Discovery of a highly potent, selective, reversible covalent inhibitor of JAK3

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ABSTRACT

Background/Purpose: Targeting of the JAK-STAT pathway has been shown to be efficacious for treatment of patients with rheumatoid arthritis through the successful use of pan-JAK inhibitors in clinical trials. To date, lack of selective JAK3 inhibitors has hindered the assessment of the role of JAK3 in autoimmune disorders. A JAK3 inhibitor has the potential benefit of alleviating undesirable side effects of JAK1 and JAK2 inhibition such as dyslipidemia and suppression of hematopoiesis, respectively. A new approach is presented to achieve potent, selective and durable inhibition of JAK3 by application of Principia's reversible covalent platform targeting a cysteine residue in the active site of JAK3 that is absent from other JAK family members. Ability of these inhibitors to block IL-2 and IL-4 signaling is presented.

Methods: Enzyme potencies were measured using the Caliper platform at Nanosyn Inc. (Santa Clara, CA). IL-2 stimulated phospho-STAT5 was measured in Ficoll separated human peripheral blood mononuclear cells (PBMCs) by flow cytometry. IL-4 stimulated STAT6 activation was measured in Ramos B cells based on a STAT6 reporter assay (Invitrogen, Madison, WI). Kinase profiling was performed at DiscoveRx (San Diego, CA).

Results: We have developed a series of molecules that are highly potent and selective for JAK3. Compound 1 inhibited JAK3 enzymatic activity with an IC_{50} of 0.5 ± 0.3 nM, but not JAK1, JAK2, or TYK2 up to a concentration of 5 uM. The selectivity among other kinases within the Cys sub-family was also high with no inhibition exceeding 60% at 1 uM. Profiling against a panel of 442 kinases confirmed the exceptional selectivity of the series. Compound 1 forms a durable yet reversible Cys interaction with JAK3 in biochemical assays with a dissociation half-life of 9 hours.
In cell-based assays, Compound 1 completely inhibited IL-2 stimulated STAT5 phosphorylation (IC_{50} = 206 ± 11 nM) in hPBMCs, IL-4 stimulated STAT6 phosphorylation (IC_{50} = 58 ± 10 nM) in Ramos B cells and IL-2 driven IFNg secretion (IC_{50} = 248 ± 8 nM) in hPBMCs. IL-6 stimulated STAT3 phosphorylation was not inhibited up to 5 uM indicating complete cellular selectivity for JAK3 over JAK1. In addition, NFAT activation downstream of TCR stimulation in Jurkat T cells was not blocked.

Conclusions: Compound 1 is a potent, selective and durable inhibitor of JAK3 and has the potential to be an efficacious treatment for rheumatoid arthritis or other T cell driven diseases with a potential for differentiation from pan-JAK inhibitors.

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INTRODUCTION

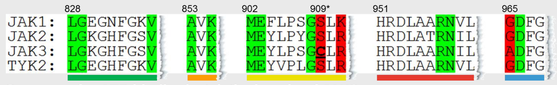
Janus Kinase 3 (JAK3) is a tyrosine kinase that links common gamma chain signaling to intracellular effector functions. This pathway regulates responses to IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Other family members include JAK1, JAK2 and TYK2 which control a wide variety of cellular functions.

JAK3 deficiency in humans is associated with a specific SCID phenotype that manifests as depletion of T cells and NK cells. B cells are present but demonstrate impaired function. Since JAK3-SCID patients have defects limited to immune cells and JAK3 deletion in mice also manifests as only in defective immune function, selective targeting of JAK3 was identified as a potential mechanism to treat immune-mediated disorders while avoiding various side effects.

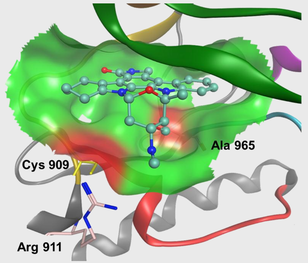
Targeting of JAK3 selectively with small-molecule inhibitors has been challenging due to the highly conserved amino acid composition of the ATP binding pocket among the JAK family members. One of the three variable amino acids in the binding pocket, Cys909, can be used to confer selectivity by targeted formation of a covalent interaction. This provides a mechanism to achieve selectivity versus JAK1, JAK2 and TYK2. By employing chemistry that allows the covalent Cys interaction to be reversible, Principia has produced selective JAK3 inhibitors that will not form protein adducts, which has been a concerning feature of previous Cys-targeting covalent drugs.

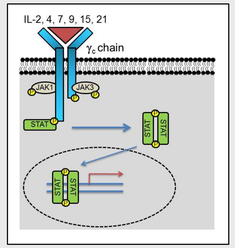
The combination of covalent modification of Cys along with targeting a unique gatekeeper amino acid has allowed the identification of a highly selective, small-molecule JAK3 inhibitor.

Targeting Cys allows JAK3 selectivity among JAK family



Sequence for ATP binding/catalytic domain of JAK3.





JAK3 signaling.

