

Pelareorep to promote the expression of a IFN-gamma-related gene signature that predicts response to checkpoint blockade therapy



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Abstract (#3089)

Background: A clinical study in patients (pts) with metastatic breast cancer (mBC) treated with pelareorep resulted in significant improvements in overall survival. Clinical studies with checkpoint blockade inhibitors (CBIs) have also resulted in noteworthy clinical responses in a small subset of mBC pts. In pts who do not respond to CBIs, the absence of IFN-γ signalling in the tumor microenvironment has been proposed as a key mediator of innate resistance. IFN-γ signalling upregulates the expression of checkpoint ligands and promotes lymphocyte activation and infiltration to the tumor microenvironment. Thus, the expression of IFN-γ-related genes can be used to both facilitate and predict response to CBIs. Given pelareorep's known capacity to promote an inflamed tumor phenotype, we hypothesised that pelareorep could also stimulate the expression of IFN-γ related genes associated with response to CBIs. **Methods:** Cell lines derived from breast cancer (BC: MCF7, T47D, MD-231), colorectal cancer (CRC: HT-29, SW620), hepatocellular carcinoma (HCC: SNU-387), and non-small cell lung cancer (NSCLC: H522) were infected with pelareorep at a multiplicity of infection equal to 50. We examined changes in gene expression and conducted cell viability assays at 6, 12, and 18 hours post-infection (including a non-infected control). To monitor changes in gene expression we employed a 780-gene panel (nanoString) to monitor for changes in the expression of key IFN-γ-related and other immunity-related genes. **Results:** All cell lines were susceptible to pelareorep induced cytopathic effect. Strikingly, BC and HCC cells lines significantly upregulated IFN-γ-related genes while CRC and NSCLC cell lines demonstrated only a modest and variable ability to promote IFN-γ pathway activation. Moreover, BC and HCC cells lines also upregulated key chemokines that are known to promote response to immunotherapy. **Conclusions:** These results suggest that various tumor types are amenable to immune priming for CBI therapy with pelareorep. The role of pelareorep in the treatment of BC and HCC deserves further investigation, particularly in combination with other immunotherapies.

Background

Pelareorep (REOLYSIN®) is an unmodified immuno-oncolytic virus. The anti-tumor activity of pelareorep is based on a two part mechanism of action (Figure 1):

1. Direct lysis of tumor cells permissive to viral replication
2. Anti-tumor immunity, comprised of: (i) the activation of an innate immune response to the infection which results in the release of chemokines/cytokines that promote lymphocyte activation and recruitment to the tumor microenvironment, particularly natural killer (NK) cells and dendritic cells (DCs); and (ii) a primary adaptive immune response mediated by cytotoxic T cells that recognize tumor- and viral-associated antigens (TAA/VAA).

