The oncolytic virus pelareorep primes the tumor microenvironment for checkpoint blockade therapy in early breast cancer patients -results from AWARE-1 study

J. Gavila [1,2], H. Loghmani [3], L. M. Manso Sanchez [4], A. Gonzalez [5,6], L. Pare Brunet [2], F. Salvador [2], T. Pascual [2,7], B. Gonzalez-Farre [6,8], M. Juan [5,6], A. Prat [2,6,7,9], T. Heineman [10]

1 Medical Oncology, Instituto Valenciano de Oncología (IVO), Valencia, Spain; 2 SOLTI Cancer Research Group, Barcelona, Spain; 3 Translational Oncology, Oncolytics Biotech Inc, Calgary, Canada; 4 Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid, Spain; 5 Immunology Department, Hospital Clinic de Barcelona, Barcelona, Spain; 6 August Pi I Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain; 7 Medical Oncology Department, Hospital Clinic de Barcelona, Barcelona, Spain; 8 Pathology Department, Hospital Clinic of Barcelona, Barcelona, Spain; 9 Medicine Department, University of Barcelona, Barcelona, Spain; 10 Medical Oncology Department, Hospital Clinic de Barcelona/SOLTI/IDIBAPS, Barcelona, Spain, 10 Clinical Development, Oncolytics Biotech, Inc, La Jolla, United States of America

ESMO Breast Cancer, May 3-5, 2022
FPN # 6P

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]. (Fig 1)
- A previous phase 2 study in metastatic breast cancer (BC) demonstrated statistically significant improvement in overall survival (OS) in patients 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, we and the SOLTI research group are conducting the AWARE-1 study (NCT04102618) in patients with early BC.

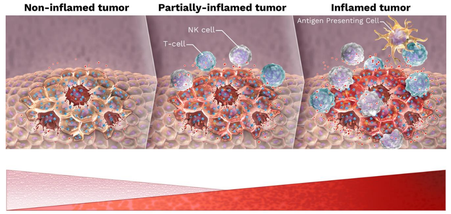


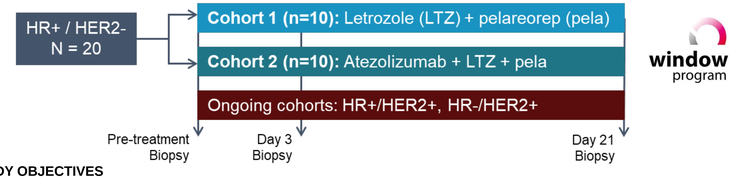
Figure 1: Pelareorep mechanism of action. Pelareorep selectively infects cancer cells leading to tumor cell lysis. The virus also mediates anti-tumor immunity by activating both innate and adaptive immune response. We hypothesize that pelareorep mediated immune responses will boost anti-PD-L1 response.

- The primary endpoint of the study is CelTIL score[3], a metric for quantitating changes in tumor cellularity and tumor infiltrating lymphocytes (TILs), for which an increase in CelTIL score is associated with favorable responses to treatment (CelTIL score = −0.8 × tumor cellularity (in %) + 1.3 × TILs (in %)[3]. Previously reported data from AWARE-1 showed that pela combined with atezolizumab (atezo) resulted in CelTIL score increases of >30% in 60% of HR+/HER2- early BC patients, thereby meeting the study's primary endpoint. Patients who received pela without atezo showed increase 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]:
  - Upregulation of PD-L1 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
  - Dramatic changes in the T cell populations in both peripheral blood and tumor
- Here we present additional translational research results from the AWARE-1 study for HR+/HER2- patients receiving 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 pela ± atezo on the tumor microenvironment (TME) and peripheral blood cell populations in 26 women with early BC (20 HR+/HER2 – patients in Cohorts 1 and 2 (fully enrolled), 6 patients in ER+/-, HER2+ population (ongoing)) (Fig 2)

Figure 2: AWARE-1 study design



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.

