Costimulatory IgM T-cell engagers with enhanced and durable cytotoxicity

Jie Xue, Keyu Li, Poonam Yakkundi, Palak Chudasama, Yi-Ting Mao, Zee Malik, Vidhya Rao, Madhura Joglekar, Kristene Mai, Gene Li, Deepal Pandya, Rodnie Rosete, Daniel Machado, Zhongde Ye, Leyla Tahrani, Jinqiu Wang, Nardeen Hanna, Elizabeth Perez, Yue Wang, Sachi Ahmed, Lusiana Widjaja, Paul Hinton, Krzysztof Bzymek, Bruce Keyt, Miho Oyasu, Liqin Liu, Hyewon Phee, Umesh S. Muchhal, Angus Sinclair

IGM Biosciences, Inc. | Mountain View, CA

Introduction

T-cell engagers (TCEs) have shown promising anti-tumor efficacy in patients with a variety of cancers by redirecting T cell cytotoxicity via a surrogate mechanism mimicking TCR Signal 1 (CD3). However, challenges have been faced balancing the efficacy, safety and tolerability in certain indications. We have developed a bispecific IgM antibody platform that provides strong target binding avidity using 10 binding domains to the antigen, with a single CD3 binding domain fused to the joining (J) chain. Due to the promising efficacy and safety of CD20xCD3 bispecific IgM Imvotamab in non-Hodgkin's lymphoma (NHL), Imvotamab is currently being clinically evaluated in autoimmune indications such as SLE, RA and myositis.

To further achieve deep and durable responses, we have built a costimulatory IgM TCE platform that engages both signal 1 (CD3) and signal 2 (CD28) on T cells (TAAxCD3xCD28). Co-stimulation of CD28 enhances T cell activation/proliferation and sustains T cell survival. This dual engagement of T-cell activation signals, along with the high avidity target binding offered by the IgM platform, may enable more effective therapeutics targeting pathogenic cells. Here we present our findings on different TAAxCD3xCD28 TCEs using in vitro and in vivo preclinical models.

Co-stimulatory IgM TCEs with both TCR signal 1+2

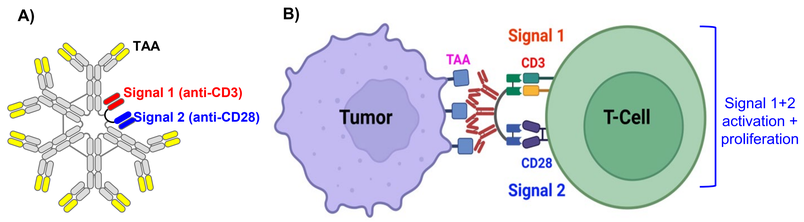

Figure 1. **A)** Structure of IgM-based costimulatory TCEs. Signal 1 (anti-CD3 scFv) and signal 2 (anti-CD28 scFv) are located at the N or C terminus of the joining (J) chain, respectively. **B)** Schematic mechanism of action of IgM-based costimulatory tri-specific TCEs in T-cell directed cytotoxicity (TDCC) assay. Note: the CD3 scFv used in these PoC molecules is different from that used in Imvotamab.

Tri-specific IgM with CD28 co-stimulation enhances *in vitro* cytotoxicity of CD20+ B lymphoma cells