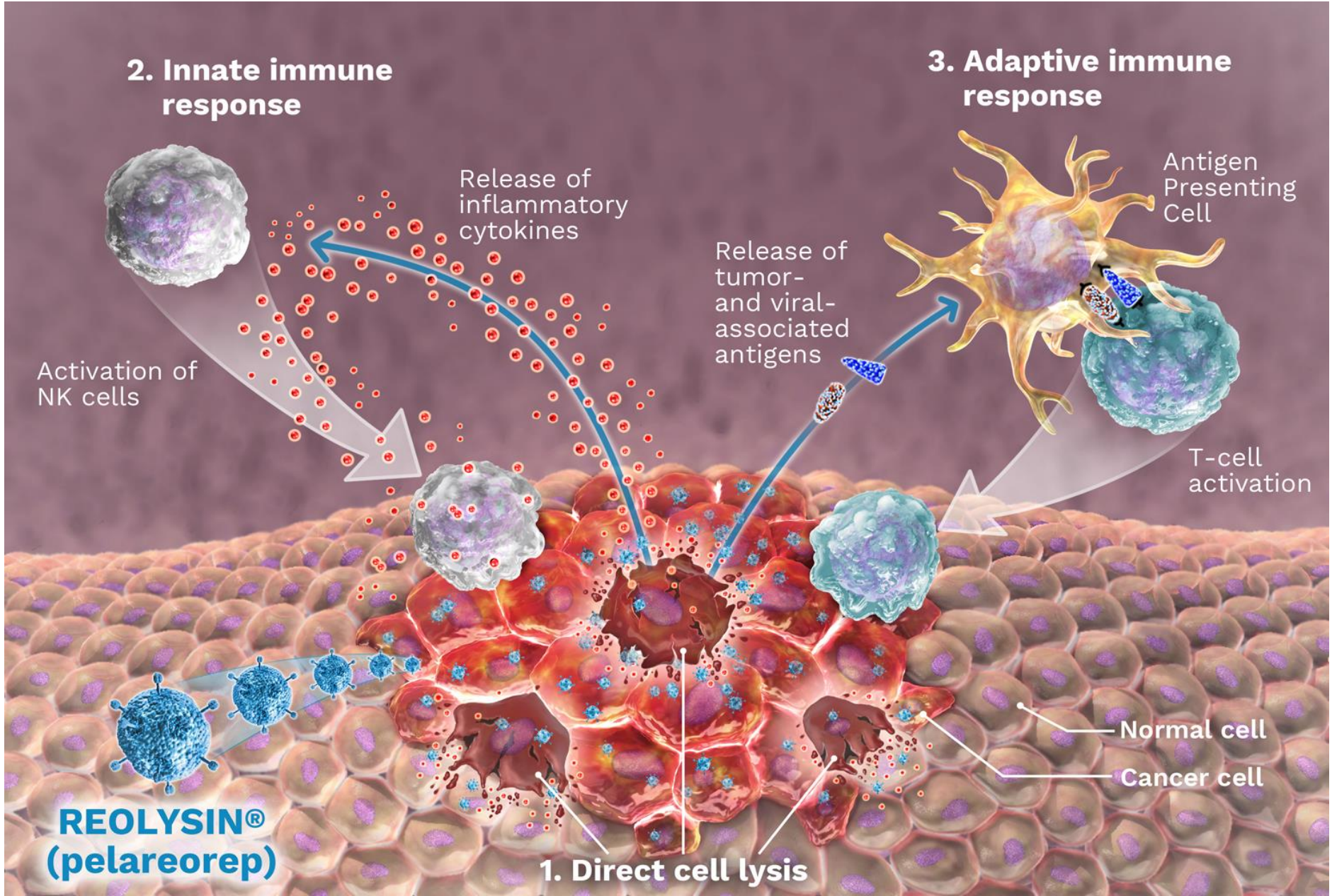


Figure 1. Pelareorep mechanism of action.

Rationale

Clinical studies with CBIs have resulted in compelling clinical responses in BC, HCC, CRC, and NSCLC, yet only a subset of patients respond [1]. In patients that do not respond to CBIs, the absence of an inflamed tumor phenotype has been proposed as a key mediator of innate resistance [2, 4]. Conversely, the presence of an inflamed tumor phenotype may both facilitate and predict response to CBIs. In fact, previous studies have identified inflammatory gene signatures that may predict response to CBI [2, 4]. In select cancer cell lines, we examined if pelareorep could promote gene expression signatures that are associated with both an inflamed tumor phenotype and predictive of response to CBIs.

Methods

Cell lines derived from hormone receptor positive (HR+) BC (MCF7, T47D), triple negative breast cancer (TNBC, MD231), NSCLC (H522), CRC (SW-620, HT29), and HCC (SNU-387) were infected with pelareorep at a multiplicity of infection equal to 50. At 0, 6, 12, and 18 hours post-infection, changes in gene expression were examined and cell viability assays conducted. Changes in gene expression were monitored with a custom 780-gene Pan Cancer Immune panel, examining genes categorized under broad immunological functions (Table 1).

Categories	# of Genes
Chemokines	25
Cytokines	22
Cell Functions	82
B-Cell Functions	13
Antigen Processing	155
Regulation	99
Cytotoxicity	15
NK Cell Functions	56
Transporter Functions	10
Pathogen Defense	38
Cell Cycle	6
Leukocyte Functions	6
T-Cell Functions	5
Adhesion	17
Complement	12
Senescence	154
Interleukins	12
Macrophage Functions	82
TLR	11
Microglial Functions	30
TNF Superfamily	22

Table 1. Summary of genes in each functional category

The majority of changes in gene expression occur after 6 hour post-infection but before significant cell lysis