RESULTS

T cell Repertoire Turnover

In all treated subjects: (Fig 3)

- A statistically significant decrease in tumor and peripheral blood T cell diversity was seen post-treatment compared to baseline (Fig 3a). Diversity was calculated as the number of unique productive rearrangements in a sample after computationally downsampling to a common number of T cells to control for variation in sample depth or T-cell fraction. Lower values in diversity are associated with more expanded clones, while higher values indicate fewer expanded clones.
- A statistically significant association was seen between the increase in CelTIL score from baseline to surgery and the decrease in clonal T cell diversity (pre- vs post-treatment) (Fig 3b)

Figure 3:

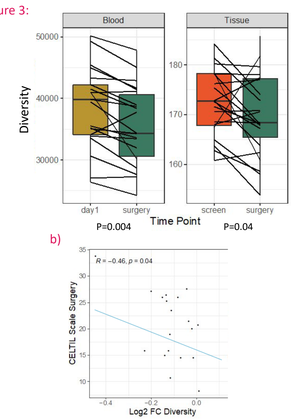
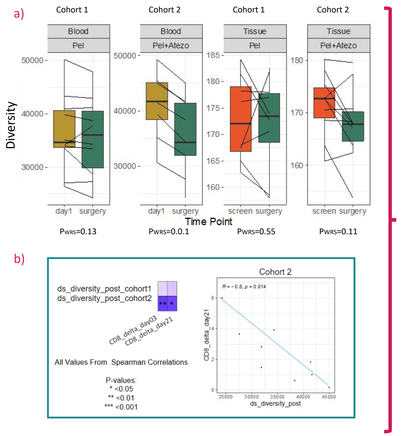


Figure 4:



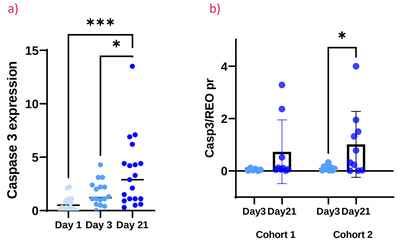
Comparative analysis of cohort 1 vs. cohort 2: (Figure 4)

- The addition of atezolizumab enhanced pela's ability to generate and expand new T cell clones[4].
- A greater decrease in pre- vs. post-treatment changes in peripheral blood T cell diversity was observed in Cohort 2 compared to Cohort 1. (Fig 4a)
- Decreased T cell diversity post-treatment negatively correlated with increases in tumor-infiltrating CD8+ T cells at Day 3 and Day 21 in both cohorts. This correlation reached statistical significance in Cohort 2. (Fig 4b)

Changes in Caspase 3 expression

- Based on the IHC data Caspase 3 expression increased in almost all patients with an average of a 4-fold increase from baseline to surgery. (Fig 5a)
- The ratio of Caspase 3-positive cells was higher in Cohort 2 vs. Cohort 1 patients when normalized to reoviral protein-positive cells (p-value=0.04). (Fig 5b)

Figure 5:



RESULTS

Changes in breast cancer subtype and risk of recurrence (PAM50 assay)

- Based on the PAM50 gene panel analysis: (Figure 6)
- There is an increase in conversion of luminal B (more aggressive) to luminal A (best prognosis) BC subtype seen in both cohorts from baseline (Day 1) to surgery (Day 21) timepoint. At the time of surgery (Day 21), 100% of the patients in Cohort 2 converted to the luminal A subtype. (Fig 6a)
- There is a decrease in Risk Of Recurrence-S (ROR-S) seen in both cohorts from baseline (Day 1) to surgery (Day 21). At the time of surgery, 100% of the patients in both cohorts show a "low" ROR-S. (Fig 6b)

Subtype (Fig 6a)

