Clayton Biotechnologies

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Technology presentation | **March 2023**

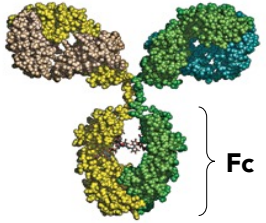
Fc Engineering Platform

For Next Generation Therapeutic Antibodies.

Fc Engineered Aglycosylated Antibodies.

- Aglycosylated IgG1 Fc domains that confer novel and/or enhanced function to therapeutic antibodies.
- Fc domains have absolute selectivity and and/or enhanced binding to one or more desired FcγRs as required for optimal therapeutic function.
- Proprietary and exclusive Fc engineering platform technology.
- Strong patent position.
- **We are seeking strategic partners to develop clinically relevant therapeutic antibodies.**

Targeted Therapy of mAbs Driven by the Fv region.
Immune Therapy of mAbs Driven by the Fc region.



The capacity to bind target antigens with versatility and specificity lies in the Fv region.

The biological effector functions of antibodies are mediated by the constant domains (Fc).

- Antibody development has traditionally focused on the Fv region
- Fc region is also an important component of the antibody for the immune function of monoclonal antibodies
- The Georgiou team has focused on engineering the Fc domain, and have generated first-in-class antibodies with novel antibody therapeutic functions.

Why Focus on the Fc region?

- Improve antibody efficacy
- Optimize the therapeutic function of antibodies
- Enhance therapeutic outcome for patients
- Overcome loss of patent exclusivity
- Engineered Fc antibodies displaying increased affinity to desired Fcg receptors generated by:
  - Glycosylation strategies (defucosylation to increase affinity to the FcgIIIa receptor- ADCC enhancement)
    - *GlycoFi (acquired by Merck); GlycArt (acquired by Roche); BioWa (multiple partnerships)*
  - Mutagenesis of the Fc domain
    - *Genentech; Xencor (multiple partnerships); Macrogenics (Lilly deal)*

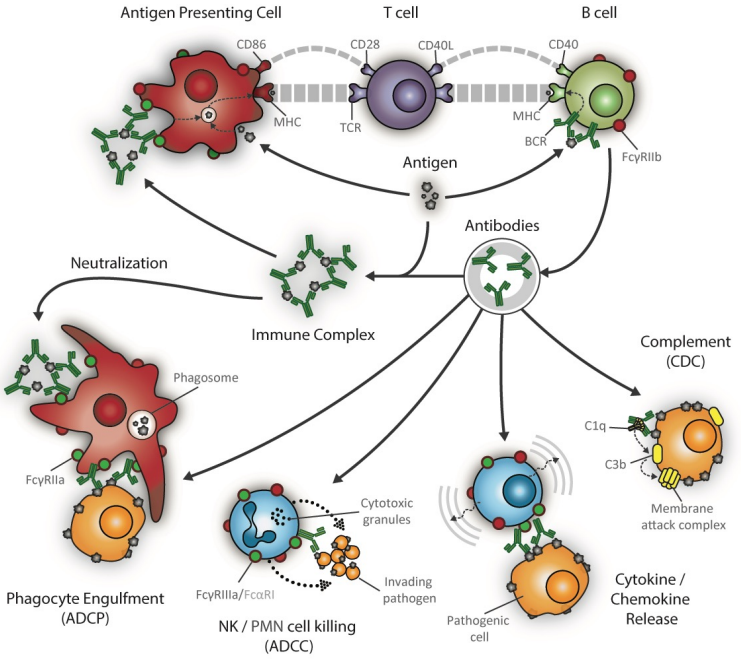
Fc Engineered Antibodies.

Limitations of Current Generation of Fc-Engineered antibodies

- Fc engineered IgGs display enhanced binding to specific FcgRs (e.g. FcgRIIb) but also retain binding to other FcgRs.
- Enhanced affinity for one receptor does not prevent engagement and signaling via other FcgRs on the cell surface.

- **Our antibodies have "absolute" selectivity for only one Fcg receptor and thereby have target specificity along with enhanced immunotherapy properties.**

Fc Engineered Antibodies.



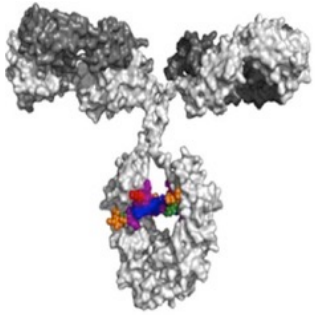
Antibodies (IgG) Bind Receptors that Positively or Negatively Signal in Various Immune Cells.

