# Abstract #2730/21: Beyond Antibodies and CAR-T: Topologically-engineered, Super-dimeric Antibody-like Molecules with Dual Fc domains for Trispecific, Bivalent Targeting of CD19, CD20, and Fc gamma Receptors

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## Abstract

**Background:** Depletion of B cells has resulted in major therapeutic benefits in autoimmune diseases and hematological malignancies. Several FDA-approved B cell-depleting antibody-based therapies are available, including antibody-drug conjugates, T cell engagers, CAR-T cells, and antibody variants with enhanced effector functions through Fc engineering. However, a significant unmet need remains in both oncology and autoimmune disease. All currently approved B cell-depleting therapies target a single B cell antigen, increasing potential for incomplete depletion and emergence of escape mutants that give rise to relapse and treatment failure.

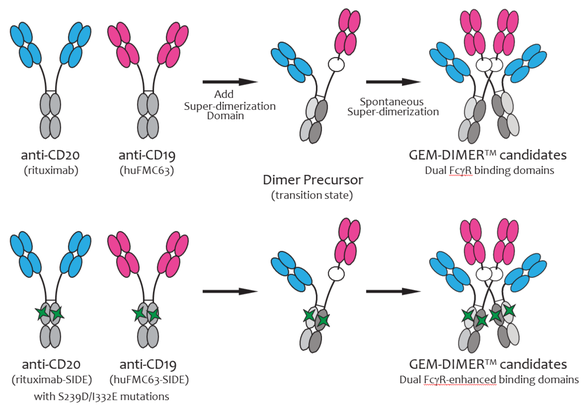
**Methods:** Here we describe trispecific antibody-like molecules generated using Hinge Bio's GEM-DIMER™ technology. In addition to bivalent target binding of both CD19 and CD20, our GEM-DIMER candidates are designed to have two Fc domains to enable powerful effector functions via cooperative binding of Fc gamma receptors. The approach is based on incorporation of a super-dimerization domain into the hinge region of an immunoglobulin heavy chain, enabling the combination of all of the components of two IgG antibodies into a single molecule. Using this approach, we generated GEM-DIMER candidates from rituximab and FMC63, the parental anti-CD19 antibody for anti-CD19 scFv used in approved CAR-T cell therapies.

**Results:** CD19/CD20-targeting GEM-DIMER molecules demonstrated binding to both CD19 and CD20 with affinities comparable to the individual parent antibodies. Importantly, CD19/CD20-targeting GEM-DIMER molecules demonstrated robust depletion of human B cells in overnight cultures of whole blood. Moreover, antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) were demonstrated in co-cultures of the human B cell lymphoma cell line Raji and human peripheral blood mononuclear cells (PBMC). As expected, due to the presence of dual Fc domains, CD19/CD20-targeting GEM-DIMER molecules exhibited enhanced binding to Fc gamma receptors, further explaining the robust effector functions observed.

**Conclusions:** CD19/CD20-targeting GEM-DIMER molecules are promising candidates to provide efficient depletion of both CD19+ and CD20+ cells, providing potential for broad and deep depletion of B cells with reduced risk of emergence of antigen escape variants. These data support the advancement of CD19/CD20-targeting GEM-DIMER molecules in multiple indications where depletion of CD19+ and/or CD20+ B cells is needed. Preparations for clinical investigation are ongoing.

## GEM-DIMER™ Technology

### Multiplying the Full Power of All Three Antibody Domains in the Form of a Single Biomolecular Therapeutic




## Results →

### CD19/CD20 GEM-DIMER™ Candidates (CD19/CD20 GD) Demonstrate High-affinity Binding to Both CD19 and CD20

| | KD (nM) | |
|---|---|---|
| **Test Article** | **CD19** | **CD20** |
| **Rituximab** | No binding | 0.24 |
| **FMC63** | 3.96 | No Binding |
| **CD19/CD20 GD** | 2.77 | 0.16 |

**Legend for table:** SPR capture kinetic experiments were performed on a Carterra LSA instrument. Test articles were captured on the Carterra CMDP chip using anti-human Fc. Recombinant human CD19 and CD20 (ACRO Biosystems) were diluted in three-fold dilution steps (500 nM-0.68 nM). Proteins were injected onto the CMD-P chip for 5 min during the association phase followed by HEPES buffer injection for another 5 min during the dissociation phase. The CMD-P chip was regenerated by flowing 0.425% phosphoric acid, 3 times with 1 min intervals after each round of analyte injection. The depicted results represent the average of six replicate experiments. Data were analyzed using the Carterra Kinetics Software (Carterra).

