Therapy of Lymphoma
Inspired by
Functional and Structural Genomics
Interplay of Functional and Structural Genomics

Genome-wide RNAi / CRISPR screens

Oncogenic somatic mutation

Essential cancer pathways
Dissecting Cancer Into Molecularly and Clinically Distinct Subtypes by Gene Expression Profiling

Diffuse Large B Cell Lymphoma (DLBCL)

Activated B Cell-like DLBCL (ABC)
Germinal Center B Cell-like DLBCL (GCB)

Subtype-specific gene expression signatures

- High Gene Expression
- Low Gene Expression

Lymphoma Biopsies

Genes

- IRF4
- PIM2
- CCND2
- FOXP1
- IL16
- CD44
- IGHM
- MME
- CR2
- KCNN3
- LRMP
- LMO2
- MYBL1
- SLAM
Dissecting Cancer Into Molecularly and Clinically Distinct Subtypes by Gene Expression Profiling

Diffuse Large B Cell Lymphoma (DLBCL)

Subtype-specific response to chemotherapy

Probability of survival

Progression-free survival (yrs) (R-CHOP Rx)

GCB DLBCL

ABC DLBCL

3-year progression-free survival

75%

40%

P = 2.27 x 10^-8
Active vs. Tonic BCR Signaling Differentiates Lymphoma Subtypes
Multiple Oncogenic Mutations in ABC DLBCL Promote Chronic Active B Cell Receptor Signaling
CD79 ITAM Mutations Are Back Seat Drivers in ABC DLBCL
Blockade of BCR Signaling in ABC DLBCL with Ibrutinib

Chronic Active BCR signaling

Ibrutinib

BTK

PKCβ

IKKγ

IKKβ

IKKα

NF-κB pathway

Survival
The Promise of Targeted Therapy in Cancer

- 52 year old female
  ABC Diffuse Large B Cell Lymphoma
- Activating Mutation in B cell receptor subunit CD79B
- Relapse following 2 prior chemotherapies
  DA-EPOCH-R + Campath  CR and relapse
  DA-EPOCH-R            CR and relapse
- Treatment with Ibrutinib, a B cell receptor signaling inhibitor
PET/CT Scan Before and On Treatment With Ibrutinib

Before Rx

On Rx: week 8

Ongoing Complete Response at > 6 years
Phase 2 Clinical Trial of Ibrutinib in Relapsed/refractory DLBCL

- Multicenter phase 2 trial
  - Relapsed/refractory DLBCL (ABC and GCB subtypes)
  - Subtype determined by immunohistochemistry and confirmed by gene expression profiling
  - Ibrutinib 560 mg p.o. daily
  - n=70
Ibrutinib is Preferentially Active in ABC DLBCL

% Response (PR + CR)

15/39

CR

PR

p=0.0062

ABC DLBCL

GCB DLBCL

1/20

PR

Ibrutinib Extends Overall Survival in Relapsed/Refractory ABC DLBCL

Median OS

- ABC: 10.32 months
- GCB: 3.35 months

p = 0.0483

4 patients with longer survival
- 1.9 yrs
- >2.7 yrs
- >3.0 yrs
- >5.0 yrs

Can Analysis of Recurrent Genetic Lesions Identify Ibrutinib Responders Within ABC DLBCL?
Influence of B Cell Receptor Mutations on Ibrutinib Response in ABC DLBCL

CD79B ITAM motif

% Response (CR + PR)

(5/9)

(10/30)
Cancers can be strongly addicted to non-genetic signaling

Mutation ≠ response
Clustering of B Cell Receptors on the ABC DLBCL Surface Suggests Antigen Engagement
Self Antigens Drive B Cell Receptor Signaling in ABC DLBCL

Self glycoprotein

Self antigen from dying cells

Self idiotope
Constitutive MYD88 Signaling in ABC DLBCL

Chronic Active BCR signaling

Constitutive MYD88 signaling

SFK
SYK
BTK
PKCβ
CARD11
MALT1
BCL10
IKKγ
IKKβ
IKKα
NF-κB pathway
Survival

MYD88 TIR domain mutation
39%
Influence of B Cell Receptor and MYD88 Pathway Mutations on Ibrutinib Response in ABC DLBCL

MYD88 TIR domain

\[
p = 0.60
\]

<table>
<thead>
<tr>
<th>% Response (CR + PR)</th>
<th>Mutant</th>
<th>WT</th>
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<tbody>
<tr>
<td>(4/12)</td>
<td>(11/26)</td>
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Influence of B Cell Receptor and MYD88 Pathway Mutations on Ibrutinib Response in ABC DLBCL

MYD88 TIR domain vs. CD79A/B ITAM motif

\[ p = 0.0038 \]

<table>
<thead>
<tr>
<th>CD79B: MYD88</th>
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<th>WT</th>
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<tbody>
<tr>
<td>Mutant</td>
<td>4/5</td>
<td>0/7</td>
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\( \% \text{ Response (CR + PR)} \)