						
	γ Receptors				α Receptor	C1q
Class	FcgRI		FcgRII	FcgRIII	FcaRI	C1qR
Subclass	FcgRI	FcgRIIa	FcgRIIb	FcgRIIIa	FcaRI	C1qR
Expression	• Macrophage • Monocytes • DCs • Eosinophils • Neutrophils	• Macrophage • Monocytes • Platelets • DCs • Neutrophils	• Macrophage • Monocytes • DCs • Eosinophils • Neutrophils • Mast cells • B cells	• Macrophage • Monocytes • DCs • Mast cells • NK cells	• Neutrophils (most abundant innate immune cells) • Monocytes • Macrophage • Eosinophils • Dendritic cells	• Macrophage
Affinity	1.67 nm	980 nM	2.5 mM	1.8 mM	300nM	

ITAM: Immunoreceptor Tyrosine-based Activation Motif
ITIM: Immunoreceptor Tyrosine-based Inhibitory Motif

Why Agylcosylated Antibodies?

Allows the Engineering of Highly Selective Fc Domains for FcγRs.



Antibodies are glycosylated at conserved positions in their constant regions.

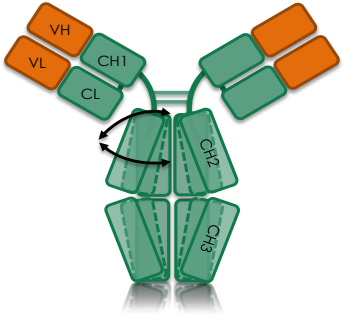
- Glycosylation (colored) at Asn-297 of the Cγ2 domains of Fc region.
- Glycosylation critical for maintaining the Fc domain in a conformation that allows its interaction with effector molecules such as FcγRs.

Antibodies produced in bacteria are aglycosylated and therefore lack FcgR-binding capacity and associated ADCC/ADCP.

- Aglycosylation retain normal pH-dependent FcRn binding and normal serum half-life in animal and humans.
- At least 5 aglycosylated antibodies are in human clinical development Phase I-III by GSK, Roche/Genentech and BMS.
- Aglycosylated antibodies can be manufactured in CHO by modifying amino acid 299.

:Rationale

- Mutations in Aglycosylated Fc may stabilize conformers that bind selectively to FcγRs, which are not accessible in Glycosylated Antibodies.



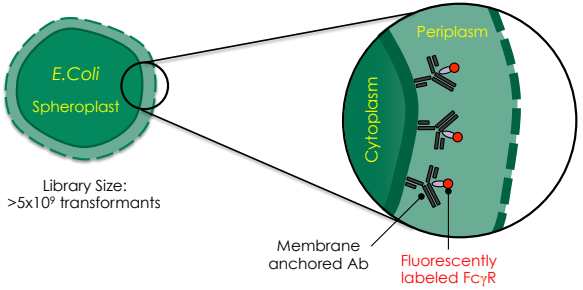
Aglycosylated Fc displays high conformational flexibility, exposing FcγR-binding sites which lie in the upper part of the CH2 domains.

Therefor they represent the ideal starting point for engineering Fc variants with novel FcγR-binding properties.

Proprietary Platform for Engineering the Fc domain of Antibodies.

- **The innovation** behind this unique technology platform stems from Prof. George Georgiou's laboratory at The University of Texas at Austin. The platform allows the identification of specific Fc engineered entities that have improved functional properties.

- **The technology** is based on a proprietary bacterial display system containing an extensive library of antibody Fc mutants. The bacterial display system allows for both positive and negative selection strategies to be applied.



The Georgiou Team has Generated a Significant Collection of Fc Domains That Augment Specific Antibody Functions.

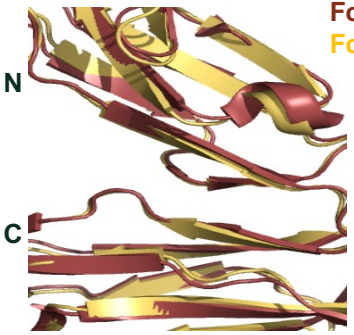
Fc Domain	Biochemical Feature
Immune Inhibitor	1. Selective binding to **FcγRIIb** with ITIM activity 2. Selective binding to **FcγRIIb** with no ITIM activity
Cytotoxic	1. Increased binding ratio **FcγRIIa / FcγRIIb** 2. Selective binding to **FcγRIIIa** 3. Binding to **FcaRI** as well as the **FcgR**
Dendritic Cell Activating	1. Selective binding **FcγRI**
Complement Activating	1. Selective binding to **C1q** 2. Increased binding to **C1q**; Binding to **activating FcRs**; no binding to inhibitory **FcγRIIb**
Half-life extension	1. pH dependent binding to **FcRn**

Biochemical Properties and Functions of Fc domains.

