The Human Kinome as Target for Autoimmune and Autoinflammatory Diseases

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Disclaimers

• NIAMS-Pfizer: Collaborative Research Agreement and Development Award

• NIH holds a US Patent: Targeting Jaks
Targeting Cytokines and Cytokine Receptors

Targeting signaling?

The complexity of signaling!
Targeting Immune Cell Signaling

- Phosphorylation – critical first step for many key receptors: TCR, BCR, FcR……

Reversible protein phosphorylation – a critical mode of signal transduction

Four Nobel Prizes:

- 1989 – Bishop and Varmus
- 1992 – Krebs and Fischer
- 2000 – Paul Greengard
- 2001 – Nurse, Hartwell, Hunt
The human kinome

- 518 protein kinases
- 8 major groups
- 90 Tyrosine kinases
- 30 families of Tyr kinases
- critical for signal transduction
- 4 Jaks

Is it possible to generate a pharmacologically useful tyrosine kinase inhibitor?
Most bind in ATP-binding site

Is it possible to develop drugs that block ATP?

Kinase Structure is Conserved

- and kinases not the only enzymes that use ATP as a substrate
**Imatinib - a strong precedent**

![Image of Imatinib](image)

### FDA-approved kinase inhibitors (some)

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Main targets</th>
<th>Indication</th>
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</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>BCR-ABL, PDGFR, SCF, KIT</td>
<td>CML, GIST Numerous trials underway</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>EGFR</td>
<td>Non-small cell lung cancer</td>
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<tr>
<td>Sorafenib</td>
<td>KIT, FLT-3, VEGFR, PDGFR</td>
<td>Hepatocellular carcinoma</td>
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<tr>
<td>Dasatinib</td>
<td>BCR-ABL, LCK, FYN, KIT, PDGFR</td>
<td>CML (Imatinib-resistant)</td>
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<tr>
<td>Sunitinib</td>
<td>PDGFR, VEGFR, KIT, FLT-3</td>
<td>GIST (Imatinib-resistant), RCC</td>
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<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>Non-small cell lung cancer</td>
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<tr>
<td>Nilotinib</td>
<td>BCR-ABL</td>
<td>CML (Imatinib-resistant)</td>
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<tr>
<td>Lapatinib</td>
<td>EGFR, HER2</td>
<td>Breast cancer (HER2)</td>
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<tr>
<td>Ruxolitinib</td>
<td>Jak2, Jak1</td>
<td>MPD-PV</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>Jak3, Jak1</td>
<td>RA</td>
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</table>
Cytokine signaling and Jaks

Key concepts:
- Four Jaks: Jak1, Jak2, Jak3, Tyk2
- Jaks work in pairs
- Different receptors bind different Jaks

How critical?
Jak inhibitors: it all started a long time ago

IL-2, 4, 7, 9, 15, 21

IL7R-SCID X-SCID

JAK1 JAK3

JAK3-SCID

Mutation of JAK3 in a Patient with SCID: Essential Role of JAK3 in Lymphoid Development


The current study further suggests that any agents that inactivate JAK3 function may be potent immunosuppressants. Moreover, the

Current Status of Jakinibs

Fast forward 19 years:

- Two Jak inhibitors FDA approved; more in development and clinical trials:
  - **Ruxolitinib**
    - Jak1/2 inhibitor – PV, MF
    - Ongoing trials in leukemia, other cancers
  - **Tofacitinib**
    - approved for RA, ongoing trials in IBD, psoriasis, renal transplant
  - **Baricitinib**
    - Jak1/2 inhibitor
    - Phase 2b – 301 patients, 12 weeks
  - **Pacritinib**
    - Jak2 inhibitor
    - Phase 2 – MF
Jakinibs block ATP binding
•Receptors don’t transmit signal
•They block ATP
•How can these drugs be specific?
•Selectivity, how important?
•Selectivity amongst Jaks?
•Kinome selectivity?

Targeting Jaks

Selectivity of Jakinibs

• Tofacitinib reasonably selective for Jaks
• Inhibits Jak3, Jak1 > Jak2 >> Tyk2
• Minimal effect on other kinases

Karaman et al Nature Biotechnology, 2005
FDA-approved Tyrosine Kinase Inhibitors: far from selective

Imatinib (Bcr-abl)
Erlotinib (EGFR)
Sunitinib
Dasatinib
Staurosporine

Karaman et al. Nature Biotechnology, 2005

Mechanism of action: Target Multiple Jaks, Multiple Cytokines

- Major effects on T and B cells
  - Th1, Th2, Th17 differentiation impaired
  - No decline in CD4 T cells
  - Metabolic effects
- Innate responses also inhibited
  - Actions of IL-6, IFN-γ, IFNα/β blocked
  - Block production of proinflammatory cytokines
- Mechanistically, first-gen Jakinibs – very similar mechanisms of action
Activated CD4⁺ T cells up-regulate glycolysis.

