Horror Autotoxicus: “the horror of self-toxicity” (Paul Ehrlich, 1901)
The body is unwilling to develop mechanism to cause its immune system
to attack itself

Autoimmune diseases arise from one’s immune system attacking healthy
tissues (self-antigens); affecting 5–8% of the population; organ specific or
systemic

More than 100 types of diseases: systemic lupus erythematosus (SLE),
rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel
disease (IBD), psoriasis, Sjögren syndrome, type I diabetes, ulcerative
colitis (UC), Crohn’s disease

Risk factors: genetics (typically polygenic; HLA allele is most significant),
environment, gender (~75% affected are women), hormone levels,
infection, and microbiota

Treatments include: NSAIDs, immunosuppressants, corticosteroids, kinase
inhibitors, monoclonal antibodies and other biologics

Kinase Inhibitors: Approved, Failed, and In the Pipeline
- Bruton tyrosine kinase (BTK) inhibitors
- Janus kinase (JAK) inhibitors
- Tyrosine kinase 2 (TYK2) inhibitors
- Phosphoinositide 3-kinase (PI3K) inhibitors
- Interleukin-2-inducible T cell kinase (ITK) inhibitors
- Spleen tyrosine kinase (SYK) inhibitors

tyrosine kinases serve as “on/off” switches, signaling cellular function via
phosphorylation of tyrosine residues on downstream proteins

Not Discussed Herein:
- Comprehensive coverage of kinase inhibitors in any disease area
- Targeting cytokines and nucleic acid sensing antagonists (Bioorg. Med.
- Monoclonal antibodies and other biologics (Nat. Rev. Drug Discovery 2019,
  18, 553-566)
- Natural products with immunosuppressive activity

Immune System:
Antibodies, Antigens, B and T cells
B cell receptors (BCRs): antibodies (immunoglobulin)
on cell membrane that bind specific antigens

upon binding an antigen: over
the course of a few days, this
B cell will mature and
proliferate to make thousands
of copies and rapidly release
antibodies into the
bloodstream

antibodies mark the toxin for destruction (opsonization),
neutralize toxins by binding to its receptors, and activate the
complement system

- Naive T cells can be activated upon binding to an antigen, proliferate, and
differentiate into T effector (T_{eff}) cells which migrate to the site of infection.
  - T helper cells bind to an antigen (foreign or infected cell) and mark it for
destruction
  - Natural Killer T cells release cytotoxins to kill a target cell
- T regulator (T_{reg}) cells are proliferated to attenuate an immune response via
release of inhibitory cytokines, cytolysis, metabolic disruption (prevent
autoimmunity, hopefully)

Relevant Group Meetings
Kinase Inhibitors: An Introduction (Peters, 2019)
Covalent Drugs: Trends, Mechanisms, and Warheads (Smith, 2022)
Fundamentals of Small Molecule Virology (Harwood, 2021)
**“Failure of Self-Tolerance”**

**Initial Causes of Autoimmunity:**
- Varies greatly across diseases, poorly understood
- 1. Abnormalities in self-antigens
- 2. Early innate immune response
- 3. Imbalance between T effector and T regulatory cells via depletion of T<sub>reg</sub> or T<sub>eff</sub> resistance

**Propagation and Presentation of Disease:**
- 1. Self-antigens driving the reaction cannot be eliminated
- 2. Epitope spreading: as tissue is damaged, new antigenic epitopes develop, leading to further immune response and mutation, creating a vicious cycle
- 3. Chronic inflammation leads to excessive cytokine, B cell, and T cell production, creating a feedback loop
- 4. Further imbalance of T<sub>eff</sub>/T<sub>reg</sub> cells

Autoantibodies (autoimmunity) can present years before clinical diagnosis (autoimmune disease) or disease may never develop.