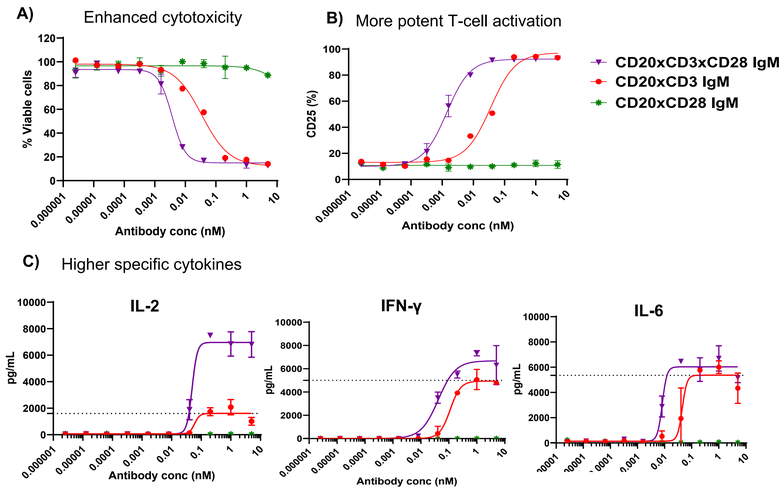

Figure 2. Anti-CD20 bi-specific and tri-specific IgM TCEs were evaluated in T cell directed cytotoxicity (TDCC) assays to profile their cytotoxicity. CD20+ B lymphoma cell line Ramos cells were co-cultured with human PBMCs at an effector-to-target (E:T) ratio at 2.5 to 1 for 96 hrs. T cell activation and cytokine release were also profiled in the same TDCC assay by flow cytometry assays. Enhanced cytotoxicity **(A)** and T cell activation **(B)** were observed with CD28 co-stimulation. Cytokine release shown in **(C)**. Data representative of one out of three independent donors.

Tri-specific IgM with CD28 co-stimulation enhances anti-tumor activity and durability in a CD20+ B lymphoma xenograft model