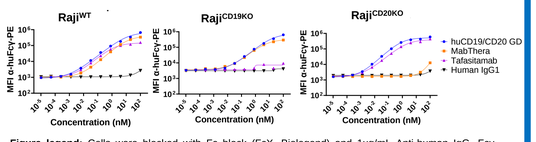



**Figure legend:** Cells were blocked with Fc block (FcX, Biolegend) and 1ug/mL Anti-human IgG, Fcγ specific Fab (Jax immunoresearch), washed and incubated with test articles for 30 minutes on ice (MabThera: Roche; tafasitamab: research-grade biosimilar). Cells were stained with Live/Dead-NIR (Thermofisher) and PE- anti human IgG, Fcγ specific polyclonal antibody (Jax immunoresearch) for 30 minutes on ice, washed, fixed with 1% PFA and analyzed on NovoCyte (Agilent) flow cytometer. Data were analyzed in FlowJo for Median Fluorescent Intensity in PE channel and triplicate Median ± SD were graphed in Prism with 4PL regression curve.

### CD19/CD20 GEM-DIMER™ Candidates Demonstrate Potent Human B Cell Depletion and NK Cell Activation

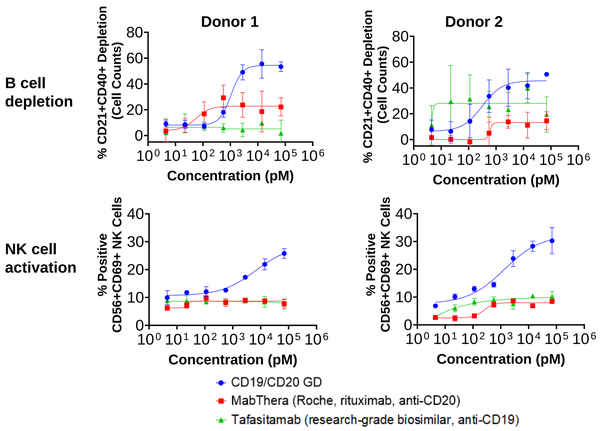



**Figure Legend:** Human whole blood (n = 2) was collected in sodium heparin and treated overnight at 37ºC with gentle shaking in the presence of test articles. Whole blood was then stained with an antibody cocktail to detect B- and NK- from T-lymphocytes before erythrocyte lysis. Harvested cells were immediately analyzed on a flow cytometer. Percent depletion is defined as: 100 x (1-sample CD21+CD40+ cell counts/no Ab CD21+CD40+ cell counts). Data were analyzed in FlowJo and triplicate mean ± SD were graphed in Prism.

### GEM-DIMER™ Candidates Demonstrate Potent ADCC Activity

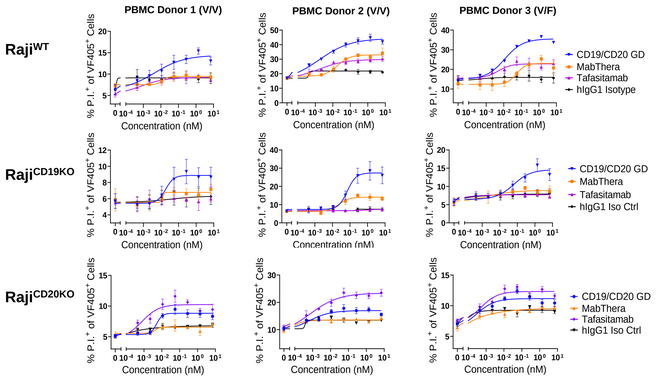



**Figure legend:** Target cells were labeled with ViaFluor 405 (VF405) and incubated with test articles for 15 min at 37°C, followed by incubation with human PBMC (25:1 E:T ratio) for 4h at 37°C. Cells were washed and stained with propidium iodide (P.I.) and analyzed by flow cytometry. Cytotoxicity was expressed as the percent of VF405+ cells positive for P.I. Data were fit to a four-parameter nonlinear regression curve. Mean ± SD is shown (CD19/CD20 GD n=6 replicate cultures; MabThera n=3; tafasitamab n=3; isotype IgG1 control n=6). Final compound concentrations are indicated.

### GEM-DIMER™ Candidates Demonstrate Potent ADCP Activity

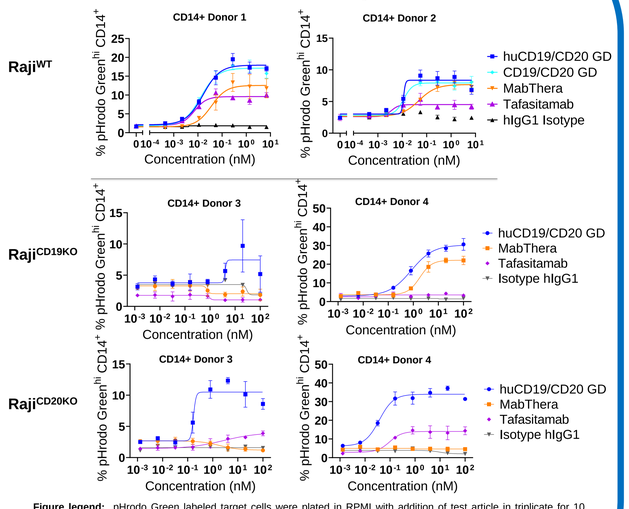
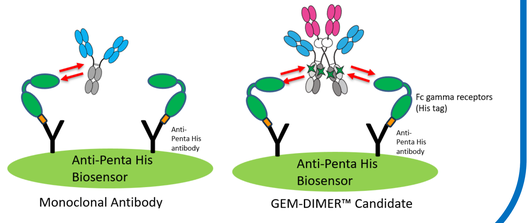



