Targeted Inhibition Of Signal Molecules as Novel Therapy For CLL

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Acknowledgements

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Learning Objectives

• Identification of signal molecules that are critical to B cell survival

• Review preclinical data supporting potential efficacy of signal molecules in CLL Therapy

• Discuss potential benefit of combining inhibitors of signal target molecules as strategies for future therapy
Signal Molecules as Targets

• Protein kinases and lipid kinases are good choices as targets of signal transduction inhibition
  – are involved in signaling pathways often related to the pathogenesis of lymphoid malignancies

• BTK (Ibrutinib)

• PI3 Kinase delta (PI3Kδ) (Idelalisib)

• Axl Receptor Tyrosine Kinase (TP-0903)
Signaling Simplified
Importance of the B-cell Receptor and Its Signaling Pathways

- The B-cell receptor (BCR) is present on all B cells and has 2 main functions:
  - Recognizing foreign antigens
  - Initiating signaling pathways for clonal selection in B-cell maturation, survival, proliferation and participation in immune response

- BCR signaling is mediated by a number of proteins including PI3Kδ, BTK, Syk, Lyn, and ZAP70

BTK (Btk)

- TEC family non-receptor tyrosine kinases that plays a dominant role in B-lineage lymphoid cells.
  - Btk is a key molecule critical to normal B-cell development.
- Demonstrated in the inherited syndrome XLA,
  - described by Dr. Ogden Carr Bruton in 1952
- XLA is a human immunodeficiency syndrome
  - failure of pre-B-cells in the bone marrow to develop into circulating mature B-cells
- Btk association with the pathogenesis of XLA supported its critical role as a key regulator of B-cell development.
Ibrutinib

- Ibrutinib (formerly PCI-32765)
  - a potent irreversible covalent inhibitor of BTK

Irreversibly binds to Cys-481 in the active site of Btk resulting in sustained inhibition. an IC$_{50}$ of 0.5 nM and high selectivity toward Btk.
mRNA BTK Levels are higher in CLL B cells vs normal B cells

Sarah E. M. Herman et al. Blood 2011;117:6287-6296
Ibrutinib Down Regulates BTK Auto phosphorylation But not BTK Protein Levels.

Lee A. Honigberg et al. PNAS 2010;107:13075-13080
Ibrutinib Induces Cytotoxicity in CLL Cells
(independent of IVGH mutational status or FISH Status)
Ibrutinib Preclinical Data in CLL

- Disrupts the environment
  - Antagonizes BCR and CD40L mediated signaling /proliferation
  - Abrogates protection mediated by soluble and contact-dependent microenvironment factors
  - Reduces CLL cell chemotaxis toward CXCL12 and CXCL13
  - NLC secretion of chemokines is down regulated
  - Disrupts adhesion of CLL cells to various substrates
    - fibronectin and VCAM-1

Herman and Byrd, Blood, 2011, 117: 6287-6296
De Rooij and Spaargaren Blood. 2012, 119(11):2590-4
Ibrutinib Preclinical Data in CLL

- BTK and Ibrutinib in mouse models:
  - Knockout of BTK prevents leukemia development in CLL mouse model
  - Targeted genetic inactivation of BTK and/or use of ibrutinib delay the onset of leukemia / improves survival in EμTCL1 mice

Ibrutinib Off Target Effects

- Inhibits interleukin 2 kinase (ITK), an essential enzyme in Th2 T cells
  - Shift the balance between Th1 and Th2 T cells and potentially enhance antitumor immune responses
  - Improvement in humoral immune status (IgM, IgA)

- Other potential off target effects
  - Defects of platelet function
    - GPVI and integrin α_{IIb}β_{3} platelet signaling deficiencies
  - Atrial fibrillation (inhibition of cardiac PI3K-Akt signaling)
    - Both BTK and Tek transcripts are present in atrial tissue
  - May decrease efficacy of anti-CD20 antibodies

**Ibrutinib Use Can Induce Signal Mutations associated with Resistance**

**Characteristics of Patients with Identified Mutations at Relapse**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>No. Prior Therapies</th>
<th>Cytogenetics</th>
<th>Study Treatment</th>
<th>Duration on Ibrutinib</th>
<th>Best Response</th>
<th>Identified Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>5</td>
<td>del(17p13.1), +12</td>
<td>560 mg qd</td>
<td>621 days</td>
<td>PR</td>
<td>C481S BTK</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>2</td>
<td>del(17p13.1), complex karyotype</td>
<td>420 mg qd</td>
<td>673 days</td>
<td>PR</td>
<td>R665W PLCγ2</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>3</td>
<td>del(11q22.3)</td>
<td>BR x 6 cycles, 420 mg qd</td>
<td>388 days</td>
<td>CR</td>
<td>C481S BTK</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>2</td>
<td>complex karyotype</td>
<td>Ofatumumab x 24 weeks, 420 mg qd</td>
<td>674 days</td>
<td>CR</td>
<td>C481S BTK</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>9</td>
<td>del(17p13.1), complex karyotype</td>
<td>840 mg qd</td>
<td>868 days</td>
<td>PR</td>
<td>C481S BTK</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>4</td>
<td>del(17p13.1), complex karyotype</td>
<td>Ofatumumab x 24 weeks, 420 mg qd</td>
<td>505 days</td>
<td>PR</td>
<td>L845F, R665W, S707Y PLCγ2, C481S BTK</td>
</tr>
</tbody>
</table>

