Molecular Profiling Reveals Anti-PD-1 Agonist Antibody-induced Changes to Key Immune Pathways

Patrick J. Halvey, Emily B. Lurier, Michael Cianci, Joanne L. Viney, Kevin L. Otipoby, Katalin Kis-Toth; Pandion Therapeutics, Watertown, MA

ABSTRACT

Co-inhibitory receptors play a critical role in the regulation of T cell activity and represent attractive targets for the restoration of immune tolerance in autoimmune disease. Activation of PD-1-mediated signaling is a promising therapeutic strategy to reduce T cell activity. We have generated a set of PD-1 agonist antibodies which inhibit activated T cells. Here we demonstrate that *in vitro* gene and protein expression molecular profiling can be used to identify pharmacodynamic biomarkers for anti-PD-1 agonists.

PD-1 agonist antibodies were evaluated in a human primary cell-based assay. RNA was extracted and conditioned media was collected. Gene expression changes in proinflammatory cytokines were measured by qRT-PCR and gene expression profiling on over 700 genes using a Nanostring autoimmune profiling panel was also carried out. Increases in proinflammatory cytokine production were measured by ELISA and a set of over 300 proteins was examined in conditioned media samples using Olink proteomic analysis. Gene set enrichment analysis showed a convergence of molecular adaptations across protein and gene measurements in response to PD-1 agonism, revealing the modulation of key immune pathways and offering a viable strategy for understanding mechanisms associated with agonism of inhibitory receptors and providing a path forward for pharmacodynamic biomarker discovery.

INTRODUCTION

- Antagonist antibodies against co-inhibitory receptors enhance immune function in T cells and have been used as therapeutic agents in oncology
- Activation of PD-1 represents an attractive strategy for suppression of activated effector T cells in autoimmune disease
- Anti-PD-1 agonist was generated and tested in a primary T cell activation assay
- Gene and protein expression analysis demonstrated pharmacodynamic effect of the PD-1 agonist

RESULTS

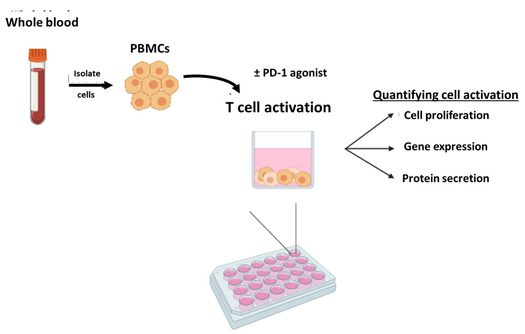

Figure 1. Primary T cell activation assay. PBMCs were isolated from healthy donor blood and activated in presence or absence of an anti-PD-1 agonist (200 nM) for 6-120 h. Supernatants were collected for protein analysis and RNA was isolated for gene expression analysis.

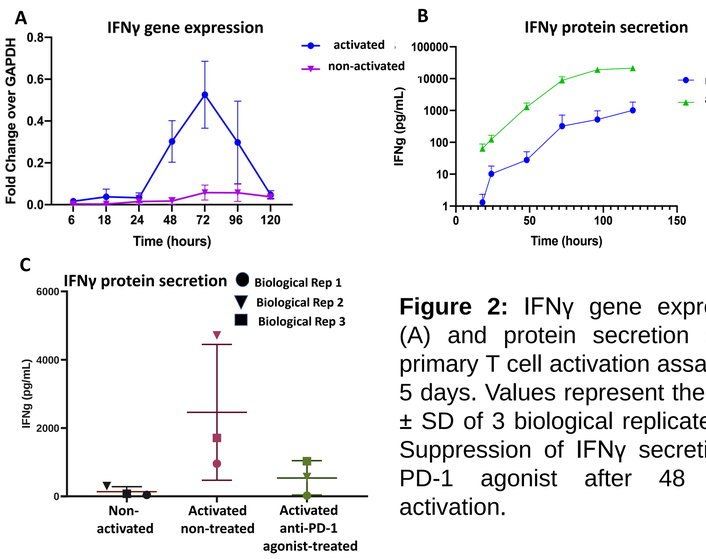

Figure 2: IFNγ gene expression (A) and protein secretion (B) in primary T cell activation assay over 5 days. Values represent the mean ± SD of 3 biological replicates. (C) Suppression of IFNγ secretion by PD-1 agonist after 48 h of activation.

RESULTS (cont.)

