Imagine A Cure: A World Without WM
Genomics/Science of WM

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What is Waldenström Macroglobulinemia?

- WM is a rare plasma cell cancer with ~1,400 cases diagnosed each year
- IgM-MGUS is precursor condition, conferring a 46-fold higher relative risk for developing WM
- B-cell lymphoproliferative pathophysiology
- Diagnosis of WM made by increased serum IgM and lymphoplasmacytic cell invasion of bone marrow (and/or organs) in conjunction with clinical symptoms

Lymphoplasmacytic lymphoma

• A unique lymphoid malignancy

• Monoclonal paraprotein is essential for diagnosis. WM if paraprotein is IgM. Malignant cell and paraprotein cause clinical disease.

• MYD88 L265P is present in >85% of WM cases. Help in diagnosis and may mediate a role in ibrutinib response

• FcγRIIIA (CD 16) and WHIM-like CXCR4 mutations and their implications

Diagnostic Criteria

• Bone marrow infiltration with small lymphocytes, plasmacytoid cells, and plasma cells

• Clonal cells are CD19+, CD20+, sIgM+
  • CD5, CD10, CD23 can be expressed in some cases, and doesn’t exclude WM

• IgM monoclonal gammopathy of any concentration
Blood cancers can develop in many different places within normal blood cell formation. The type of blood cancer that results has to do with where normal cell development is blocked. This picture shows the cell type where different blood cancers arise.

- Myeloid stem cells
  - Myelodysplastic syndrome
  - Acute myeloid leukemia (AML)
  - Various precursor myeloid cells

- Lymphoid stem cells
  - Acute lymphoblastic leukemia (ALL)
  - Blast cells
  - Various precursor lymphoid cells
  - Various lymphoid cells
  - Mature cells
  - Plasma cells

- B lymphocytes
  - Chronic lymphocytic leukemia (CLL)
  - B-cell non-Hodgkin lymphoma
  - Hairy cell leukemia
  - Hodgkin lymphoma

- T lymphocytes
  - T-cell non-Hodgkin lymphoma
  - T-cell large granular lymphocytic (SLL) leukemia

- Natural killer cells
  - NK-cell non-Hodgkin lymphoma
  - NK-cell large granular lymphocytic (SLL) leukemia
WHO Classification of Waldenstrom’s Macroglobulinemia: IgM-Secreting Lymphoplasmacytic Lymphoma

**B-Lymphobocyte Cells**
CD19+, CD20+, CD38-

**Plasma Cells**
CD19-, CD20-, CD38+

**Lymphoplasmacytic Cells**
CD19+, CD20+, CD38+/-
**WM Genomics**

Gene Expression Profiling (GEP) of WM

- Normal plasma cells
- WM & Chronic lymphocytic leukemia
- Multiple myeloma

WM shares a similar GEP to CLL

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**MYD88 and CXCR4**

- **MYD88** (Myeloid differentiation Primary Response Gene 88)
  - Mediates NF-kB and JAK/STAT activation → increased WM cell growth and survival
  - Overexpressed in ~90% WM patient cells

- **CXCR4** Expressed in high levels by WM cells
  - Mediates interaction of WM cells with their microenvironment
  - Activation of downstream cell survival pathways (PI3K/mTOR/AKT)

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Treon SP, et al. NEJM 2012;367.
MYD88 L265P by AS-PCR Can Help Distinguish WM From Overlapping Entities

Pro-Survival Signaling by Mutated MYD88 in Waldenstrom's Macroglobulinemia
WHIM-like CXCR4 Mutations in Waldenstrom’s Macroglobulinemia

Most common mutation is S338X

CXCL12/SDF-1a (AA 4-20; 187; 288)

Mutated CXCR4 Permits Ongoing Pro-Survival Signaling by CXCL12

30-40% of WM patients

CXCR4 receptor remains up with mutation

CXCL12

Bone Marrow Stroma

WM Cell

Drug resistance

### Bendamustine Plus Rituximab in Newly Diagnosed WM Patients – French Innovative Leukaemia Organization (FILO)

![Graph showing progression-free survival over time (months) for patients with different MYD88 and CXCR4 statuses.]

