Pelareorep primes the tumor for checkpoint inhibition therapy by activating the interferon-gamma signaling pathway and tumor inflammation signature in early breast cancer patients - results of the AWARE-1 trial

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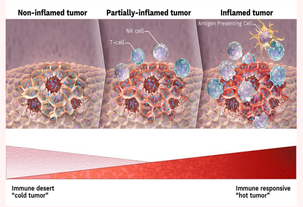
BACKGROUND

- The status of the tumor microenvironment (TME) can profoundly affect the response to immune-based therapies for the treatment of cancer. For example, it has been shown that increased activation of the interferon-gamma (IFN-γ) signaling pathway as well as the activation of the Tumor Inflammation Signature (TIS) genes can predict response to checkpoint blockade therapy.[1-2]
- Pelareorep (pela) is an intravenously delivered unmodified oncolytic reovirus that can selectively replicate in tumor cells and induce an inflamed TME characterized by increased T cell infiltration (Fig 1).[3] To examine if pela can mediate the priming of an anti-tumor immune response and induce a proinflammatory TME that would respond better to checkpoint blockade therapy, the AWARE-1 study (NCT04102618) was conducted in HR+/HER2- newly diagnosed, early breast cancer (BC) patients. Patients were enrolled into two cohorts: Cohort 1 (C1): pela + letrozole; and Cohort 2 (C2): pela + letrozole + atezolizumab (atezo).

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.



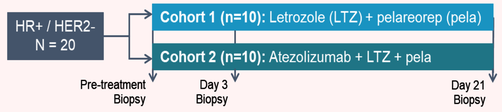
- Previously reported AWARE-1 data showed that pela combined with atezo resulted in CelTIL score increases of >30% in 60% of patients, thereby meeting the study's primary endpoint. Patients who received pela without atezo showed increases of >30% in CelTIL score in 40% of patients.[4] The CelTIL score is a metric for quantitating changes in tumor cellularity and tumor-infiltrating lymphocytes (TILs), and an increased CelTIL score is associated with favorable responses to treatment.[5]
- Increased CelTIL scores were accompanied by a favorable immunologic response observed in both the tumor and the blood as demonstrated by:[4,6-7]
  - Upregulation of PD-L1 and caspase 3 expression in tumor tissue
  - Increased CD8+ memory T cells, a favorable CD8:Treg ratio, along with an increase in markers of T cell activation and no significant change in markers of T cell exhaustion in the TME
  - Changes in the T cell populations including decreases in clonal T cell diversity, which was associated with increased CelTIL scores and TILs at surgery
  - Decreased expression of markers of T cell exhaustion (CD39, LAG3, and TIM-3) in the blood
- Together these results demonstrated that pela, alone or in combination with atezo, modified the inflammatory state of the TME and that these pela effects were enhanced by the addition of atezo.
- Here we present additional gene expression and pathway analyses from AWARE-1 in order to elucidate the mechanism of how pela primes the TME to respond to atezo.

STUDY DESIGN & METHODS

- AWARE-1 is a window-of-opportunity study designed to evaluate the safety and efficacy of pela ± atezo on the TME and peripheral blood cell populations (Fig 2).
- Newly diagnosed HR+/HER2- early BC patients were enrolled into two cohorts: C1: pela + letrozole (n=10); and C2: pela + letrozole + atezolizumab (n=10). Pela was intravenously administered on days 1, 2 and 8, 9, and atezolizumab was given on day 3.
- Here we report **day 3** data. At that timepoint, patients in both cohorts had received identical treatment that included pela but not atezo. Therefore, the data from C1 and C2 were pooled. For this analysis, tumor biopsies (FFPE samples) collected pre-treatment (D1) and on day 3 (D3, prior to the atezo administration) were examined by GeoMx digital spatial profiling (DSP, using Nanostring's Cancer Transcriptome Atlas [CTA]). Moreover, the expression of 760 immune-related genes was analyzed using a specific immune panel. Gene Set Enrichment Analysis (GSEA v4.1.0) was used to assess pela-induced activated pathways.

