TCGA Workflow for Integrative Molecular Analysis of Cancer

1. Tumor biopsy

2. Pathology QC

3. DNA & RNA isolation, QC

4. DNA sequence: exomes, whole genomes

5. RNA expression, copy number, DNA methylation

6. Data and results storage & QC

7. Integrative computational analysis

8. Essential cancer pathways
Integration of a TCGA-like Pipeline Into Cancer Clinical Trials Has the Potential to Change Clinical Care
Diffuse large B cell lymphoma

~40% of Non-Hodgkin lymphomas

~23,000 new diagnoses/yr

~50% cure rate

~10,000 deaths/yr
Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma

Activated B Cell-like DLBCL (ABC)

Germinal Center B Cell-like DLBCL (GCB)

Primary Mediastinal B Cell Lymphoma

Genes

Lymphoma Biopsies

Gene Expression

High

Low
Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma

Probability of survival

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

P = 2.27 \times 10^{-8}

GCB DLBCL

ABC DLBCL

3-year progression-free survival

75%

40%
Oncogenic Activation of NF-κB in ABC DLBCL
Chronic Active B Cell Receptor Signaling in ABC DLBCL
Chronic Active B Cell Receptor Signaling in ABC DLBCL

Chronic Active BCR signaling

10%

CARD11 coiled-coil mutation

NF-κB pathway

Survival
Chronic Active B Cell Receptor Signaling in ABC DLBCL
Chronic Active B Cell Receptor Signaling in ABC DLBCL
The B Cell Receptors in ABC DLBCLs Are Clustered and Immobile

ABC DLBCL

GCB DLBCL

Cumulative probability

ABC DLBCL

GCB DLBCL

B cell receptor diffusion (mm²/s)
Chronic Active B Cell Receptor Signaling in ABC DLBCL

Chronic Active BCR signaling

CD79A/B ITAM mutation

21%

BTK

PKCβ

CARD11 MALT1 BCL10

IKKγ IKKβ IKKα

IκB kinase

NF-κB pathway

Survival
Constitutive MYD88 Signaling in ABC DLBCL

Chronic Active BCR signaling

Constitutive MYD88 signaling

IκB kinase

IκB kinase

Survival
Constitutive MYD88 Signaling in ABC DLBCL

Chronic Active BCR signaling

Constitutive MYD88 signaling

39%
Significant Overlap of CD79B/A and MYD88 L265P Mutations in ABC DLBCL

MYD88 TIR domain

ABC DLBCL (n=154)
- MYD88 L265P (29%) - 19%
- CD79B/A (23%) - 10%
- CD79B/A or MYD88 L265P (42%) - 13%
Blockade of BCR Signaling in ABC DLBCL with Ibrutinib

Chronic Active BCR signaling

SFK
SYK

BTK

PKCβ

CARD11
MALT1
BCL10

IKKγ
IKKβ
IKKα

NF-κB pathway

Survival
Ibrutinib Covalently Binds to the BTK Active Site
The BTK Inhibitor Ibrutinib is Toxic for ABC DLBCLs With Chronic Active B Cell Receptor Signaling
Clinical Trials of Ibrutinib in Relapsed/refractory DLBCL

- Pilot trial (NCI)
  - Relapsed/refractory DLBCL (ABC subtype)
  - Subtype determined by immunohistochemistry and confirmed by gene expression profiling
  - Ibrutinib 560 mg p.o. daily
  - n=10 (completed)
Patient #2 on Pilot Trial of Ibrutinib in Relapsed/refractory ABC DLBCL

- 52 year old female
  ABC DLBCL

- CD79B Y196C mutation
  MYD88 wild type

- Relapse following 2 prior chemotherapies
  DA-EPOCH-R: Complete response and relapse
  DA-EPOCH-R + Campath: Complete response and relapse

- Single agent treatment with ibrutinib

- Complete response at week 8 by CT and PET scan

- Sustained complete response at > 2 years on ibrutinib
Complete Remission of ABC DLBCL in Patient #2 on Pilot Trial of Ibrutinib

Before Rx

On Rx: week 8
Patient #9 on Pilot Trial of Ibrutinib in Relapsed/refractory ABC DLBCL

