Translating Genomic Advances into Novel Therapies for Waldenström Macroglobulinemia

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Bing Center for Waldenstrom’s Macroglobulinemia
Dana-Farber Cancer Institute
Harvard Medical School
Advances in the Biology of Waldenstrom’s Macroglobulinemia

Treatment Approach

Ixazomib
Bortezomib
Carfilzomib
Benda
GA101
CAL101
Pom
Len
RAD001

Someday, all this will be yours.

WM

© Guy & Rodd/Distributed by Universal Uclick via CartoonStock.com
### WM–centric toxicities with commonly used therapies

<table>
<thead>
<tr>
<th>Agent</th>
<th>WM Toxicities</th>
</tr>
</thead>
</table>
| **Rituximab**       | • IgM flare (40-60%)-> Hyperiscosity crisis, Aggravation of IgM related PN, CAGG, Cryos.  
                      | • Hypogammaglobulinemia-> infections, IVIG  
                      | • Intolerance (15-20%)                                                                                                                        |
| **Nucleoside Analogues** | • Hypogammaglobulinemia-> infections, IVIG  
                      | • Transformation, AML/MDS (15%)                                                                                                                |
| **IMIDS**           | • Peripheral Neuropathy (60% >grade 2 with Thalidomide)  
                      | • Aggravated IgM flare (Revlimid and Pomalidomide)  
                      | • Severe anemia (Revlimid)                                                                                                                                 |
| **Bortezomib**      | • Grade 2+3 Peripheral neuropathy (60-70%); High discontinuation (20-60%)                                                                 |
New directions in WM
MYD88 L265P Somatic Mutation in WM

C to G at position 38186241 at 3p22.2

MYD88^{L265P} confirmed by AS-PCR in 95% WM patients, 50-80% IGM MGUS.

Acquired UPD at 3p22.

Treon et al, NEJM 367:826, 2012
# MYD88 L265P Somatic Mutation in WM

<table>
<thead>
<tr>
<th>METHOD</th>
<th>TISSUE</th>
<th>WM</th>
<th>IGM MGUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treon WGS/Sanger</td>
<td>BM CD19⁺</td>
<td>91%</td>
<td>10%</td>
</tr>
<tr>
<td>Xu AS-PCR</td>
<td>BM CD19⁺</td>
<td>93%</td>
<td>54%</td>
</tr>
<tr>
<td>Gachard PCR</td>
<td>BM</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>Varettoni AS-PCR</td>
<td>BM</td>
<td>100%</td>
<td>47%</td>
</tr>
<tr>
<td>Landgren Sanger</td>
<td>BM</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>Jiminez AS-PCR</td>
<td>BM</td>
<td>86%</td>
<td>87%</td>
</tr>
<tr>
<td>Poulain PCR</td>
<td>BM CD19⁺</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>Argentou PCR-RFLP</td>
<td>BM</td>
<td>92%</td>
<td>1/1 MGUS</td>
</tr>
<tr>
<td>Willenbacher Sanger</td>
<td>BM</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>Mori AS-PCR/BSiE1</td>
<td>BM</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>Ondrejka AS-PCR</td>
<td>BM</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Ansell WES/AS-PCR</td>
<td>BM</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Patkar AS-PCR</td>
<td>BM</td>
<td>85%</td>
<td></td>
</tr>
</tbody>
</table>

> 50 confirmatory studies worldwide!!
Lymphoproliferative Disorders driven by mutated MYD88

Waldenstrom’s Macroglobulinemia (95-97%)  
IGM MGUS (50-80%)  
Lymphoplasmacytic Lymphoma (non-IGM) (100%)  
Primary CNS Lymphoma (80-90%)  
Immune Privileged Lymphomas (80-90%)  
ABC DLBCL (20-40%)  
Marginal Zone Lymphoma (6-10%)  
CLL (4-8%)

MYD88 Mutations in B-cell Malignancies

MYD88 mutations transactivate NFκB

Ngo et al, Nature 2011
Treon et al, NEJM 2012

MYD88 L265P mutated WM cells
Viruses/Bacteria $\rightarrow$ Toll Receptor $\rightarrow$ IL-1 Receptor $\leftarrow$ Inflammation

