Beyond Antibodies and CAR-T: Topologically Engineered, Superdimeric Antibody NK Engagers and T Cell Engagers for B Cell Depletion with Cooperative Binding to Target and Effector Cells

DJ Capon, LA Troitskaya, ME Fomin, U Edman, B Frank, BZ Capon, B Law, SJ Chapin, GM Lewis, ML Gefter, J Punnonen, NLS Chan

Hinge Bio, Inc., Burlingame, CA 94010

Abstract 1857184

Abstract

Background/Purpose The dramatic demonstration of CD19 CAR-T efficacy in systemic lupus erythematosus (SLE), idiopathic inflammatory myositis, and systemic sclerosis by Georg Schett and colleagues (F. Muller et al., N Engl J Med 2024 Feb 22;390(8):687-700) has opened the possibility that autoimmunity in such diseases may be reset through the depletion of B cells leading to durable remissions. Given the challenges of deploying CAR-T at large scale and in a diverse patient population whose disease severity varies considerably, there is greatly renewed interest in next-generation NK and T cell engagers to safely achieve deep depletion of autoantibody producing cells.

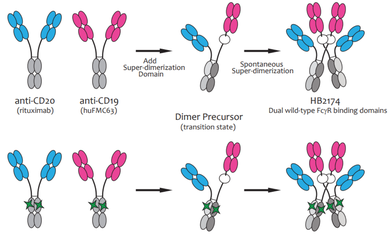
Methods CD19/CD20 dual-targeting GEM-DIMER NK/monocyte engagers demonstrated enhanced binding to target and effector cells, as well as increased induction of apoptosis, ADCC, and ADCP compared with the parent antibodies. We observe a dramatic increase in binding to low affinity Fcγ receptors (e.g., FcγRIIIa CD16a/158V and CD16a/158F) of two to three orders of magnitude. In contrast, binding to high affinity Fcγ receptors (FcγRI CD64) modestly increased, indicating a significant shift in Fcγ receptor binding specificity in favor of effectors such as NK cells and monocytes expressing such low affinity Fcγ receptors. Multivalent FolRα-targeting GEM-DIMER T cell engagers with four anti-FolRα domains demonstrated enhanced binding to FolRα-expressing IGROV-1 target cells of up to one order of magnitude greater than a FolRα-targeting 2+1 T cell engager with only two anti-FolRα domains, indicating increased specificity and presumed safety for the disease target.

Results CD19/CD20 dual-targeting GEM-DIMER NK/monocyte engagers demonstrated enhanced binding to target and effector cells, as well as increased induction of apoptosis, ADCC, and ADCP compared against the parent antibodies. We observe a dramatic increase in binding to low affinity Fcγ receptors (e.g., FcγRIIIa CD16a/158V and CD16a/158F) of two to three orders of magnitude. In contrast, binding to the high affinity Fcγ receptor (FcγRI CD64) was modestly increased, indicating a significant shift in Fcγ receptor binding specificity in favor of effectors such as NK cells and monocytes expressing such low affinity Fcγ receptors. Multivalent FolRα-targeting GEM-DIMER T cell engagers with four anti-FolRα domains demonstrated enhanced binding to FolRα-expressing IGROV-1 target cells of up to one order of magnitude greater than a FolRα-targeting 2+1 T cell engager with only two anti-FolRα domains, indicating increased specificity and presumed safety for the disease target.

Conclusion The ability of GEM-DIMER NK/monocyte engagers to potently and selectively engage low affinity FcγR-expressing cells such as NK cells and monocytes, and the ability of GEM-DIMER T cell engagers to bind more selectively to disease targets offers new opportunities beyond those possible with conventional antibodies and CAR-T. CD19/CD20 and CD19 targeting tetravalent GEM-DIMER NK/monocyte engagers and T cell engagers demonstrating cooperative binding to disease targets and effector cells are promising candidates for broad and deep depletion of B cells with reduced risk of re-emergence of autoimmune-reactive variants.

A New Class of NK/Monocyte Engagers

GEM-DIMER™ technology was used create candidate HB2198, combining bivalent anti-CD19 and bivalent anti-CD20 targeting, with dual enhanced Fc domains, to treat autoimmune disease via B cell depletion

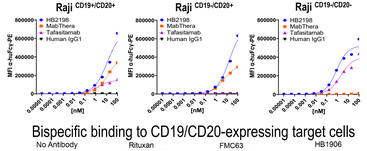


HB2198: Potent Target Binding

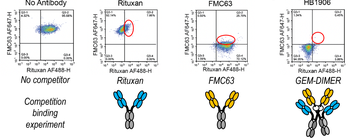
HB2198 monovalent binding to CD19 and CD20 is as potent as the MabThera (rituximab) and huFMC63 parent antibodies

	CD19		CD20		
	k_D (M)	Fold diff	k_D (M)	Fold diff	
MabThera	N/A	N/A	5.70E-10	1.0	Determined by SPR
huFMC63	3.43E-09	1.0	N/A	N/A	
Tafasitamab	1.38E-09	2.5	N/A	N/A	
HB2198	2.45E-09	1.4	2.53E-10	2.3	

