B cell receptor signaling pathways in health and disease

Dimitar Efremov, MD, PhD
Molecular Hematology Unit
International Centre for Genetic Engineering & Biotechnology
Trieste, Italy
# BCR signaling inhibitors

<table>
<thead>
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<th>Drug</th>
<th>Disease</th>
<th>Stage of development</th>
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<tr>
<td>SYK</td>
<td>Fostamatinib</td>
<td>CLL, NHL, RA, AIHA, ITP</td>
<td>Phase 2, Phase 3</td>
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<td>Entospletinib</td>
<td>CLL, NHL</td>
<td>Phase 2</td>
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<td>Cerdulatinib</td>
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<td>BTK</td>
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<td>Phase 2</td>
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<td>BTK</td>
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<td>pan-PI3K</td>
<td>Pilaralisib</td>
<td>CLL, NHL</td>
<td>Phase 1</td>
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Questions to be addressed:

- What is the evidence for a role of the BCR pathway in the pathogenesis of CLL and other B cell malignancies?

- How is the BCR pathway activated in CLL and other B cell malignancies?

- What are the differences in BCR signaling between CLL and normal B cells?

- Are there differences between BCR signaling inhibitors?
B cell receptor (BCR) pathway
Evidence for a role of the BCR pathway in the development of CLL

- Expression of stereotyped BCRs, i.e. BCRs encoded by similar or identical immunoglobulin variable region gene combinations, in over 30% of the cases

Evidence for a role of the BCR pathway in the development of CLL

- High levels of BCR target genes in freshly isolated CLL cells
  - Herishanu Y et al. Blood 2011

- Expression of constitutively active BCR signaling molecules:
  - LYN, SYK, PI3K, BTK, PKCβ, ERK, NF-kB, NF-AT

Contri A et al, J Clin Invest. 2005;115:369-78
Muzio M et al, Blood. 2008;112:188-95
Ringshausen I et al, Blood. 2002;100:3741-8
Abrams St et al, Blood. 2007;109:1193-201
Evidence for a role of the BCR pathway in the progression of CLL

- Strong association between clinical course and BCR-related features:
  - Mutational status of IGHV genes
  - Responsiveness to BCR stimulation

Treatment-free survival in CLL patients stratified according to IGHV mutation status

Treatment-free survival in CLL patients stratified according to response to anti-IgM stimulation

Damle RN et al, Blood 1999
Hamblin TJ et al, Blood 1999
Le Roy C et al, Blood 2012
Cesano A et al, Haematologica 2013
Iacovelli S et al, Blood 2015
D'Avola A et al, Blood 2016
Evidence for a role of the BCR pathway in the pathogenesis of other B cell malignancies

- **Restricted IGHV gene repertoire (BCR stereotypy):**
  - Mantle cell lymphoma
  - Splenic marginal-zone lymphoma

- **Constitutive activation of BCR signaling molecules:**
  - Mantle cell lymphoma (SYK/BTK/NF-kB)
  - ABC Diffuse large B cell lymphoma (SYK/BTK/NF-kB)
  - GC Diffuse large B cell lymphoma (SYK/PI3K/AKT)
  - Burkitt lymphoma (SYK/PI3K/AKT)

References:

Mechanisms of BCR pathway activation in CLL

- Classical BCR signal generated by binding to external autoantigens:
  - low-affinity autoantigens generated during apoptosis or oxidation (e.g., non-muscle myosin heavy chain IIA, vimentin, oxidized LDL, dsDNA, etc)

Chu CC et al, Blood. 2008
Lanemo Myhrinder A et al, Blood. 2008
Steininger C et al, Blood. 2012
Mechanisms of BCR pathway activation in CLL

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  - Chu CC et al, Blood. 2008
  - Lanemo Myhrinder A et al, Blood. 2008
  - Steininger C et al, Blood. 2012

- Cell autonomous BCR signal generated by interactions between the HCDR3 region of one CLL BCR with intrinsic motifs in the immunoglobulin molecule of another BCR
Establishment of transgenic mouse models to study the impact of antigen specificity on CLL development in vivo

- **Eμ-TCL1 transgenic mice:**
  - Overexpress TCL1 oncogene in B cell compartment
  - Develop CLL-like leukemias at 8-13 months of age
Establishment of transgenic mouse models to study the impact of antigen specificity on CLL development *in vivo*

- IgHEL BCR (mutated VH) / sHEL (High-affinity soluble autoantigen)
- IgHEL BCR (mutated VH) / mHEL-KK (High-affinity membrane autoantigen)
- IgHEL BCR (mutated VH) / pHEL-CpG (High-affinity foreign antigen)
- IgPtC BCR (unmutated VH) / PtC (Low-affinity membrane autoantigen)
- IgSm BCR (unmutated VH) / Sm (Low-affinity membrane autoantigen)
- IgHEL BCR (mutated VH) (No antigen)