Ibrutinib-sensitive and -resistant Forms of ABC DLBCL

ABC DLBCL

GCB DLBCL

Ibrutinib (nM)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HBL1</th>
<th>TMD8</th>
<th>Ly10</th>
<th>HLY1</th>
<th>SUDHL2</th>
<th>BJAB</th>
<th>Ly1</th>
<th>Ly8</th>
</tr>
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<tbody>
<tr>
<td>Live cells (% DMSO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYD88 mutation</td>
<td>L265P</td>
<td>L265P</td>
<td>L265P</td>
<td>S219C</td>
<td>S222R</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>BCR mutation</td>
<td>CD79B</td>
<td>CD79B</td>
<td>CD79A</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
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p = 0.0038

ABC DLBCL (n=155)
- MYD88 L265P: 19%
- CD79B: 13%

1.5x enrichment (p=0.03)

MYD88 Inhibition Decreases Proximal B Cell Receptor Signaling in ABC DLBCL Cells

IMG-2005
MyD88 inhibitor: + + + + +
Control peptide: + + + + +

pY (4G10)
pY416 Src

ABC DLBCL line: DLBCL2 LY10 HBL1 TMD8
MyD88 mutation: L265P L265P L265P L265P
BCR mutation: CD79B CD79A CD79B CD79B
Seeing Protein Interactions in Cells: The Proximity Ligation Assay
Colocalization of Phosphorylated CD79A and MYD88 in the Cytoplasm of ABC DLBCL Cells

pY182 CD79A x MYD88 Proximity Ligation Assay
The CRISPR Revolution

Cas9-induced mutation

Matching genomic sequence

Guide RNA

Genomic DNA

Cas9

Repair
CRISPR-Cas9 Screening Identifies Essential Genes In ABC DLBCL Cell Lines

sgRNA toxicity in TMD8 ABC DLBCL cells

sgRNAs: **MYD88**

Fold change day 21 vs. day 0 (log2)
TLR9 is Required in ABC DLBCL Lines With MYD88 L265P
Colocalization of IgM and TLR9 in Cytoplasmic Vesicles in ABC DLBCL
Colocalization of IgM and TLR9 in Cytoplasmic Vesicles in ABC but not GCB DLBCL

HBL1 (ABC DLBCL)  BJAB (GCB DLBCL)

IgM x TLR9 Proximity Ligation Assay
NF-κB Pathway Activation in ABC DLBCL Occurs in the Lysosomal Compartment Containing TLR9 and IgM

HBL1 (ABC)  BJAB (GCB)

TLR9 × p-IκBα

IgM × p-IκBα
NF-κB Activation by BCR and TLR/MYD88 Signaling is Colocalized in the Cytoplasm
The BCR-MYD88 Superpathway in ABC DLBCL

- PLCγ2
- BTK
- BLNK
- SFK
- SYK
- Ca²⁺
- DAG
- PKCβ
- TLR9
- MYD88
- CARD11
- MALT1
- BCL10
- IKKγ
- IKKβ
- IKKα
- NF-κB pathway

BCR signaling

Endolysosome

Endosome

BCR recycling
Two Pathogenetic Pathways to ABC DLBCL?

Hyperaddiction to BCR signaling

Extreme sensitivity to ibrutinib

BCR-dependent
Ibrutinib-sensitive

CD79A/B WT
MYD88 WT

↑ NF-κB

CD79A/B Mutant
MYD88 WT

↑ NF-κB

CD79A/B Mutant
MYD88 Mutant

↑ NF-κB

Hypermethylation of MYD88

↑ NF-κB

MYD88-dependent
BCR-independent
Ibrutinib-resistant

CD79A/B WT
MYD88 Mutant
High Prevalence of MYD88 L265P Mutation in Extranodal Lymphomas with an ABC DLBCL Phenotype

<table>
<thead>
<tr>
<th>MYD88 L265P (% cases)</th>
<th>ABC DLBCL</th>
<th>1° cutaneous lymphoma, leg type</th>
<th>1° testicular lymphoma</th>
<th>1° CNS lymphoma</th>
</tr>
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<tr>
<td></td>
<td>28</td>
<td>55</td>
<td>71</td>
<td>56</td>
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Primary Central Nervous System Lymphoma is Enriched For Mutations in MYD88 and the BCR Subunit CD79B

ABC DLBCL (n=250)
- MYD88 L265P (28%)
- Both (11%) 17%
- CD79B ITAM (24%) 13%

PCNSL (n=213)
- MYD88 L265P (56%)
- Both (37%) 19%
- CD79B ITAM (53%) 17%

59%

24%
Hypothesis:
Extranodal DLBCLs are hyper-addicted to BCR signaling
=> Will respond frequently to ibrutinib
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