Immunotherapy with Long-Lived Anti-CD20 × Anti-CD3 Bispecific Antibodies Stimulates Potent T Cell-Mediated Killing of Human B Cell Lines and of Circulating and Lymphoid B Cells in Monkeys: A Potential Therapy for B Cell Lymphomas and Leukemias

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Introduction
- CD20 is highly expressed on normal and malignant B cells, and is a well-established target of antibody therapeutics for B cell leukemias and lymphomas.
- However, one limitation of approved anti-CD20 antibodies such as rituximab, ofatumumab, and obinutuzumab is that they are unable to stimulate T cell-mediated killing of CD20+ B cells.
- To exploit the potent activity intrinsic to T cell immunotherapy while maintaining the favorable dosing regimen of a therapeutic antibody, we have designed novel humanized bispecific antibodies that bind to both CD20+ B cells and CD3+ T cells.
- Such bispecific antibodies act via "redirected T cell cytotoxicity" (RTCC), a mechanism that stimulates targeted T cell-mediated killing regardless of T cell antigen specificity.
- Unlike other bispecific formats, these antibodies possess a full Fc domain that binds to human FcRn (to maintain long serum half-life) and spontaneously forms stable heterodimers that are readily manufactured.

C Bispecific antibodies kill human B cells

1. Killing by purified human T cells
   - Target: Human B cell line
   - Lysis induced by CD3 stimulation
   - CD20+ B cell target cells
   - CD3+ T cell effectors
   - T cell responses: CD20+ B cell lysis

2. Killing by human PBMCs
   - Target: Human B cell line
   - Lysis induced by CD3 stimulation
   - CD20+ B cell target cells
   - CD3+ T cell effectors
   - T cell responses: CD20+ B cell lysis

3. T cells are serial killers even when outnumbered by target cells
   - Log10 [Ab] vs. Time (Hours)
   - T cell responses: CD20+ B cell lysis

D Bispecific antibodies prevent human immunoglobulin production

1. Bispecific antibodies suppress human Igs in PBMC-engrafted mice
   - PBMC engraftment of NSG mice
   - XmAb13676 (5 mg/kg IV)
   - IgM and IgG levels

E Bispecifics deplete B cells in blood & lymphoid organs in monkeys

1. Long half-life
   - Blood B cells
   - Bone marrow B cells
   - Lymph node B cells

2. Blood B cells
   - 3 cynomolgus per group
   - Single IV infusion on Day 0

3. Bone marrow B cells
   - XmAb13676
   - 300 & 3000 µg/kg doses

F B cell depletion correlates with T cell redistribution & activation

1. Redistribution from blood
   - CD69 induction
   - Cytokine release

Summary
The anti-CD20 + anti-CD3 antibodies XmAb13676 and XmAb13677:
- Incorporate a human Fc domain for long half-life
- Recruit T cells to kill B cells in vitro with ~50 and ~2 ng/ml potency, respectively
- Safely & effectively deplete B cells in monkeys at single doses as low as 30 µg/kg
- Deplete lymphoid B cells in bone marrow & lymph nodes
- Show T cell-mediated toxicity at higher doses (1 of 3 @ 3 mg/kg XmAb13676; 2 of 3 @ 0.3 mg/kg XmAb13677)
- Are efficiently manufactured using standard antibody production methods

These results support clinical testing of these bispecific antibodies in patients with CD20+ B cell leukemias and lymphomas.