HB2198 binds potently to cells expressing CD19, CD20, or both



Bispecific binding to CD19/CD20-expressing target cells



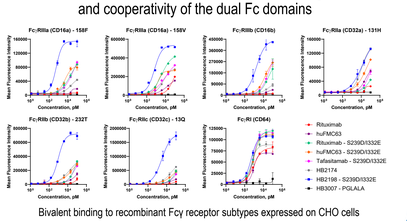
HB2198 Binds Cooperatively to Low Affinity Fcγ Receptors

HB2198 monovalent binding reflects the SD/IE enhancing mutation

	Type	FcγR Binding	CD16a-158F		CD16a-158V		CD16b		CD32a-131H		CD32a-131R		CD32b/c		CD64	
			k_D (M)	Fold	k_D (M)	Fold	k_D (M)	Fold	k_D (M)	Fold	k_D (M)	Fold	k_D (M)	Fold	k_D (M)	Fold
Rituximab (MabThera)	mAb	wt	1.32E-06	1.0	3.09E-07	1.0	4.32E-06	1.0	3.99E-07	1.0	6.35E-07	1.0	8.89E-06	1.0	5.47E-11	1.0
huFMC63		wt	1.56E-06	1.0	3.16E-07	1.0	3.67E-06	1.2	3.64E-07	1.1	7.09E-07	0.9	4.09E-06	2.2	9.93E-11	0.6
Rituximab S239D/I332E		high	1.76E-08	86.6	4.32E-09	71.3	6.68E-08	64.8	1.15E-07	3.5	7.68E-08	8.3	1.38E-07	64.1	2.68E-11	2.1
huFMC63 S239D/I332E		high	1.72E-08	88.3	4.53E-09	68.1	8.63E-08	50.1	1.34E-07	3.0	9.76E-08	6.5	2.05E-07	43.4	<3.00E-11	>2.7
Tafasitamab S239D/I332E		high	1.53E-08	99.5	4.19E-09	73.7	6.21E-08	69.6	6.11E-08	6.5	3.91E-08	16.2	9.94E-08	89.3	<3.00E-11	>2.7
HB2174	GEM-DIMER	wt	2.31E-06	0.7	4.97E-07	0.6	8.91E-06	0.5	8.32E-07	0.6	9.01E-07	0.7	4.48E-06	2.0	1.18E-10	0.5
HB2198 S239D/I332E		high	1.57E-08	96.9	4.19E-09	73.7	5.92E-08	43.5	1.92E-07	2.1	1.28E-07	5.0	2.83E-07	31.4	<3.00E-11	>2.7

Monovalent binding to recombinant Fcγ receptor subtype molecular targets measured using SPR

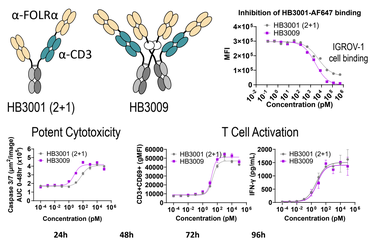
HB2198 bivalent binding reflects the SD/IE enhancing mutation and cooperativity of the dual Fc domains



Bivalent binding to recombinant Fcγ receptor subtypes expressed on CHO cells

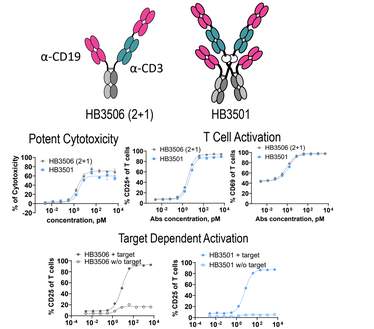
A New Class of T Cell Engagers

Tetravalent anti-FOLRα x anti-CD3 T cell engagers demonstrate potent cytotoxicity and superior binding to FOLRα target cells



IGROV-1 (FOLRα+) ovarian cancer cells were incubated with test articles and primary human T cells (10:1 E:T ratio) for 48h at 37°C. Apoptotic cells were measured kinetically via caspase3/7 activity over 48hrs in IncuCyte. CD69 upregulation and soluble IFNγ was analyzed by flow cytometry and MSD.

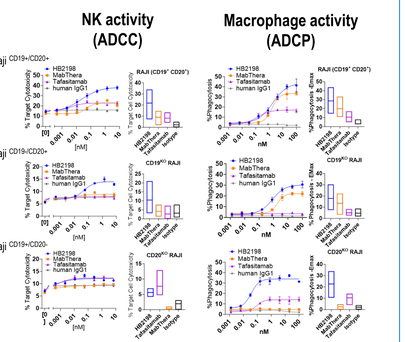
Tetravalent anti-CD19 x anti-CD3 T cell engagers demonstrate potent cytotoxicity and target cell-specific T cell activation



Toledo target cells were incubated with test articles and primary human T cells (10:1 E:T ratio) for 24h at 37°C. Percentage of dead target cells was determined using LDH release assay. B) Percent of T cells expressing CD69 and CD25 was analyzed by flow cytometry.

