Pelareorep promotes the expression of a chemokine signature that predicts response to immunotherapy

G. Wilkinson[1], A. Piar[2], Z. Cesarz[2], H. Tran[1], R. Chakrabarty[1], A. A. Gutierrez[1], M. Coffey[1]
[1]Oncolytics Biotech Inc. [2]Almac Diagnostics.

ONCOLYTICS BIOTECH INC.

Abstract

Introduction: It has been proposed that the presence of inflamed tumor phenotypes, characterized by the presence of infiltrating lymphocytes and the expression of specific chemokines and cytokines, can predict response to immunotherapy and result in better patient outcomes [1, 2]. We hypothesized that pelareorep, an immuno-oncolytic virus (IOV), may elicit predictive proinflammatory gene signatures in select cancer cell lines permissive to viral infection.

Methods: Cell lines derived from non-small cell lung cancer (NSCLC, H522), colorectal cancer (CRC, SW-620), and hepatocellular carcinoma (HCC, SNU-387) were infected 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 pelareorep infection (including a non-infected control). To monitor changes in gene expression we employed a custom 780-gene Pan Cancer Immune panel developed by nanoString Technologies and specifically monitored for changes in the expression of key interferon and NF-κB signalling genes, immune checkpoint ligands, and a 12-gene chemokine signature predictive of a positive response to immunotherapy identified by Messina et al. [2].

Results: All cell lines examined were susceptible to pelareorep induced cytopathic effect. Strikingly, principal component analysis revealed that the changes in gene expression were unique and different for each cell line. Of the cell lines examined, only HCC cells infected with pelareorep promoted an inflammatory signature, similar to the one used to predict response to immunotherapy in melanoma [2].

Conclusions: This study demonstrates that pelareorep can prime or promote a predictive inflamed tumor phenotype in HCC, which correlates with the innate response recently described in HCC-animal models treated with pelareorep [3]. The role of pelareorep in the treatment of HCC deserves further investigation, particularly in combination with other immunotherapies.

Background

Pelareorep (REOLYSIN®) is a first-in-class immuno-oncology-viral agent that has been delivered intratumorally and intravenously in preclinical and clinical oncology studies. Pelareorep's anti-tumor activity is based on a dual mechanism of action, which is complementary, but not interdependent (Figure 1):

1. Direct oncolytic activity of tumor cells permissive to viral replication
2. Induction of anti-tumor immunity through:
   - Activation of innate immunity against virally infected tumor cells and upregulation of inflammatory cytokines.
   - Increased presentation of tumor- and viral-associated epitopes by antigen-presenting cells (APCs, e.g., dendritic cells), allowing for the generation of an adaptive antitumor immune responses.

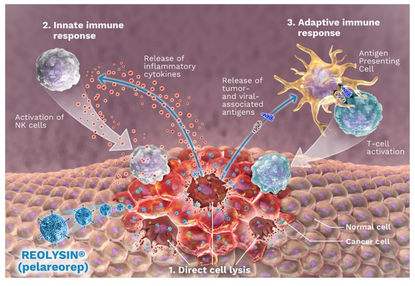


Figure 1. Pelareorep mechanism of action

Rational

Clinical studies with checkpoint blockade inhibitors (CBIs) have resulted in noteworthy clinical responses in 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 NSCLC (H522), CRC (SW-620), and HCC (SNU-387) were infected with pelareorep at 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 category

Results

Global changes in gene expression are unique and different for each cell line following pelareorep infection

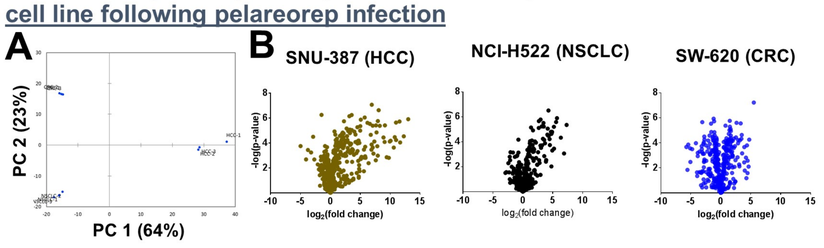
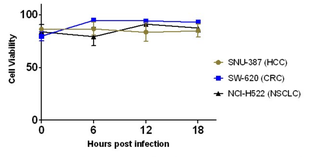


Figure 2. Principle component (A) and volcano plot analysis (B) revealing changes in gene expression following pelareorep treatment, 18 hours post-infection.