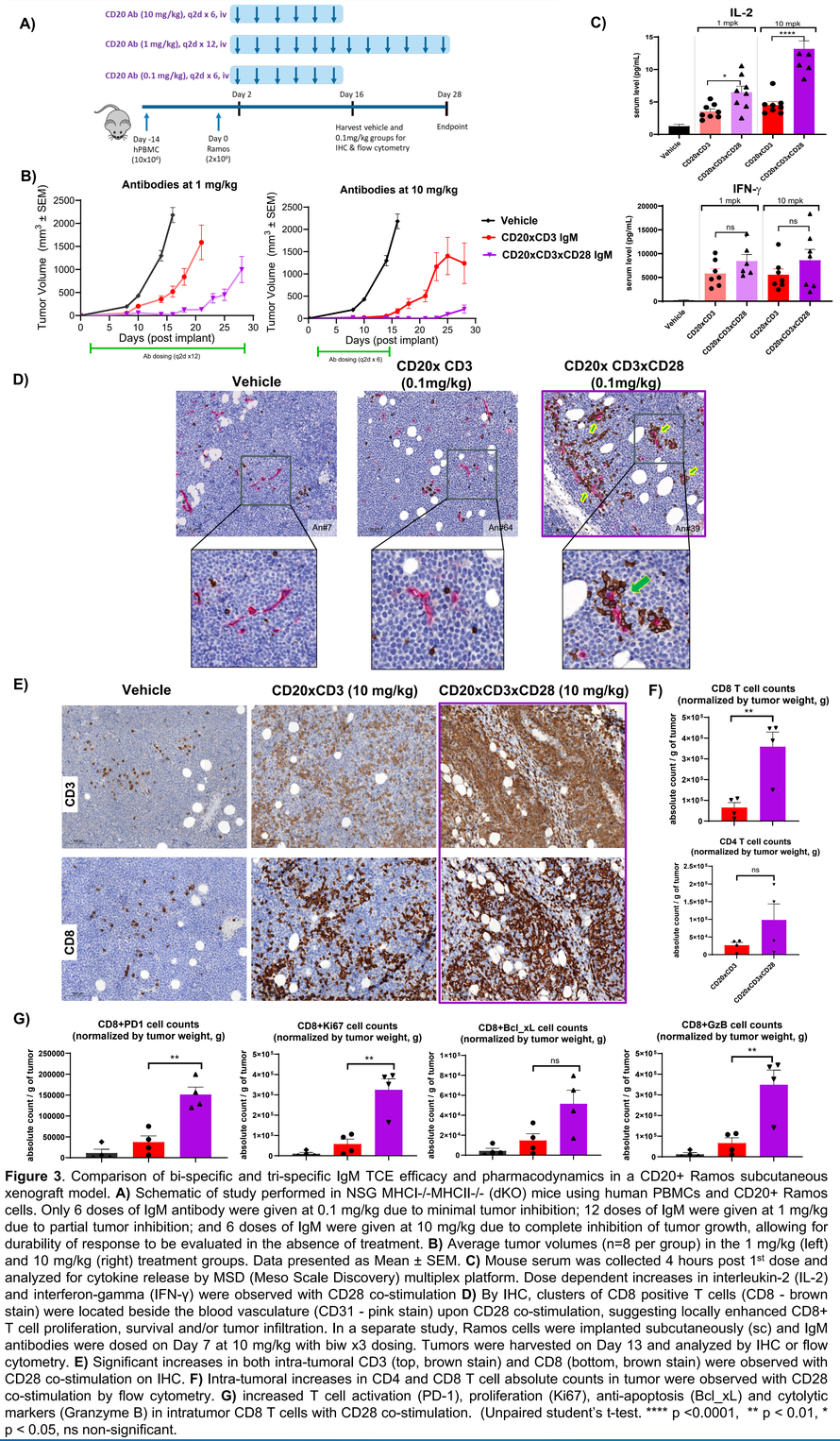

Figure 3. Comparison of bi-specific and tri-specific IgM TCE efficacy and pharmacodynamics in a CD20+ Ramos subcutaneous xenograft model. **A)** Schematic of study performed in NSG MHCI-/-MHCII-/- (dKO) mice using human PBMCs and CD20+ Ramos cells. Only 6 doses of IgM antibody were given at 0.1 mg/kg due to minimal tumor inhibition; 12 doses of IgM were given at 1 mg/kg due to partial tumor inhibition; and 6 doses of IgM were given at 10 mg/kg due to complete inhibition of tumor growth, allowing for durability of response to be evaluated in the absence of treatment. **B)** Average tumor volumes (n=8 per group) in the 1 mg/kg (left) and 10 mg/kg (right) treatment groups. Data presented as Mean ± SEM. **C)** Mouse serum was collected 4 hours post 1st dose and analyzed for cytokine release by MSD (Meso Scale Discovery) multiplex platform. Dose dependent increases in interleukin-2 (IL-2) and interferon-gamma (IFN-γ) were observed with CD28 co-stimulation **D)** By IHC, clusters of CD8 positive T cells (CD8 - brown stain) were located beside the blood vasculature (CD31 - pink stain) upon CD28 co-stimulation, suggesting locally enhanced CD8+ T cell proliferation, survival and/or tumor infiltration. In a separate study, Ramos cells were implanted subcutaneously (sc) and IgM antibodies were dosed on Day 7 at 10 mg/kg with biw x3 dosing. Tumors were harvested on Day 13 and analyzed by IHC or flow cytometry. **E)** Significant increases in both intra-tumoral CD3 (top, brown stain) and CD8 (bottom, brown stain) were observed with CD28 co-stimulation on IHC. **F)** Intra-tumoral increases in CD4 and CD8 T cell absolute counts in tumor were observed with CD28 co-stimulation by flow cytometry. **G)** increased T cell activation (PD-1), proliferation (Ki67), anti-apoptosis (Bcl_xL) and cytolytic markers (Granzyme B) in intratumor CD8 T cells with CD28 co-stimulation. (Unpaired student's t-test. **** p <0.0001, ** p < 0.01, * p < 0.05, ns non-significant.

Tri-specific IgM with CD28 co-stimulation enhances *in vivo* activity in mesothelin positive gastric cancer cells