HB2198 Demonstrates Enhanced Effector Cell Functions

HB2198 broadly targets CD19 and/or CD20 via ADCC and ADCP



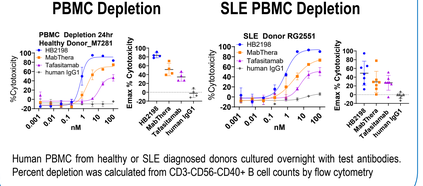
ADCC and ADCP Assays using Raji target cells and either PBMCs or differentiated macrophages as effector cells, respectively. Cytotoxicity and phagocytosis were determined by flow cytometry.

HB2198 Demonstrates Versatile Target and Effector Profile

HB2198 targets CD19 and CD20 via multiple mechanisms

	Whole Blood	CD19+/CD20+ cells				CD19+/CD20- cells			
	B cell Depletion	ADCC	ADCP*	CDC	Direct Killing	ADCC	ADCP*	CDC	Direct Killing
CD19/CD20 HB2198	++++	++++	++++	+++	+	+++	+++	+	nd
Rituximab (MabThera)	++	+++	++	++++	+	-	-	-	nd
Tafasitamab (Minjuvi)	++	+++	+	-	+/-	+++	+	-	nd

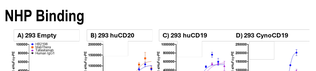
HB2198 superior B cell depletion in SLE donor PBMCs



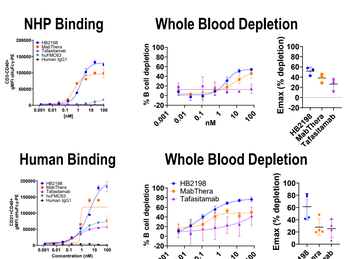
Human PBMC from healthy or SLE diagnosed donors cultured overnight with test antibodies. Percent depletion was calculated from CD3-CD56-CD40+ B cell counts by flow cytometry

HB2198 Demonstrates Potent *ex vivo* Human and NHP B Cell Depletion

NHP Binding

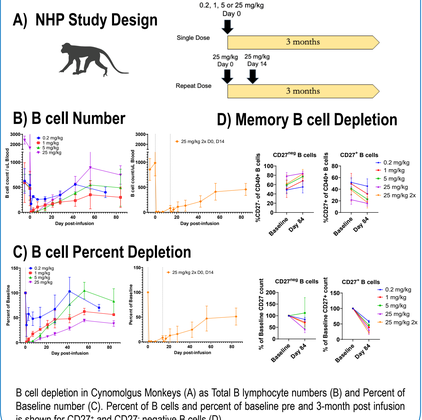


HB2198 shows comparable binding to and depletion of human and cynomolgus B cells



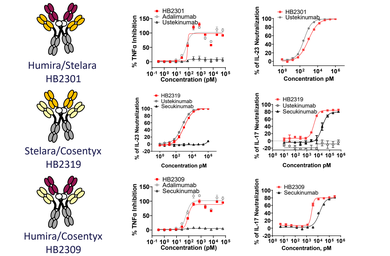
Human or Cynomolgus whole blood incubated overnight in the presence of test antibodies

HB2198 Demonstrates Potent *in vivo* B Cell / Memory B Cell Depletion

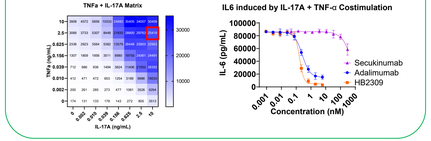


B cell depletion in Cynomolgus Monkeys (A) as Total B lymphocyte numbers (B) and Percent of Baseline number (C). Percent of B cells and percent of baseline pre and 3-month post infusion is shown for CD27+ and CD27- negative B cells (D).

GEM-DIMER Candidates for Potent, Dual Cytokine Blockade



HB2309 potently blocks the synergistic effect of TNFα and IL-17A inducing IL-6 secretion from primary human dermal fibroblasts



Summary & Conclusions

GEM-DIMER Technology represents a robust and versatile platform for the generation of multiple classes of therapeutics demonstrating cooperative binding of disease targets and effector cells. Such novel therapeutic candidates include NK/monocyte engagers, T cell engagers, and multi-cytokine inhibitors.

HB2198, a CD19/CD20-targeting GEM-DIMER candidate with dual enhanced Fc domains exhibited cooperative binding to Fcγ receptors, resulting in increased ADCC and ADCP effector functions over conventional antibodies targeting CD19 or CD20. Importantly, HB2198 retained potent activity in the presence of physiologic levels of competing human IgG. HB2198 depleted memory B cells from healthy and Systemic Lupus Erythematosus (SLE) patient donors in *ex vivo* cultures. Infusion of HB2198 in cynomolgus monkeys led to potent *in vivo* depletion of B cells. Together these data demonstrate the potential of HB2198 for broad and deep depletion of autoantibody producing cells with application to therapeutic indications where depletion of CD19+ and/or CD20+ B cells would provide clinical benefit.