T Cell-engaging Antibodies for Cancer Therapy

iSBTc Workshop on Monoclonal Antibodies in Cancer

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Bispecific T Cell-engaging (BiTE) Antibodies Allow All Cytotoxic T Cells Recognition of a Surface Antigen

Act Independently of Specificity of T Cell Receptor

Allow T Cells Recognition of Tumor-associated Surface Antigen

Do not Require MHC Class I and Peptide Antigen
BiTE Technology Teaches Antibodies to Engage T Cells

α-Tumor Antibody

BiTE

α-CD3 Single-chain Antibody

Single-chain Antibody

Linker

Human in Sequence

Crossreactive with Primates
BiTE-engaged T Cells Form Cytolytic Synapses


Confocal Microscopy

Stainings:
- Perforin
- Lck
- LFA-1 (CD11a)
BiTE Mode of Action

Clinical Proof of Concept with CD19/CD3-bispecific BiTE Antibody Blinatumomab (MT103)
Blinatumomab: First BiTE to Enter Clinical Trials

- Bispecific for CD19 and CD3
- CD19 is pan-B cell antigen absent from stem cells and plasma cells but present on most human B cell malignancies
- Ongoing phase 1 trial in patients with refractory/relapsed non-Hodgkin's lymphoma (NHL)
- Completed phase 2 study in patients with minimal residual B-precursor acute lymphocytic leukemia (B-ALL)
- Initiated pivotal study in minimal residual B-ALL, and phase 2 study in relapsed/refractory ALL of adults

\[ K_D = 10^{-9} \text{ M} \]
\[ K_D = 10^{-7} \text{ M} \]

Mol. Wt.: 55 kDa
T1/2 β: ca. 2 h
Produced by CHO cell-based process
Cytokine Release from T Cells by Blinatumomab Is Dependent on CD19-expressing Target Cells

Blinatumomab Triggers Potent Lysis of Lymphoma Cells by Previously Unstimulated Human T Cells

Human B Lymphoma Line MEC-1

4 Human T Cell Donors Tested

- PBMC 381
- PBMC 511
- PBMC 515
- ABi 014

1 ng/ml in Serum Gives PRs and CRs

E:T ratio = 10:1
Assay time: 24 Hours
Target: Cells: MEC-1 labelled with PKH-26

EC$_{50}$ Range: 15–40 pg/ml = 0.27 - 7.2 pM
Serial Lysis by Blinatumomab-Engaged T Cells


Effector (E) = Unstimulated human CD8⁺ T Cells
Target (T) = Human Pre-B ALL Line NALM-6

**24-hour Cytotoxicity Assay**

- EC₅₀ = 150 pg/ml (= 2.7 pM)
Blinatumomab Induces T Cell Proliferation

Key Hallmarks of Blinatumomab and Other BiTE Antibodies

- **Strictly target cell-dependent activation of resting T cells**
  - Monovalent binding of BiTE to CD3 does not activate TCR complex

- **Highly potent redirected lysis of target cell**
  - At femtomolar concentrations
  - CD8⁺ CD4⁺ and effector memory T cells contribute
  - Lysis of dividing and non-dividing target cells

- **Serial lysis by BiTE-activated T cells**
  - Activity at low E:T ratios <1

- **Proliferation of BiTE-activated T Cells**
  - Contribution to in-vivo efficacy

- **No internalization of target antigens or CD3**
  - Monovalent binding does not modulate surface expression
Ongoing Phase 1 Study in NHL Patients with Blinatumomab

- **Study Population**
  - Relapsed/refractory NHL patients
  - Mostly follicular and mantle cell lymphoma
  - Median of 3 previous chemo/immunotherapies (some up to 12)
  - 86% pretreated with rituximab (up to 3 different rituximab-based single agent or combination regimens per patient)

- **Design**
  - 3+3 patient dose escalation
  - Thus far dose levels ranging from 0.0005 – 0.090 mg/m² per day
  - Continuous i.v. infusion via port with portable pump over 4-8 weeks (out-patient as of week 3)
  - Steroids at infusion start
  - Objectives: Safety and tolerability, PK, PD, anti-tumor activity
Safety of Blinatumomab in NHL Patients