Subtype				
Patinet ID	Cohort	DAY 1	DAY 3	DAY 21
SF957	1	LumB	LumB	Normal
SK837	1	LumB	LumA	LumB
DG746	1	LumB	LumA	LumA
SA526	1	LumA	Normal	LumA
PO124	1	LumA	LumA	LumA
TR584	1	LumA	LumA	LumA
HJ996	1	LumA	LumA	LumA
LK521	1	Normal	LumB	LumA
DT474	1	Normal	LumA	LumA
WE748	1	No tissue	No tissue	No tissue
C1 subtypes		40%LumA, 30%LumB, 20%Norm, 10%NA	70%LumA, 10%LumB, 10%Norm,10%NA	70% LumA, 10%LumB, 10%Norm,10%NA
TV682	2	LumA	LumA	LumA
EF328	2	LumA	LumA	LumA
GU874	2	LumA	LumA	LumA
FD541	2	LumA	LumA	LumA
SR444	2	LumA	LumA	LumA
LD432	2	LumA	LumA	LumA
JK7482	2	LumA	LumA	LumA
SQY418	2	LumB	LumB	LumA
KP963	2	LumB	LumA	LumA
FQ901	2	LumB	LumB	LumA
C2 subtypes		70% LumA, 30%LumB	80%LumA, 20%LumB	100% LumA
C1 & C2 pooled subtypes		55% LumA, 30% LumB, 10% Normal, 5%NA	75% LumA, 15% LumB, 5% Normal, 5%NA	85% LumA, 5% LumB, 5% Normal, 5%NA

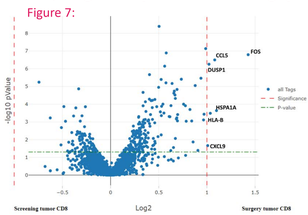
Risk of Recurrence-S (ROR-S) (Fig 6b)

ROR-S				
Patinet ID	Cohort	DAY 1	DAY 3	DAY 21
SF957	1	med	med	low
SK837	1	med	med	low
DG746	1	med	low	low
SA526	1	low	low	low
PO124	1	low	low	low
TR584	1	low	low	low
HJ996	1	low	low	low
LK521	1	low	med	low
DT474	1	med	low	low
WE748	1	No tissue	No tissue	No tissue
C1 subtypes		0%high, 40%med, 50% low, 10%NA	0%high, 30%med, 60% low, 10%NA	90%low, 10%NA
TV682	2	med	med	low
EF328	2	low	low	low
GU874	2	med	med	low
FD541	2	low	low	low
SR444	2	low	low	low
LD432	2	low	low	low
JK7482	2	low	low	low
SQY418	2	low	low	low
KP963	2	med	med	low
FQ901	2	high	high	low
C2 subtypes		10%high, 30%med, 60% low	10%high, 30%med, 60% low	100%low
C1 & C2 pooled subtypes		5%high, 35%med, 55% low, 5%NA	5%high, 30%med, 60% low, 5%NA	95%low, 5% NA

Changes in tumor T cell phenotype (GeoMx, Digital Spatial Profiling (DSP))

- DSP analysis on the CD8 population within the tissue samples showed a profile consistent with T cell activation from baseline to surgery (Day 21) in both cohorts.
- The volcano plot shows 6 significantly differentiated genes that indicate a pattern of T cell activation (listed in green in the table), while well-known markers of T cell exhaustion were not increased by therapy (listed in red). (Fig 7)

Figure 7:



Gene	Log2 (D21/D1)	-log10 pvalue
FOS	1.43	6.79
HSPA1A	1.10	3.64
CCL5	1.08	6.50
HLA-B	1.03	3.49
DUSP1	1.02	6.25
CXCL9	1.01	3.66
LAG3	0.44	1.66
CD244	0.12	1.07
TIGIT	0.14	0.94
PDCD1 (PD-1)	0.06	0.28
CTLA4	-0.02	0.09

CONCLUSION

- Previously we showed that Cohort 2 met the study's success criterion of ≥30% increase in CelTIL score in at least 50% of the patients[4].
- The additional translational research results presented here show that pelareorep and atezolizumab act synergistically to establish a favorable immunologic response in both the tumor and the blood as demonstrated by:
  - Changes in the T cell populations including decreases in clonal T cell diversity, which is associated with increased CelTIL scores and TILs at surgery (Day 21) (Figs 3 and 4)
  - Upregulation of caspase 3 in tumor samples (Fig 5)
  - A favorable change from luminal B to luminal A subtype (better prognosis) and decrease in risk of recurrence (ROR) (Fig 6a and 6b)
  - An increase in markers of T cell activation and no significant change in markers of T cell exhaustion (Fig 7)
- These data illustrate pela's ability to induce an inflamed tumor phenotype and demonstrate its synergy with atezo. Moreover, they 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). Disclosures of J. Gavila : Astra-Zeneca, Pfizer, Novartis, Roche. Email: jogagre@hotmail.com