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The Immunogenomic Impact of Indoximod on the Tumor Microenvironment of Patients with Advanced Melanoma

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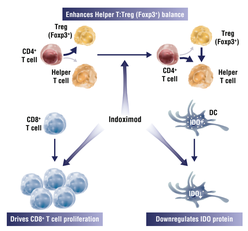
INTRODUCTION

- The indoleamine 2,3-dioxygenase (IDO) pathway mediates immunosuppressive effects through the metabolism of tryptophan (Trp) into kynurenine (Kyn), triggering downstream signaling through the Trp sensors GCN2 and mTOR as well as through the aryl hydrocarbon receptor (AhR), which senses Kyn[1-4]
- IDO is upregulated in many human tumors and tumor-draining lymph nodes, including melanoma[5-9]
- Indoximod is an orally administered, small-molecule IDO pathway inhibitor that reverses the immunosuppressive effects of low Trp and high Kyn that result from IDO activity[10]
- Preclinical and clinical data support evaluating the combination of a checkpoint inhibitor with an IDO pathway inhibitor as potential treatment for advanced melanoma[10,11]

Indoximod Mechanism of Action

- Indoximod has immunostimulatory effects involving 4 main cell types:
  - Reverses the effects of low Trp by increasing proliferation of effector ($CD8^+$) T cells
  - Directly reprograms Treg into helper T cells
  - Downregulates IDO expression in dendritic cells (DCs)

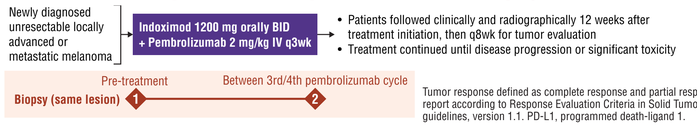


OBJECTIVES

- To determine impact of indoximod + pembrolizumab on the tumor microenvironment (TME)
- To understand the contribution of indoximod in combination treatment
- To examine pharmacodynamic effects of indoximod related to its proposed MOA
- To identify predictive biomarkers for patient stratification in future trials

METHODS

Phase 2, Single-Arm, Open-Label Study (NCT02073123)



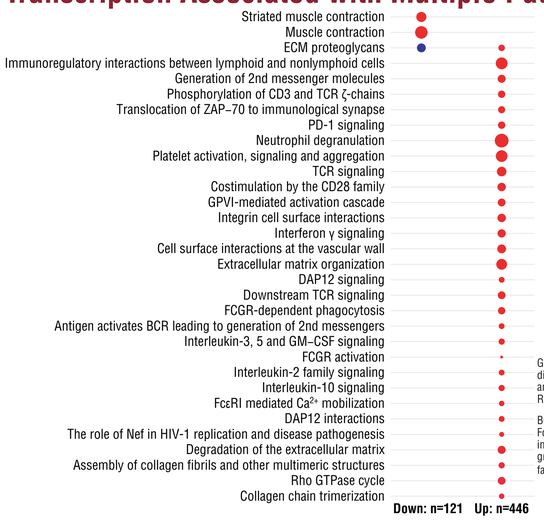
Tumor response defined as complete response and partial response by site report according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, version 1.1. PD-L1, programmed death-ligand 1.

- RNA-seq analysis, multiplex immunofluorescence staining for IDO1 (Millipore, MAB10009), Ki67 (Biocare, CRM325A); PD-L1 expression was determined by immunohistochemistry (IHC) using a validated assay for clone 22C3 as previously described[12]

RESULTS

- Patients comprised the efficacy evaluable population (received ≥1 study medication dose and ≥1 post-baseline response evaluation)
  - Samples for immunofluorescence: pre-treatment, n=14; on treatment, n=11
  - Samples for RNA sequencing: pre-treatment, n=14; on treatment, n=14
  - Samples for PD-L1 and IDO1 staining: pre-treatment only, n=38

Indoximod + Pembrolizumab Modulated Gene Transcription Associated with Multiple Pathways



Gene Ontology (GO) and Reactome enrichment of differentially expressed genes (DEGs) between pre- and on-treatment samples (FDR ≤0.05). Data from RNAseq.

BCR, B cell receptor; ECM, extracellular matrix; FcεRI, high-affinity receptor for Fc region of immunoglobulin E; FCGR, Fcg receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPVI, glycoprotein VI.

