Engineering Bispecific Antibodies for Cancer therapy – A Unique Perspective for Pediatric Solid Tumors Spanning Three Decades

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Curing Metastatic Solid Tumor (neuroblastoma): An unmet need

Chemotherapy → Remission
Radiation Surgery

? How to prevent relapse
Antibody-based Immunotherapy: Fc dependent and independent mechanisms

Disialoganglioside: GD2

\[ \text{Ceramide} \rightarrow \text{Gal-Cer} \rightarrow \text{Gal-Cer} \rightarrow \text{GM}_2 \]

\[ \downarrow \quad \downarrow \quad \downarrow \]

\[ \text{Glc-Cer} \quad \text{Gal-Glc-Cer} \quad \text{GM}_3 \]

\[ \downarrow \quad \downarrow \quad \downarrow \]

\[ \text{LacCer} \quad \text{GalNAc GalGlc Cer} \quad \text{GD}_3 \quad \text{GT}_3 \]

\[ \downarrow \quad \downarrow \quad \downarrow \quad \downarrow \]

\[ \text{GA}_2 \quad \text{GA}_1 \quad \text{GA}_1 \quad \text{GA}_1 \]

\[ \downarrow \quad \downarrow \quad \downarrow \quad \downarrow \]

\[ \text{GM}_2 \quad \text{GM}_1 \quad \text{GD}_2 \quad \text{GT}_2 \quad \text{GT}_1c \quad \text{GT}_1b \quad \text{GQ}_1c \quad \text{GM}_1b \quad \text{GD}_1a \quad \text{GQ}_1b \quad \text{GP}_1c \]

\[ \text{Asialo-series} \quad a\text{-series} \quad b\text{-series} \quad c\text{-series} \]

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Anti-GD2 mAb 3F8 (mouse IgG3) targets exquisitely to neuroblastoma in patients
IgG based Immunotherapy of high risk stage 4 neuroblastoma:
(≥18 months of age at diagnosis or MYCN amplified tumor)

- 3F8 is a mouse IgG3 specific for antigen GD2
devolved by hybridoma technique
(It mediates ADCC and CMC)

- It was developed to treat high risk stage 4
neuroblastoma

- As a single antibody against a single antigen,
it has curative potential

- It causes acute pain side effects, with no long
term sequelae

- The addition of $^{131}\text{I}-3\text{F8}$ to naked $3\text{F8}$ did not
improve survival.
Treating High Risk Metastatic Neuroblastoma:
anti-GD2 3F8 (mouse IgG3) + GM-CSF

87% CR of marrow disease, 69% CR by MIBG
Cheung et al., Int J Cancer 135: 2199-2205, 2014

Kushner et al., Oncoimmunology 4(7):e1016704. eCollection 2015

1st remission
Cheung et al., JCO 30:3264, 2012

Brian Kushner
Shakeel Modak

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Minimal residual disease (MRD) measurement by Quantitative RT-PCR: Early outcome predictor

(Marker panel: GD2 synthase, PHOX2B, CCND1, ISL1)

Dx → Induction Therapy +/- ASCT → 1st CR/VGPR; Primary refractory; 2nd remission → BM test

3F8 ± GM–CSF x 2 cycles → BM test → 3F8 ± GM–CSF x 2 years ± CRA x 6 cycles

Pre-immunotherapy

Post-2 cycles

Years from start of 3F8 immunotherapy

Proportion surviving progression-free

Marker panel negative

Marker panel positive

PFS

Marker panel negative

Marker panel positive

PFS

Cheung et al. JCO 33:755, 2015

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Compartmental radioimmunotherapy (cRIT) using intrathecal (intra-Ommaya) $^{131}$I-mAb is potentially curative for CNS metastasis.

