ACTIVITY OF RITUXIMAB AND OFATUMUMAB AGAINST MANTLE CELL LYMPHOMA (MCL) IN VITRO IN MCL CELL LINES BY COMPLEMENT DEPENDENT CYTOTOXICITY (CDC) AND ANTIBODY-DEPENDENT CELL MEDIATED CYTOTOXICITY ASSAYS (ADCC)

Dr. Gopichand Pendurti M.B.B.S

Mentor: Dr. Francisco J. Hernandez-Illizaliturri MD
Overview of presentation

• Introduction to mantle cell lymphoma.
• Concept of minimal residual disease.
• Anti CD 20 antibodies.
• $^{51}$Cr release assays.
• Flow cytometry on cell lines.
• Results.
• Future.
MANTLE CELL LYMPHOMA

• Mantle cell lymphoma is characterized by abnormal proliferation of mature B lymphocytes derived from naïve B cells.

• Constitutes about 5% of all patients with Non Hodgkin's lymphoma.

• Predominantly in males with M:F ratio 2.7:1 with onset at advanced age (median age 60yrs).

• It is an aggressive lymphoma with median survival of patients being 3-4 years.

• Often presents as stage III-IV with lymphadenopathy, hepatosplenomegaly, gastrointestinal involvement, peripheral blood involvement.

• Genetic hallmark is t(11:14)(q13;q32) translocation leading to over expression of cyclin D1 which has one of the important pathogenetic role in deregulating the cell cycle.

• Other pathogentic mechanisms include molecular and chromosomal alterations that

  ➢ Target proteins that regulate the cell cycle and senescence (BMI1, INK4a, ARF, CDK4 AND RB1).

  ➢ Interfere with cellular response to DNA damage (ATM, CHK2 and p53).

MORPHOLOGY

- Spectrum of variants from classic type to blastoid and pleomorphic types

Classic MCL
- Small–medium-sized lymphocytes with irregular nuclei and inconspicuous nucleoli

Blastoid MCL
- Rounded nuclei, finely dispersed chromatin and inconspicuous nucleoli

Pleomorphic MCL
- Larger cells with irregular and pleomorphic nuclei and distinct small nuclei

Immunophenotype

• Mature B-cell phenotype with moderate to strong expression of surface immunoglobulins (Ig M and Ig D) predominantly lambda.

• B-cell-associated antigens such as **CD20**, CD22, CD79, and the T-cell-associated antigen CD5.

Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunotherapy: a European MCL intergroup study.

- Molecular remission is an independent prognostic factor for response duration.

- In spite of upfront high dose chemotherapy induction with auto stem cell transplantation about 44% of patient still have minimal residual disease.

Figure 1. Diagram of the 2 randomized EU-MCL network trials. (A) Mantle cell lymphoma (MCL) Younger and (B) MCL Elderly with the respective minimal residual disease (MRD) sampling time points. MRD is assessed until clinical relapse or death. Maintenance treatment in both arms of the elderly protocol is given until progression or death.
Minimal residual disease quantification by RQ-PCR of 190 patients before, during and after induction.

Response duration (RD) according to MRD status after combined immunochemotherapy.

RD according to MRD status in PB and/or BM after end of induction in MCL Younger and MCL Elderly patients

RD duration according to MRD status assessed in the PB after induction treatment in both trials.

RD duration according to MRD status assessed in the BM after induction treatment in both trials.

RD-Response duration
MRD-Minimal residual disease

Pott et al. Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunotherapy European MCL intergroup study. Blood 2010;115(16):3215-3223
RD according to MRD status assessed in PB and/or BM within the first 12 months after ASCT in MCL Younger patients.

RD according to MRD status assessed in PB and/or BM during the first year of maintenance in MCL Elderly patients.

RD-Response duration
MRD-Minimal residual disease
ASCT- Autologous stem cell transplantation

“Can the use of new anti CD20 monoclonal antibodies like ofatumumab lead to molecular remission in patients with mantle cell lymphoma, ultimately increasing the response duration along with upfront high dose induction therapy and auto stem cell transplantation?”.
Mechanisms of Action of Anti-CD20 Antibodies

**OFATUMUMAB**

• TYPE I human IgG1K antibody with molecular weight of 149 Kda.

• Ofatumumab binds to novel epitope of CD20 which encompasses small extracellular loop.

• Ofatumumab lyses Raji cells, Daudi cells better than rituximab through CDC whereas ADCC results were comparable.

• CDC with ofatumumab is not dependent on cell surface expression of complement region molecules.

• CDC occur even at lower density of CD20 on cell surface than with rituximab.

Binding site of Ofatumumab to CD 20 on the B-cells
Ofatumumab induces cell lysis by CDC
• Beum et al and Taylor et al compared the c3b deposition and cell killing by ofatumumab and rituximab.

Complement activation

Induces membrane blebbing

Generates streamers (long, thin structures)

Their extent of formation correlates with CDC

• Ofatumumab causes more rapid and greater blebbing and streamer formation than rituximab.

• Ofatumumab is most promising in patients with CLL who have fludarabine-and alemtuzumab-refractory disease and in those with bulky disease who experienced treatment failure with fludarabine therapy.

51 Cr release assays to compare the biological activity of various monoclonal antibodies targeting CD 20 in MCL cell lines

Material and methods:

- Mantle cell lymphoma cell lines-JeKo, Mino, Rec-1, Z-138 were used
- Radioactive 51 Cr
- Ofatumumab (10ug/ml)
- Rituximab (10ug/ml)
- Herceptin or trastuzumab (10ug/ml)-Isotype
- Serum or peripheral blood mononuclear cells.

