A window-of-opportunity Study with atezolizumab and the oncolytic virus pelareorep in early Breast Cancer (REO-027, AWARE-1)

Luis Manso[1], Patricia Villagrasa[2], Nuria Chic[3], Juan Miguel Cejalvo[4], Yann Izarzugaza[5], Blanca Cantos[6], Salvador Blanch[7], Manel Juan[3], Blanca Gonzalez[3], Rita Laeufle[8], Gerard Nuovo[9], Grey Wilkinson[8], Matt Coffey[8], Azucena Gonzalez[3], Débora Martínez[3], Laia Paré[2], Fernando Salvador[2], Xavier Gonzalez[10], Aleix Prat[2,3]Joaquín Gavilá[7].

1 Hospital Universitario 12 de Octubre, Madrid; 2 SOLTI Breast Cancer Research Group, Barcelona; 3 Hospital Clinic, Barcelona; 4 Hospital Clínico Universitario de Valencia ; 5 Hospital Universitario Fundación Jiménez Díaz ; 6 Hospital Universitario Puerta de Hierro-Majadahonda ; 7 Instituto Valenciano de Oncología (IVO) ; 8 Oncolytics Biotech Inc.; 9 Ohio State University Arthur G. James Cancer Hospital and Richard J. Solove Research Institute, Columbus, OH. ; 10 Hospital Universitari General de Catalunya, Sant Cugat del Vallés, Spain.

BACKGROUND

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

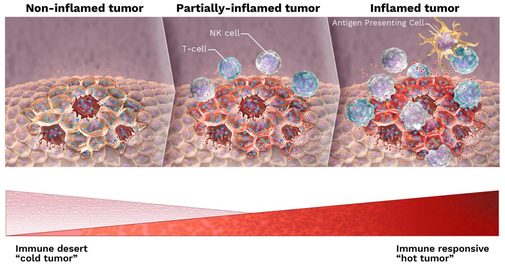

Figure 1. Pelareorep mechanism of action. Pelareorep selectively infects cancer cells leading to tumor cell lysis. The virus also mediates anti-tumor immunity by activating both innate and adaptive immune response. We hypothesize that pelareorep mediated inflammation will boost anti-PD-L1 response.

- A previous phase 2 study in metastatic breast cancer (BC) compared treatment with pela, in combination with paclitaxel (PTX) versus PTX alone[2]. This study demonstrated a statistically significant improvement in overall survival (OS). We hypothesized that the OS benefit from pela + PTX may be attributed to an adaptive immune response triggered by pela.
- To test this hypothesis, we designed a window of opportunity study (**AWARE-1**) within the "Window Program" of SOLTI, which is currently enrolling, to assess the biological activity of pela in different BC types in combination with anti-PD-L1 therapy, atezolizumab, and other BC therapies (NCT04102618).
- The **primary endpoint of the study is CelTIL score[3]**, a metric for quantifying the changes in tumor cellularity (Cel) and tumor infiltrated lymphocytes (TILs), where an increase in CelTIL is associated with a favorable response to treatment.

STUDY DESIGN

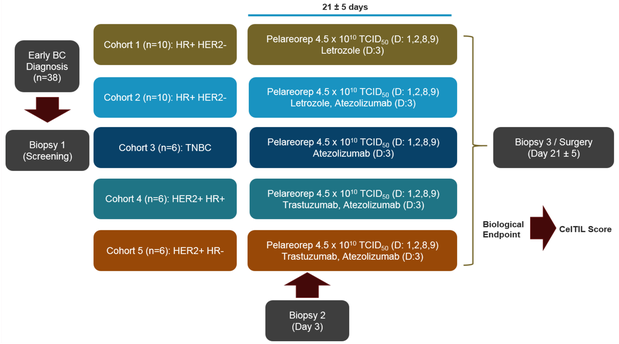

Figure 2. Study design. Patients are treated with pela on days 1, 2, 8, and 9, while atezolizumab is administered on day 3 (excluding cohort 1). Tumor biopsies are collected at diagnosis, day 3, and day ~21.

STUDY OBJECTIVES

- **PRIMARY OBJECTIVE:** to **evaluate CelTIL score** increase at 3 weeks of treatment of each cohort.
- **KEY SECONDARY AND EXPLORATORY OBJECTIVES:**
  - To describe **safety and tolerability** of the different drug combinations.
  - To evaluate **biological changes to predict response** to study drug(s), including 60 breast cancer-related genes and a panel of 770 immune-related genes.
  - To examine **CD4 and CD8-T cell reactivity** between baseline and treated samples.
  - DNA seq of **T-cell receptor repertoire**.
  - To evaluate whether pelareorep with different therapies induce **different immune blood markers,** such as changes in peripheral blood mononuclear cells.