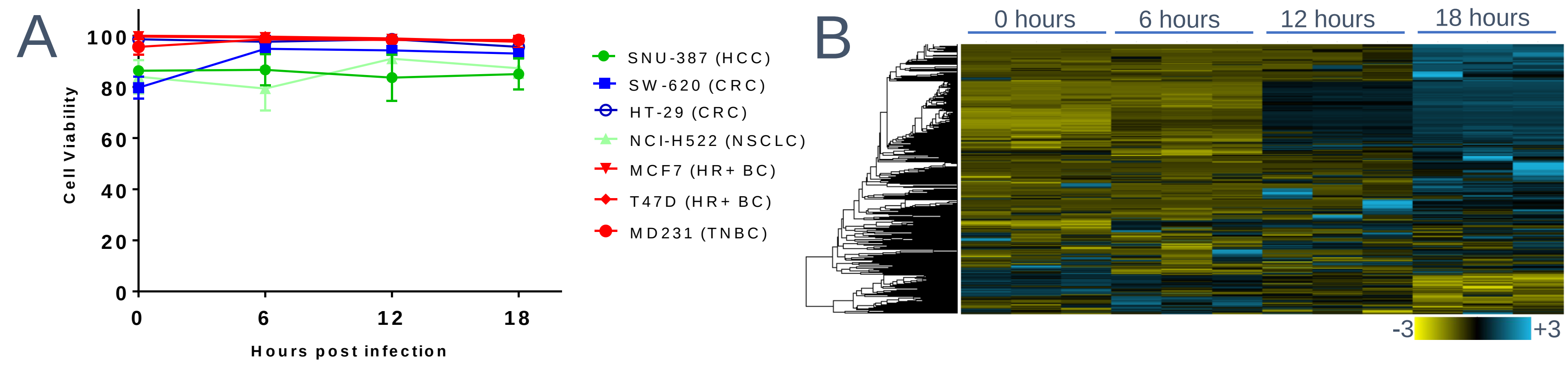


Figure 2. (A) Cell viability assessment following pelareorep infection in BC, HCC, CRC, and NSCLC cell lines at 0 (non-infected control), 6, 12, 18 hours post-infection. (B) Representative heat map analysis of temporal changes in gene expression in MCF7 cells after pelareorep infection.

Pelareorep promotes the expression of gene signatures that predict response to immunotherapies in HR+ BC cells

Gene signature for pembrolizumab [4]							
	HCC (SNU-387)	CRC (SW-620)	CRC (HT29)	NSCLC (H522)	HR+ BC (MCF7)	HR+ BC (T47D)	TNBC (MD231)
IDO1	***	ns	ns	ns	**	****	**
CXCL10	****	ns	****	ns	****	****	****
CXCL9	ns	ns	**	ns	****	ns	ns
HLA-DRA	ns	ns	ns	ns	***	***	ns
STAT1	****	**	****	****	****	****	**
IFNG(R1)	ns	** (down)	****	****	***	***	ns
Gene signature for immunotherapy [2]							
	HCC (SNU-387)	CRC (SW-620)	CRC (HT29)	NSCLC (H522)	HR+ BC (MCF7)	HR+ BC (T47D)	TNBC (MD231)
CCL2	***	Ns	ns	**	****	****	**
CCL3	***	ns	*	ns	ns	****	****
CCL4	****	ns	**	ns	****	****	****
CCL5	****	ns	****	****	****	****	****
CCL8	***	ns	ns	ns	ns	ns	ns
CXCL9	ns	ns	**	ns	****	ns	ns
CXCL10	****	ns	****	ns	****	****	****
CXCL11	****	ns	****	ns	****	****	****

Table 2. Activation of gene signatures that predict response to immunotherapies, 18 hours post-infection, relative to non-infected cells. Genes expressed in PBMCs are excluded. ns, $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Results