Iacovelli S et al, Blood. 2015; 125:1578-88
Analysis of cell-autonomous BCR signaling capacity

Clone IGHV and IGLV genes in expression vector

Co-transfect IGHV/IGLV genes in the BCR-negative BLNK-inducible murine B-cell line TKO

Add tamoxifen to activate BLNK and analyze intracellular Ca$^{2+}$ flux

All Eμ-TCL1 leukemia-derived BCRs have cell-autonomous signaling capacity

**Eμ-TCL1/IgPtC tg mice**

**anti-PtC BCRs**
- T1 leukemia #337
- T1 leukemia #002
- T1 leukemia #48

**Eμ-TCL1/IgSm tg mice**

**anti-Sm BCRs**
- T1 leukemia #165
- T1 leukemia #121
- T1 leukemia #166

**Eμ-TCL1/IgHEL tg mice**

**anti-HEL BCR**
- T1/IgHEL

**normal B cells**

**endogenous BCRs**
- T1 leukemia #947 (endog. HC/tg LC)
- T1 leukemia #603 (endog. HC/tg LC)
- T1 leukemia #356 (endog. HC/tg LC)

Iacovelli S et al, Blood. 2015; 125:1578-88
Interactions with low-affinity autoantigens and cell-autonomous BCR interactions are positively selected during CLL development

**IgPtC BCR / PtC**
(Low-affinity membrane autoantigen)

**IgSm BCR / Sm**
(Low-affinity membrane autoantigen)

**IgHEL BCR / pHEL-CpG**
(High-affinity foreign antigen)

**IgHEL BCR / sHEL**
(High-affinity soluble autoantigen)

**IgHEL BCR / mHEL-KK**
(High-affinity membrane autoantigen)

**IgHEL BCR**
(no external autoantigen)

Iacovelli S et al, Blood. 2015; 125:1578-88
anti-PtC and anti-Sm BCRs differ in the intensity of signals generated by cell-autonomous and cell-extrinsic interactions.

**TKO cells with anti-PtC BCR**
- + PtC

**TKO cells with anti-Sm BCR**
- + Sm

**Leukemic cells with anti-PtC BCR**
- + PtC

**Leukemic cells with anti-Sm BCR**
- + Sm

Iacovelli S et al, Blood. 2015; 125:1578-88
Sm and PtC are internalized by the leukemic BCRs

Iacovelli S et al, Blood. 2015; 125:1578-88
Leukemias that respond to external antigen develop more rapidly than leukemias that do not respond or do not bind to external antigen.

**Time to overt leukemia development in E\(\mu\)-TCL1 tg mice**

- **Weak cell-autonomous signal, responsive to external antigen**
  - \(E\mu\)-TCL1/IgPtc
  - \(P < 0.001\)

- **Strong cell-autonomous signal, not responsive or not reactive with external antigen**
  - \(E\mu\)-TCL1/IgHEL
  - \(E\mu\)-TCL1/IgSm
  - \(E\mu\)-TCL1/IgPtc vs. IgHEL: \(P < 0.001\)
  - \(E\mu\)-TCL1/IgPtc vs. IgSm: \(P < 0.001\)
  - \(E\mu\)-TCL1/IgSm vs. IgHEL: \(P = \text{n.s.}\)

*Iacovelli S et al, Blood. 2015; 125:1578-88*
Mechanisms of BCR pathway activation in aggressive and indolent CLL

**Indolent CLL (M-CLL)**
- **Strong** cell-autonomous BCR-BCR interactions

**Aggressive CLL (U-CLL)**
- **Weak** cell-autonomous BCR-BCR interactions
- **Low-affinity external autoantigens**

- Basal Ca\(^2+\) signaling is increased in CLL compared to normal B cells and is significantly higher in M-CLL compared to U-CLL cells.

Muggen AF et al, Leukemia 2015;29:321-8
Distinct homotypic B-cell receptor interactions shape the outcome of chronic lymphocytic leukemia

Stereotyped subset #4
(IGHV4-34/IGKV2-30)
- Indolent disease
- Unresponsive

Strong cell-autonomous BCR-BCR interactions

Stereotyped subset #2
(IGHV3-21/IGLV3-21)
- Aggressive disease
- Responsive

Weak cell-autonomous BCR-BCR interactions

Stereotyped subset #8
(IGHV4-39/IGKV1-39)
- Aggressive disease
- Responsive
Mechanisms of BCR pathway activation in other B cell malignancies

**External autoantigens** (FL, MCL):
- 20% of FL and 50% of MCL Igs bind to vimentin

**Cell-autonomous BCR-BCR interactions** (ABC DLBCL):
- Clustered BCRs in >20% of ABC DLBCL
- Mutations in CD79A and CD79B