MYD88 L265P Signal Pathway

Yang et al, Blood 2013
**MYD88 binds to the active form of Bruton’s Tyrosine Kinase (BTK) in L265P expressing WM cells**

### A.

<table>
<thead>
<tr>
<th></th>
<th>BCWM.1</th>
<th>MWCL-1</th>
<th>OCI-Ly19</th>
<th>Ramos</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP: IgG</td>
<td>BTK</td>
<td>P-BTK</td>
<td>BTK</td>
<td>P-BTK</td>
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<td></td>
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<td>BTK</td>
<td>P-BTK</td>
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<td></td>
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<td></td>
<td>BTK</td>
<td>P-BTK</td>
</tr>
</tbody>
</table>

**IB:** MYD88

**IB:** BTK

**Ratio:** MYD88 / BTK (%)

15.1 81.8 32.8 89.0 18.9 17.0 13.1 24.4

**IB:** MYD88

**IB:** BTK

**IgG - heavy chain**

### B.

<table>
<thead>
<tr>
<th></th>
<th>BCWM.1</th>
<th>MWCL-1</th>
<th>OCI-Ly19</th>
<th>Ramos</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP: IgG</td>
<td>MYD88</td>
<td>MYD88</td>
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<td>MYD88</td>
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</tbody>
</table>

**IB:** MYD88

**IB:** BTK

**IgG - heavy chain**

### C.

<table>
<thead>
<tr>
<th></th>
<th>BCWM.1</th>
<th>MWCL-1</th>
<th>OCI-Ly3</th>
<th>OCI-Ly19</th>
<th>Ramos</th>
<th>RPMI-8226</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP: IgG</td>
<td>DMSO</td>
<td>DMSO</td>
<td>Ibrutinib</td>
<td>DMSO</td>
<td>Ibrutinib</td>
<td>DMSO Ibrutinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DMSO</td>
<td>Ibrutinib</td>
<td>DMSO</td>
<td>Ibrutinib</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>DMSO</td>
<td>Ibrutinib</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DMSO</td>
<td>Ibrutinib</td>
<td>DMSO</td>
<td>Ibrutinib</td>
</tr>
</tbody>
</table>

**IB:** MYD88

**IB:** BTK

**IB:** MYD88

MYD88 L265P induces BTK in WM cells

WHIM-like CXCR4 C-tail mutations in WM

Warts, Hypogammaglobulinemia, Infection, and Myelokathexis.

- 30-40% of WM patients
- > 30 Nonsense and Frameshift Mutations
- Almost always occur with MYD88\textsuperscript{L265P}

## MYD88 and CXCR4 MUTATIONS in B-CELL MALIGNANCIES

<table>
<thead>
<tr>
<th></th>
<th>(N=)</th>
<th>MYD88 $^{L265P}$</th>
<th>CXCR4 $^{WHIM}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Donors</td>
<td>32</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>IgM MGUS</td>
<td>12</td>
<td>6 (50%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Non-IgM MGUS</td>
<td>7</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Untreated WM</td>
<td>102</td>
<td>97 (95%)</td>
<td>44 (43%)</td>
</tr>
<tr>
<td>Treated WM</td>
<td>62</td>
<td>57 (92%)</td>
<td>21 (34%)</td>
</tr>
<tr>
<td>MZL</td>
<td>20</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>CLL</td>
<td>32</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>14</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Xu et al, British J. Hematol. 2015
Not all WM cells carry the CXCR4 mutation and some patients even carry multiple CXCR4 mutations!

Cancer cell fraction analysis of CXCR4\textsuperscript{S338X} showed that mutations were primarily subclonal, with highly variable clonal distribution (median 45.1\%, range 1.2\%-97.5\%).