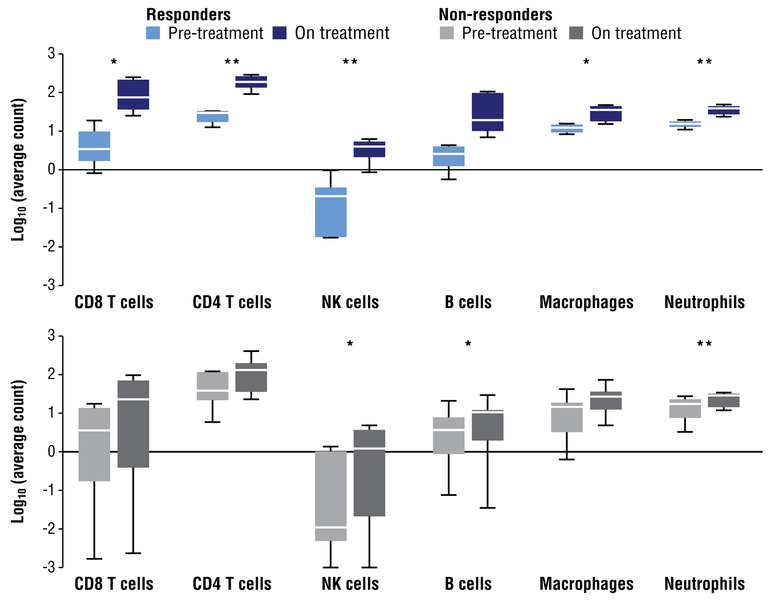
Genes Used in Analyses for Gene Expression Profiles

Immune cell types	CD8 T cells	*CD8A, CD8B*
	CD4 T cells	*CD4*
	NK cells	*KLRF1, KLRC1, NCR1*
	B cell	*CD79B, BTLA, FCRL3, BANK1, CD79A, BLK, RALGPS2, FCRL1, HVCN1, BACH2*
	Macrophages	*FUCA1, MMP9, LGMN, HS3ST2, CLEC5A, GPNMB, C11orf45, CD68, CYBB*
	Neutrophils	*KDM6B, HSD17B11, EVI2B, MNDA, MEGF9, SELL, NLRP12, TRANK1*
Immune activity	Cytotoxicity	*GNLY, CD226, GZMA, GZMB, GZMH, GZMK, GZMM, PRF1, FASLG, LAMP1*
	T cell activation	*CD247, IL2RA, CD27, CD28, CD44, TREML2, GRB2, PLCG2, PDE4B, LAT, LAT2, NEDD4, CD5, CD6, PDE4C, PDE4D, TNFRSF9, TNFRSF25, TNFRSF18, TNFRSF8, CD226, ZAP70, CD69, ICOS, TNFRSF4, LCP2, SYK, TYROBP, LCK, SLAMF8, CD84, LY9, CD40LG*
	T cell exhaustion	*ADORA2A, BTLA, CTLA4, LAG3, PDCD1, HAVCR2, TIGIT, PTPN6, CEACAM1, INPPL1, PTPN11, INPP5D, CBL, TIMD4, PTPN3, PTPN22*
	Costimulatory	*ICOSLG, CD70, TNFSF14, CD40, TNFSF9, TNFSF4, TNFSF15, TNFSF18, TNFSF8, SLAMF1, CD58*
	Coinhibitory	*PDCD1LG2, CD274, HLA-G, LGALS9, TNFRSF14, CD200, NT5E, IDO1, IDO2, PVR*
TME	Antigen presentation	*PSMB8, PSMB9, PSMB10, HSPA5, CANX, CALR, PDIA3, TAPBP, TAP1, TAP2, HLA-A, HLA-B, HLA-C, B2M, IFNG, IFNGR1, IFNGR2, JAK1, JAK2, STAT1*
	Proinflammatory	*IL23A, IL6, TNF, IFNG, IL1B, IL15, IL18, IL11, IL7*
	Anti-inflammatory	*EBI3, TGFB1, TGFB2, IL10*
	Chemokine	*CCL5, CXCL8, CCR1, CCL3, CCR4, CCR5, CCR7, CCR9, CCR10, CCL28*
	Melanoma	*MITF, MLANA, PMEL, TYR*

Expression scores were calculated as the geometric mean of FPKM values.