RESULTS

- Up to date, 13 patients from 6 different hospitals in Spain have been included in the study. Here, we report initial translational results of the first 6 patients:

Pelareorep replication and immunological changes within the tumor microenvironment (TME)

- Analysis of CelTIL show an increase in four of the six patients.
- Productive viral replication in day 3 and day ~21 biopsies (surgery) was very high.
- Immunohistochemistry analysis revealed an increase in CD8+ T-cells and upregulation of PD-L1 on day 3 and day 21 biopsies for all patients

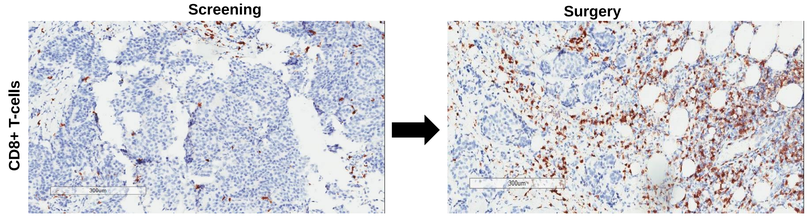

Figure 3. Representative histologic analysis of changes of CD8+ T-cell infiltration from screening to surgery in patient SE957 (cohort 1, HR+/HER2-) treated with pelareorep and letrozole.

Patient	Cohort	Viral replication, % (SD) of tumor cells at surgery	% change in CelTIL score	Fold change in PD-L1+ cells (surgery/ screening)	Fold change in CD8+ cells (surgery/ screening)
DG756	1	75.2 % (11.1)	+76%	11.0	4.3
SK837	1	83.9 % (6.9)	-48%	2.0	1.6
SE957	1	85.9 % (6.2)	+29%	3.1	11.2
TV482	2	51.9 % (8.9)	+17%	2.8	4.6
FG901	2	2.1 % (0.8)	-65%	2.7	1.6
AX353	3	64.9 % (8.4)	+22%	1.3	1.6

Table 1. Percentage of virus positive cells, percentage of change in CelTIL score and fold change in PD-L1 + cells and CD8+ cells (surgery *vs* screening).

RESULTS

Pelareorep replication and immunological changes within the TME continued

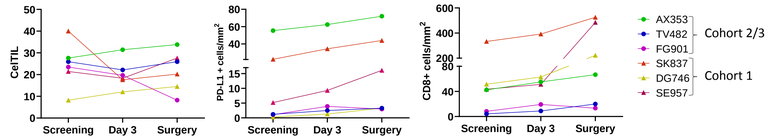

Figure 4. Changes in CelTIL score and immune cells

T-cell receptor (TCR) immunosequencing and CelTIL

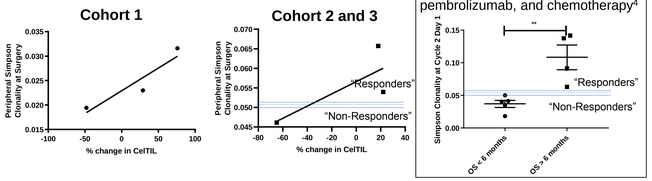

Figure 5. Peripheral T-cell clonality correlation with % change in CelTIL score. Box, prior study demonstrating a similar threshold to separate potential responders and non-responders utilizing a different clinical endpoint[4].

Overlap Between Peripheral and Tissue Expanded T-cells clones

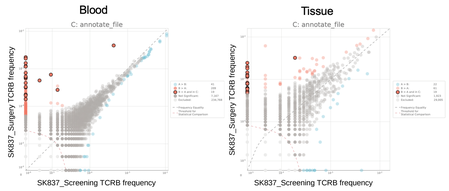

Figure 6. Overlap in T-cell clones between tissue and the periphery in patient SK837

Patient	Cohort	Blood expanded	Tissue expanded	Expanded overlap
DG756	1	287	2	2
SK837	1	209	61	19
SE957	1	56	0	0
TV482	2	99	1	1
FG901	2	457	4	3
AX353	3	520	91	71

Table 2. Overlap in total expanded clones between the tissue and the periphery.

CONCLUSIONS

- The degree of viral replication was consistent with changes in CelTIL and within immunological change in the TME, mainly CD8 T-cell infiltration and PD-L1 expression.
- Preliminary data from the first six patients in AWARE-1 demonstrate pela-mediated priming of an adaptive immune response, helping to validate our hypothesis that the extended OS observed in our prior mBC study can be attributed to pela-mediated T-cell priming
- Following initial treatment (~3 weeks), peripheral T-cell clonality may be correlated with changes in CelTIL and clinical response as seen in prior studies[4]

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] Mahalingam et al. Clinical Cancer Research (2020) 26, 71-81.