* No mutations identified in kinases with homology at C481

Woyach, NEJM 2014
BTK Inhibition (Ibrutinib)

Summary

• Targets a key signaling molecule vital to B cell survival

• Extensive *in vitro* and preclinical model efficacy

• Caveats
  – Drug resistance
  – Off target effects
PI3Kinase Delta (δ) Inhibition
PI3Kδ-dependent Signaling Pathways in B-cell Malignancies

Signaling pathways critical for survival, proliferation, and homing in many B-cell malignancies are dependent upon PI3Kδ.
Phosphoinositide 3’-kinase (PI3K) Inhibitors

• Several different PI3K inhibitors are in various stages of development:
  – Isoform-specific (e.g., PI3Kδ)
  – Pan-PI3K inhibitors
  – Dual inhibitors targeting both PI3K and mammalian target of rapamycin (mTOR)

• PI3Kδ is the isoform predominantly involved in BCR signaling
  – Expressed primarily in circulating lymphocytes neutrophils and monocytes and in lymphoid tissues, including spleen, lymph nodes, and thymus

• Idelalisib (previously CAL-101) selectively and reversibly inhibits the δ isoform of PI3K thus sparing other isoforms

Idelalisib: A Selective Inhibitor of PI3Kδ

- *In vitro* protein kinase-based assay data for idelalisib:
  - Idelalisib is 110-fold more potent at inhibiting PI3Kδ relative to PI3Kγ, with a greater selectivity for PI3Kα and PI3Kβ isoforms

<table>
<thead>
<tr>
<th>Class I PI3K Isoform</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x $K_m$ ATP (μM)</td>
<td>100</td>
<td>500</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>IC$_{50}$ (nM)</td>
<td>8,600</td>
<td>4,000</td>
<td>2,100</td>
<td>19</td>
</tr>
<tr>
<td>IC$_{50}$-Based PI3Kδ Fold Selectivity</td>
<td>453</td>
<td>211</td>
<td>110</td>
<td>1</td>
</tr>
</tbody>
</table>

Can it target PI3Kδ-dependent pathways in malignant B-cells?

Idelalisib Modulates Malignant B Cell Signaling

- Patient-derived malignant B-cells displayed constitutive levels of pAKT$_{T308}$
  - significantly reduced by idelalisib

- Treatment of patient CLL or MCL cells with sCD40L or BCR crosslinking caused rapid induction of pAKT$_{S473}$
  - inhibited by idelalisib at 0.1 to 1.0M

PI3Kinase δ Inhibition By Idelalisib Inhibits The Chemokines CCL3 and 4

NLC cultured with CLL B cells induces secretion of CCL3/4

PI3Kinase δ Inhibition By Idelalisib reduces CLL Cell Chemotaxis and Migration

PI3Kinase δ Inhibition
Off Target Effects

• The bad
  – Increased incidence of diarrhea, colitis \(^1\)
    • Seen in PI3Kinase delta knockout mice \(^2\)
  – Transaminitis
  – Pneumonitis
    • Can be seen with other mTOR inhibitors (everolimus)

• The good
  – Targeting PI3Kδ does not inhibit ADCC mediated by anti-CD20 antibodies \(^3\)
  – May stimulate anti tumor responses \(^4\)
    • Reduction of T Regulatory function

2. Gastroenterology 2010; 139: 1642 – 1653
Pl3Kinase δ Inhibition (Idelalisib) in B-cell Malignancies: Summary

• Inhibition of key signaling pathways in CLL B cells (plus MCL)
  – Decreased activation of AKT, mTOR and other downstream effectors

• This is associated with:
  – Reduced growth, proliferation, survival and homing to tissue sites for CLL B cells\textsuperscript{1-4}

• Provides Preclinical rationale for its use in therapy

Axl RTK studies
Why Look For Alternative Signal Inhibitors?