- 59 year old female
  ABC DLBCL

- CD79B wild type
  MYD88 wild type

- Primary refractory disease
  R-CHOP x 6: No response
  R-ICE x 2: No response
  Oxaliplatin + gemcitabine x 3: No response

- Single agent treatment with ibrutinib

- Near complete response at week 3 by CT and PET scan
Rapid Normalization of LDH Following Ibrutinib Treatment
Partial Remission of ABC DLBCL in Patient #3 on Pilot Trial of Ibrutinib
Clinical Trials 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 (accrual complete)
Higher Response Rate to Ibrutinib in ABC DLBCL Than GCB DLBCL

\[ p = 0.004 \]

% Response (CR + PR)

\[
\begin{align*}
\text{ABC DLBCL} & : 12/29 \\
\text{GCB DLBCL} & : 1/20
\end{align*}
\]
Complete and Partial Responses to Ibrutinib Are Enriched For ABC DLBCLs

% Baseline tumor size (sum of perpendicular diameters)

ABC DLBCL
GCB DLBCL
Ibrutinib Responses Can Extend Life in Patients With Relapsed/Refractory ABC DLBCL For > 6 Months
Can Analysis of Recurrent Genetic Lesions Identify Ibrutinib Responders Within ABC DLBCL?
CD79B Mutant ABC DLBCL Predicts a High Rate of Response to Ibrutinib

% Response (CR + PR) vs CD79B Mutant

5/7
Ibrutinib Response in ABC DLBCL Does Not Require B Cell Receptor Mutation

% Response (CR + PR)

CD79B: Mutant 10/29

CD79B: WT 5/7
MYD88 L265P Plus CD79B Mutations Identify Ibrutinib-responsive ABC DLBCL

% Response (CR + PR)

CD79B: Mutant - 5/7  
MYD88:  
Mutant Mutant 4/5

10/29
MYD88 L265P Without CD79B Mutation Predicts Ibrutinib Resistance in ABC DLBCL

% Response (CR + PR)

<table>
<thead>
<tr>
<th>CD79B:</th>
<th>MYD88:</th>
<th>Mutant</th>
<th>WT</th>
<th>Mutant</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant</td>
<td>-</td>
<td>5/7</td>
<td>10/29</td>
<td>4/5</td>
<td>0/4</td>
</tr>
</tbody>
</table>
CARD11 Mutant ABC DLBCL Does Not Respond To Ibrutinib
Homozygous Deletion of INK4a/ARF is Recurrent in ABC DLBCL and is Associated With Unfavorable Outcome

CDKN2A  
CDKN2B  
INK4a/ARF homozygous deletion  

![Bar Chart and Survival Curve]

- **Bar Chart:**
  - CDKN2A
  - CDKN2B
  - INK4a/ARF homozygous deletion

- **Survival Curve:**
  - WT
  - INK4a/ARF deletion

**Overall Survival (years)**

**Probability**

\[ p = 0.017 \]
Homozygous Deletion of the INK4a/ARF Locus Predicts Ibrutinib Response

% Response (CR + PR)

INK4a/ARF: Homozygous deletion

5/8

WT

0/7
The Heterogeneity of Human Cancer Necessitates Analysis of Large Numbers of Biopsies

- May need to extend genetic analysis to \( n > 10,000 \) to see patterns of co-occurrence and exclusion among genetic lesions.
Integrative Analysis Will Be Key to Deciphering Response / Resistance to Therapy in Cancer

- Pathway-centric view of genetic lesions
- Gene expression signatures of response / resistance
- Pathway activity assessment by protein modifications
Towards Precision Medicine in Routine Cancer Care

• Need to make molecular diagnostic tests widely available
• Develop of a Cancer Genome Commons database to accelerate precision medicine
Acknowledgements

Metabolism Branch, CCR, NCI
Wyndham Wilson
Yandan Yang
Sameer Jhavar
Roland Schmitz

Frederick National Laboratory, NCI
Jason Lih
Mickey Williams

Laboratory of Pathology, CCR, NCI
Stefania Pittaluga

CIT, NIH
Wenming Xiao
John Powell

Biometric Research Branch, DCTD, NCI
George Wright

Pharmacyclics
Jesse McGreivy
Lori A. Kunkel
Sriram Balasubramanian
Mei Cheng
Davina Moussa
Joseph J. Buggy

