JAK-STAT platform for immune-related drug Discovery

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Background

The Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathways are evolutionarily conserved transmembrane signaling mechanisms that ensure the ability of normal cellular communication with the external environment. Various cytokines, interferons, growth factors, and other specific molecules can activate JAK-STAT signaling to drive a range of physiological and pathological processes, including proliferation, metabolism, immune response, inflammation, and malignancy. However, the JAK-STAT signaling pathway and related gene mutations are closely associated with aberrant immune activation and cancer progression. Through extensive research into the structure and function of the JAK-STAT pathway, a variety of clinical drugs have emerged and been approved for the treatment of disease, primarily targeting cytokines and their receptors, JAK inhibitors and STAT inhibitors.

Method

Both JAK and STAT, once activated, cause STAT phosphorylation and the amount of downstream cytokines secreted. Therefore by using different cytokines such as IL-6, IL-23, IFNα, TPO or GM-CSF, etc., stimulating PBMC, T cells or whole blood, and detecting STAT phosphorylation as well as secreted cytokines, the activity of various drugs on the JAK-STAT pathway can be assessed.

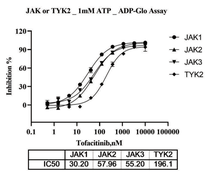
Reporter assay: The reporter cells were pre-treated with compound for 1 hour or 24 hours and then stimulated by various stimulating factors, the STAT-inducible signals were detected via Tecan Sunrise.

STAT phosphorylation assay: The primary cells, including PBMC, sorted subtypes of cells, or the cell line were pre-incubated with compound and stimulated with cytokines, the cells were collected and lysed, intracellularly phosphorylated STAT (pSTAT) was detected by ELISA or AlphaLISA.

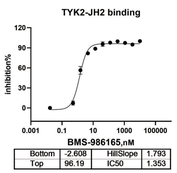
For the whole blood, once it has been stimulated, the samples were simultaneously lysed for erythrocytes and fixed for leukocytes, then permeabilized and stained with pSTAT and phenotypic markers to monitor phosphorylation level in different immune subsets.

Cytokine release assay: Compound treated PBMC, NK92, naïve CD4 T cells or whole blood were stimulated with stimulating factor and the cellular supernatant or plasma were collected for quantification of secreted cytokines

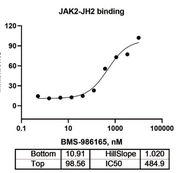
Results 1: Biochemistry and SPR



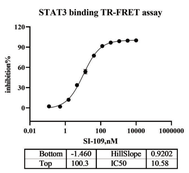
	JAK1	JAK2	JAK3	TYK2
IC50	30.20	57.96	55.20	196.1



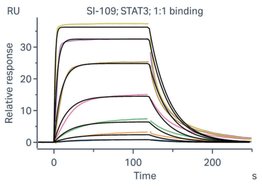
Bottom	-2.608	HillSlope	1.793
Top	96.19	IC50	1.353

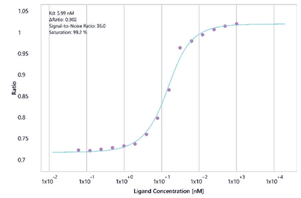


Bottom	10.91	HillSlope	1.020
Top	98.56	IC50	484.9



Bottom	-1.460	HillSlope	0.9202
Top	100.3	IC50	10.58



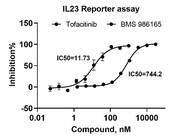


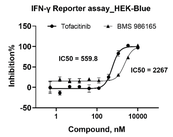
JAK-STAT screening strategies for Hit Identification

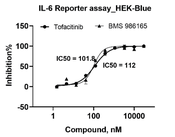
Several biochemistry with biophysical strategies can be used for a drug discovery program, and it is the target properties which drive the selection of the most suitable technology. The SPR, Spectral Shift ,NanoDSF was the most appropriate method for the STAT drug-discovery program.

