Daratumumab, a novel human CD38 monoclonal antibody for the treatment of B-cell Non-Hodgkin Lymphoma
B cell lymphoid malignancies seen through the lymph node

Bone marrow
- Precursor B cell
- Naive B cell

Lymph Node
- Mantle
- Germinal center
- Marginal zone

Peripheral B cell malignancies (mature)
- Chronic lymphocytic leukemia
- Mantle cell lymphoma
- Follicular lymphoma
- Burkitt lymphoma
- Diffuse large B cell lymphoma
- MALT lymphoma
- Lymphoplasmacytic lymphoma
- Memory B cell
- Plasma cell
- Multiple myeloma

Precursor B cell malignancies
- Leukemia/lymphoma lymphoblastic/o B
  - del 13q
  - Del 11q
  - Del 17p
  - NOTCH1
  - SF3B
- Chronic lymphocytic leukemia
  - t(11;14)
  - Cyclin D1
  - P53
  - ATM
  - P16
  - BMI
- Mantle cell lymphoma
  - t(14; 18)
  - BCL2
CD38 appears to be a global molecular bridge to the environment, promoting survival/proliferation over apoptosis of B cells on their way to and after neoplastic transformation.
Daratumumab (DARA): Human CD38 mAb

- In development for Multiple Myeloma (MM)
- Induces potent ADCC (Antibody-dependent Cellular Cytotoxicity) in Burkitt lymphoma and MM-derived cell lines as well as in patient MM cells (both with autologous and allogeneic effector cells).
- Strong ability to induce CDC (Complement-dependent Cytotoxicity) in patient MM cells.
- Under evaluation in phase I/II clinical trials in patients with MM
- Our primary goal is to evaluate the activity of daratumumab against CD38 expressing tumor cells in B-cell lymphomas:
  - Mantle cell lymphoma (MCL)
  - Chronic lymphocytic leukemia (CLL)
  - Follicular lymphoma (FL)

High CD38 levels
Heterogeneous CD38 levels
1. To understand the mechanisms of DARA–induced cytotoxicity in MCL, FL and CLL cell lines and primary cells.

2. To evaluate the effect of DARA in combination with lenalidomide on ADCC in CLL cells.

3. To analyze the effect of DARA in SDF1α/CXCL12-induced migration in vitro and in vivo homing in CLL models.

4. To explore in vivo antitumoral effect of DARA in MCL, FL and CLL xenograft mouse models.
1. Mechanisms of DARA–induced cytotoxicity in MCL, FL and CLL cell lines and primary cells: ADCC

- PBMCs from healthy donors
- Ratio T:E = 1:50, 4h treatment
- Calcein release assay

NK cells

Monocytes /macrophages

Daratumumab induces ADCC in the presence of external effectors in MCL, FL and CLL
2. Effect of lenalidomide on DARA-Induced ADCC in primary CLL cells

Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab

Michael. S. van der Veer,1 Michel de Weers,2 Berris van Kessel,1 Joost M. Bakker,2 Shulamiet Wittebol,1 Paul W.H.I. Parren,2 Henk M. Lokhorst,4 and Tuna Mutis1

- PBMCs from healthy donors (pretreated with lenalidomide 3uM 72h)
- Calcein release assay

Lenalidomide pretreatment of PBMCs significantly increases Daratumumab ADCC activity
3. Effect of DARA in SDF1α/CXCL12-induced migration *in vitro* and *in vivo* homing in CLL

**CD38 increases CXCL12-mediated signals and homing of chronic lymphocytic leukemia cells**

T Vaisitti¹,², S Aydin¹,², D Rossi³, F Cottino¹,², L Bergui⁴, G D’Arena⁵, L Bonello², AL Horenstein¹,², P Brennan⁶, C Pepper⁵, G Gaidano⁴, F Malavasi¹,² and S Deaglio¹,²

*In vitro*
Daratumumab interferes with *in vitro* CLL migration and *in vivo* homing to spleen
4. *In vivo* antitumoral effect of DARA in MCL, FL and CLL xenograft mouse models

**FL (RL cell line)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Condition</th>
<th>Tumor Volume (mm³)</th>
<th>RFU (relative fluorescence units)</th>
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<tbody>
<tr>
<td>1</td>
<td>10x10^6 RL cells sc</td>
<td>0</td>
<td><strong>6.3x10^6</strong></td>
</tr>
<tr>
<td>7</td>
<td>20 mg/kg Dara</td>
<td>200</td>
<td><strong>6.17x10^6</strong></td>
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<td>14</td>
<td>10 mg/kg Dara</td>
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<tr>
<td>21</td>
<td>10 mg/kg Dara</td>
<td>1200</td>
<td><strong>1.44x10^6</strong></td>
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<tr>
<td>28</td>
<td>10 mg/kg Dara</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Sacrifice</td>
<td>1000</td>
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</tr>
</tbody>
</table>

**Ab treatment**

**n=5**

**p<0.01**

**Tumor weight (g)**

- **IgG1-B12**: 2.5 ± 0.2
- **Daratumumab**: 0.5 ± 0.1

**IRDye800 probe (LI-COR, Odyssey)**
Daratumumab inhibits tumor growth in FL and MCL mouse models.
Daratumumab increases overall survival in a systemic CLL mouse model
Conclusions

• DARA induces ADCC in MCL, FL and CLL primary cells and cell lines in the presence of PBMC effector cells.

• Lenalidomide pretreatment of effector PBMCs significantly enhances ADCC induced by DARA in CLL primary cells.

• DARA significantly reduces SDF1α/CXCL12-induced migration in vitro and in vivo homing to spleen of CLL cells.

• In vivo, DARA inhibits tumor growth in MCL and FL sc mouse xenograft models and improves overall survival of a CLL systemic mouse model.
Thanks for your attention!

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Vanina Rodriguez
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Gael Roué
Armando López-Guillermo
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Joost M. Bakker
Wim Bleekeer

Parul Doshi

Adrian Wiestner
Daratumumab does not induce CDC in MCL, FL or CLL cells: possible explanations

**Table A**

<table>
<thead>
<tr>
<th>Sample</th>
<th>CD38</th>
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<td>HBL2</td>
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<td>JEKO</td>
<td>52.8</td>
<td>96.22</td>
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<td>REC</td>
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<td>93.34</td>
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<td>MINO</td>
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<td>95.12</td>
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<td>CLL5</td>
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<td>82.88</td>
<td>74.92</td>
<td>72.9</td>
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**Graph B**

A scatter plot showing % lysis for different samples with IgGl as a control.

**Graph C**

A graph showing CD38 molecules per cell for different samples.
**Models of study & CD38 immunotherapy**

**Chronic lymphocytic leukemia (CLL)**

- Limited efficacy of anti-CD20 antibodies
- High CD38—marker of **bad prognosis** and **disease progression**
- CD38 is upregulated in the **proliferating fraction** (*Damle RN, Blood 2007*)
- CD38 identifies CLL with **high migratory potential** (*Deaglio S, Blood 2007*)
- CD38 cooperates with **CXCR4**-induced migration and in sustaining BCR-mediated signals (*Vaisitti et al, Leukemia 2010*)

**Mantle cell lymphoma (MCL)**

- Blastoid variant shows higher CD38 expression
- Bortezomib resistant MCL cells express higher CD38 levels (*Pérez-Galán, P. Blood 2011*)
- Possible role in **migration** and **microenvironment** interactions as found in CLL

**Follicular lymphoma (FL)**

- Invariable high CD38 expression (GC cell)
- Alternative to anti-CD20 therapies in relapse setting
- Possible role in **migration** and **microenvironment** interactions as found in CLL