CCL8	MX1	CD80	IFITM1
CCL13	SERPING1	HIST1H3G	CFP
IL6	CMKLR1	IFNG	CCL18
CXCL1	RSAD2	IFI44	LILRB1
CXCL10	OAS1	HERC5	HERC6
CD163	IFI44L	TMEM176A	IFI35
IL17F	CTSC	TNFSF10	ISG20
CCL2	IL22	C1QA	SERPINA1
IFI27	IFIT3	OAS2	LAG3
CXCL9	S100A12	C2	GBP5
CCL19	CXCL8	CXCL2	CD274
SIGLEC1	CD209	GZMB	CGAS
IFITM3	FCN1	FCGR2B	IL1B
THBS1	PDCD1LG2	CLEC4E	
IDO1	IL17A	TLR2	
ISG15	CFB	LYN	
CXCL5	C1QB	PDPN	
IFIT1	CD14	S100A9	

Table 1. Differentially expressed genes in anti-PD-1 agonist-treated activated PBMCs compared to vehicle treated. Gene expression was assessed using NanoString autoimmune profiling panel. Gene count values were normalized to housekeeping gene expression. Genes were filtered for log2 fold-change of vehicle-treated over anti-PD-1 agonist-treated activated PBMCs (n=3, log2FC <-1).

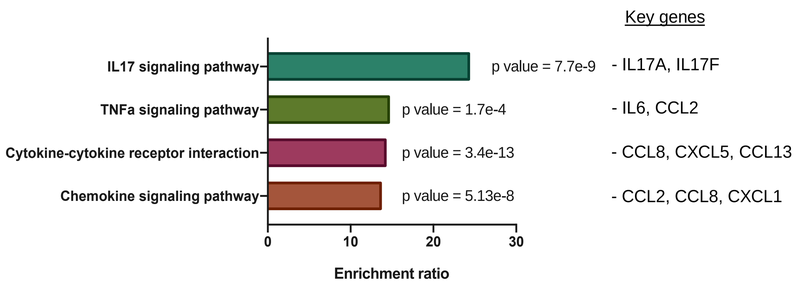

Figure 3. Pathway analysis for differentially expressed genes. Genes which showed at least a 2-fold expression difference between vehicle-treated and PD-1 agonist-treated activated cells were included in the analysis. Webgestalt was used to perform overrepresentation (ORA) analysis with the KEGG pathways database. Enrichment ratios are shown for selected pathways (adjusted p-value, Benjamini-Hochberg FDR < 0.05).

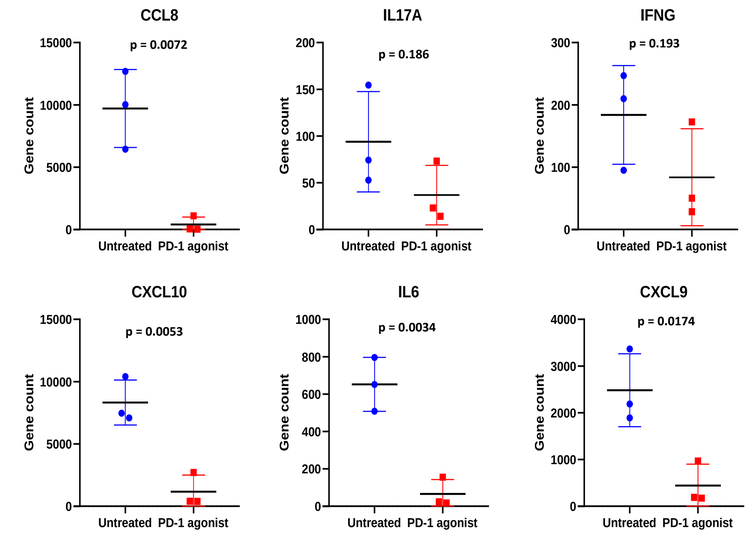

Figure 4: Selected genes differentially expressed in vehicle-treated and PD-1 agonist-treated activated cells. NanoString gene expression values after 48h (n=3 biological replicates). Unpaired t-test was used to test significance.

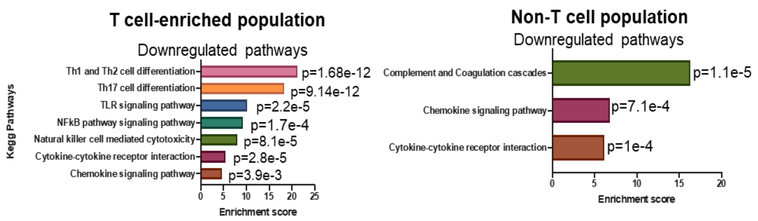

Figure 5. Pathway enrichment analysis in T cell and non-T cell populations. A primary T cell activation assay (n=3) was carried out in the presence or absence of anti-PD-1 agonist. CD3-positive T cells were separated from non-T cells and NanoString gene expression profiling was carried out. Webgestalt was used to perform overrepresentation (ORA) analysis with the KEGG pathways database. Enrichment ratios are shown for selected pathways (Benjamini-Hochberg FDR < 0.05).