J&J
Deb Ricci

Ibrutinib DLBCL Trial Consortium
John Gerecitano
Andre Goy
Sven DeVos
Vaishalee P. Kenkre
Paul Barr
Kristie A. Blum
Andrei Shustov
Ranjana Advani
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL

- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required

- MYD88 L265P mutations cooperate with CD79B mutations to enhance BCR signaling addiction
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required
- MYD88 L265P mutations cooperate with CD79B mutations to enhance BCR signaling addiction
- ABC DLBCLs with CARD11 mutations or MYD88 L265P without CD79B mutation resist ibrutinib
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required
- MYD88 L265P mutations cooperate with CD79B mutations to enhance BCR signaling addiction
- ABC DLBCLs with CARD11 mutations or MYD88 L265P without CD79B mutation resist ibrutinib
- INK4a/ARF homozygous deletion is common in R/R ABC DLBCL and associated with ibrutinib response
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required
- MYD88 L265P mutations cooperate with CD79B mutations to enhance BCR signaling addiction
- ABC DLBCLs with CARD11 mutations or MYD88 L265P without CD79B mutation resist ibrutinib
- INK4a/ARF homozygous deletion is common in R/R ABC DLBCL and associated with ibrutinib response
- Larger ABC DLBCL cohorts are needed to understand the relationship of genetic events to ibrutinib response
Targeted Therapy of ABC DLBCL

- Ibrutinib induces complete and partial responses in relapsed/refractory ABC DLBCL but the response rate is low in GCB DLBCL
- CD79B mutations enrich for ibrutinib activity in ABC DLBCL but are not required
- MYD88 L265P mutations cooperate with CD79B mutations to enhance BCR signaling addiction
- ABC DLBCLs with CARD11 mutations or MYD88 L265P without CD79B mutation resist ibrutinib
- INK4a/ARF homozygous deletion is common in R/R ABC DLBCL and associated with ibrutinib response
- Larger ABC DLBCL cohorts are needed to understand the relationship of genetic events to ibrutinib response
- ABC DLBCL is a good biomarker of ibrutinib response
Ibrutinib Treatment Related Toxicities

- Diarrhea (grade 1)
- Nausea (grade 1)
- Fatigue (grades 1 and 2)
- Time-dependent decrease in B cell numbers
  Maintenance of serum immunoglobulin levels
Patient #3 on Pilot Trial of Ibrutinib in Relapsed/refractory ABC DLBCL

- 48 year old male
  ABC DLBCL

- CD79B wild type
  MYD88 wild type

- Multiple prior relapses following chemotherapy and radiation
  R-CHOP x 6
  R-ESHAP
  Autologous bone marrow transplant

- Single agent treatment with ibrutinib

- Complete response at week 10 by CT and PET scan
Complete Remission of ABC DLBCL in Patient #3 on Pilot Trial of Ibrutinib
Patient on Phase 2 Trial of Ibrutinib in Relapsed/refractory DLBCL

- 71 year old male
  ABC DLBCL

- CD79B Y196H mutation
  MYD88 L265P mutation

- R-CHOP + genasense + radiation: Partial response
  Ofatumamab + lenalidomide: No response
  ICE => No response
  R-DHAP => No response

- Single agent treatment with BTK inhibitor (PCI-32765)

- Complete response at week 12 by CT and PET scan
Complete Remission of ABC DLBCL in Phase 2 Trial of Ibrutinib

Before Rx

On Rx: week 12
A Gene Expression-based Classifier of ABC vs. GCB DLBCL Using FFPE Biopsies

![Graph showing the relationship between FFPE Cross-validated Score and Original Frozen biopsy score for ABC DLBCL and GCB DLBCL. The graph is divided into quadrants, with points marked by blue triangles for Good, green triangles for Marginal, and red triangles for Poor array quality. The diagonal line represents the expected perfect correlation.](graph.png)
Molecular Pathogenesis of Diffuse Large B Cell Lymphoma

GCB DLBCL
- Germinal center B cell
- NF-kB → IRF4

ABC DLBCL
- Plasmablast
- Differentiation arrest
- Plasma cell