Xu et al, British J. Hematol. 2015

Multiple CXCR4 mutations can be found in some individual WM patients!!
MYD88 and CXCR4 Transcriptome

Supervised Clustering of 3,103 genes found to be significantly differentially expressed between MYD88^{L265P}CXCR4^{WT} (N=29) and MYD88^{L265P}CXCR4^{WHIM} (N=23) WM patients

Hunter et al, BLOOD (In press)
CXCR4 Signaling in WM Patients with WHIM mutations

Yang Cao

Cell

SDF-1

Plerixafor

Ulucuplomab

CXCR4

Ser

346/7

GRK 2/3

ERK

AKT

B-arrestins

SURVIVAL

DRUG RESISTANCE

Busillo et al, JBC 2010
Mueller et al, PLOS ONE 2013
Cao et al, Leukemia 2014
Rocarro et al, Blood 2014
Cao et al, BJH 2015
Constitutive pAKT expression in CXCR4 S338X WM patients on Ibrutinib

Cao et al, Leukemia 2014
Activating mutations trigger survival signaling in Waldenstrom’s Macroglobulinemia.
Targeting Actionable Mutations

MYD88
Multicenter study of Ibrutinib in Relapsed/Refractory WM (≥1 prior therapy)

Screening
Registration

420 mg po qD Ibrutinib

Progressive Disease (PD) or Unacceptable Toxicity

Stop Ibrutinib

Event Monitoring

Stable Disease or Response
Continue

Event Monitoring

www.clinicaltrials.gov
NCT01614821
# Baseline Characteristics for Study Participants (n=63)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>63</td>
<td>44-86</td>
</tr>
<tr>
<td>Prior therapies</td>
<td>2</td>
<td>1-9</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>10.5</td>
<td>8.2-13.8</td>
</tr>
<tr>
<td>Serum IgM (mg/dL)</td>
<td>3,520</td>
<td>724-8,390</td>
</tr>
<tr>
<td>B₂M (mg/dL)</td>
<td>3.9</td>
<td>1.3-14.2</td>
</tr>
<tr>
<td>BM Involvement (%)</td>
<td>60</td>
<td>3-95</td>
</tr>
<tr>
<td>Adenopathy &gt;1.5 cm</td>
<td>37 (59%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Splenomegaly &gt;15 cm</td>
<td>7 (11%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Treon et al, NEJM 372: 1430, 2015
Serum IgM and Hb Levels Following Ibrutinib

Best IgM Response: 3,520 to 880 mg/dL; p<0.001

Best Hemoglobin Response: 10.5 to 13.8; p<0.001
Best Clinical Responses to Ibrutinib
Median duration of treatment: 19.1 (range 0.5-29.7) months

ORR: 91%  Major RR (≥ PR): 73%

<table>
<thead>
<tr>
<th>Response</th>
<th>(N=)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGPR</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>PR</td>
<td>36</td>
<td>57</td>
</tr>
<tr>
<td>MR</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

Median time to ≥ MR: 4 weeks
Median time to ≥ PR or better: 8 weeks

Progression-free and overall survival for 63 previously WM patients treated with ibrutinib.

Ibrutinib Related Adverse Events in previously treated WM patients

FDA News Release
FDA expands approved use of Imbruvica for rare form of non-Hodgkin lymphoma
First drug approved to treat Waldenstrom’s
January 29, 2015

EMA Approval for symptomatic previously treated and chemoimmunotherapy unsuitable frontline WM
First ever for Waldenstrom’s
July 8, 2015

Health Canada Santé Canada
April 5, 2016
Responses to ibrutinib are impacted by MYD88 (L265P and non-L265P) and CXCR4 mutations.

<table>
<thead>
<tr>
<th></th>
<th>MYD88\textsuperscript{MUT} CXCR4\textsuperscript{WT}</th>
<th>MYD88\textsuperscript{MUT} CXCR4\textsuperscript{WHIM}</th>
<th>MYD88\textsuperscript{WT} CXCR4\textsuperscript{WT}</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=</td>
<td>36</td>
<td>21</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Overall RR</td>
<td>100%</td>
<td>85.7%</td>
<td>60%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Major RR</td>
<td>91.7%</td>
<td>61.9%</td>
<td>0%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

2 patients subsequently found to have other MYD88 mutations not picked up by AS-PCR.

Kinetics of major responses following ibrutinib therapy in genotyped WM patients.