Results

Compound 1: Biochemical screening shows selectivity for JAK3

Alignment of JAK3 Cys family kinases

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JAK3: PELRLVMEYLPSGCLRDFLQRHRAR
BTK:  RPIFIITEYMANGCLLNYLREMRHR
BMX:  YPIYIVTEYISNGCLLNYIRSHGKG
TEC:  KPIYIVTEFMERGCLLNFLRQRQGH
TXK:  KPLYIVTEFMENGCLLNYLRENKGK
ITK:  APICLVTEFMEHGCLSDYLRTQRGL
EGFR: STVQLITQLMPFGCLLDYVREHKDN
ERB2: STVQLVTQLMPYGCLLDHVRENRGR
ERB4: PTIQLVTQLMPHGCLLEYVHEHKDN
BLK:  EPIYIVTEYMARGCLLDFLKTDEGS
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JAK3 shares Cys with 9 other kinases.

Conserved Cysteine Panel

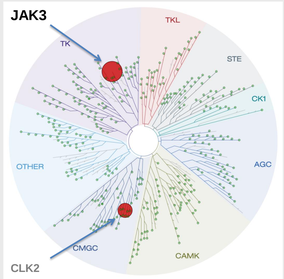
Kinase	Percent Inhibition (1 uM)	Kinase	Percent Inhibition (1 uM)
JAK3	98 ± 2.6	TEC	19 ± 0.8
BLK	59 ± 5.0	BTK	16 ± 0.8
ITK	51 ± 1.6	ERB-B4	7 ± 0.9
TXK	35 ± 3.2	EGFR	4 ± 0.7
BMX	30 ± 3.8	ERB-B2	3 ± 0.9

JAK3 selective among Cys family - likely due to unique gatekeeper residue Met and reversible covalent binding.

JAK Family Selectivity

Kinase	IC50 (uM)
JAK3	0.0005
JAK1	> 5
JAK2	> 5
TYK2	> 5

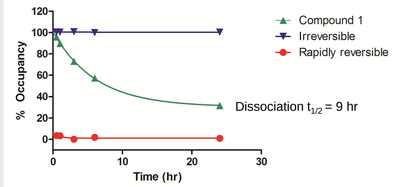
High selectivity for JAK3 among other JAK family members.



Kinomescan: High selectivity vs kinome (tested against 451 kinases @ 1 uM). CLK2 60x less potent and reversible.

Reversible covalent JAK3 inhibitor demonstrates slow off-rate kinetics

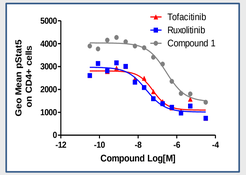
Determination of biochemical off-rate for JAK3 inhibitors



Off-rate determined by TR-FRET with a high affinity reversible probe following dilution of the compounds to allow reversibility

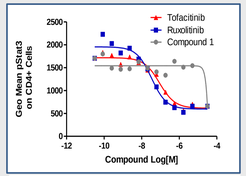
Results

Compound 1: Cell based assays demonstrate potency and selectivity for JAK3



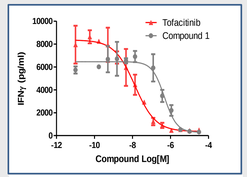
	IC50 (uM)
Tofacitinib	0.040
Ruxolitinib	0.026
Compound 1	0.21

IL-2 induced pSTAT5 in human PBMC. Human PBMC were stimulated with 20 ng/ml IL-2 for 30 min. CD4+ cells were gated for phospho-stat5.



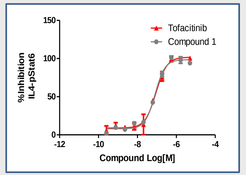
	IC50 (uM)
Tofacitinib	0.089
Ruxolitinib	0.033
Compound 1	> 5

IL-6 induced pSTAT3 in human PBMC. Human PBMC were stimulated with 20 ng/ml IL-6 for 30 min. CD4+ cells were gated for phospho-stat3.



	IC50 (uM)
Tofacitinib	0.030
Compound 1	0.248

IL-2 induced IFNγ in human PBMC. Human PBMC were stimulated with αCD3 and αCD28 followed by 50 ng/ml IL-2 overnight. IFNγ analyzed by ELISA



	IC50 (uM)
Tofacitinib	0.065
Compound 1	0.058

IL-4 induced pSTAT6 in Ramos B cells. Ramos cells were stimulated with IL-4 and 5 hours later a reporter for Stat6 activation was measured (Invitrogen).

Cell-based off-target assays

Assay	IC50 (uM)
TCR-induced NFAT in Jurkat cells[1]	>5
HCT-116 proliferation[2]	2.3

[1]Invitrogen NFAT reporter assay.
[2]Cell viability after 72hrs.

CONCLUSIONS

Cysteine targeting by covalent binding allows complete JAK3 selectivity within the JAK family of kinases as well as excellent kinome selectivity.

Use of reversible covalent chemistry produces inhibitors with slow off-rate kinetics allowing durable inhibition of the target.[1]

Principia has identified a highly potent and selective inhibitor of JAK3 that can be utilized to probe the effects of selective JAK3 inhibition.

Selective JAK3 inhibition is sufficient to completely block IL-2 induced signaling and function in primary human T cells as well as IL-4 induced signaling in Ramos B cells.

Selective, potent, durable JAK3 inhibitors have the potential to be differentiated from pan-JAK inhibitors on the basis of JAK1,2 driven side effects.

References

1. Serafimova IM, Pufall MA, Krishnan S, Duda K, Cohen MS, Maglathlin RL, McFarland JM, Miller RM, Frödin M, Taunton J. Reversible targeting of noncatalytic cysteine with chemically tuned electrophiles. Nat Chem Biol. 8:471-6, 2012.