COMPARATIVE BIOMARKER PROFILES OF PACRITINIB, MOMELOTINIB, PEXIDARTINIB, AND RUXOLITINIB USING BIOMAP® DIVERSITY PLUS PANEL

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INTRODUCTION

- Pacritinib (PAC) is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R[1,2]
  - PAC has demonstrated significant and sustained spleen volume reduction and symptom control in patients with myelofibrosis regardless of baseline platelet count[3]
- The objective of this analysis was to compare the biomarker activity profile for PAC, with those of the JAK inhibitors, ruxolitinib (RUX) and momelotinib (MOM), and the CSF1R inhibitor pexidartinib (PEX) using the BioMAP® Diversity PLUS panel of human primary cell-based systems modeling vascular, epithelial, stromal, skin, lung, inflammation, wound-healing and tissue remodeling biology related to tissue and disease states[4] (**Table 1**; https://www.discoverx.com)
  - At clinically relevant concentrations, phenotypic profiles for 4 test agents were compared to each other as well as a a database of reference benchmark profiles and potential dose-dependent effects were identified

Table 1. Systems in BioMAP Diversity PLUS

System Name	Icon	Cell	Disease	Biomarker Readouts
3C		Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, ICAM-1, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, SRB
4H		Venular endothelial cells	Asthma, Allergy, Autoimmunity	MCP-1, Eotaxin-3, VCAM-1, P-selectin, uPAR, SRB, VEGFRII
LPS		Peripheral blood mononuclear cells, Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sPGE2, SRB, sTNFα
SAg		Peripheral blood mononuclear cells, Venular endothelial cells	Autoimmune Disease, Chronic Inflammation	MCP-1, CD38, CD40, E-selectin, CD69, IL-8, MIG, PBMC Cytotoxicity, Proliferation, SRB
BT		Peripheral bloon mononuclear cells, B cells	Asthma, Allergy, Oncology, Autoimmunity	B cell Proliferation, PBMC Cytotoxicity, Secreted IgG, sIL-17A, sIL-17F, sIL-2, sIL-6, sTNFα
BF4T		Bronchial epithelial cells, Dermal fibroblasts	Asthma, Allergy, Fibrosis, Lung Inflammation	MCP-1, Eotaxin-3, VCAM-1, ICAM-1, CD90, IL-8, IL-1α, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-1, SRB, tPA, uPA
BE3C		Bronchial epithelial cells	Lung Inflammation, COPD	ICAM-1, uPAR, IP-10, I-TAC, IL-8, MIG, EGFR, HLA-DR, IL1α, Keratin 8/18, MMP-1, MMP-9, PAI-1, SRB, tPA, uPA
CASM3C		Coronary artery smooth muscle cells	Cardiovascular Inflammation, Restenosis	MCP-1, VCAM-1, TM, TF, uPAR, IL-8, MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-1, Proliferation, SAA, SRB
HDF3CGF		Dermal fibroblasts	Fibrosis, Chronic Inflammation	MCP-1, VCAM-1, ICAM-1, Collagen I, Collagen III, IP-10, I-TAC, IL-8, MIG, EGFR, M-CSF, MMP-1, PAI-1, Proliferation_72hr, SRB, TIMP-1, TIMP-2
KF3CT		Keratinocytes, Dermal fibroblasts	Psoriasis, Dermatitis, Skin Biology	MCP-1, ICAM-1, IP-10, IL-8, MIG, IL-1α, MMP-9, PAI-1, SRB, TIMP-2, uPA
MyoF		Lung fibroblasts	Fibrosis, Chronic Inflammation, Wound Healing, Matrix Remodeling	a-SM Actin, bFGF, VCAM-1, Collagen-I, Collagen-III, Collagen-IV, IL-8, Decorin, MMP-1, PAI-1, TIMP-1, SRB
/Mphg		Venular endothelial cells, Macrophages	Cardiovascular Inflammation Restienosis, Chronic Inflammation	MCP-1, MIP-1α, VCAM-1, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sIL-10, SRB, SRB-Mphg