Changes in gene expression occur before significant cell lysis

Figure 3. Cell viability assessment following pelareorep infection in HCC, CRC, and NSCLC cell lines at 0 (non-infected control), 6, 12, 18 hours post-infection.



Pelareorep differentially promotes the expression of innate and adaptive immunity related genes in HCC, CRC, NSCLC cell lines

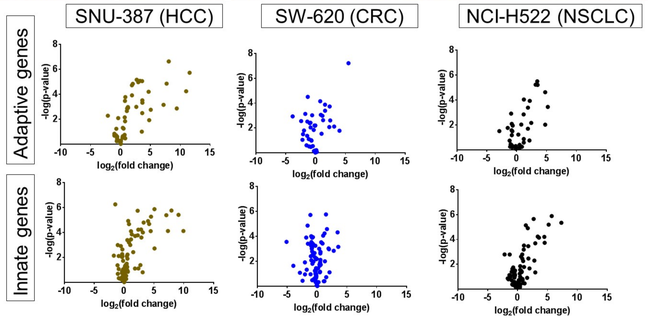


Figure 4. Changes in gene expression following pelareorep treatment, in innate and adaptive immunomodulatory genes, 18 hours post-infection.

Results

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

HCC (SNU-387)	
GO biological process	**Fold Enrichment**
positive regulation of natural killer cell chemotaxis (GO:2000503)	> 100
regulation of natural killer cell chemotaxis (GO:2000501)	> 100
regulation of MHC class I biosynthetic process (GO:0045343)	> 100
negative regulation by host of viral transcription (GO:0043922)	> 100
T cell chemotaxis (GO:0010818)	> 100
positive regulation of monocyte chemotactic protein-1 production (GO:0071639)	> 100
eosinophil chemotaxis (GO:0048245)	> 100
regulation of chronic inflammatory response (GO:0002676)	> 100
regulation of lymphocyte chemotaxis (GO:1901623)	> 100
positive regulation of lymphocyte chemotaxis (GO:0140131)	> 100
eosinophil migration (GO:0072677)	> 100
T cell migration (GO:0072678)	> 100

Table 2. GO analysis of top 20 differentially expressed genes 18 hours after infection, relative to baseline control. False Discovery Rate < 0.05.

Pelareorep promotes the expression of gene signatures that predict response to immunotherapies in HCC cells

Gene signature developed by Messina et al. 2012 [2] for immunotherapy

	SNU-387 (HCC)	SW-620 (CRC)	H522 (NSCLC)
CCL2	***	ns	**
CCL3	***	ns	ns
CCL4	****	ns	ns
CCL5	****	ns	****
CCL8	***	ns	ns
CXCL9	ns	ns	ns
CXCL10	****	ns	ns
CXCL11	****	ns	ns

Gene signature developed by Ayers et al. 2017 [4] for pembrolizumab

	SNU-387 (HCC)	SW-620 (CRC)	H522 (NSCLC)
IDO1	***	ns	ns
CXCL10	****	ns	ns
CXCL9	ns	ns	ns
HLA-DRA	ns	ns	ns
STAT1	****	***	****
IFNG(R1)	ns	**(down)	****

Table 3. 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, $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Conclusion

This study demonstrates that pelareorep can promote the expression of gene signatures that are predictive of response to CBIs in a HCC cell line. While these effects are modest in CRC and NSCLC they do not rule out the possibility of synergistic pelareorep-ICB combination therapy in discrete CRC and NSCLC subtypes, for example, subtypes that harbor a high mutational burden (TMB). Thus the role of pelareorep in the treatment of HCC as well as TMB high CRC and NSCLC deserves further investigation, particularly in combination with other immunotherapies.

References

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