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**Bruton’s Tyrosine Kinase (BTK) Inhibitors**

**Non-receptor (cytostolic) tyrosine kinase in Tec family**
- 1. BCR activation leads to activation of Lyn and SYK
- 2. BTK is phosphorylated at Tyr551
- 3. BTK then autophosphorylates at Tyr223 and is fully activated

**BTK Structure**

**ibrutinib (Imbruvica)**
- First-in-class BTKI
- $8.3 billion in sales in 2022, third top selling cancer drug and twelfth overall
- Used in treatment for over 270,000 patients worldwide

Approved for mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), Waldenström’s macroglobulinemia, marginal zone lymphoma, and GvHD

- Developed at Celera Genomics to study BTK
- Pharmacycics (now AbbVie) acquired the BTK discovery program
- Johnson & Johnson helped co-develop the drug once it was in Phase II
- Off-target reactivity with other Tec proteins containing Cys481, including RLK, BMX, and TEC.

**High risk of bleeding, rash, atrial fibrillation, diarrhea due to promiscuity**

**BTK IC<sub>50</sub> = 0.5 nM**

(suggested reading: *For Blood and Money*, Nathan Vardi)

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**Curr. Topics Med Chem. 2022, 22, 1647-1691; Nat. Chem. Rheum. 2014, 10, 212-228**
Horror Autotoxicus: Small Molecule Kinase Inhibitors in Immunology

Alex Rerick

Baran Group Meeting 06/03/2023

3 FDA approved BTKIs:
- ibrutinib (2013)
- acalabrutinib (2017)
- zanubrutinib (2020)

International BTKI approvals:
- tirabrutinib (2020, Japan)
- orelabrutinib (2020, China)

Reversible Covalent Inhibitors
- Attenuate the binding time with softer electrophile
- Even if a reversible covalent inhibitor binds endogenous thiol, they can unbind
- Selectivity within kinome: ability to attenuate non-covalent interactions to increase selectivity

Irreversible Non-Covalent Inhibitors
- Covalent inhibitors of the Cys481 residue have inherent selectivity risk: 10 other kinases have an equivalent Cys residue in their active site
- Also possess high haptenization risk

- Attenuate the binding time with softer electrophile
- Even if a reversible covalent inhibitor binds endogenous thiol, they can unbind
- Selectivity within kinome: ability to attenuate non-covalent interactions to increase selectivity

Fenebrutinib (GDC-0853)
Genentech

BTK is not a disease driver, inhibitors only interrupt the downstream effects of inflammation in autoimmune disease, requiring chronic treatment: 
attenuation of side effects is critical

Fenebrutinib: covalent binding of Cys481
key interaction at Tyr551

J. Med. Chem. 2018, 61, 2227−2245

G-278
BTK IC_{50} = 4 nM
observed hepatotoxicity in mice & dogs

BTK IC_{50} = 8.4 nM
reliable safety profile

- Phase II reached primary and secondary endpoints for multiple sclerosis (MS) (announced May 16th, 2023)
- Only reversible inhibitor in Phase III for MS

J. Med. Chem. 2022, 65, 5300−5316

37% mech?

1. NaH, BrMeCN
2. H_2, Pd/C

3 steps

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2. H_2, Pd/C
3 steps

37% mech?

72% mech?

J. Med. Chem. 2018, 61, 2227−2245
Increasing BTK Selectivity

- Benzyl amide sits in H3 pocket, near non-conserved residues; lipophilic groups increase binding affinity.

- Early leads:
  - BTK IC$_{50}$ for R = H: 3700 nM
  - BTK IC$_{50}$ for R = t-butyl: 142 nM

- BII068, Biogen:
  - BTK IC$_{50} = 1$ nM
  - Favorable in vivo pharmacokinetics
  - Poor ex vivo pharmacodynamics
  - Halted in phase I

Discovery Route

- 1. BH$_3$
- 2. Boc$_2$O
- 3. B$_2$pin$_2$, [Pd]: 58% 3 steps

Process Route

- A: Pd free, acidic conditions, no exogenous acid and same pot Boc removal
- BII068, precipitated free base