• Current agent issues
  – Targeted therapies of the BCR signaling pathway (ibrutinib,idelalisib,IPI-145) offer promise, these agents are not curative and resistance develops
  – Issues for patients who discontinue Ibrutinib
  – Low grade but chronic toxicities
  – Emergence of Richter’s Syndrome
Signal Target Search Strategy

- We explored whether CLL B-cells express other constitutively active RTKs and their functional implication in CLL B-cell survival
  - If present wanted to know if they share common signal mediators, e.g., Lyn (Src), PI3K,

- We have detected constitutively active Axl RTK and basic fibroblast growth factor receptor 3 (FGFR3) RTKs in CLL B-cells

Ghosh 2011 Blood 117:1928
Expression of Axl in CLL B-Cells (constitutively phosphorylated)

Flow cytometric analysis

[Graphs showing flow cytometric analysis of normal B and T cells, followed by CLL B and T cells for both P1 and P2 samples.]

Western blot analysis

[Western blot images showing P-Axl, Total Axl, and Actin for CLL B samples labeled P1 to P10.]

Confocal Microscopy

Axl RTK

- Axl is a member of the TAM (Tyro3, Axl, MER) family of RTKs

- The word “Axl” was derived from “Anexelekto” – Greek for “Uncontrolled”

- The human Axl gene is located on chromosome 19q13.2 and encodes a protein of MW 100-140 kD depending on glycosylation

- Growth arrest specific gene 6 (Gas6) is the natural ligand

Cold Spring Harb Perspect Biol. 2013 Nov 1;5(11):
Transl Lung Cancer Res. 2013 Jun;2(3):152-9
Axl in Human Cancers

- Depending on cell-types, activation of Axl initiates various signaling pathways
  - Cell survival, proliferation, apoptosis inhibition, migration, cell adhesion and cytokine production

- This is mediated via interactions with a spectrum of signaling molecules
  - PI3K, PLCγ, Grb2, c-Src, Lck, Ras, Raf1

- Importantly, Axl has been reported to be overexpressed in a number of human malignancies predicting poor survival

Leukemia. 2014 Jun;28(6):1252-8
Clin Cancer Res. 2014 Jan 1;20(1):164-75
Ann Surg Oncol. 2013 Dec;20 Suppl 3:S467-76
Axl is Physically Associated With Cellular Kinases/Lipase

1. CLL B cell lysates were found to contain Axl along with PI3K, c-Src, Syk, PLCγ2, ZAP-70, Lyn

2. We immunoprecipitated Axl RTK from the lysates and examined for co-localization by WB for these proteins

Ghosh 2011 Blood 117:1928
Axl Inhibition Induces Robust Apoptosis In CLL B-Cells

TP-0903 Targets P-Axl

Sinha et al., Clin Cancer Res. 2015 May 1;21(9):2115-26.
TP-0903 Reduces Apoptotic Proteins and Induces Expression of Pro-apoptotic BIM

A. Status of Apoptotic Proteins

B. Expression status of BIM

Sinha et al., Clin Cancer Res. 2015 May 1;21(9):2115-26.
Summary

- CLL B-cells express constitutively active but variable levels of Axl RTK

- Axl acts as a docking site for multiple non-receptor RTKs
  - Lyn, Syk kinases, ZAP70, lipase PLC\(_{\gamma}2\) and PI3K/AKT signaling axis

- Inhibition of Axl induces robust apoptosis in CLL B-cells including those from high-risk 17p-/-11q- CLL

- Axl inhibition results in significant reduction of the anti-apoptotic Bcl-2, XIAP and Mcl-1 and up regulation of the pro-apoptotic protein BIM in CLL B-cells
Can Axl RTK Inhibitor Synergize with Other Signal Inhibitors?

• Signal inhibitors can synergize
  – Idelalisib and Ibrutinib inhibit BCR-controlled mediated adhesion

• Tested two BTK inhibitors with and without Axl inhibition (TP-0903)
  • Ibrutinib (irreversible)
  • TP-4216 (reversible)

Concurrent Administration of TP-0903 With BTK Inhibitors

Using Combination Index analysis 6 of 10 CLL patients had synergy or additive effects on apoptosis when combined with the reversible BTK inhibitor

Sinha et al., Clin Cancer Res. 2015 May 1;21(9):2115-26.
Therapeutic Opportunity

- Axl Inhibition can induce robust CLL B cell apoptosis
- Axl RTK inhibitor in combination with reversible BTK inhibitor has robust additive or synergistic \textit{in vitro} activity on CLL B cell apoptosis
- These inhibitors are orally bioavailable and would be candidates for clinical trial testing
Summary

- Signal molecule transduction biology in CLL B cells provides clear rationale for targeting

- No perfect target yet!
  - Off target issues
  - Drug resistance

- Remaining issues include:
  - How to best combine these inhibitors?
  - Further enhance selectivity of target
  - Develop inhibitors that minimize drug resistance
  - Prevent emergence of Richter’s transformation