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

Figure 3: Volcano plot and corresponding table of top activated pathways

GeoMx DSP showed that therapy significantly activated IFN-γ signaling from D1 to D3 (Normalized Enrichment Score [NES] = 3.3, p-values <0.02) in the cytokeratin-positive subset of the tumor samples from all patients in cohort 1 and 2. The most significantly activated paths are highlighted on the volcano plot on the right and listed in the left table.

Pathway description	Pathway size	NES	pValue
Interferon alpha/beta signaling	70	5.35	0.01
Interferon Signaling	200	5.32	0.01
Antiviral mechanism by IFN-stimulated genes	81	3.65	0.01
Interferon gamma signaling	91	3.37	0.01
ISG15 antiviral mechanism	73	3.09	0.01
ER-Phagosome pathway	85	3.06	0.01
Antigen processing-Cross presentation	100	3.03	0.01
Cytokine Signaling in Immune system	883	2.89	0.01
Initial triggering of complement	23	2.70	0.01
Innate Immune System	1066	2.43	0.01

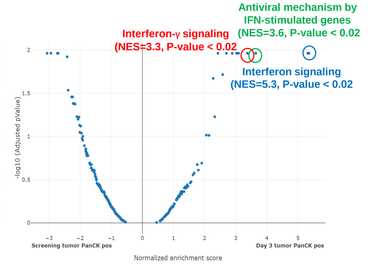


Figure 4: Heatmap of upregulated genes in IFN-gamma signaling pathway

GSEA of the immune dataset (730 immune genes + 30 housekeeping genes) from the whole tissue samples from D1 and D3 also showed a significant upregulation of IFN-gamma signaling pathway genes listed at the right of the heatmap below (FDR <25%, p-value<0.001). Each column represents one patient in C1 and C2.

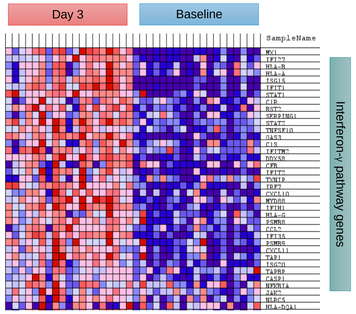
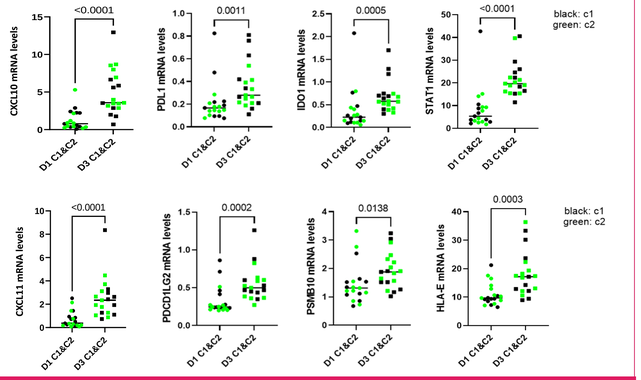


Figure 5: Examples of upregulated genes in IFN-gamma signaling pathway and the TIS pathway

In addition to the upregulation of genes involved in the IFN-gamma signaling pathway, in the whole tumor samples obtained from patients in C1 and C2, many genes of the TIS pathway were also upregulated at D3 compared to baseline, several of which are graphed below.



CONCLUSION

Here we show gene expression and pathway analyses comparing baseline to day 3 in tumor samples from AWARE-1 patients (Cohorts 1 and 2). These results demonstrate that pela mediates priming of the TME. Pela appears to prime the tumor for response to checkpoint blockade therapy by activating the IFN-gamma signaling pathway as well as by activating several genes in the TIS pathway. Together with previously reported AWARE-1 results, these data illustrate pela's ability to induce an inflamed tumor phenotype and may explain the ability of atezo to enhance the intratumoral inflammatory effects of pela. In summary, the AWARE-1 results support pela's immune-based mechanism of action and suggest that combining pela with atezo may improve clinical outcomes in BC patients.

References: [1] Damotte et al. J Transl Med 2019 [2] Ayers et al. J Clin Invest 2017 [3] Samson et al. Sci Transl Med 2018;10 [4] Manso et al. AACR 2021 [5] Nuciforo et al. Ann Oncol (2018), 29: 170-77 [6] Gavila et al. ESMO BC 2022 [7] González-Navarro et al. SITC 2022