- 1. SOCl$_2$, cat. DMF
- 2. AlCl$_3$: 81% 2 steps

BII091, BTK IC$_{50} = <0.5$ nM

Phase I for MS

References:

- J. Med. Chem. 2020, 62, 12526-12541
- J. Med. Chem. 2022, 65, 1206-1224
**Horror Autotoxicus:**
Small Molecule Kinase Inhibitors in Immunology

Alex Rerick
Baran Group Meeting
06/03/2023

BMS returns to irreversible covalent inhibitors

How to overcome the toxicity associated with ibrutinib?
- Covalent inhibitors suffer from higher risk of more significant and prolonged off-target reactivity and idiosyncratic adverse drug reactions
- Non-covalent binding provides selectivity
- Irreversible covalent binding provides high coverage with low dosing

A covalent drug (1) reversibly binds in the active site, then (2) covalently reacts with a nucleophilic residue
- Non-covalent binding provides selectivity
- Irreversible covalent binding provides high coverage with low dosing

Drug needs to inactivate its target faster than it's cleared, but its elimination must be rapid enough to avoid off target effects

**Discovery Route**

1. H₂, Pt/C
2. NaNO₂, NaSO₃

**Mechanism**

**Four Step Process Route**

- BMS-986142: BTK IC₅₀ = 0.5 nM
- branebrutinib (BMS-986195): BTK IC₅₀ = 0.1 nM


Horror Autotoxicus:
Small Molecule Kinase Inhibitors in Immunology

Janus kinase (JAK)

Approved JAK Inhibitors for autoimmune diseases

tofacitinib (Xeljanz)
NIH and Pfizer
Approved 2014, first approved JAK inhibitor for immunological disease (RA, UC, psoriatic arthritis)
“JAK3 inhibitor” - but highly promiscuous

Pan-JAK Inhibitors

baricitinib

peficitinib

JAK family: JAK1, JAK2, JAK3, TYK2
- Non-receptor kinases that mediate cytokine signaling
- High level of homology in family:
  - JH1 (kinase domain) ATP is bound here, many JAK inhibitors bind at this site
  - JH2 (pseudo-kinase domain) aids in binding at JH1 but does not actively phosphorylate
- Conserved homology hinders ability to develop kinase specific drugs

delgocitinib (Corectim)
Japan Tobacco
Approved in Japan for atopic dermatitis, 2020, as a topical treatment
FDA Phase III, Fast Track for hand eczema

extensive screening: pyrolopyrimidine maintains selectivity

Discovery Route: stereoselective spirocycle synthesis

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Discovery Route: stereoselective spirocycle synthesis
Developing non-permeable small molecules for intestinal disorders

Strategies:
1. "Soft drugs" with intentionally metabolically vulnerable positions leading to high-first pass metabolism
2. Special release formulation
3. Pro-drug converted in the intestines
4. Intentionally break Lipinski’s Rule of 5?

**JAK Inhibitors for Inflammatory Bowel Disease (IBD)**

Inflammatory bowel disease (IBD) is caused by dysfunctional immune response to the microbiome and not by attacking self-antigens

Can we design a non-systemic drug without empirical screening?

**Izencitinib (TD-1473)**
Theravance Biopharma

non-systemic Pan-JAK inhibitor, high efflux ratio high colonic tissue and low plasma concentration mechanism of selectivity unclear, potentially a "soft drug"

failed to achieve clinical efficacy in phase II

**Janssen**

STAT4 IC_{50} = 54 nM

Discovery route

low systemic exposure, high colonic

satisfies Lipinski’s rule

cLogP = 0.8
MW = 409 Da
HBD = 3 and HBA = 8

**Izencitinib**

\[
\text{cLogP} = 3.4 \\
\text{MW} = 403 \text{ Da} \\
\text{HBD} = 3 \text{ and HBA} = 8
\]

2 steps

87% 2 steps

87% 68% 99%

1. NaOH
H\text{2}O/MeOH

2. DIPEA

H\text{2}Pd/C

95%

Can we design a non-systemic drug without empirical screening?