*MYD88=MUT; CXCR4=Wt = Mutated; CXCR4=MUT = Wild type.*

### Ibrutinib Activity in Previously Treated WM: Update of the Pivotal Trial (median f/u 59 mos)

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>MYD88\text{MUT}</th>
<th>MYD88\text{MUT}</th>
<th>MYD88\text{WT}</th>
<th>MYD88\text{WT}</th>
<th>P Value</th>
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<tr>
<td>N</td>
<td>63</td>
<td>36</td>
<td>22</td>
<td>4</td>
<td>N/A</td>
<td></td>
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<tr>
<td>Overall response rate, n (%)</td>
<td>90.5%</td>
<td>100%</td>
<td>86.4%</td>
<td>50%</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Major response rate, n (%)</td>
<td>79.4%</td>
<td>97.2%</td>
<td>68.2%</td>
<td>0%</td>
<td>&lt;.0001</td>
<td></td>
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</tbody>
</table>

**Categorical responses**

- **Minor responses, n (%):** 11.1% (1.2%), 2.8% (2.8%), 18.2% (18.2%), 50% (50%), <.01
- **Partial responses, n (%):** 49.2% (50%), 50% (50%), 59.1% (59.1%), 0% (0%), .03
- **Very good partial responses, n (%):** 30.2% (47.2%), 9.1% (9.1%), 0% (0%), <.01

**Median time to response (months)**

- **Minor response (≥ minor response):** 0.9 (0.9), 0.9 (0.9), 0.9 (0.9), .38
- **Major response (≥ partial response):** 1.8 (1.8), 4.7 (N/A), .02

*One patient had MYD88 mutation, but no CXCR4 determination and had SD.*
Ibrutinib in Previously Treated WM: Updated PFS

All patients

5 year PFS: 54%
5 year OS: 87%

MYD88 and CXCR4 Mutation Status

5 year PFS: 54%
5 year OS: 87%

Updated from Treon et al. N Engl J Med. 2015

Responses in iNOVATE Study (Ibrutinib+Rituximab vs. Placebo+Rituximab): Update

*Following modified 6th IWWM Response Criteria (NCCN 2014); required 2 consecutive assessments.
Progression-Free Survival Benefit: Impact of MYD88/CXCR4 Genotype

- Improved PFS with ibrutinib
- 36-month PFS rates
  - MYD88 WT/CXCR4 WT: 82% vs 44%
  - MYD88 WT/CXCR4 WHIM: 64% vs 26%
  - MYD88 WT/CXCR4 WT: 84% vs 29%

Covalent BTK Inhibitors in WM

Ibrutinib | Acalabrutinib | Zanubrutinib | Tirabrutinib

<table>
<thead>
<tr>
<th>IC50 (nM)</th>
<th>Ibrutinib</th>
<th>Acalabrutinib</th>
<th>Zanubrutinib</th>
<th>Tirabrutinib</th>
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<tr>
<td>BTK</td>
<td>5.1 ± 1.0</td>
<td>1.5 ± 0.2</td>
<td>3.3 ± 0.5</td>
<td>0.3 ± 0.0</td>
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<tr>
<td>TEC</td>
<td>12.8 ± 11</td>
<td>10.3 ± 1.2</td>
<td>28.4 ± 4</td>
<td>44 ± 3.9</td>
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<tr>
<td>ITK</td>
<td>9.6 ± 1.2</td>
<td>9.6 ± 1.2</td>
<td>29 ± 4</td>
<td>3.9 ± 3.3</td>
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<tr>
<td>TXK</td>
<td>368 ± 141</td>
<td>2.0 ± 0.5</td>
<td>9.3 ± 2.7</td>
<td>2.1 ± 0.6</td>
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<tr>
<td>BAX</td>
<td>4.6 ± 2.2</td>
<td>0.8 ± 0.5</td>
<td>3.6 ± 2.4</td>
<td>4.2 ± 0.4</td>
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<tr>
<td>EGF</td>
<td>&gt;1000</td>
<td>5.2 ± 1.3</td>
<td>109 ± 35</td>
<td>7.5 ± 1</td>
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<tr>
<td>ERBB2</td>
<td>&gt;1000</td>
<td>6.4 ± 1.4</td>
<td>&gt;1000</td>
<td>88 ± 26</td>
</tr>
<tr>
<td>ERBB4</td>
<td>38.5</td>
<td>3.4 ± 1.4</td>
<td>49 ± 12</td>
<td>0.9 ± 0.0</td>
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<tr>
<td>ILK</td>
<td>&gt;1000</td>
<td>0.1 ± 0.0</td>
<td>131 ± 27</td>
<td>2.5 ± 0.4</td>
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<tr>
<td>JAK3</td>
<td>&gt;1000</td>
<td>32 ± 1.1</td>
<td>5.4 ± 5.1</td>
<td>1377 ± 258</td>
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<tr>
<td>NF1</td>
<td>2.9 ± 0.2</td>
<td>0.6 ± 0.0</td>
<td>7.4 ± 0.7</td>
<td>0.9 ± 0.3</td>
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<tr>
<td>VAV</td>
<td>0.2 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>240 ± 65</td>
<td>7.4 ± 0.4</td>
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</tbody>
</table>

