New Anti-CD20 Monoclonals

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Conflicts of interest

- **Active patents for new monoclonal antibodies**
  - N° WO 03/035904, N° WO2005/118854, GB0822345.5: Licensed to Clinical Data
  - PCT/EP2013/050787

- **With companies developing monoclonal antibodies**
  - Takeda Millenium: Honorarium
  - Roche/Genentech: Honorarium/consultancy
  - GSK: Honoraires
  - Celgene: Honorarium/consultancy
  - Sanofi: Honorarium
Aims of this talk

- To understand the different mechanisms of action of anti-CD20 mAbs
- To understand different ways to improve anti-CD20 mAbs activity
- To know differences between the « new anti-CD20 mAbs » (obinutuzumab, ublituximab and ofatumumab) compared to rituximab
Therapeutic antibodies: a very old story

- 1891. *Emil von Behring* infused and cured a child with anti-diphteria sera

- 1893. Paul Gibier « take the juice of tumor, infuse in a horse and then infuse the serum of the horse to the patient »

- 1895. Jules Héricourt and Charles Richet infused the first patient with sarcoma
How rituximab works *in vivo*?

Complement-dependent cytotoxicity (CDC)

Antibody-dependent cellular cytotoxicity (ADCC)

Phagocytosis

Lysis

Ways to improve rituximab efficacy

Epitope/function

Fc/function

Compartment

Effector cells

CD20
**Type 1 or Type 2 mAb**

<table>
<thead>
<tr>
<th>Type I mAbs</th>
<th>Type II mAbs</th>
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<tbody>
<tr>
<td>Rituximab, Ofatumumab, Veltuzumab, Ublituximab</td>
<td>Tositumomab, Obinutuzumab</td>
</tr>
<tr>
<td>Localize CD20 into lipid rafts ➔ CDC</td>
<td>Do not localize CD20 into lipid rafts ➔ No CDC</td>
</tr>
<tr>
<td>No homotypic adhesion</td>
<td>Homotypic adhesion</td>
</tr>
<tr>
<td>ADCC</td>
<td></td>
</tr>
<tr>
<td>Phagocytosis</td>
<td></td>
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## Type 1 or Type 2 mAb

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</tr>
<tr>
<td>No homotypic adhesion</td>
<td>Homotypic adhesion</td>
</tr>
<tr>
<td>Caspase dependant cell death</td>
<td>Non-caspase dependant cell death</td>
</tr>
<tr>
<td>Bind CD20 molecules</td>
<td>Bind half of CD20 molecules/type I</td>
</tr>
<tr>
<td>CD20 modulation</td>
<td>No CD20 modulation</td>
</tr>
<tr>
<td>Trogocytosis</td>
<td>ADCC</td>
</tr>
<tr>
<td></td>
<td>Phagocytosis</td>
</tr>
</tbody>
</table>
CD20 structure and organisation

Small loops: 72-80
Large loops: 142-184

Proteins associated with CD20: MHC I, MHC II, CD53, CD81, CD82, CD40, BCR,...
CD20 migration into lipid rafts induces CDC
CD20 type I epitope

Ofatumumab induces higher CDC

Ofatumumab, a discontinuous epitope

Type I mAbs

Rituximab, 2H7, LT20

Ofatumumab

Ofatumumab, a discontinuous epitope

Type I mAbs

Rituximab, 2H7, LT20

Ofatumumab

Ofatumumab binding site

A ring of hydrophobic residues surrounding a deep positively charged pocket

Ofatumumab CD20 epitope

Ofatumumab exhibits higher CDC against CD20\textsuperscript{low} cells

Ofatumumab concentration-effect relationship


Anti-CD20 mAbs induces homotypic adhesion differently

Homotypic adhesion induces peripheral localisation of actin and mitochondria

Raji cells expressing Ac-GFP-labelling actin + Tos

JC-1 labeled cells

Cell death induces by type II mAbs differs from apoptosis

Involvement of lysosomes in type II mAbs cell death

Type II mAbs bind half of CD20 molecules compared to type I mAbs

Comparison of the obinutuzumab-CD20 epitope-binding with other CD20 mAbs

CD20 Type II epitope

**Type I mAbs**

- Rituximab, 2H7, LT20

```
KISH FLKM ESLN FIRAH TPY IN IYN CEPA A nP SEKN SPST QYCY
```

**Type II mAbs**

- Obinutuzumab, tositumomab

```
KISH FLKM ESLN FIRAH TPY IN IYN CEPA NP SEkN SPST QYCY
```