RESULTS (cont.)

CCL13	CCL11	IL13
CCL8	IL12	CD8A
IL6	IL10	IL15
CXCL1	CCL23	FLT3LG
CXCL6	GZMA	FASLG
CXCL10	CCL20	TNFSF10
CCL7	IL12B	OSM
CCL19	CXCL5	CD274
IL17A	LIF	TNFSF12
CXCL9	LTA	CCL17
GZMB	TNFRSF9	CD70
IFNG	IL2	TNFRSF11B
IL24	TNFRSF4	
CXCL11	CCL4	

Table 2. Differentially expressed proteins in anti-PD-1 agonist-treated activated cells compared to vehicle-treated cells. Supernatants were collected and proteomic analysis was carried out using the Olink platform. Proteins were filtered for mean log2 fold-change of vehicle-treated over anti-PD-1 agonist-treated (n= 3 , log2FC < -1).

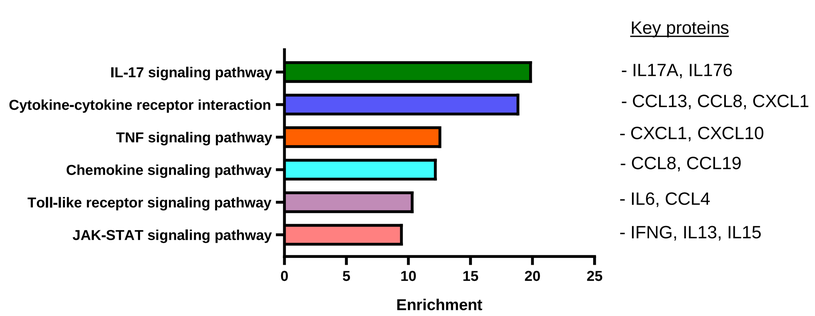

Figure 7. Pathway analysis for differentially expressed proteins. Proteins which showed at least a 2-fold difference were included. Webgestalt was used to perform overrepresentation (ORA) analysis with the KEGG pathways database. Enrichment ratios are shown for select pathways above (adjusted p-value, Benjamini-Hochberg FDR < 0.05).

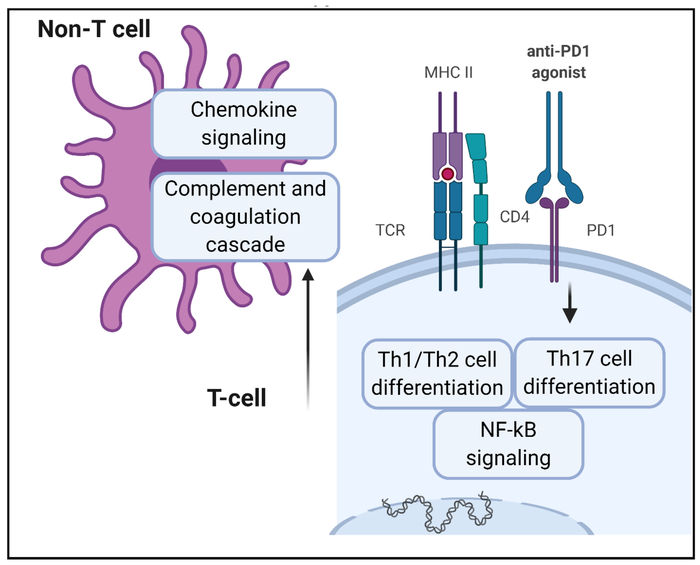

Figure 8. Anti-PD-1 agonist induced suppression of T cell activity

SUMMARY

- Gene and protein expression profiling reveal changes to key immune pathways in response to anti-PD-1 agonist
- Suppression of genes associated with Th1/Th2 and Th17 differentiation in T cells was found
- Suppression of chemokine signaling in non-T cell population was detected
- Downregulation of key markers seen at protein and gene level, including CCL8, IFNG, IL17A

REFERENCES

1. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Bing Zhang , Stefan Kirov, Jay Snoddy. Nucleic Acids Res. 2005 Jul 1;33.