**Mannose-binding lectins** (FL):
- somatic hypermutation introduces sites for abnormal mannose glycosylation in >80% FL Igs
- mannosylated Igs interact with lectins expressed by DCs, macrophages and common bacteria

**Mutations in transcription factor TCF3** and its negative regulator ID3 in >70% of BL:
- Enhanced expression of BCR pathway components
- Reduced expression of SHP1
- Enhanced tonic BCR signaling

Cha SC et al, J Immunol. 2013
BCR signaling in CLL vs normal B cells

- Reduced BCR signaling capacity
- Downmodulation of surface IgM

Interactions with external antigen
Cell-autonomous BCR-BCR interactions
Interactions with soluble or FcμR-bound serum IgM

BCR internalization
IL-4 enhances surface IgM expression and BCR signaling capacity of CLL cells

**Blood**

- CLL cell
- IL-4R
- BCR

**Lymph node**

- T cell
- IL-4
- IL-4R
- Follicular dendritic cell
- Antigen
- CLL cell
- BCR

IL-4 downregulates Fc\(\mu\)R expression on CLL cells

**Blood**

- Fc\(\mu\)R
- BCR
- IL-4R

**Lymph node**

- Fc\(\mu\)R
- BCR


cell

- Follicular dendritic cell
- Antigen
- T cell

Blood

- Surface Fc\(\mu\)R
- Surface IgM

Lymph node

- Surface Fc\(\mu\)R
- Surface IgM

Gobessi et al, 2016, unpublished
Stimulation of Fc\(\mu\)R with soluble IgM or Fc\(\mu\) increases CLL cell survival but inhibits proliferation

**Blood**

- Serum IgM

**Lymph node**

- IL-4R
- BCR
- CD40L
- CD40
- TLR
- Fc\(\mu\)R

Stimulation of Fc\(\mu\)R with soluble IgM or Fc\(\mu\) increases CLL cell survival but inhibits proliferation.

Gobessi et al, 2016, unpublished
BCR signaling in CLL vs normal B cells

- Reduced BCR signaling capacity
- Downmodulation of surface IgM
- Aberrant expression of molecules involved in BCR signal transduction:
  - overexpression of ZAP-70, LCK, LYN, PKCβ, PTPN22 and TCL1
  - reduced expression of PHLPP1 and p66Shc
**In vivo studies of the role of ZAP-70 and PTPN22 in CLL development and behavior**

- **ZAP-70 expression:**
  - T cells, NK cells, CLL B cells, low levels in normal naive B cells

- **PTPN22 expression:**
  - T cells, myeloid cells, CLL B cells, low levels in normal B cells
CLL B cells from $E_\mu$-TCL1 transgenic mice overexpress PTPN22 but do not express ZAP-70

PTPN22 knockout allele

ZAP-70 transgene
PTPN22 knockdown accelerates leukemia development in Eμ-TCL1 transgenic mice

TCL1

TCL1/PTPN22−/−

Ibrahimoglu et al, 2016, unpublished
Ectopic expression of ZAP-70 does not influence leukemia development in E\(\mu\)-TCL1 tg mice

Gobessi et al, 2016, unpublished
Are the effects of targeting SYK, BTK and PI3Kδ the same?

**Downstream BCR signaling molecules are not equally inhibited by SYK, BTK and PI3Kδ inhibitors**

**Inhibitor:**
- anti-IgM: - + - + - + - +
- Fos: - + - + - + - +
- Ibr: - + - + - + - +
- Idel: - + - + - + - +

**Inhibitors:**
- Fos, fostamatinib (SYK inhibitor)
- Ibr, ibrutinib (BTK inhibitor)
- Idel, idelalisib (PI3Kδ inhibitor)

Bojarczuk K et al, Blood. 2016;127:3192-201
Mechanism of action of Venetoclax (ABT-199)

Pro-apoptotic multidomain effectors
- BIM
- BID
- PUMA

Pro-apoptotic BH3-only activators
- BAX
- BAK

Pro-apoptotic BH3-only sensitizers
- BAD
- BMF
- NOXA

Anti-apoptotic
- BCL-2
- BCL-xL
- BCL-W
- MCL-1
- BFL-1

Venetoclax

Apoptotic signal

Cytochrome c release
Caspase 9 activation
Caspase 3 activation
Apoptosis
BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to Venetoclax

- Sustained BCR engagement with external ligand (anti-IgM) induces resistance to Venetoclax

- BCR-mediated resistance to Venetoclax is mediated primarily by induction of Mcl-1

- SYK inhibitors downregulate MCL-1 more effectively than inhibitors of BTK or PI3Kδ
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