Changes in T cell clonality in AWARE-1 study, a window-of-opportunity study with atezolizumab and the oncolytic virus pelareorep in early breast cancer

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BACKGROUND

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

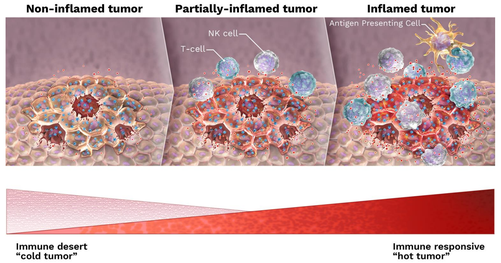


Figure 1. Pelareorep mechanism of action. Pelareorep selectively infects cancer cells leading to tumor cell lysis. The virus also mediates anti-tumor immunity by activating both innate and adaptive immune response. We hypothesize that pelareorep mediated immune responses will boost anti-PD-L1 response.

- A previous phase 2 study in metastatic breast cancer (BC), known as IND.213, 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 and tumor infiltrated lymphocytes (TILs), where an increase in CelTIL is associated with a favorable response to treatment.

STUDY DESIGN

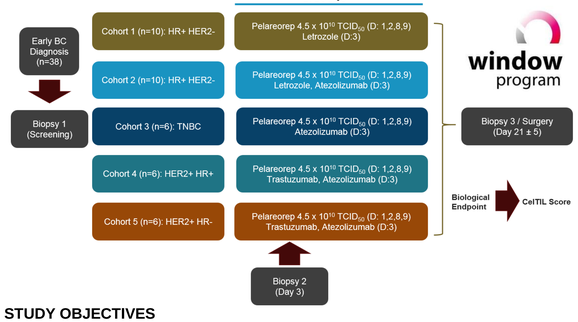


Figure 2. Study design. Patients are treated with pela on days 1, 2, 8, and 9, while atezolizumab is administered on day 3 (excluding cohort 1). Tumor biopsies are collected at diagnosis, day 3, and day ~21.

STUDY OBJECTIVES

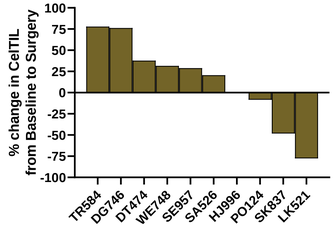
- Primary objective: to evaluate CelTIL score increase following 3 weeks of treatment in each cohort.
- Secondary objective: to evaluate immunological changes within the tumor and peripheral blood.

RESULTS

- To date, 23 patients from 13 different hospitals in Spain have been included in the study. Here, we report initial translational results focusing on cohort 1 (n=10):

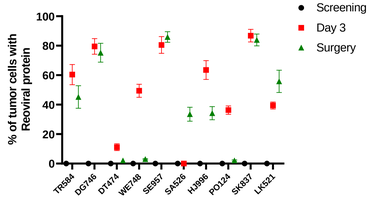
CelTIL and pelareorep replication in tumor biopsies following IV delivery

Figure 3. CelTIL score from cohort 1. CelTIL is calculated with the following equation: 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 scores is then scaled to reflect a range from 0 to 100 points.



- An increase in CelTIL is associated with better treatment outcomes.
- There is a 70% CelTIL response rate for cohort 1.

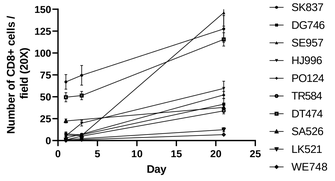
Figure 4. Pelareorep replication assessed by immunohistochemistry.



- Productive reoviral infection is seen in all post-treatment tumor biopsies, averaging 52% of tumor cells at day 3 and 42% at surgery.
- Viral replication was not observed in adjacent normal tissue.
- Viral dsRNA (an immune adjuvant) is observed in a higher proportion of tumor cells than is viral protein (not shown).

Treatment with pelareorep promotes the recruitment of CD8+ T cell to the tumor microenvironment

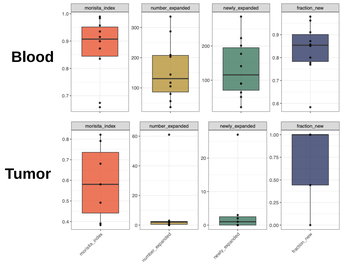
Figure 5. Changes in CD8 T cell infiltration



- All patients had an increase in CD8+ T cells at surgery (day 21).
- On average there was a 14-fold increase in intratumoral CD8 T cells from baseline to surgery (ranging from 0.6 to 41).

Treatment with pelareorep promotes the creation of new T cell clones

Figure 6. Clonal expansion in peripheral blood and tumor

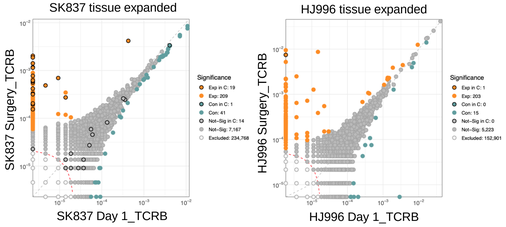


- There is a high degree of turnover or change in the T cell population within the tumor and peripheral blood, as measured by the Morisita index. This index calculates sample similarity taking into account both overlap of clones and frequency. Values below 0.95 indicate a high degree of turnover.
- The majority of clonal expansion in both the periphery and tumor is from newly detected clones, consistent with previous pelareorep studies[4].