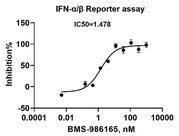
Results 2: JAK-STAT inhibition in Reporter cell

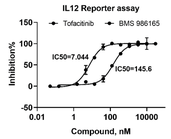
Reporter Cell	Signaling pathway
IFN-α/β Reporter Cell	TYK2/JAK1
IFN-γ Reporter Cell	JAK1/JAK2
IL-12 Reporter Cell	JAK2/TYK2
IL-23 Reporter Cell	JAK2/TYK2
IL-22 Reporter Cell	TYK2/JAK1
GM-CSF Reporter Cell	JAK2
IL-6 Reporter Cell	JAK1/JAK2/TYK2

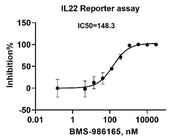


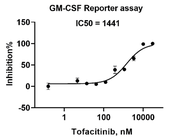


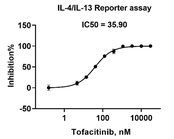




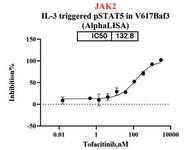


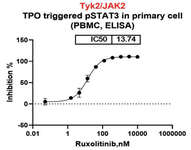


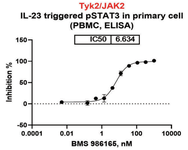


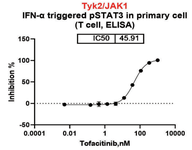


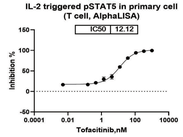
Results 3: STAT phosphorylation in cells

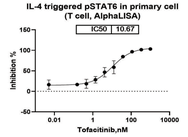


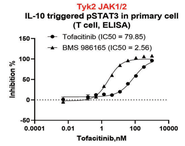


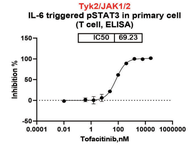




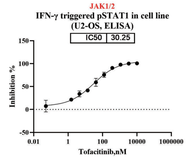




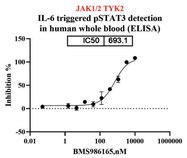


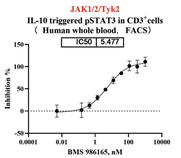


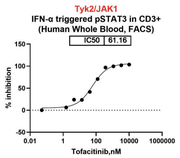
Based on the signaling pathways of different cytokines, we selected different cells or treated the cells accordingly to evaluate the inhibitory activity of various JAK inhibitors by detecting the downstream STAT phosphorylation level.

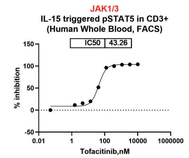


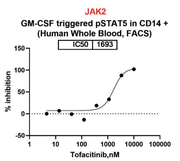
Results 4: STAT phosphorylation in whole blood

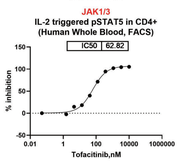


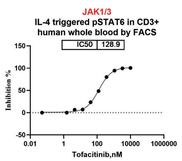


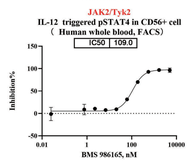




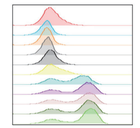




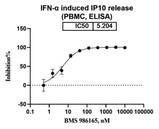


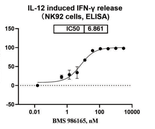


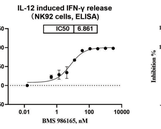
Unlike direct in vitro cellular assays, the detection of phosphorylation in whole blood needs to be based on circling specific cell populations before analysis. We have developed a comprehensive flow assay system to evaluate the activity of various relevant inhibitors in whole blood.

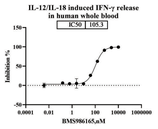


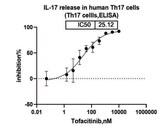
Results 5: Cytokine release inhibition











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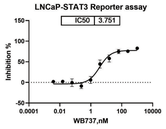

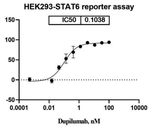

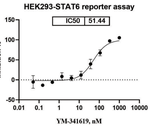

Summary

Both in PBMC and in sorted T cells as well as whole blood, various JAK-STAT-related inhibitors can be validated for their activity by assaying STAT phosphorylation as well as cytokines. Currently, ICE has established various in vitro assays for the JAK-STAT signaling pathway to facilitate drug discovery.