Glycolysis-dependent energy production is maintained in the presence of IL-2.

Does tofacitinib affect T cell metabolism?
Tofacitinib changes cell metabolism in response to IL-2

- Naive T cells (CD4+, CD62L+, CD44low) → Anti CD3/CD28, 3 Days → IL-2, 24 hr → Tofacitinib

### Basal Respiration
- Non-mitochondrial respiration
- Proton Leak

### Maximal Respiration
- H+ driven ATP turnover
- Oligomycin
- FCCP
- AntimycinA+
- Rotenone

### OCR (pMoles/min)
- Non-glycolytic Acidification
- Maximal Glycolysis
- Glucose
- Oligomycin
- 2DG

### ECAR (mP Holmes/min)
- Time (min)

- IL-2
- Anti-IL-2
- IL-2 + Tofacitinib (1uM)

Tofacitinib changes expression of OXPHOS- and glycolysis-related genes

### TCA cycle
- Aldh18a1
- Got1
- Idh1
- Slc3a2
- Slc7a5
- Aldoa
- Aldoc
- Hk2
- Ldha
- Glut1

### Log2 of RPKM
- WT
- WT + Tofacitinib

**OXPHOS**
- Glut1
- HK2
- G6P
- F6P

**Glycolysis**
- Aldoa
- Aldolase
- Pyruvate
- Lactate
**Tofacitinib effects on T cell metabolism**

- Tofacitinib does not inhibit mitochondria-dependent respiration (OXPHOS) and glycolysis in response to TCR activation
- Tofacitinib inhibits both OXPHOS and glycolysis in response to IL-2
- Inhibition of T cell metabolism is one aspect of mechanism of action of tofacitinib
- OXPHOS- and glycolysis-related genes are down regulated in tofacitinib-treated cells

**Clinical use of Jakinibs in the future?**

- Selectivity of next gen selective Jakinibs?
  - Jak3, Tyk2, Jak1, Jak2?
  - GLPG0634 – Jak1 selective
    - Efficacy in phase 2a
  - VX-509 – Jak3 selective
  - Pacritinib – Jak2 selective
- How should be thinking about this class of drugs in terms of maximal benefit/safety?
  - Like biologics? Like steroids? Steroid sparing? Steroid replacing?
- Use with other modalities?
- Utility of both less selective and more selective Jakinibs?
  - Utility of multikinase inhibitors?
  - What diseases?
  - Different phases of disease?
Toxicity of Jakinibs

- Infection, few opportunistic infections in RA trials, Herpes zoster
- Cytopenias: anemia, leukopenia
  - Presumably related to Jak2 inhibition
- Tumors?
- Increased lipids
  - Lipid effect seen with tocilizumab (anti-IL-6R)
  - Mechanism? Significance?
- Increased creatinine, transaminases, GI perforations
  - Mechanism? Related to Jaks? Unrelated?

Autoinflammation: CANDLE

- Mendelian autoinflammatory disorder
- Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature
- Mutations of immunoproteasome (PSMB8 and others)
- IFN signature
- Ongoing trial with baricitinib in CANDLE
**Healthy control**

Stimulation with IFN-γ 10 IU/ml

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<tr>
<th>Gray area</th>
<th>Solid line</th>
<th>Dashed line</th>
<th>Dotted line</th>
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</thead>
<tbody>
<tr>
<td>unstimulated</td>
<td>IFN-γ 10 IU/ml</td>
<td>IFN + tofacitinib 0.1 μmol</td>
<td>IFN + tofacitinib 0.5 μmol</td>
</tr>
</tbody>
</table>

**Dose dependent decrease of STAT-1 phosphorylation by tofacitinib**

**Individual response to treatment of the 13 IFN-regulated genes**

**Patient 1**

**Patient 2**

**Patient 3**

Visits

1mg 3mg

RPKM

0 5 10 15

ATF3
CEACAM1
HERC6
SPATS2L

Visits

1mg 3mg

RPKM

0 2 4 6 8 10

IFIT3
IFI6
MX1
OAS1
OAS2
OAS3
OASL
TNFSF10
XAF1

Visits

3mg
How about other -kinibs?

