Phenotypic Evaluation and Discrimination of Clinical JAK2 Inhibitors for Myeloproliferative Disorders

Abstract
Small molecule inhibitors of Janus kinases (JAKs) represent a promising treatment for myeloproliferative disorders (MPD), including polycythemia vera (PV), essential thrombocytosis (ET), and myelofibrosis (MF). Currently, the JAK2 inhibitors ruxolitinib (RUX) and fedratinib (FED) are approved for the treatment of MPD, while momelotinib (MK0518) and ganetespib (GAN) remain in clinical development. The primary target of JAK inhibitors, the JAK-STAT pathway, plays a critical role in multiple clinical disease models, relevant for myeloproliferation, including survival, differentiation, and proliferation. However, as approved JAKs are reportedly promiscuous and inhibit kinases beyond the JAK family, the overall clinical impact of each likely reflects the sum of its target activities and downstream secondary pharmacology. This impacts both efficacy and safety. We evaluated these JAK2 inhibitors by phenotypic profiling in the BioMAP Diversity RPPS Panel. The BioMAP platform consists of 12 human primary cell lines co-cultured in single model to recapitulate key aspects of tissue and disease states. Activity profiles were generated on the modulation of 146 protein biomarkers by each JAK2 inhibitor at concentrations that span clinical exposure levels. Comparison of drug profiles at the top two concentrations revealed multiple common activities, including anti-inflammatory effects on T and B cells consistent with clinical efficacy in MPD. Additionally, all four drugs decreased multiple inflammation and immune biomarkers. Pairwise correlation analysis revealed that RUX, FED, and GAN clustered together, indicating they share mechanistically relevant similarity, consistent with their shared primary JAK2 target. In contrast, MMID did not cluster at any concentration, indicating it is mechanistically distinct, likely related to unique secondary targets. Overall, this study directly relates to clinical versus secondary pharmacology of four JAK2 inhibitors, and provides a key outcome relevant to clinical application.

Methods
BioMAP systems are composed of human primary cells pooled from healthy donors, in mono- or co-culture conditions, stimulated to capture the complexity of human disease biology. Four JAK2 inhibitors, ruxolitinib, fedratinib, momelotinib, and ganetespib, were assessed for their concentration-dependent impacts on a panel of 146 protein-based and clinically relevant biomarkers of inflammation, immune biology, tissue remodeling, homeostasis, proliferation, and cell cytostasis. Bioactivity data is used to generate a BioMAP profile that is then analyzed to gain insight on mechanism of action, efficacy-related activities, and safety-relevant secondary pharmacology to help guide clinical applications and predict human outcomes. Advanced analytical tools enable similarity searching, reference benchmark overlays, and mechanism-driven clustering. Detailed protocols for the BioMAP Diversity RPPS Panel of 12 disease-relevant systems is published (1).

Overview of the BioMAP Phenotypic Platform

BioMAP Systems
• Primary Human Cells
• Disease Relevant Stimuli
• Protein Biomarkers
• In Vitro Disease Models
• Translational Activities

Data Analytics
• BioMAP Annotated Profiles
• Mechanistic Signatures Database
• Profiles of ~4500 Components
• Efficacy & Safety Signatures
• Approved and Failed Drugs
• Small Molecules, Tool Compounds

Reference Database
• Biomarker Identification
• Biologics, Peptides, Cytokines, etc.

BioMAP Diversity RPPS Panel
12 human primary cell lines co-cultured in 384-well arrays to assess the activity of small molecule inhibitors of JAK kinases.

High Level of Physiological Relevance
Translational Programs for Program Success and Unparalleled Level of Validation and Guidance

Figure 1. The BioMAP Platform provides superficial and standardized human disease models spanning a broad scope of tissue and disease biology, with human primary cells co-cultured and stimulated to mimic relevant disease-relevant environments. The primary use of the BioMAP Platform include human primary cell-tissue models, disease models, advanced assay tools, data visualization, and the comprehensive BioMAP Platform Information of over 4,500 compounds. The BioMAP Platform uniquely provides physiologic disease arrays across a variety of pathologic settings and is an attractive alternative to human patient-derived or genetically modified mouse models.