Figure 1: Extraction of PBMC from whole blood.
• MCL cell lines were counted and centrifuged at 2000rpm for 5 minutes.

• Removed the supernatant and added 100µl of $^{51}$Cr to the cell pellets, incubated them for 2 hrs at 37°C, 5% CO$_2$.

• Washed the cell lines to remove excess of chromium using RPMI media.

• Re-suspended the cells in media to get a final concentration of $10^6$ cells/ml.

• Placed 100µl of cell suspension in each well.

• Treated cells with 50µl of oftamumab, rituximab, isotype, serum, PBMC or media.

• Incubated for 6 hrs, after that detergent was added to maximum release row.
Model of the 96 well plate prepared for the experiments
• Centrifuged the plate for 5 min at 2000rpm.

• Collected 100µl of supernatant, avoided touching the bottom of the well.

• Read the amount of radioactivity using a beta counter reader.

• Calculated the percent lysis using the formula

\[
\% \text{ lysis} = \frac{[^{51}\text{Cr release from sample} - ^{51}\text{Cr release from control}]}{[^{51}\text{Cr release from maximum release} - ^{51}\text{Cr release from control}]} \times 100
\]
FLOW CYTOMETRY

- Flow cytometry was performed on the cell lines for the expression of CD 20 and complement inhibitory proteins (CIP)- CD 55 and CD 59.

Why Complement inhibitory proteins?

- Rituximab resistant Raji cells had increased expression of CD 55 and CD 59.
- We compared the flow cytometry results with the flow data available on the rituximab sensitive and rituximab resistant Raji cells.
STATISTICS

• SPSS 16 was used for the independent t-Test to calculate the significance of the lysis between the two anti CD 20 antibodies.
• Ofatumumab induced significantly higher levels of cell lysis compared to rituximab in CDC assays.

<table>
<thead>
<tr>
<th>MCL Cell line</th>
<th>Ofatumumab</th>
<th>Rituximab</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC-1</td>
<td>25.4%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Z-138</td>
<td>56.4%</td>
<td>0.65%</td>
</tr>
<tr>
<td>Mino</td>
<td>65.9%</td>
<td>0.5%</td>
</tr>
<tr>
<td>JeKo</td>
<td>43.9%</td>
<td>13.3%</td>
</tr>
</tbody>
</table>

p-value significant at <0.001
CDC on MCL cell lines

Graphs showing % Lysis for different cell lines and mAbs (10 μg/ml)
CDC on MCL cell lines
• Ofatumumab and rituximab have comparable levels of cell lysis in ADCC assays.

<table>
<thead>
<tr>
<th>MCL Cell line</th>
<th>Ofatumumab</th>
<th>Rituximab</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC-1</td>
<td>12%</td>
<td>14%</td>
</tr>
<tr>
<td>Z-138</td>
<td>14%</td>
<td>12%</td>
</tr>
<tr>
<td>Mino</td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>JeKo</td>
<td>12%</td>
<td>12%</td>
</tr>
</tbody>
</table>

p value not significant - 0.264
• Ofatumumab and rituximab showed comparable level of cell lysis in ADCC assays.
ADCC on MCL cell lines
Surface expression of CD 20 in MCL cell lines

Histograms of the flow cytometry for CD20 surface expression.
Comparison of the CD20 in the rituximab sensitive Raji cells and rituximab resistant Raji cells with JeKo and Z138 MCL cell lines.
Figure: comparing the CD 20 among the MCL cell lines and Raji rituximab sensitive and rituximab resistant cells.
Surface expression of CD 59 in MCL cell lines

- JeKo
- REC-1
- Z138
- MINO

Legend:
- Unstained
- CD59
- FITC Isotype
Comparison of the CD59 in the rituximab sensitive Raji cells and rituximab resistant Raji cells with JeKo and Z138 MCL cell lines.
Figure: comparing the CD59 among the MCL cell lines and Raji rituximab sensitive and rituximab resistant cells.
Surface expression of CD 55 in MCL cell lines

- **REC-1**
- **JeKo**
- **MINO**
- **Z-138**
Comparison of the CD55 in the rituximab sensitive Raji cells and rituximab resistant raji cells with JeKo and Z138 MCL cell lines.
Figure: comparing the CD55 among the MCL cell lines and Raji rituximab sensitive and rituximab resistant cells.
CONCLUSION

• Ofatumumab induced significantly higher levels of cell lysis compared to rituximab in CDC assays in all MCL cell lines.

• Ofatumumab and rituximab have comparable levels of cell lysis in ADCC assays in all MCL cell lines.

• Flow cytometry showed similar levels of CD 20 expression in all the MCL cell lines and when compared with rituximab sensitive Raji cells also.

• Complement inhibitory proteins particularly CD 55 were higher and are comparable to rituximab resistant Raji cells explaining the difference between the activity of rituximab and ofatumumab.
Further studies on the pre clinical activity of ofatumumab and rituximab in MCL cell lines.

- Imagestream analysis and western blot techniques were used to accurately delineate the surface expression of CD 20 and complement inhibitory proteins.

- Expression of complement inhibitory proteins (CIPs) CD55 and CD59 was determined by Imagestream analysis and Western blot.

- In primary tumor cells, OFA and RTX demonstrated similar activity.

- SCID mice were inoculated SQ with $10 \times 10^6$ Z-138 cells. Once tumors were established, mice were assigned to observation versus 4 doses of either OFA or RTX, and anti-tumor activity was measured by changes in tumor volume.
THANK YOU

Dr. Matthew Barth MD

Cory Mavis MS

Dr. Myron Cuczman MD

Dr. Francisco J. Hernandez-Illizarutti MD

Cancer is a word, not a sentence.

John Diamond