Pelareorep differentially promotes the expression of innate and adaptive genes

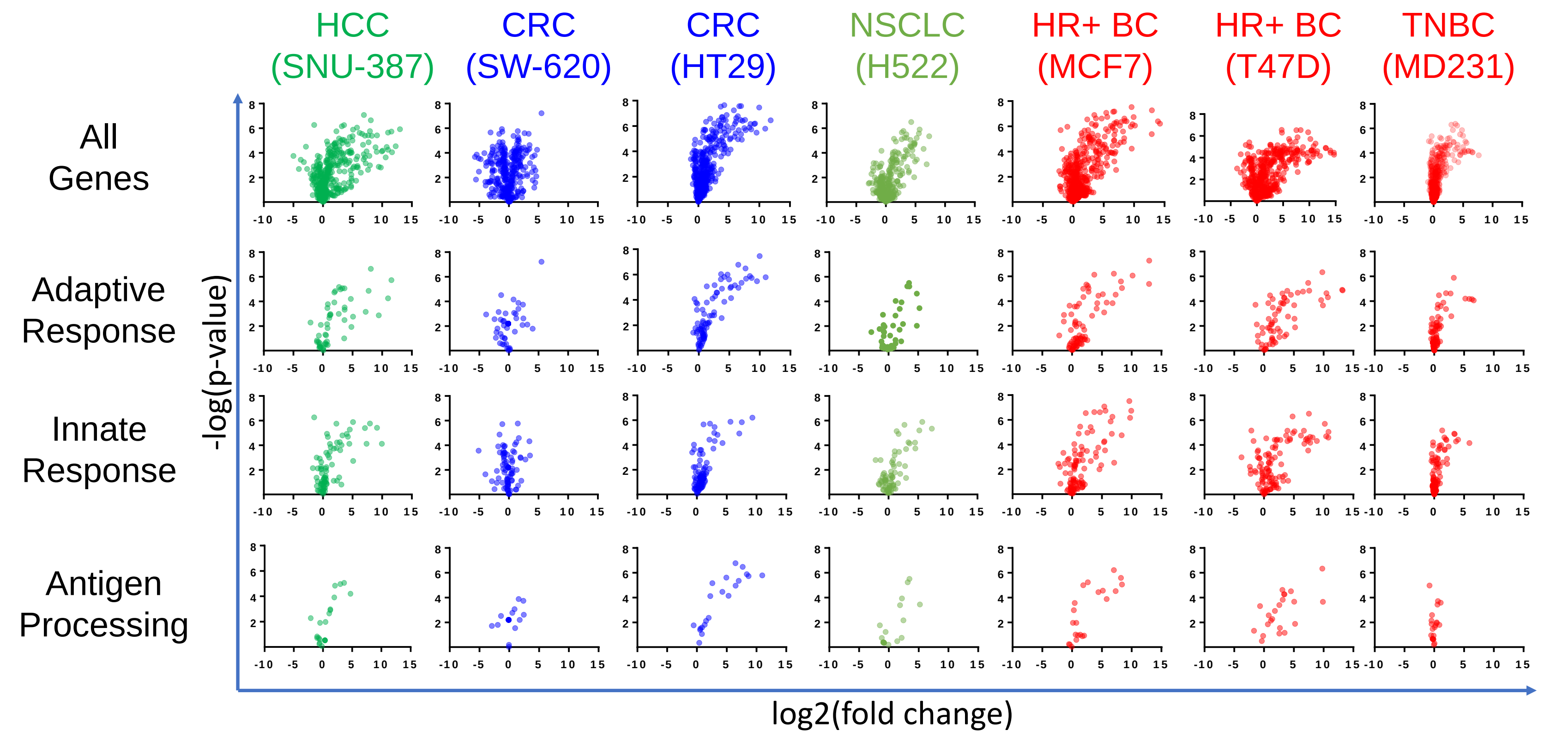


Figure 3. Volcano plot analysis revealing changes in gene expression following pelareorep treatment, 18 hours post-infection, examining genes involved in innate and adaptive response as well as antigen processing.

Gene ontology (GO) analysis reveals that the top differentially expressed genes promote T and NK cell recruitment and activation

HR+ BC (MCF7)		
GO biological process complete	Fold Enrichment	P-value
regulation of natural killer cell chemotaxis (GO:2000501)	> 100	1.20E-07
positive regulation of natural killer cell chemotaxis (GO:2000503)	> 100	2.39E-05
regulation of chronic inflammatory response (GO:0002676)	> 100	2.08E-07
regulation of MHC class I biosynthetic process (GO:0045343)	> 100	3.07E-05
Pos. reg. of leukocyte adhesion to vascular endothelial cell (GO:1904996)	> 100	3.07E-05
regulation of lymphocyte chemotaxis (GO:1901623)	> 100	6.20E-09
T cell migration (GO:0072678)	> 100	7.38E-09
cellular response to exogenous dsRNA (GO:0071360)	> 100	7.03E-07
regulation of T cell chemotaxis (GO:0010819)	> 100	6.64E-05

Table 3. GO analysis of top 20 differentially expressed genes 18 hours after infection, relative to baseline control. False Discovery Rate < 0.05.

Conclusion

We demonstrate that pelareorep can promote the expression of gene signatures that are predictive of response to CBIs in select cell lines, namely HCC and HR+ BC. These results warrant further investigation of CBI + pelareorep combination therapy, particularly in HR+ BC where patients are often refractory to CBI therapies. Moreover, the pronounced inflammatory effects of pelareorep in HR+ BC cell lines may help to explain the prominent increase in overall survival in HR+ BC patients treated with pelareorep in a phase 2 clinical study [5, 6] (Figure 4).

References

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[6] Thorsson et al. The immune Landscape of Cancer. Immunity, 2018. 48, 812-830

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