- To date, no cytokine storm, no autoimmunity, no lymphoproliferative disorder, no immune response to drug, no drug-related death
- Most frequent clinical adverse events (AEs) were flu-like: Pyrexia, chills, headache
- Most frequent laboratory AEs were as expected by mode of action: Lymphopenia and leukopenia
- Dose-dependency for certain AEs, e.g., pyrexia, chills, and CRP and D dimer increases
- 50% frequency of AEs during first three days, 50% during following 4-8 weeks (first dose phenomena)
- Most significant AEs leading to discontinuation were CNS-related AEs, such as aphasia, confusion, ataxia, seizure; occur shortly after treatment start; all fully reversible within days; no findings by MRI
- CNS events predominantly seen in patients with very low peripheral B cell counts (=> biomarker)
- CNS events can be mitigated by sneak-in dosing regimen
Activation and Selective Expansion of Effector Memory T Cells upon Start of BiTE Infusion

B Cell Depletion in Patient with Mantle Cell Lymphoma

Dose Level: 30 µg/m²/24 h
Dose-dependent Activity of Blinatumomab in NHL Patients

- Peripheral T Cell Redistribution
- Complete and Sustained B Cell Depletion
- Bone Marrow Clearance; First PR/CR
- RR >90%
- MTD?

Dose Levels Tested  [µg/m²/Day]
Dose-dependent Clinical Responses in NHL Patients in a Phase 1 Study  (ASH Dec. 2009)

- By Cheson criteria and independent review of CT scans
- Mainly follicular and mantle cell lymphoma (MCL) patients

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Patients (N = 50)</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>Overall Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5, 1.5 and 5 µg/m² per Day</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0/13</td>
</tr>
<tr>
<td>15 and 30 µg/m² per Day</td>
<td>20</td>
<td>2</td>
<td>2</td>
<td>4/20</td>
</tr>
<tr>
<td>60 µg/m² per Day</td>
<td>13</td>
<td>5</td>
<td>7</td>
<td>12/13*</td>
</tr>
<tr>
<td>90 µg/m² per Day</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2/4#</td>
</tr>
</tbody>
</table>

*One patient not evaluable due to treatment discontinuation after 2 days
#Two patients not evaluable due to DLTs
Response Assessment at 60 µg/m²/d (EHA June 2010)

Constant Dosing

- 2
- 8
- 4
- 8
- 4
- 8
- 8
- 8

Step-up Dosing (15 to 60; or 15, 30 to 60)

- 4
- 8
- 8
- 4
- 8
- 8

% Decrease in $\Sigma$ Longest Diameter at 8 Week

Treatment Duration in Weeks

- 0
- 10
- 20
- 30
- 40
- 50
- 60
- 70
- 80
- 90
Durability of Responses in FL and MCL for Constant Dosing at 60 and 90 µg/m² per Day

EHA June 2010

Responses at 60 µg:
- Median duration 21+ months
- Duration up to 30 months
- 4 patients = 2 years duration
- Three out of 8 ongoing

Responses at 90 µg:
- Two out of 2 ongoing

- MCL
  - PR
  - CR
- FL
  - PR
  - CR
  - PR
  - PR
  - CR
Response in a Patient with Bulky Mantle Cell Lymphoma

- Patient with MCL, stage IV A, 42 years, male
- Blinatumomab treatment at 60 µg/m²/d (monotherapy)

Cumulative Dose after 8-Week Treatment = 6 mg
Status of Phase 1 Study in NHL Patients

- Favorable safety profile
- Very high response rate at dose levels $\geq 60 \, \mu g/m^2$ per day
- Ongoing responses in half of the patients without further treatment or alternative therapies
- Study ongoing for optimization of dose and schedule and for exploration of other CD19$^+$ B cell malignancies
Completed Phase 2 Study in Patients with B-lineage Leukemia (B-ALL)

- **Patient population**: B-ALL patients with high risk of relapse due to remaining bone marrow disease after standard therapy (= minimal residual disease; MRD); detectable by PCR
- **Patients treated**: 21, with the following MRD marker:
  - Bcr/abl neg. (individ. rearrangements) 14 patients
  - Bcr/abl neg., t(4;11) 2 patients
  - Bcr/abl pos. 5 patients
- **Median age**: 48 y (20-77); 12 female, 9 male patients
- **Dosing**: 15 μg blinatumomab/m²/day by repeated 4-week continuous infusions; at least 3 consolidation cycles post positive MRD response with 2-week intervals
- **Prior treatment**: At least induction/consolidation chemotherapy I (some up to consolidation V)
- **17 patients** had never achieved MRD negativity on prior treatments
Course of Minimal Residual Disease During Frontline Consolidation Chemotherapy of ALL

Example of Patient #109-002
Effective Treatment of Minimal Residual Disease (MRD) with Blinatumomab

Patient #109-002

Leukemia

Chemotherapy GMALL (Elderly) Protocol

Blinatumomab

MRD pos.