DKK, DKK: lymphoproliferative, BAX, bone marrow tyrosine kinase gene in chromosome X; ERBB2, erb-b2 receptor tyrosine kinase; ERBB4, erb-b4 receptor tyrosine kinase; ITK, interakinin-2 inducible T-cell kinase; JAK3, Janus kinase 3; TEC, tyrosine kinase expressed in hepatocellular carcinomas; TEC, T and K cell expressed kinase.
Phase I/II Trial of Ulocuplumab and Ibrutinib in CXCR4-Mutated Patients With Symptomatic WM

Schema

Ibrutinib

Until PD or Intolerance

Weekly Ulo

Biweekly Ulo

STOP

4 weeks

20 weeks

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Ibrutinib</th>
<th>Ulocuplumab Cycle 1</th>
<th>Ulocuplumab Cycles 2-6</th>
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<tbody>
<tr>
<td>Level 1 –Starting dose</td>
<td>420 mg PO DQ</td>
<td>400 mg weekly</td>
<td>800 mg every other week</td>
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<tr>
<td>Level 2</td>
<td>420 mg PO DQ</td>
<td>800 mg weekly</td>
<td>1200 mg every other week</td>
</tr>
<tr>
<td>Level 3</td>
<td>420 mg PO DQ</td>
<td>800 mg weekly</td>
<td>1600 mg every other week</td>
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</table>

ClinicalTrials.gov Identifier: NCT03225716

Mavorixafor in Combination With Ibrutinib in CXCR4-Mutated WM

- Non-competitive, allosteric, small molecule antagonist of CXCR4
- Orally Bioavailable; mean t₁/₂ of ~23 hours
- High volume of distribution
Bcl-2 family proteins: Guardians of the mitochondria

Pro-apoptotics
- Bax
- BIM
- NOXA

Anti-apoptotics
- Bcl-xL
- Bcl-2
- Mcl-1

Cell survival

Bcl-2 inhibitor
- Bax
- Bcl-2
- Mcl-1

Mcl-1 inhibitor

Cell death
Venetoclax (ABT-199) Augments Ibrutinib-Induced Apoptosis

Higher BCL2 levels in MYD88-mutated WM

Response | N = 31 | Prior BTK inhibitor | CXCR4 mutations
---|---|---|---
Overall (≥ minor) | 27 (90%) | 14 (93%) | 13 (93%)
Major (≥ partial) | 25 (83%) | 13 (87%) | 12 (86%)
Very good partial | 6 (20%) | 5 (33%) | 4 (29%)
Partial | 19 (63%) | 8 (54%) | 8 (54%)
Minor | 2 (7%) | 1 (6%) | 1 (6%)
Time to response | 1.9 months | 1.1 months | 1.3 months

BM involvement
At baseline, median 40% (4-95%).
At best response, median 3% (0-50%).
Ibrutinib and Venetoclax in Treatment-Naive WM
Ongoing Clinical Trial at Dana Farber Cancer Institute

24 months

Ibrutinib
420 mg/day
x 4 weeks

Add Venetoclax
100 mg/day week 5
200 mg/day week 6
400 mg/day weeks 7,8

Ibrutinib
420 mg/day
And
Venetoclax
400 mg/day

Observation

4 weeks
4 weeks
22 months
Follow to PD or off study

Personal communication

Ibrutinib and Venetoclax
in Treatment-Naive WM
NCT0480602

Patients with Newly Diagnosed WM
Randomize

Arm 1: Ibrutinib 420 mg PO daily for Cycle 1-24 + Ritasimab 375 mg/m² weekly in Cycles 1 and 6