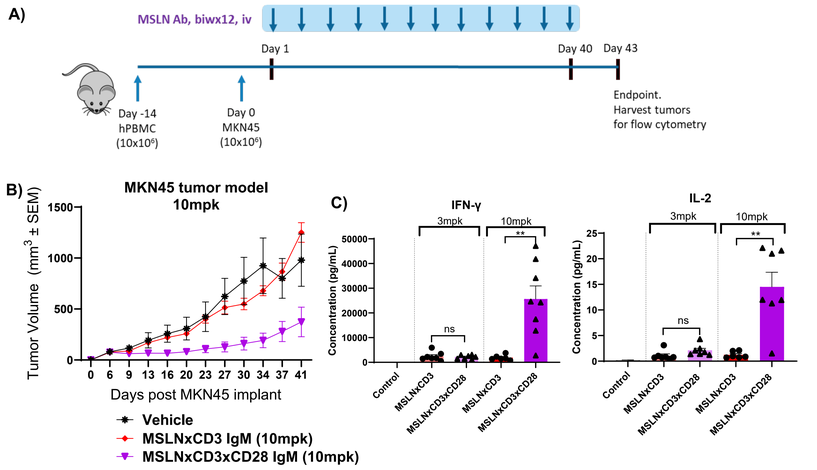

Figure 4. Comparison of bi-specific and tri-specific IgM TCE efficacy and pharmacodynamics in a MSLN+ (mesothelin) MKN45 subcutaneous (sc.) xenograft model. **A)** Schematic of study performed in NSG (dKO) mice using human PBMCs and MSLN+ MKN45 cells. **B)** The graph depicts the average tumor growth (n=8 per group) at 10 mg/kg. Data presented as Mean ± SEM. Tumor suppression was also observed at 3 mg/kg group (data not shown). **C)** Mouse serum was collected 4 hours post 1st dose and analyzed by MSD (Meso Scale Discovery) multiplex platform. Dose dependent increases in interleukin-2 (IL-2) and interferon-gamma (IFN-γ) were observed with CD28 co-stimulation. As seen in the CD20+ Ramos study, similar intra-tumoral increases in CD4 and CD8 T cell absolute counts in tumor were observed with CD28 co-stimulation by flow cytometry, along with increases in T cell activation (PD-1), proliferation (Ki67), anti-apoptosis (Bcl_xL) and cytolytic markers (Granzyme B) in intratumor CD8 T cells (data not shown). Significant differences were determined by unpaired student's t-test. ** p < 0.01, * p < 0.05, ns non-significant.

Tri-specific IgM with CD28 co-stimulation enhances *in vitro* & *in vivo* activities in TROP-2 positive cells

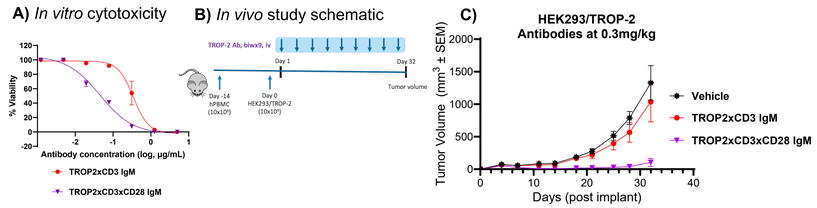

Figure 5. Comparison of the *in vitro* and *in vivo* activities of bi-specific and tri-specific IgM TCEs in TROP-2 transduced HEK293T (HEK293/TROP-2) cells. **A)** IgM T cell engagers with or without CD28 co-stimulation were evaluated in a T cell directed cytotoxicity (TDCC) assay. Human PBMCs were co-cultured with HEK293T/TROP-2 cells in the presence of serial dilutions of IgM antibodies. A luminescence-based viability assay was used to assess cell viability after incubation. The percent viability following antibody treatment was calculated by normalization of viable cells from no treatment group. The *in vivo* efficacy of bi-specific and tri-specific IgM TCEs were also evaluated in HEK293/TROP-2 subcutaneous (sc.) xenograft model. **B)** Schematic of study performed in NSG (dKO) mice using human PBMCs and HEK293T/TROP-2 cells. **C)** The graph depicts the average tumor growth (n=7 per group) at 0.3 mg/kg. Data presented as Mean ± SEM. Tumor suppression was also observed for bi-specific and tri-specific IgMs at 1 and 3 mg/kg groups (data not shown).

Summary

- We developed a next generation tri-specific IgM T cell engager (TCE) that co-engages both CD3 and CD28 while maintaining avidity to antigens.
- Compared with a corresponding bi-specific IgM TCE, the tri-specific IgM TCE enhanced cytotoxicity and T cell activation.
- Tri-specific IgM TCEs also increased in vivo efficacy with durable responses. Pharmacodynamic studies demonstrated increased T cells in tissues, as well as enhanced T cell activation, proliferation, cytolytic killing, and anti-apoptotic features.
- Taken together, these results suggested that the next generation tri-specific IgM TCE platform has potential in targeting pathogenic cells in a solid tumor setting and potentially autoimmune disease.