Figure 2. Individual BioMAP profiles reveal impacts of JAK2 inhibitors on canonical biomarkers in human-on-human disease models. Note that all four profiles have bulk cytokine and upregulation of cytokine receptor molecule within individual receptor moment. TheProfilas for the quantification of protein-based biomarkers measured in each system. The Proteins represent a log transformation of the biomarker results for the drug doses tested (t = 0.13) each vehicle control (s = 0.13). The gray region around the x-axis represents the 10% significance margin generated from a false positive control. Bioactivity data are analyzed to draw a more comprehensive picture of the dynamic interactions of the significant biomarker responses and time and dose used to correlate with an effect size “p” (29% effect). Not all protein effects are detected with a gray area.

Figure 3. Comparative profiling analysis reveals activities common to all four agents. Distinctive profiles from BioMAP Diversity RPPS profiles were compared, 3D content, and nearest neighbor analysis were used to identify the highest activity. These profiles were analyzed with MetaCore Pathway Analysis software (GeneGo, Inc.) and Ingenuity Pathway Analysis (Ingenuity Systems, Inc.) to identify canonical and pathway-based biomarkers and downstream target pathways, and secondary regulatory effects. These activity-based data provide the capability to identify potential toxicity, safety, and efficacy profiles.

Figure 4. Comparative overview analysis clustered ruxolitinib similarly to ruxolitinib and fedratinib. Ruxolitinib and fedratinib did not cluster with MK0518 or GAN. BioFactors and GAN (63%) inhibiting, it remains relatively different from the other inhibitors, with the exception of MK0518 and individual JAK2 inhibitors with a low concentration (1.1 µM) with similar size differences.

Figure 5. Differential Activities Indicate Distinct Secondary Pharmacology

BioMAP Toxicity Signature Analysis Reveals Differential Safety Pharmacology

Overview of BioMAP Toxicity Profile

BioMAP Toxicity
• Efficacy & Safety Signatures
• Acute Toxicity
• Inflammation
• Tissue Impairment
• Organ Toxicity
• Skin Reactivity
• Skin Irritation
• Tissue Reactivity
• Vascular Toxicity

Common Activites Identity Potential JAK2 Efficacy Factors

Figure 6. Comparative summary analysis clusters from two representative mechanism classes identifies 27 differentiating activities that were annotated criteria. Bioactivity profiles analysis of RUX and MMID of representative concentrations revealed distinct impacts on acute toxicity potential, cellular disruption, biofilm formation, and collagen synthesis. However, bioactivity profiles analysis of RUX, FED, and GAN also flag independent mechanisms associated with inflammation and tissue impairment. In contrast, MMID had no effect on RUX, vehicle, TPA, and PPAR-1. Differential outcomes on finding novel signatures for pharmacology with potential implications for efficacy and safety.

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Momelotinib is Mechanically Unique Within the JAK2 Inhibitor Class

Phenotypic activities for RUX and MMID are consistent with the acute toxicity potential of the compounds. However, momelotinib’s efficacy is consistent with the anti-inflammatory potential but not the efficacy of the compounds. The BioMAP Diversity RPPS panel was analyzed for each concentration and profile toxicity Signatures are complemented in biological activities correlated to an increased risk of similar adverse outcomes or worse.

Summary
Bioactivity-based rates for JAK2 inhibitors are high, mainly due to lack of response, loss of efficacy, intolerance, and drug-induced cytopenias, supporting the need for new approaches that can report early on preclinical efficacy and safety. BioMAP Phenotypic Profiling of the four JAK2 inhibitors RUX, FED, MMID, and GAN at concentrations relevant for clinical exposure reveals a shared mechanistic signature that includes anti-inflammatory effects on T and B cells, consistent with efficacy in MPD, and multiple immune and inflammatory biomarkers, consistent with inhibition of immune cell activation. These results have potential to represent a sentinel signature for this class that can serve as an efficacy profile signature for MPD. Differentiating activities related to tissue remodeling and inflammation biomarkers indicate distinct secondary pharmacology with implications for safety-related outcomes. These data demonstrate the value of profiling preclinical candidates and approved drugs for biological impact in conditions that present: the complex crosstalk and feedback mechanisms relevant to in vivo outcomes.

References
4. See abstract 828 for related work using the BioMAP Platform from Eurofins Discover.