Kramer et al. 2016
Empowering Antibodies

✓ Natural Killer cells
✓ T lymphocytes
2+2 platforms to build bispecific antibodies

Wu and Cheung, Pharmacology & Therapeutics 2017
Building next generation humanized antibodies
Combination immunotherapy and radioimmunotherapy

NK cells → T cell → Tumor

Fc-enhanced Antibody (+/- adoptive NK cells) ← IgG1 → Bispecific Antibody to retarget T cells (+/- ICI, +/- adoptive T cells)

Bispecific Antibody for PRIT (+/- radiation repair inhibitors)

DOTA-BsAb → Anti-CD3

T-BsAb

DOTA

Anti-DOTA

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Using T–BsAb (hu3F8–BsAb) to drive T cells to target carbohydrates (e.g. ganglioside GD2)
In vitro properties of hu3F8-BsAb

- Mediates TDCC against GD2(+) tumors at femtomolar EC50, irrespective of tumor type
- $>10^5$ margin of safety for non-neuronal cells
- Induces immune synapse on T cells
- Mediates cytokine release (IFNγ, IL6, IL10, TNFα, IL2) only if GD2(+) tumors are present
- No Activation induced cell death (AICD), unlike CAR T cells

*Xu et al., Cancer Immunol Res 3:266, 2015*
*Hoseini et al., Oncoimmunology, 2017, PMID: 28680755*
In vivo properties of hu3F8-BsAb

- Drives T cell infiltration into GD2(+) tumors
- Ablates GD2(+) cell line tumors in humanized SCID mice
- Ablates GD2(+) PDX in humanized SCID mice
- Ablates GD2(+) murine tumors in huCD3 transgenic mice with no cytokine storm
- Overcomes PD1 or PDL1 in the tumor microenvironment (TME)
- No AIID or CD4:CD8 shift, unlike CAR T cells

Yu et al., Cancer Immunol Res 3:266, 2015
Hoseini et al., Oncoimmunology, 2017 (in press)
Besides GD2, T–BsAb can drive circulating T cells into tumors bearing endocytosing antigens (e.g. HER2) for tumor ablation

- Ablates HER2(+) cell line xenografts (breast CA and ovarian CA) in humanized SCID mice
- Ablates HER2(+) PDX in humanized SCID mice (breast CA and gastric CA)
- Overcomes PD1 and PDL1 in the TME
- At subtherapeutic doses, synergizes with anti–PDL1
Empowering Antibodies with Radioisotopes as Liquid Radiation
Radioactive particles break DNA
Radioactive particles ($\alpha, \beta, \gamma$) differ in size, energy and path length

- **Gamma Particle**: 0.1–1 MeV, (Single DNA strand break)
- **Beta Particle**: 1,000–10,000 µm range, 0.1–1 MeV, (Single DNA strand break)
- **Alpha Particle**: 50–80 um range, 5–8 MeV, (Double DNA strand breaks)
Radioisotopes—the Payload for Liquid Radiation

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Physical $T_{1/2}$ (days)</th>
<th>Trade Name</th>
<th>Particles</th>
<th>Particle energy mean (keV)</th>
<th>Tissue Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radium–223</td>
<td>11.4</td>
<td>Alpharadin</td>
<td>Alpha</td>
<td>5850</td>
<td>&lt;0.1*</td>
</tr>
<tr>
<td>Actinium–225</td>
<td>10.0</td>
<td>–</td>
<td>Alpha</td>
<td>5935</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lead–212</td>
<td>0.4</td>
<td>–</td>
<td>Alpha</td>
<td>1335</td>
<td>&lt;0.1**</td>
</tr>
<tr>
<td>Astatine 211</td>
<td>0.3</td>
<td>–</td>
<td>Alpha</td>
<td>7450</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Bismuth–213</td>
<td>0.03</td>
<td>–</td>
<td>Alpha</td>
<td>5982</td>
<td>&lt;0.1**</td>
</tr>
<tr>
<td>Strontium–89</td>
<td>50.5</td>
<td>Metastron</td>
<td>Beta</td>
<td>580</td>
<td>6</td>
</tr>
<tr>
<td>Iodine–131</td>
<td>8.0</td>
<td>–</td>
<td>Beta</td>
<td>610</td>
<td>0.8</td>
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<tr>
<td>Lutetium–177</td>
<td>6.7</td>
<td>–</td>
<td>Beta</td>
<td>497</td>
<td>0.7</td>
</tr>
<tr>
<td>Samarium–153</td>
<td>1.9</td>
<td>Quadramet</td>
<td>Beta</td>
<td>233</td>
<td>3</td>
</tr>
</tbody>
</table>

* Small bowel retention
** Kidney damage

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Why conventional IgG radioconjugates fail

