Assessment of Cytokine Release for MEDI-565 (AMG 211), a novel CEA/CD3-bispecific single-chain BiTE® antibody

Patricia Ryan, PhD
Fellow Toxicologist
Biologics Safety Assessment
MedImmune
1. Background on CEA BiTE® MoA
2. Species Selection and MABEL Approach
3. In vitro Nonclinical Safety Assessment
4. FTIH Dose Selection
MEDI-565/AMG 211 - CEA BiTE® (Bispecific T-Cell Engager): Mechanism of Action

◆ Potent bispecific scFv T-cell engager (BiTE®) binds carcinoembryonic antigen (CEA) highly expressed on cancer cells and CD3 on T-cells causing T cell activation, cytokine release and tumor cell lysis

◆ Currently in Phase I clinical development for treatment of gastrointestinal adenocarcinomas

BiTE® is a registered trademark of Amgen)
In Vitro MEDI-565 Induced T Cell Killing of CEA+ Cells

Methods: Target cells: CHO/huCEA and CHO; Effector cells: human CD3+ T cells; E:T ratio=10:1; Incubation time: 72 h, Measure: FACS Assay

Results: CEA expression is necessary for MEDI-565 to induce T cell lysis of target cells.

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**CEA positive cells**

- Control BiTE®
- MEDI-565

**CEA negative cells**

- Control BiTE®
- MEDI-565

**Graphs:**

- Specific Lysis [%]
- Specific Cytotoxicity [%]

**CHO/huCEA dhfr-**
Effects of MEDI-565 on T Cells: In Vitro Release of Cytokines

Methods: Effector Cells: human CD3+ T cells; Targets: LS174T colon tumor cell line; E:T Ratio = 5:1; BiTE® @ 100 ng/mL; Incubation time 24 h

Results: Multiple cytokines secreted by T cells when MEDI-565 co-engages CEA on target tumor cell and CD3 on effector T cells.
MEDI-565 Antitumor Activity Evaluated in Mouse Models

BiTE

Human-specific

Mouse
NOD/SCID, immunocompromised

Tumor
Human

T Cells
Human, mixed with tumor

- Dose-dependent, significant inhibition of LS174T colon tumor growth (20 µg ≈ 1 mg/kg)
- Schedule independent

Immediate Treatment

Delayed Treatment (1 mg/kg)
No relevant animal model exists for testing the human test material, MEDI-565, in animal toxicity studies.

Murine and cynomolgus monkey surrogate molecules were inadequate based on non-CEA mediated T cell activation.

Alternative approach used was to perform a detailed in vitro study to estimate minimum anticipated biological effect level (MABEL).
Proposed Nonclinical Safety Plans

◆ No relevant animal model exists for testing the human test material in in vivo toxicity studies.
  – Murine and cynomolgus monkey surrogate molecules inadequate based on non-CEA mediated T cell activation.

◆ Alternative approach is to perform a detailed in vitro study to estimate minimum anticipated biological effect level (MABEL).
  – Establish a dose response for in vitro activity to determine MABEL.
  – Identify most sensitive measure to achieve 20% maximal effect (EC20) for cytokine release, lysis, T cell activation, etc.

◆ Conduct a PK study of MEDI-565 in monkeys
  – Use allometric scaling approach to model human PK parameters
  – Extrapolate MABEL dose from in vitro data to establish an appropriate first time in human dose using available monkey PK data.
FDA Interactions

° Given lack of relevant tox model, MedImmune proposed not to conduct in vivo toxicity studies with MEDI-565.
  – Instead, FTIH dose will be based on MABEL in vitro experiments and PK modeling.

° FDA agreed to proposed approach and requested additional information be included in the IND
  – Provide all data and justification for lack of relevant tox model.
  – Strongly recommended assessing receptor occupancy as part of MABEL approach.
  – Monitor the in vitro ability of MEDI-565 to induce cytokine release and proliferation from at least three human donors.
  – Cytokine assays should measure the production of IFNγ, TNFα and IL-6, as well as any other relevant cytokines.
  – Recommended to include conditions where the BiTE is 'dry coated' onto plates (see Stebbings et al., 2007 J. Immunol. 179:3325) and a positive control antibody, such as OKT3, which is a known inducer of cytokine release.
Characterization of In vitro Activity of MEDI-565

- Apply principles outlined in Duff Report for high risk biologics (Experts Scientific Group on Ph I Clinical Trials, 2006).
- Determine affinity/theoretical receptor occupancy
- **MABEL studies**: Characterize MEDI-565 in vitro activity on cocultures of human cells (mixtures of T cells and CEA+ tumor cells)
  - T cell activation
  - Cytotoxicity (specific lysis)
  - Cytokine release
- **In Vitro Safety Studies**: Assess T cell proliferation and cytokine release using variety of conditions (e.g. “dry coat”) compared to a positive control mab (OKT3).
Theoretical Fractional Receptor Occupancy

A Fractional Occupancy CD3

B Fractional Occupancy huCEA

Calculations:
\[ F = \frac{[\text{mAb}]}{([\text{mAb}] + \text{KD})} \]
\[ [\text{mAb}] = \frac{F \times \text{KD}}{100 - F} \]

Receptor | 20% Occupancy
---|---
CD3 | 4200
CEA | 73

Values in ng/mL
MABEL studies: MEDI-565 mediates T cell Activation and Specific Lysis

MEDI-565 induced upregulation of the T cell activation markers CD69 and CD25 and specific lysis of ASPC-1 tumor cells in cocultures with CD3+-enriched human PBMC preparations.