Arm 2: Ibrutinib 420 mg PO daily for Cycle 1-24 + Venetoclax 400 mg PO Daily Ramp-up Dosing** for Cycle 1-24

Stable disease or better at the end of 24 cycles
Progression of disease at any time between Cycles 3-24

CROSSOVER to Arm 2
Continue Ibrutinib and Ritasimab as per Arm 1 + Venetoclax PO Daily Ramp-up Dosing**

Stable disease or better at the end of 24 cycles after adding Venetoclax
Progression of disease at any time within 24 cycles after adding Venetoclax

Event Monitoring

*Cycle=28 Days
**Venetoclax Ramp-up dosing:
Cycles 1-17: Venetoclax 200 mg PO daily, Cycles 1-18: Venetoclax 400 mg PO daily, Cycles 1-19: Venetoclax 400 mg PO daily
Cycles 2 onwards: Venetoclax 800 mg PO daily (or 400 mg PO daily, if 800 mg not tolerated)
Phase I clinical trial of APG-2575 (NCT03537482)

Baseline characteristics of patients

- **Age, yr**
  - Median (range): 70.0 (39–89)
- **≥70, no. (%)**: 19 (52.8)
- **Gender, no. (%)**
  - Male: 26 (72.2)
  - Female: 10 (27.8)
- **Type of cancer, no. (%)**
  - CLL/SLL: 15 (41.7)
  - MM: 6 (16.7)
  - WM: 5 (13.9)
  - Acute myeloid leukemia: 1 (2.8)
  - Mantle-cell lymphoma: 1 (2.8)
  - Diffuse large B-cell lymphoma: 1 (2.8)
  - Follicular lymphoma: 5 (13.9)
  - Multiple myeloma: 1 (2.8)
  - Myelodysplastic syndrome: 1 (2.8)
  - Hairy-cell leukemia: 1 (2.8)
- **Median (range) no. of previous therapies**: 2.0 (1–13)

36 patients

- APG-2575 (lisaftoclax) was well tolerated up to 1,200 mg/day
- MTD not yet reached
- No significant new or unmanageable safety findings

Phase I clinical trial of APG-2575 (NCT03537482)

- **CLL patient cohort n=15**

Cycles to PR (partial remission)

- Most CLL patients achieved PR within 2 - 4 cycles
New Driver Mutations Identified in MYD88 WT WM

Principal component analysis of top 500 high variance genes.

NFKB Signaling Cascades in MYD88 WT WM

Genomic-Based Treatment Approach to Symptomatic Treatment-Naive WM

- MYD88 Mut
  - CXCR4 WT
  - Rapid Response
  - BTK inhibitor (monotherapy)
  - Alternatives: Benda-R, PI-based regimen
- MYD88 WT
  - CXCR4 Mut
  - Rapid Response
  - Plasmapheresis for severe HV, CAGG, CRYOS, rapidly progressing IGM PN
  - Benda-R or PI-based regimen
- MYD88 Mut
  - CXCR4 Mut
  - Rapid Response Not Required
  - BTK inhibitor plus rituximab
  - Alternative: Benda-R, PI-based regimen
- MYD88 WT
  - CXCR4 WT
  - Benda-R, PI-based regimen

- Rituximab should be held for serum IgM ≥4,000 mg/dL
- Benda-R for bulky adenopathy or extramedullary disease
- PI-based regimen for symptomatic amyloidosis, and possible ASCT as consolidation
- Rituximab alone, or with ibrutinib if MYD88 Mut or bendamustine for IgM PN depending on severity and pace of progression
- Maintenance rituximab may be considered in patients responding to rituximab-based regimens

Genomic-Based Treatment Approach to Symptomatic Relapsed or Refractory WM

- MYD88 Mut
  - CXCR4 WT
  - First and second relapse or refractory
  - BTK inhibitor alone (if BTKi naive)
  - Alternatives: Benda-R, PI-based regimen
- MYD88 WT
  - CXCR4 Mut
  - Plasmapheresis if severe HV, CAGG, CRYOS, rapidly progressing IGM PN
  - First and second relapse or refractory
  - BTK inhibitor plus rituximab (if BTKi naive)
  - Alternative: Benda-R, PI-based regimen
- MYD88 WT
  - CXCR4 WT
  - Benda-R, PI-based regimen
  - Third or later relapse or refractory
  - BTK inhibitor alone (if BTKi naive)
  - Alternatives: venetoclax, NA1, everolimus