Conventional Radioimmunotherapy

Pretargeted Radioimmunotherapy (PRIT)


Orcutt et al. Protein Eng Des Sel 23:221, 2010
Mol Can Ther 11:1365, 2012
The components for Multistep Targeting DOTA–BsAb
DOTA–BsAb Pretargeted Radioimmunotherapy (PRIT)

**Step 1 (Day)**
- Bispecific antibody

**Step 2 (Hours)**
- Hapten/Dextran clearing agent

**Step 3 (Minutes)**
- M-DOTA complex

M    Affinity  
Y    15.4 ± 2.0 pM  
Lu   10.8 ± 2.5 pM


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Curing GD2(+) Neuroblastoma using DOTA–BsAb PRIT: No Toxicity at 33 MBq or 0.9 mCi $^{177}$Lu/mouse

<table>
<thead>
<tr>
<th>Tissues</th>
<th>cGy/MBq</th>
<th>cGy/mCi</th>
<th>AUC Tumor/tissue ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.6</td>
<td>22</td>
<td>142</td>
</tr>
<tr>
<td>Tumor</td>
<td>84.9</td>
<td>3141</td>
<td>1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.7</td>
<td>26</td>
<td>121</td>
</tr>
<tr>
<td>Lung</td>
<td>3.5</td>
<td>129</td>
<td>24</td>
</tr>
<tr>
<td>Liver</td>
<td>2.1</td>
<td>78</td>
<td>40</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.0</td>
<td>74</td>
<td>42</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.9</td>
<td>33</td>
<td>94</td>
</tr>
<tr>
<td>Sm. Intestine</td>
<td>0.8</td>
<td>30</td>
<td>106</td>
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<tr>
<td>Lg. Intestine</td>
<td>2.1</td>
<td>78</td>
<td>40</td>
</tr>
<tr>
<td>Kidneys</td>
<td>3.7</td>
<td>137</td>
<td>23</td>
</tr>
<tr>
<td>Muscle</td>
<td>5.5</td>
<td>203</td>
<td>15</td>
</tr>
<tr>
<td>Bone</td>
<td>0.7</td>
<td>26</td>
<td>121</td>
</tr>
</tbody>
</table>

Cheal et al., Mol Cancer Therapeutics 13:1803, 2014
Curing GPA33(+) CRC using DOTA–BsAb PRIT: No Toxicity at 165 MBq or 4.4 mCi $^{177}$Lu/mouse

A. Graph showing tumor volume over days post-inoculation with different treatments.

B. Non-invasive nanoSPECT/CT (55 MBq/cycle).

C. Non-invasive $^{86}$Y PET.

For 3 cycle treatment, 165 MBq of $^{177}$Lu–DOTA–Bn

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rads</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>150</td>
<td>93</td>
</tr>
<tr>
<td>tumor</td>
<td>14,000</td>
<td></td>
</tr>
<tr>
<td>kidney</td>
<td>875</td>
<td>16</td>
</tr>
</tbody>
</table>

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Proof of Concept of DOTA–BsAb PRIT
5 different targets, 5 different cancer diagnosis

✓ GPA33 in Colorectal Cancer (CRC)
   Cheal et al., EJNMMI 43:925, 2016
   Cheal et al., J Nuclear Medicine 2017 (in press)

✓ GD2 in neuroblastoma
   Cheal et al., Mol Cancer Therapeutics 13:1803, 2014

✓ HER2 in Breast Cancer
   Cheal et al., World Molecular Imaging Congress, Vol 18, 2015

✓ CD20 in Lymphoma
   Green et al., Cancer Research 76:6669, 2016

✓ CEA in Carcinoma
   Yazaki et al., Protein Eng Des Sel 26:187, 2013
BsAb in Pediatric Cancers

- Further optimization of PK to improve therapeutic ratio

- Combination of T-BsAb and Immune checkpoint inhibitors

- Combination of DOTA-BsAb PRIT with inhibitors of DNA repair

- Understanding the tumor microenvironment
Disclosures

• 3F8, hu3F8 and hu3F8–BsAb were licensed to YmAbs Therapeutics, Inc. by Memorial Sloan Kettering Cancer Center (MSK)

• HER2–BsAb was licensed to Abpro, Inc. by MSK

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