**Figure legend:** pHrodo Green labeled target cells were plated in RPMI with addition of test article in triplicate for 10 minutes prior to the addition of CellTraceViolet labeled, M-CSF differentiated, CD14+ primary human M2 macrophages as effectors cells in a 1:2 ratio. Cells were then co-cultured for 2 hours at 37C. CD19KO and CD20KO cultures were performed in the presence of physiological concentration of huIgG (10 mg/mL). Uptake was measured as the percentage of CTV+ macrophages that are pHrodo Green+ by Flow cytometry (Novocyte). Data were analyzed in Flowjo with Mean +- SD and 4 PL curve fit graphed in Prism vs final compound concentration.

### GEM-DIMER™ Candidates Demonstrate Potent Binding to Fc gamma Receptors

| | CD16a 158F | | CD16a 158V | | CD16b | | CD32a167H | | CD32a 167R | | CD32 b/c | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample ID** | Kd (nM) | Fold Diff. | Kd (nM) | Fold Diff. | Kd (nM) | Fold Diff. | Kd (nM) | Fold Diff. | Kd (nM) | Fold Diff. | Kd (nM) | Fold Diff. |
| **Rituximab** | 304.5 | 1.0 | 267.2 | 1.0 | 1252.6 | 1.0 | 583.5 | 1.0 | 496.7 | 1.0 | 1008.4 | 1.0 |
| **Rituximab-SIDE** | 28.6 | 10.6 | 13.4 | 20.0 | 54.2 | 23.1 | 140.7 | 4.1 | 147.4 | 3.4 | 162.7 | 6.2 |
| **HuFMC63** | 186.2 | 1.6 | 202.5 | 1.3 | 405.2 | 3.1 | 446.7 | 1.3 | 441.5 | 1.1 | 343.3 | 2.9 |
| **HuFMC63-SIDE** | 24.8 | 12.3 | 14.8 | 18.0 | 51.0 | 24.6 | 107.8 | 5.4 | NA | NA | 132.3 | 7.6 |
| **huCD19/CD20GD (w/o SIDE)** | 15.9 | 19.2 | 7.1 | 37.7 | 29.2 | 42.8 | 17.2 | 33.9 | 18.2 | 27.3 | 29.0 | 34.8 |
| **huCD19/CD20 GD** | 4.0 | 75.9 | 3.4 | 77.8 | 6.6 | 189.5 | 15.1 | 38.6 | 8.9 | 56.1 | 13.6 | 74.3 |

**Legend for table:** Kinetic binding analysis of test articles to Fc gamma receptors was performed using an Octet Red384 (Sartorius) system with 384-well plates. All steps were performed at 26°C. Polyhistidine-tagged Fc gamma receptor ectodomains (R&D Systems) were captured on Anti-Penta-His biosensors (Sartorius) in PBS-B (PBS with 1 mg/mL BSA, pH 7.4). After washing with PBS-B, the biosensors with captured Fc gamma receptors were incubated with varying concentrations of testing articles for 15 seconds followed by a 60 second dissociation period in PBS-B. Biosensors were regenerated by stripping with 0.425% phosphoric acid at the end of each cycle. Data analysis was conducted using a standard 1:1 binding model. NA = not available




## Summary & Conclusions

### *Summary of Results*

- ✓ CD19/CD20 GEM-DIMER candidates resulted in robust depletion of human B cells and activation of NK cells in cultures of human whole blood
- ✓ GEM-DIMER candidates demonstrated enhanced binding to Fc gamma receptors relative to parental antibodies
- ✓ Potent ADCC activity on human B cell lymphoma cell line Raji was observed, whether wild-type Raji or variant cell lines lacking CD19 or CD20
- ✓ Potent phagocytosis of human B cell lymphoma cell line Raji was observed, whether wild-type Raji or variant cell lines lacking CD19 or CD20

### *Conclusions*

- ✓ Efficient depletion of both CD19⁺ and CD20⁺ cells provides potential for broad and deep depletion of B cells with reduced risk of emergence of antigen escape variants
- ✓ CD19/CD20 GEM-DIMER candidates are advancing in preclinical development with significant potential in both oncology and autoimmunity

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