RESULTS

Overlap of expanded tumor and peripheral T cells

Figure 7. New T cell clones are expanded in the tumor and periphery



Patient ID	Tissue Expanded	Tissue And Peripheral Expanded
LK521	1	0
SK837	61	19
DG746	2	2
SA526	3	3
HJ996	2	1

- Many of the expanded clones in the tissue are also expanded in the periphery.
- The majority of expanded clones in the tissue are 'new' clones from the periphery.
- Clonal expansion in pts SK837, LK521, and HJ996 suggests the expansion of anti-tumor clones.

Common clones were expanded in multiple patients

Amino Acid	V Gene Name	J Gene Name	n	Subjects
CASSITGTGELFF	TCRBV19-01	TCRBJ02-02	5	SK837,DT474,SA526,PO124,AX353
CASSLTGGTDTQYF	TCRBV07-03	TCRBJ02-03	5	SE957,DT474,SA526,HJ996,AX353
CASSLEANTEAFF	TCRBV11-X	TCRBJ01-01	3	LK521,AX353,FG901
CASSLESGAGNTIYF	TCRBV07-09	TCRBJ01-03	3	LK521,SA526,PO124
CASSQDRAANQPQHF	TCRBV04-01	TCRBJ01-05	3	PO124,TR584,AX353
CASSAORDNTEAFF	TCRBV02-01	TCRBJ01-01	2	HJ996,AX353
CASSAKVNTEAFF	TCRBV09-01	TCRBJ01-01	2	SK837,HJ996
CASSLESGSGNTIYF	TCRBV07-09	TCRBJ01-03	2	LK521,PO124
CASSLGGNSGANVLTF	TCRBV28-01	TCRBJ02-06	2	DG746,TR584
CASSLGGSYEQYF	TCRBV11-01	TCRBJ02-07	2	SK837,TR584
CASSLTGTGELFF	TCRBV19-01	TCRBJ02-02	2	SK837,SA526
CASSPDRDNTEAFF	TCRBV02-01	TCRBJ01-01	2	DT474,AX353
CASSPTGGTDTQYF	TCRBV07-03	TCRBJ02-03	2	SA526,AX353
CASSQDGAGGDEQYF	TCRBV04-02	TCRBJ02-07	2	SK837,AX353
CASSQTGGTDTQYF	TCRBV07-03	TCRBJ02-03	2	SK837,AX353
CASSRTGGTDTQYF	TCRBV07-03	TCRBJ02-03	2	HJ996,AX353
CASSSTGGTDTQYF	TCRBV07-03	TCRBJ02-03	2	SK837,HJ996
CASSVGGGSYEQYF	TCRBV06-05	TCRBJ02-07	2	SE957,PO124
CASSYSGHYGYTF	TCRBV06-05	TCRBJ01-02	2	PO124,AX353
CASSYSGPNTEAFF	TCRBV06-02/06-03	TCRBJ01-01	2	SA526,AX353
CSAALAATQYF	TCRBV20-01	TCRBJ02-03	2	SA526,PO124

- In total, 2,634 unique TCRs expanded across all 10 patients (blood or tissue). 21 were expanded in 2 or more patients.
- Clones shared a common motif CASS**X**TGGTDTQYF and the same V and J genes.
- Of the 21 most common clones, only 1 was present at baseline, which suggests a specific response to pelareorep.
- This motif was also expanded in patients from prior studies with pelareorep[4].

CONCLUSION

- Following a previous mBC study with pelareorep, we hypothesized that the survival advantage of patients treated with pelareorep + PTX was due to pelareorep's ability to create an anti-viral or anti-tumor T cell response in breast cancer that promotes therapeutic efficacy. Preliminary data from cohort 1 of AWARE-1 supports this hypothesis.
- Within the expanded pool of new T cell clones following pelareorep therapy, we identified both presumptive anti-tumor and anti-viral T cell clones.
- Given the absence of checkpoint blockade therapy in cohort 1, a 70% CelTIL response rate is encouraging. Interestingly, the degree of viral replication (protein) does not correlate with changes in CelTIL. One explanation for this observation is that viral dsRNA, rather than viral protein, may be a better correlative marker since dsRNA is a known immune adjuvant that can trigger inflammatory signalling pathways[5]. This research, along with additional correlative studies with be the topic of future publications.
- Results from IND.213[2], AWARE-1, & BRACELET-1[6] studies will be used to inform a future registration study in mBC.

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. [5] Chattopadhyay, S. and G.C. Sen. Journal of interferon & cytokine research, 2014. 34(6): p. 427-436. [6] clinicaltrials.gov: NCT04215146.