### Parameters and EC
table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EC&lt;sub&gt;20&lt;/sub&gt;</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;</th>
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<tr>
<td>CD69</td>
<td>0.44</td>
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<tr>
<td>CD25</td>
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<tr>
<td>Lysis</td>
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</tbody>
</table>

Mean values in ng/mL
MABEL studies: MEDI-565 Mediates Cytokine Release

MEDI-565 induced release of cytokines from CD3+-enriched human PBMC preparations co-cultured with ASPC-1 tumor cells.
MABEL Study Conclusions

◆ **MABEL assay development**
  – Co-cultures of human donor CD3+-enriched PBMC and CEA-expressing cells
  – Parameters evaluated include cell lysis, T cell activation markers (CD25 and CD69), and cytokine release.

◆ **MEDI-565-induced lysis of tumor cells was the most sensitive measure for MABEL**

◆ **MEDI-565-induced activation of T cells and concomitant cytokine release were less sensitive measures for MABEL**

◆ **Serum levels to achieve fractional receptor occupancy of 20% were well above the EC20 value for lysis.**
In Vitro Safety Studies: T Cell Proliferation and Cytokine Release

- Adapted from Stebbings et al., JI 2007;179:3325
- Six protocols used for presentation of TGN1412 to white blood cells
MEDI-565 In Vitro Proliferation and Cytokine Release Study Parameters

◆ Human PBMC (n = 3 donors)

◆ Test article
  – MEDI-565 dose titration (in solution, wet coat, dry coat)

◆ Controls
  – CHO and CHO/huCEA cells
  – OKT3 (10 µg/ml in solution, wet coat, dry coat)
  – Phytohaemagglutinin (PHA, 2 µg/ml) + rhIL-2 (10 U/ml)

◆ Read-outs
  – Cytokine release (24 hrs; IFNγ, TNFα, IL-2, IL-6, IL-10)
  – Proliferation (96 hrs; CFSE)
IFNγ Release Required Co-engagement of both CEA and CD3 by MEDI-565 in Solution

- Positive controls were 10 µg/ml OKT3; 2 µg/ml PHA+ 10 U/ml IL-2
- Soluble MEDI-565 above 0.51 ng/ml induced IFNγ release by PBMC only in presence of CEA+ cells.
  - Low or no release of TNFα, IL-2, IL-6, IL-10 was seen (not shown).
- Immobilized MEDI-565 above 14 ng/ml (wet or dry) induced release of all cytokines (not shown).
T cell Proliferation Induced by MEDI-565

- Mean proliferation of CD4+ and CD8+ T cells in PMBC stimulated with soluble and immobilized MEDI-565 compared to OKT3 and PHA + IL-2 controls.

- Proliferation required CD3 on T cells to be cross-linked by MEDI-565 either through concurrent binding to CEA, or when MEDI-565 was immobilized (plate-bound).
In Vitro Safety Study Conclusions

◆ Results supported mechanism of action

◆ Cross-linking of CD3 and binding to CEA by MEDI-565 was required to induce T cell proliferation and cytokine release.
  - T cells in PBMCs were induced to proliferate and release cytokines only when CD3 on T cells was cross-linked by MEDI-565 through concurrent binding to CEA, or when MEDI-565 was immobilized to a solid-phase surface (plate-bound).

◆ Cell proliferation was accompanied by increase in IFN\(\gamma\) with modest increases in TNF\(\alpha\) and IL-6 and no increases in IL-2 and IL-10.

◆ Since MEDI-565 monovalently binds CD3 on T cells, no cross-linking is expected in vivo in the absence of cells expressing CEA.
PK modeling to Predict Human PK Parameters

◆ Conducted single IV/SC dose PK study in cynomolgus monkeys (cross-over design)

◆ Three compartment model used to fit cynomolgus monkey PK data

◆ Results demonstrate large volume of distribution (Vd), high SC bioavailability (BA) and long terminal elimination half-life (t1/2z) compared to similar molecules.

◆ Human PK parameters predicted using simplified allometric scaling.
Strategy for FTIH Dose Selection

- Human PK parameters of MEDI-565 were predicted using allometric scaling based on monkey PK data
- Phase I dose regimen projected based on simulated C-t profiles and in vitro MABEL results

**Human PK parameters of MEDI-565**

- Predicted efficacious dose trough above EC50.
- Starting dose Cmax below EC20.
Summary

◆ No relevant animal model was available for in vivo toxicity studies of MEDI-565

◆ A cynomolgus monkey PK study was conducted to model human PK

◆ In vitro cytokine release and T cell proliferation was assessed using recommended assay format dry-coated on wells ("Stebbings protocol")
  – Cytokine release only occurred with cross-linking of CD3 and binding to CEA

◆ In addition, established a dose response for in vitro activity using human cells to calculate minimum anticipated biological effect level (MABEL)
  – Theoretical Receptor Occupancy (concentration to achieve 20% occupancy for both receptors)
  – T cell activation and cytokine release in presence of tumor cells
  – Cytotoxicity (lysis of tumor cells)

◆ MEDI-565-induced lysis of tumor cells was the most sensitive measure for MABEL
  – Human starting dose was calculated to give predicted $C_{\text{max}}$ below the EC20 for lysis

◆ Currently in Phase I for treatment of gastrointestinal adenocarcinoma
Acknowledgements

- Scott Hammond
- Stacy Fuhrmann
- Mike Oberst
- Kathy Mulgrew
- Meina Liang
- Amy Schneider
- Raffaella Faggioni
- Song Ren
- Rakesh Dixit
- Lorin Roskos
- Salah Kivilingh

Amgen
- Patrick Baeuerle
- Benno Rattel