Elbow hinge substitution

Model of type I or type II mAbs-CD20 interactions

**Type I**
- Inter tetramer binding
  - More CD20 bound
  - Translocation into lipid rafts
    - CDC
  - «open» configuration of CD20
    - Caspase dependant cell-death

**Type II**
- Intra tetramer binding
  - Half CD20 bound
  - No translocation into lipid rafts
    - No CDC
  - «closed» configuration of CD20
    - No caspase dependant cell-death
  - Homotypic adhesion
Ways to improve rituximab efficacy

Epitope/function

** Epitope/Function

CD20

142

1

219-225

KISH FLKM ESLN FIRAH TPY IN IYN CEP A NP SEKN SPST QYCYN

TLKH FLKM RRLE LIQTS KPY VD IYDCEP NIS SEKN SPST QYCYN

homme

souris

Fc/function

Complement

C1q

FcγRs
Type I and type II mAbs exhibit similar Fc-mediated functions

ADCC:
- NK cell
- Granzyme
- Perforine
- CD20
- B-lymphoma cell
- FcγRs
- Antibody

ADPC:
- B-lymphoma cell
- CD20
- FcγRs
- Macrophage
- Antibody
## FcγRs expression

<table>
<thead>
<tr>
<th></th>
<th>FcγRI</th>
<th>FcγRIIa</th>
<th>FcγRIIb</th>
<th>FcγRIIc</th>
<th>FcγRIIa</th>
<th>FcγRIIb</th>
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</thead>
<tbody>
<tr>
<td>Monocytes/macrophages</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NK cells</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Neutrophils</td>
<td>+/-</td>
<td>+</td>
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<td></td>
<td>+</td>
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<tr>
<td>B lymphocytes</td>
<td></td>
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<td>+</td>
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<tr>
<td>Dendritic cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastocytes</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td></td>
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</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
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</tr>
</tbody>
</table>
FCGR3A polymorphism influences IgG1 binding to NK-cells

Human IgG1 have a better affinity for FcγRIIIa-158VV NK-cells than FcγRIIIa-158FF NK cells

Amino acid 158 interacts with Fc arm
FcγRIIIa-158VF influences rituximab response in human

Is FcγRIIIa-158VF influences all IgG1 mAbs response?

<table>
<thead>
<tr>
<th>Response</th>
<th>V/V No.</th>
<th>%</th>
<th>V/F No.</th>
<th>%</th>
<th>F/F No.</th>
<th>%</th>
<th>Carrier* No.</th>
<th>%</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>No.</td>
<td>11</td>
<td>26</td>
<td>17</td>
<td>43</td>
<td>9</td>
<td>42</td>
<td>6</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>CR + PR</td>
<td>9</td>
<td>82</td>
<td>11</td>
<td>42</td>
<td>6</td>
<td>35</td>
<td>17</td>
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<tr>
<td>SD + PD</td>
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<td>18</td>
<td>15</td>
<td>58</td>
<td>11</td>
<td>65</td>
<td>26</td>
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</table>

Trastuzumab

Ways to improve ADCC

- Low fucose
- Mutation Fc
Effets of glycoengineering and Fc mutations

sFcyRIIIαV binding

sFcyRIIIαF binding

Target cells: WIL2-S

**Superior ADCC: The effect of glycoengineering**

**Graph A:**

- **Z138 (PBMC: V/V):**
  - Obinutuzumab
  - Rituximab
  - Ofatumumab

**Legend:**
- **Black square:** Ublituximab
- **Red diamond:** Rituximab

**Antibody concentration (ng/mL):**
- 0.064
- 0.32
- 1.6
- 8
- 40
- 200
- 1000

**Antibody-dependent killing (%):**
- 0
- 20
- 40
- 60
- 80
- 100

**PBMC: V/V = peripheral blood mononuclear cells expressing human FcγRIIIa V/V.**

Herter S, *et al.* ASH 2010. Abstract 3925 (Poster presentation)

De Romeuf C *et al.* *Br J Haematol* 2008; 140: 635-643
Conclusion

Arbitrary scale: Rituximab referenced with 3 for all *in vitro* activities.
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