Immune Cell Infiltration Increased From Baseline in Responders

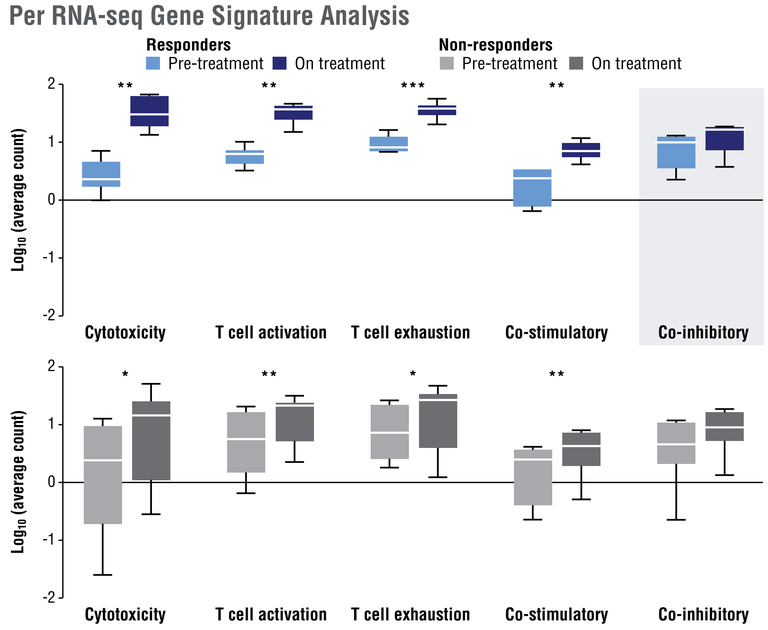
Per RNA-seq Gene Signature Analysis



Significance indicated as *p<0.05; **p<0.01, determined by paired *t*-test.

Cell Activity Increased From Baseline in Responders

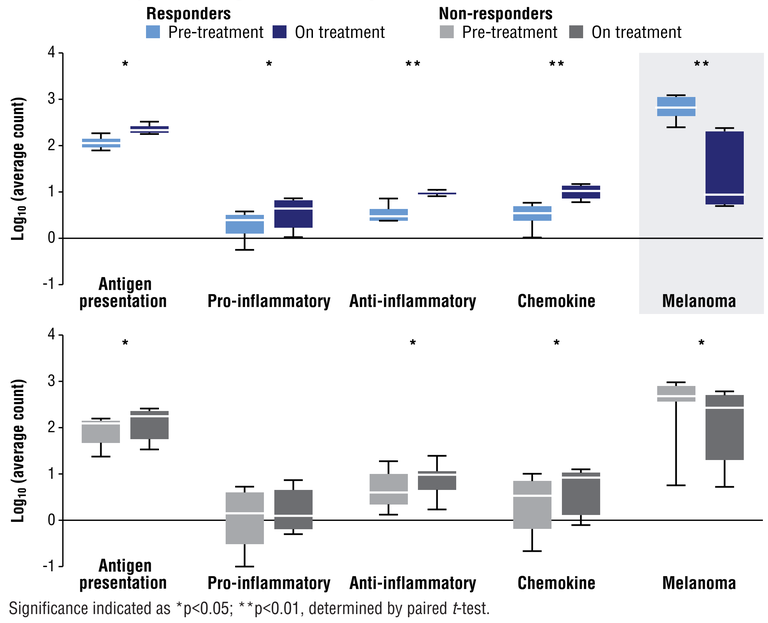
Per RNA-seq Gene Signature Analysis



Significance indicated as *p<0.05; **p<0.01; ***p<0.001, determined by paired *t*-test.

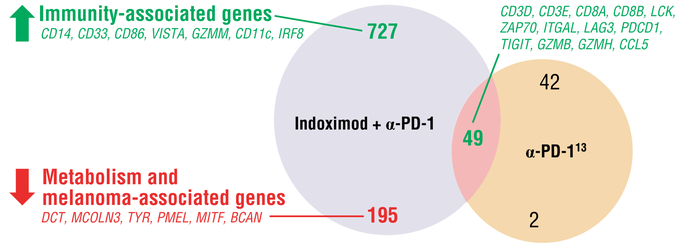
Expression of Pro-inflammatory Genes Increased and Melanoma-Related Genes Decreased in Responders