Treon et al, NEJM 372: 1430, 2015
Targeting Actionable Mutations

CXCR4
Phase I Study of Ulocuplumab in Relapsed/Refractory CXCR4^{WHIM} WM Patients

Screening, Informed Consent, Registration

Ulocuplumab Weekly for 4 weeks, then every other week for 24 weeks.

Progressive Disease or Unacceptable Toxicity

Stop Ulocuplumab

Event Monitoring

SD or Response Continue

Event Monitoring
Targeting Signaling Pathways

BCL2 Signaling
BCL-2 is overexpressed in primary WM patient cells by next generation sequencing (RNAseq) in MYD88 and CXCR4 mutated and unmutated patients.

Hunter et al, ASH 2015; Abstract 128
Venetoclax (ABT-199)

- Highly selective BCL2 inhibitor
- Approved for the treatment of 17p-deleted CLL
- Under investigation in CLL (other indications) and other B-cell malignancies.
- Tumor lysis syndrome, neutropenia.
The anti-BCL2 agent Venetoclax (ABT-199) shows pre-clinical and clinical activity in WM.


N=4
ORR=100%; all major responders
PFS: 18, 25, 38+, 40+ months.

Cao et al, BJH 2015;
Phase I/II Study of ABT-199 in Previously Treated WM

Screening

Informed Consent and Registration

ABT-199 200 ➔ 800 mg a Day

Progressive Disease or Unacceptable Toxicity

Stop ABT-199

Event Monitoring

SD or Response

Continue

Event Monitoring
Targeting Signaling Pathways

HCK Signaling
Hematopoietic Cell Kinase (HCK)

- SRC family kinase
- Expressed at early stages of B-cell development
- GEP in WM shows HCK among the most aberrantly over-transcribed genes. (2 highest HCK, IL6)
- In MM cells, HCK transactivated by IL6 through IL6ST.
- HCK among 285 genes down-regulated in MYD88 knockdown HBL-1 MYD88 mutated cells.

Hematopoietic Cell Kinase (HCK) is induced by Mutated MYD88 in WM and ABC DLBCL cells.

Yang et al, ASH 2015
HCK is activated in MYD88 Mutated Cell Lines and Primary WM Patient Cells.

Yang et al, ASH 2015
IL-6 transactivates HCK in MYD88 Mutated Cell Lines and Primary WM Cells

Yang et al, ASH 2015
Signaling Pathways Driven by Mutated MYD88 in Waldenström's Macroglobulinemia

Yang et al, Blood 2013; ASH 2015, Abstract 705
Ibrutinib and A419259 bind to and block HCK kinase activity.
Transduction with HCK gatekeeper mutant (T333M) promotes resistance in MYD88 mutated WM cells treated with Ibrutinib or A419259

D.

**Ibrutinib**

![Ibrutinib graph]

**A419259**

![A419259 graph]

**CC-292**

![CC-292 graph]

Yang et al, ASH 2015
MYD88 Signaling is dependent on TIR domain homodimerization.

DD: death domain  
ID: intermediate domain  
TIR: TIR domain
MYDDOSOME ASSEMBLY in MYD88 Mutated WM

Liu et al, BJH 2016
Targeting Myddosome Assembly

Collaboration
TREON/WALENSKY LABS

Synthesize library of stapled peptides

Screen library of stapled peptides to identify optimal binders

MYD88

Apply cell-permeable stapled peptides in cellular analyses

WM Cells

In vivo efficacy and mechanism of action studies in cancer models
MYD88 and CXCR4 mutations are the most common mutations present in WM patients, respectively. Other mutations exist in WM at lower frequencies, and remain to be investigated.

MYD88 triggers BTK, and the BTK inhibitor ibrutinib is highly active in patients with MYD88 mutations. Lower response activity is observed in patients with CXCR4 mutations.

Ulocuplumab is a novel CXCR4 inhibitor that will be entering Phase I testing in WM patients.

BCL2 is overexpressed in WM, the BCL-2 inhibitor venetoclax has entered a dedicated Phase I/II Study in WM.

New therapeutics under development include HCK and Myddosome assembly inhibitors.
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www.wmworkshop.org