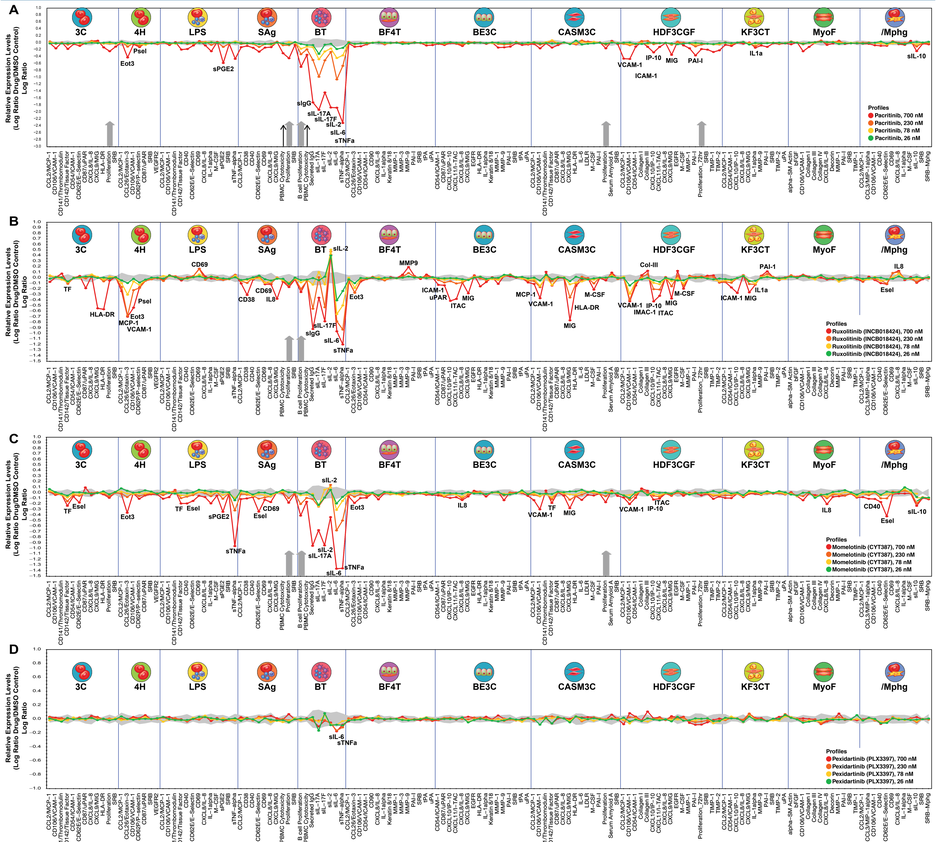
METHODS

- Phenotypic activity profiles for 4 concentrations (26 nM, 78 nM, 230 nM, and 700 nM) of each test agent (PAC, RUX, MOM, PEX) were generated with key biomarker changes assessed
  - Differentiating and common biomarkers identified by overlaying the profiles of the test agents at selected concentrations
  - Using a reference database of >4000 agents, activity profiles most similar to those for the test agents were mathematically computed
- Diversity PLUS systems contain human primary cell types cultured as mono- or co-cultures (**Table 1**), stimulated and incubated for 24h (except the BT system for 72h with sIgG at 6 days and MyoF for 48h)
  - Cells were plated in 96- or 384-well plates containing test agents (PAC, RUX, MOM, PEX) and appropriate drug controls, negative controls, and vehicle controls
- Direct ELISA was used to measure biomarker levels of cell-associated and cell membrane targets while soluble end-points were quantified from supernatants using HTRF® detection, bead-based multiplex immunoassays, or capture ELISA
- Results are presented as a log ratio of biomarker levels in the presence of the test agent relative to the averaged vehicle controls on the same plate
- Overt adverse effects on cell proliferation and viability were measured by sulforhodamine B for adherent cells and alamarBlue® for suspension cells
- For proliferation assays, individual cell types are cultured at subconfluence and measured at time points optimized for each system (48-96 hours) relative to the vehicle control samples tested in parallel