Horror Autotoxicus:
Small Molecule Kinase Inhibitors in Immunology

Alex Rerick

Baran Group Meeting
06/03/2023

Deucravacitinib
(Sotyktu)
BMS-986165

TYK2 JH2 IC\textsubscript{50} = 0.2 nM
First-in-class oral, selective, allosteric TYK2 Inhibitor
FDA approved September 2022 for plaque psoriasis


- PI3K\textgamma (in macrophages and granulocytes) and PI3K\textdelta (also in B and T cells) are implicated in autoimmune disease
- PI3K\textalpha and PI3K\textbeta are cancer targets

Phosphoinositide 3-kinase (PI3K)

HM5023507 attenuates T\text{eff} cell activation, reducing interleukin and interferon production, without reducing T\text{reg} function (in vitro)

IDealisib (Zydelig)
Gilead
FDA approved 2014 for CLL
PI3K\textdelta selective

HM5023507 Janssen
IC\textsubscript{50} (\mu M)
PI3K\textgamma = 0.004
PI3K\textdelta = 0.005
PI3K\textbeta = 0.590

**Spleen Tyrosine Kinase (SYK)**

**Non-receptor (cytostatic) kinase expressed in hematopoietic cells**
- Downstream of B and T cell receptor (B/TCR) signaling
- Overexpression of SYK leads to upregulation of B/TCRs
- Phosphorylates BTK, upregulates Treg

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**fostamatinib (R788)**
- Rigel Pharmaceuticals
- FDA approved for adult immune thrombocytopenia (ITP)
  - off-target activity
  - trials for RA and SLE discontinued - high dosing, insufficient efficacy

**GSK2646264**
- topical application (tissue selective)
  - failed in Phase I

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**gusacitinib (ASN002)**
- Asana BioSciences
  - first oral dual SYK/JAK inhibitor for dermatitis
  - FDA fast track designation
  - Phase II for chronic hand eczema (CHE)
  - Phase I for SLE

**Discovery Route**

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**IC₅₀ SYK = 5nM**
- JAK1 = 46 nM
- JAK2 = 4 nM
- JAK3 = 11 nM
- TYK2 = 8 nM

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*Int. Immunopharmacol. 2021, 90, 107168*

Interleukin-2-inducible tyrosine kinase (ITK)
- Non-receptor (cystostolic) tyrosine kinase in the Tec family expressed in T cells, mast cells, and natural killer cells
- Activated downstream of TCR by LCK and recruited to the membrane by PI3K
- Upon Tyr512 phosphorylation, ITK autophosphorylates Tyr180 and phosphorylates PLCγ1
- ITK positively regulates T helper cells, aids in migration of autoreactive T cells, and mediates cytokine secretion

Scaffold Inspiration: Avitinib, an EGFR tyrosine kinase inhibitor, Phase III, Sorrento Pharmaceuticals
ITK IC₅₀ = 1 µM

Balancing activity & selectivity
- position points toward a pocket: increase selectivity for noncovalent binding step?
- saturated ring attenuates reactivity to retain selectivity
- displaced a water molecule unique to ITK

Reversible ATP Competitive ITK Inhibitors
Elucidating better SAR for selective ITKIs?

Corvus Pharmaceuticals: CPI-818 (structure undisclosed) is a selective ITKI in phase III for T Cell Lymphoma and early development for autoimmune disease

Moving Beyond Symptomatic Treatment: Therapeutic Interventions to Halt Disease?
- More selective, tolerable drugs: lowering side effects
- Drugs to halt disease progression
- Earlier intervention and better screening

Biologics may be used in conjunction with small molecules or as second line treatment

Tumor necrosis factor (TNF) Inhibitors (Biologics)
Monoclonal antibodies: infliximab, adalimumab, and others
Rituximab: monoclonal antibody for B cell depletion

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