MRD neg.

07-07 09-07 11-07 01-08 03-08 05-08 07-08 09-08 11-08

Induction Cons. 1 Cons. 2 Cons. 3 Cons. 4 MT103 MT103 MT103 MT103
### Response Data

<table>
<thead>
<tr>
<th>Number of Patients Included in Study</th>
<th>Number of Patients Evaluable for Response Assessment</th>
<th>Number of Patients Reaching MRD Negativity</th>
<th>MRD Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>20*</td>
<td>16</td>
<td>80%</td>
</tr>
</tbody>
</table>

*One patient not evaluable due to less than one treatment cycle and lack of response assessment*
Hematological Relapse-Free Survival
Non-transplanted Patients  (EHA 2010)

- One patient was not evaluable due to early treatment stop (AE), one patient (who responded to treatment) withdrew consent
- TKI – tyrosine kinase inhibitor given at indicated time
- Current median relapse-free survival (RFS) is 10+ months
Blinatumomab provides an active therapy that permits time to arrange for allogeneic transplant.

Patients receiving blinatumomab prior to transplant tolerate allogeneic transplant well.

Current median RFS is 13+ months; no clinical relapses encountered to date.
Summary of Phase 2 Study in Patients with Minimal Residual B-lineage ALL (EHA 2010)

- Complete molecular response in 80% (16 out of 20) of evaluable ALL patients
- Relapse free survival (RFS) so far up to 22 months, and ongoing; no median reached after 408 days; historical median RFS in this patient population is only 200 days
- Responses also observed in patients with tyrosine kinase inhibitor-refractory bcr/abl (T_{315}I), and with (4;11) translocation
- No mortality upon subsequent transplantation (N=8)
- Very favorable safety profile
<table>
<thead>
<tr>
<th>BiTE Target (Development Partner)</th>
<th>Indication/Target Tissue</th>
<th>Developmental Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>B cell malignancies and disorders</td>
<td>Pivotal</td>
</tr>
<tr>
<td>EpCAM</td>
<td>EpCAM+ solid tumors</td>
<td>Phase 1</td>
</tr>
<tr>
<td>CEA (MedImmune/AZ)</td>
<td>CEA+ solid tumors</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>N.d. (Bayer Schering Pharma)</td>
<td>Solid tumors</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>N.d. (Sanofi-aventis)</td>
<td>Solid tumors</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>N.d. (Boehringer Ingelheim)</td>
<td>Multiple myeloma</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>EGFR</td>
<td>EGFR+ solid tumors</td>
<td>In-vivo PoC (monkey, mouse)</td>
</tr>
<tr>
<td>CD33</td>
<td>AML, CML, MDS</td>
<td>In-vivo PoC (monkey, mouse)</td>
</tr>
<tr>
<td>MCSP</td>
<td>Melanoma</td>
<td>In-vivo PoC (monkey, mouse)</td>
</tr>
<tr>
<td>EphA2</td>
<td>EphA2+ solid tumors</td>
<td>In-vivo PoC (mouse)</td>
</tr>
<tr>
<td>PSCA</td>
<td>Prostate cancer</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>FAP-alpha</td>
<td>Sarcoma, stromal fibroblasts</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>IGF-1R+ solid tumors</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>Her-2/neu</td>
<td>Breast and gastric cancer</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>Endosialin</td>
<td>Neovasculature</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>Carboanhydrase IX</td>
<td>Renal cancer</td>
<td>In-vitro activity shown</td>
</tr>
<tr>
<td>cMet</td>
<td>cMet+ solid tumors</td>
<td>In-vitro activity shown</td>
</tr>
</tbody>
</table>
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