RESULTS

- Biomarker activities observed for all 4 test agents are shown in **Figure 1** and **Table 2**
  - PAC, RUX, and MOM had broader activities than PEX across the Diversity Plus panel
  - At clinically relevant concentrations (i.e., 230 nM), PAC had the strongest inhibitory activities on sIL-17A, sIL-2 and sIL-6 in the BT system, while RUX had the most potent inhibitory activities in other Diversity PLUS systems such as 4H, BE3C, and CASM3C
  - At the highest concentration (700 nM), PAC was cytotoxic to PBMCs in the SAg and BT systems (thin black arrows)
  - Peak systemic levels of free PAC observed clinically do not exceed 200 nM
- Similarity clustering using pairwise correlation analyses at all non-cytotoxic concentrations indicates the phenotypic signature of each agent is maintained across a wide range of concentrations
- PAC, RUX, and MOM were all antiproliferative to B cells, but only PAC was antiproliferative to endothelial cells and fibroblasts, effects typically observed with agents used for oncology indications (**Table 3**)
- Using the reference database, the profile of PAC was most similar to Axl and PI3Kδ inhibitors, at clinically relevant concentrations
  - MOM was similar to Syk inhibitors and A2A antagonists
  - RUX was similar to other JAK inhibitors

Figure 1. BioMAP Profiles for (A) Pacritinib, (B) Ruxolitinib, (C) Momelotinib, and (D) Pexidartinib



X-axes list the quantitative protein biomarker readouts as well as proliferation and viability endpoints measured in each system
Y-axes show log-transformed ratios of biomarker readouts for each test agent/vehicle controls
The grey region around the Y-axis shows the 95% significance envelope generated from historical vehicle controls used a confidence interval to identify activities of each test agent
Biomarker activities are annotated when ≥2 consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have ≥1 concentration with an effect size > 20%
Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others.
Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow.

Table 2. Summary of Biomarker Activities

	Biomarker	PAC	RUX	MOM	PEX
Inflammation-related	Eotaxin 3	↓	↓	↓	
	E-selectin		↓	↓	
	VCAM-1	↓	↓	↓	
	sTMFα	↓	↓	↓	↓
	I-TAC		↓	↓	
	MIG	↓	↓	↓	
	IL-8		↔	↓	
	IP-10	↓	↓	↓	
	sPGE2	↓		↓	
	MCP-1		↓		
	ICAM-1	↓	↓		
	IL-α	↓	↓		
	P-selectin	↓	↓		
Immunomodulatory-related	CD40			↓	
	sIL-10	↓		↓	
	sIL-17A	↓		↓	
	sIL-6	↓	↓	↓	↓
	CD69		↔	↓	
	sIL-2	↓	↑	↑	
	sIgG	↓	↓		
	HLA-DR		↓		
	M-CSF		↓		
	CD38		↓		
	sIL-17F	↓	↓		
Tissue remodeling-related	uPAR		↓		
	uPA				
	Collagen III		↔		
	PAI-1	↓	↔		
	TIMP-2				
	MMP-9		↔		
Hemostasis-related	TF		↓	↓	

↑ indicates an increase relative to vehicle control; ↓ indicates decrease relative to vehicle control; ↔ indicates where an increase was observed in some but not in others.
Note: concentrations containing detectable cytotoxicity were removed for this analysis.

Table 3. Summary of Antiproliferative Activites

	PAC	RUX	MOM	PEX
B cells	✓	✓	✓	
T cells		✓		
Endothelial cells	✓			
Coronary artery smooth muscle cells	✓		✓	
Fibroblasts	✓			

CONCLUSIONS

- At clinically relevant concentrations, PAC had the strongest inhibitory activities on sIL-17A, sIL-2 and sIL-6 in the BT system and was anti-proliferative to B cells suggesting this compound could be used as a treatment for B cell neoplasia and may also have potential as an immunomodulating therapy in inflammation-related diseases
  - RUX had the broadest scope of inhibitory activities in other Diversity PLUS systems such as 4H, BE3C and CASM3C
- While both RUX and PAC were antiproliferative to B cells, only RUX also blocked T cell proliferation
  - PAC was also antiproliferative to non-hematopoietic endothelial cells and fibroblasts, which are effects more commonly observed with general anti-cancer agents
- The BioMAP Diversity PLUS panel is useful for evaluating comparative potential effects of targeted compounds in conditions that preserve the complex crosstalk and feedback mechanisms that may be relevant to *in vivo* outcomes
- The distinct phenotypic profiles of PAC, RUX, MOM, and PEX illustrate divergent biological effects consistent with subtleties of biological effects of competitive ATP binding site agents developed against a single target (JAK2) and suggest clinical responses and outcomes are also likely to be distinct for each agent

References

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