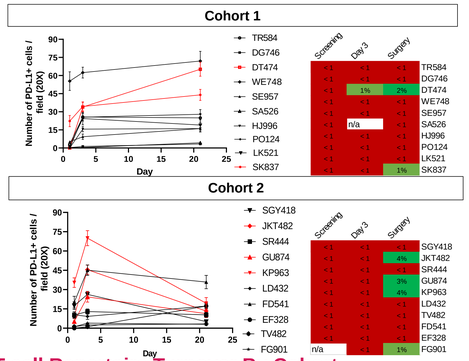
Changes in CD8+ T cell infiltration

- Intratumoral CD8+ T cells increased in all patients at surgery (day 21) with an average of an 11-fold increase in CD8+ T cells from baseline to surgery (range: 1.1 to 24.3).
- The ratio of CD8+ T cells to FOXP3+ regulatory T cells (CD8/FOXP3) was higher in cohort 2 patients compared to cohort 1, when normalized to reoviral protein + cells.
- At surgery/day 21, the numbers of memory T cells (CD45RO) and CD8+ T cells was also numerically higher in cohort 2 patients compared to cohort 1, when normalized to reoviral protein + cells.



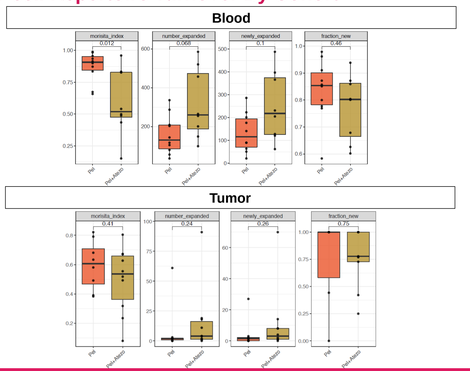
RESULTS

Pelareorep upregulates PD-L1 expression in the VENTANA (SP142) and 28-8 Assay



- (Left) Plots of PD-L1 expression were obtained with the 28-8 anti-PD-L1 clone quantifying expression in both tumor tissue and immune cells.
- Analysis with the 28-8 anti-PD-L1 clone shows that nearly all patients have increased PD-L1 expression in the TME by day 21 compared to baseline. Red lines indicate patients that have "converted" to PD-L1 positive (>1%) with the SP142 assay.
- (Right) Heat maps show changes in PD-L1 expression with the Ventana PD-L1 (SP142 assay), quantifying expression in immune cells only. 20-40% of HR+/HER2- patients become PD-L1 positive (>1%) during treatment.
- No patients were considered PD-L1 positive prior to treatment.

T cell Repertoire Turnover By Cohort



- There is more T cell repertoire turnover, as indicated by the Morisita index in patients in cohort 2 compared to patients in cohort 1, indicating more overall change in the T cell population during treatment in both peripheral blood and tumor tissue.
- Cohort 2 patients also have more expanded T cell clones and more newly created clones relative to cohort 1 in both blood and tumor tissue (see number expanded and newly expanded).
- Regardless of cohort, treatment with pelareorep transforms the T cell populations in both blood and tumor tissue where > 75% of the T cells are considered 'new' (see fraction new).

CONCLUSION

- Cohort 2 met the study's success criterion of ≥30% increase in CelTIL score in at least 50% of the patients
- In addition, pelareorep + atezolizumab act synergistically to establish a favorable immunologic response in both the tumor and the blood as demonstrated by:
  - Upregulation of PD-L1 in tumor tissue
  - Increased CD8+ and memory T cells in tumor tissue
  - A favorable CD8:Treg ratio, indicating a less immunosuppressive tumor microenvironment
  - Dramatic changes in the T cell populations in both peripheral blood and the tumor
- Therefore, the combination of pelareorep and atezolizumab warrants further investigation as a potential breast cancer treatment approach

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.