Per RNA-seq Gene Signature Analysis



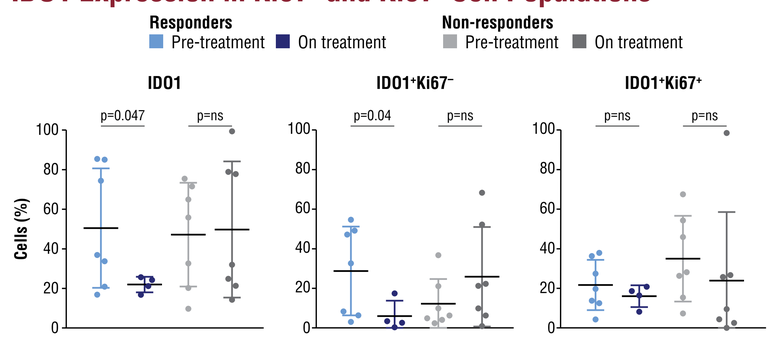
Significance indicated as *p<0.05; **p<0.01, determined by paired *t*-test.

Contribution of Indoximod to Immunologic and Metabolic Changes



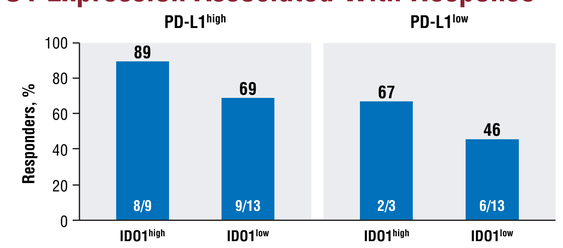
RNA-seq data compared against published studies[13] to illustrate shared and unique genes upregulated and downregulated upon treatment with indoximod + anti-PD-1 (purple) or anti-PD-1 (orange). Gene lists with FDR adjusted p-value < 0.05 were compared.

IDO1 Expression in $Ki67^-$ and $Ki67^+$ Cell Populations



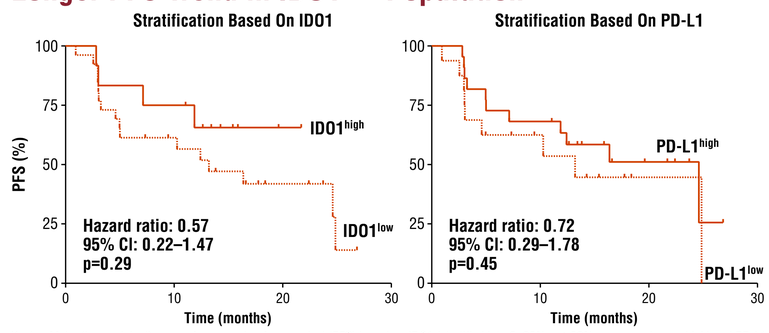
Percentage of $IDO1^+$ cells, $IDO1^+Ki67^-$ cells and $IDO1^+Ki67^+$ cells in pre- and on-treatment biopsies of responders and non-responders using immunofluorescence staining. Welch t tests were used for statistical analyses.

High IDO1 Expression Associated With Response



A histogram depicts the proportion of ORR among patients in groups defined by a composite variable of IDO1 (stratified into 0–20% or ≥20% groups as low vs high) and PD-L1 expression (stratified into 0–1% or ≥1% groups as low vs high) at baseline. IDO1 measured by immunofluorescence and PD-L1 measured by IHC.

Longer PFS Trend in $IDO1^{high}$ Population



Kaplan-Meier (log-rank test) curve of progression-free survival (PFS) based on IDO1 (stratified into 0–20% or ≥20% groups as low vs high) and PD-L1 expression (stratified into 0–1% or ≥1% groups as low vs. high) at baseline. IDO1 measured by immunofluorescence and PD-L1 measured by IHC.

CONCLUSIONS

- Indoximod + pembrolizumab induced multiple immunologic and metabolic changes in the TME
- Comparison against published studies suggests indoximod combination therapy contributed to immunologic and metabolic changes in a markedly different manner than α-PD-1 alone
  - Including gene expression consistent with enhanced cytotoxicity and innate immune cell infiltration and activation (*CD14, CD33, CD86, GZMM, CD11c,* and *IRF8* among others)
- Decreased IDO1 in $Ki67^-$ cells, most likely representing decreased IDO1 expression in DCs in responders, supports indoximod MOA
- Indoximod-based combination therapy in patients with high baseline IDO1 expression may have a better prognosis

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