- Nucleoside analogs (NA) should be avoided in younger patients, and candidates for ASCT1
- ASCT may be considered in patients with multiple relapses, and chemo-sensitive disease

Treon et al. JCO. 2020.
Development of biological tools
(In vitro and in vivo models of WM)

Establishment and characterization of first molecularly validated Waldenstrom macroglobulinemia (WM) cell line

- BCWM-1
  - MYD88 mutation
  - Normal karyotype
  - No verification

- MWCL-1
  - MYD88 mutation
  - Rearranged karyotype
  - Resembles patient
  - Identical IGHV seq

- RPC1-WM1
  - MYD88 mutation
  - Rearranged karyotype
  - Resembles patient
  - Identical IGHV seq

Drexler et al, Leuk Lymph 2013.
Creation of Waldenstrom macroglobulinemia digital avatars using machine-learning and systems biology algorithms exposes novel and clinically relevant therapeutic opportunities

Aneel Paulus MD¹,², Sharoon Akhtar MPhil¹, Shumail M. Paulus MBBS¹, Yamima Bashir MBBS¹, Hassan Yousaf MBBS¹, Davite Cogen¹, Shireen Vali PhD³, Ansu Kumar⁴, Neeraj K. Singh⁴, Shweta Kapoor⁴, Taher Abbasi³, Vivek Roy MD², Sikander Ailawadhi MD² and Asher Chanan-Khan MD¹,²

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Predictive Modeling Workflow

- Mutations
- CNV
- Methylation
- Microenvironment

- Big data integration

Actionable insights

<table>
<thead>
<tr>
<th>Drug</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sensitive</td>
</tr>
<tr>
<td>B</td>
<td>Resistance</td>
</tr>
<tr>
<td>C</td>
<td>Resistance</td>
</tr>
<tr>
<td>D + A</td>
<td>Sensitive</td>
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- Cancer cells
- Data collection
- Molecular signature
- Machine-learning algorithms
- Digital avatar
- Drug library
- Non-Clinical validation
- Clinical translation
Ibrutinib-Resistant WM Cells as a Biological Validation Tool

A. Selection of ibrutinib-resistant clones

% Growth (MTS assay, 72h)

ibrutinib (µM)

0 0.1 0.2 0.4 0.8 1.6 2.4 2.8 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 5.0 5.2 5.4 5.6 5.8 6.0 6.2 6.4 6.6 6.8 7.0 7.2 7.4 7.6 7.8 8.0 8.2 8.4 8.6 8.8 9.0 9.2 9.4 9.6 9.8 10.0

RPCI-WM1
RPCI-WM1/IR (2-fold)
RPCI-WM1/IR (4-fold)

B. RPCI-WM1/IR

DMSO
ibrutinib 20µM

C. RPCI-WM1/IR

D. Relevant gene analysis

MYD88 L265P present

BTK Cys481S mutation absent

E. CXCR4 WHIM-Like mutations absent
deln: 136872465 – 136872352

Digital Avatar of Ibrutinib-Resistant RPCI-WM1/IR WM Cells

High CNV

Low CNV

Various processes
General cell proliferation
Cell cycle

Various processes
BCR/Lymphocyte Proliferation signaling
Cell cycle

Mutations

3% 4% 4% 3% 4% 38% 42% 3% 3% 7% 76%

Various processes
Apoptosis
Autophagy
Cell cycle

CNV

(69)

Mutations

(89)
In silico modeling predicts MEK1/2 as a targetable driver of RPCI-WM1/IR cells

Key Takeaways

- Better understanding of WM biology is shaping our work in drug development and new treatments.
- WM treatment should no longer be a “one size fits all” concept.
- Newer studies are focusing on how to change the natural history of WM and get deeper responses / fixed-duration therapy with non-traditional drug approaches.
- Better and more targeted regimens can lead to development of more effective and tolerable agents/regimens for WM treatment.
Questions & discussion
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