- 518 kinases
- Fostamatinib (and other sykinibs?)
- Btk
- PKC
- PI-3K/PKB/mTor
- MAPKs
  - p38
  - ATP antagonists vs allosteric inhibitors

Fostamatinib – Syk/multi-kinase inhibitor

- Syk associated with immunoreceptors in macrophages, B cells, neutrophils, mast cells
- Phase II RA
  - 57-67% ACR20
Fostamatinib – Phase II RA

Weinblatt et al, NEJM 2010

Fostamatinib – adverse effects

- Diarrhea
- Hypertension
- Neutropenia
- Infections
- No opportunistic infections
Fostamatinib – ongoing trials

- T cell lymphoma
- B cell lymphoma
- Advanced colorectal cancer
- Non-small cell, head and neck, renal cancers
- ITP
- Phase II study – 3 month Phase II trial in patients who failed biologics – no diff from placebo; differences in secondary endpoints (*Genovese A&R, 2010*)

Targeting Intracellular Signaling in Immune Cells

**Calcineurin inhibition**
- CsA
- Tacrolimus

**mTOR inhibition**
- Sirolimus
- Everolimus
Targeting MAPKs

- High frequency of BRAF mutations in melanoma
  - First and second generation B-Raf inhibitors under investigation
  - TCR and IL-2 activate Raf
  - Utility in rheumatological diseases?
- p38 MAPK
  - Activated by proinflammatory cytokines
  - 36 clinical trials completed or ongoing
  - 12 studies in RA completed
  - Clinical efficacy not observed for three compounds
  - BMS-582949 p38 inhibitor, added to MTX
    - ACR20 – 53%
    - reduced CRP

Kinase inhibitors approved or on clinical trials (some)

- Monoclonal antibodies (RTKs)
  - Bevacizumab
  - Cetuximab
  - Panitumumab
  - Trastuzumab
- mTOR inhibitors
  - Sirolimus
  - Everolimus
- Small molecules
  - Imatinib*
  - Erlotinib*
  - Sorafenib*
  - Nilotinib*
  - Lapatinib*
  - Dasatinib*
  - Sunitinib*
  - Gefitinib*
  - Pazopanib*
  - Bosutinib*
- Small molecules
  - Pegatinib
  - Masatinib
  - Mubritinib
  - Vandetinib
  - Ranibizumab
  - Lestaurtinib
  - Tofacitinib*
  - Ruxolitinib*
  - Fostmatinib
  - Dovitinib
  - Toracenib

*FDA approved
STAT inhibitors: up and coming

<table>
<thead>
<tr>
<th>Name</th>
<th>Target</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Stattic</td>
<td>STAT3</td>
<td>Chem Biol- 2006</td>
</tr>
<tr>
<td>ML116</td>
<td>STAT3</td>
<td>Probe Reports from the NIH Molecular Libraries Program- 2010</td>
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<tr>
<td>Pimozide</td>
<td>STAT5</td>
<td>Blood- 2011</td>
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<td>BP-1-102</td>
<td>STAT3</td>
<td>PNAS- 2012</td>
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<tr>
<td>OPB-31121</td>
<td>STAT3</td>
<td>Cancer Lett- 2103</td>
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<td>STX-0119</td>
<td>STAT3</td>
<td>Int J Oncol-2013</td>
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Acknowledgements

NIAMS
Yasuko Furumoto
Kamran Ghoreschi
Arian Laurence
John O'Shea
Raphaela Goldbach-Manšky
Adriana Almeida de Jesus
Yin Liu

Pfizer
Paul Changelian
Ed Thomas
Gary Chan
Sam Zwillich
Nandini Kishore
Mike Jesson
Xiong Li
Debby Meyer
Richard Head
Tofacitinib decreases OXPHOS and glycolysis only in response to IL-2

Naïve T cells (CD4+, CD62L+, CD44low) → Anti CD3/CD28, 3 Days → IL-2, 24 hr → Tofacitinib

**OCR**

TCR + + +
anti-IL-2 - + -
Tofacitinib - - +

**ECAR**

TCR + + +
anti-IL-2 - + -
Tofacitinib - - +

**OCR**

IL-2 + - -
Anti-IL-2 - + +
Tofacitinib - - +

**ECAR**

IL-2 + - -
Anti-IL-2 - + +
Tofacitinib - - +

Maximum OCR (pmoles/min)

Maximum ECAR (mpH/min)
Fostamatinib – Syk/multi-kinase inhibitor

- Syk associated with immunoreceptors in macrophages, B cells, neutrophils, mast cells

p38 MAPK

- Stress (anisoosmolarity, reactive oxygen species and ultraviolet radiation)
- Pro-inflammatory cytokines (TNF, IL-1 and TGFβ)
- Edotoxin

MAPK cascade

- Small GTPases (RAC1 and CDC42)
- MAPKKs (such as MTK1, TAK1 and ASK1)
- MAPKKs (such as MKK3, MKK4 and MKK6)
- MAPK (p38) (dual phosphorylation of Thr-Gly-Tyr)
- Substrates (transcription factors and kinases)

Biological effects (inflammation, T<sub>H</sub>-cell polarization, adhesion and chemotaxis)
Jakinibs: Lessons learned?

- Strong rationale – genetics
- but – drug not equivalent to k/o
  - Jak2
- pathway reasonably clear
- robust enzymatic assay
- Strong correlate with cellular substrates, preclinical and clinical readouts
  - STAT inhibitors?