Fc domains selectively increase or decrease binding to individual activating or inhibitory FcγRs or C1q, with a dramatic effect on the effector function of the antibody.

Fc Domain	Fc ID	Biochemical Feature	Target Ag (Fv)	Main Optimized Function
Immune Inhibitor	**CHL13/21**	Selective binding to **FcγRIIb** with or without ITIM activity	CD40, TNF family	Increased agonistic activity for TNF-sFR Ligands; Blocks activation of immune cells
Cytotoxic	**Fc1004**	Increased binding ratio **FcγRIIa / FcγRIIb**	Her2, others	Increased ADCP by monocytes/macrophages
	TH409	Selective binding to **FcγRIIIa**	TBD	Increased ADCC. ADCP
	IgGA.3	Binding to **FcaRI** as well as the **FcgR**	Her2, CD20	Highly potent ADCC and ADCP mediated cell killing
Dendritic Cell Activating	**Fc5**	Selective binding **FcγRI**	Her2, others	Dendritic cell activation
Complement Activating	**Fc801/802**	Selective binding to **C1q**	CD20, others	Increased CDC and opsonization
	Fc805	IgG1-like binding to **activating FcγRs**; increased binding to **C1q**; no binding to inhibitory **FcγRIIB**	TBD	Increased ADCC. ADCP & CDC
Half-life Extension	**DHS**	Gylcosylated or agylcosylated binding to **FcRn**; binding to	TBD	Increased serum half-life

Engineering Antibodies with Exquisite Affinity and Selectivity for FcγRIIb – A Challenge Due to High Homology Between IIb and IIa.



FcγRIIa = Red (Activating)
FcγRIIb = Gold (Inhibitory)

96% Identity

The Georgiou technology platform enables the enrichment of Fc domain mutants with unique and selective differential binding preferences between highly homologous receptors.

"Antibody binding through its Fc domain to FcγRIIb, a receptor on B cells, blocks that activation of immune pathways, which has an important function in autoimmune, allergic and inflammatory diseases."

J of Mol Biol 2001, 309(3):737-749.

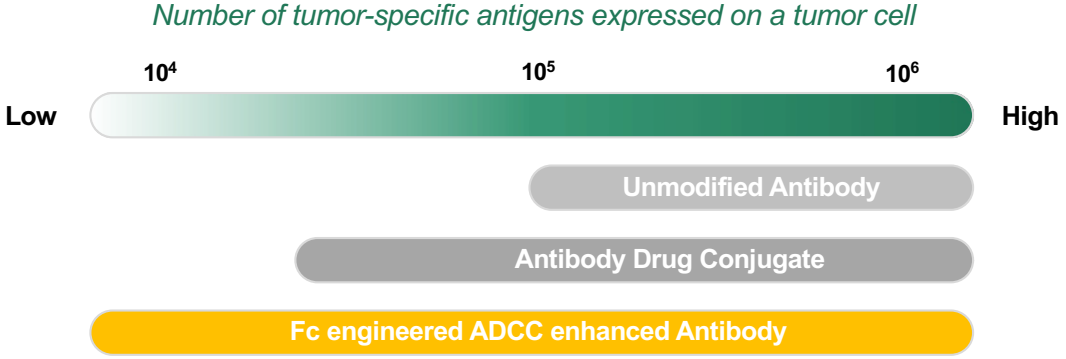
Immune Inhibitor Domains - CHL13/21:

Fc Domains with Selective Binding to FcγRIIb.

Antibody	FcγRI	FcγRIIa^{R131}	FcγRIIa^{H131}	FcγRIIb	FcγRIIIa ^{V158}	FcγRIIIa ^{V158}
WT	1	1	1	1	1	1
EF Xencor	1.2	1	>500	430	0.4	ND
V12 Chugai	0.02	0.1	2	217	0	0
CHL13 Clayton	0.04	0	0	225	0	0
CHL21 Clayton	0	0	0	48	0	0
CHL25 Clayton	0.02	0	0	14	0	0

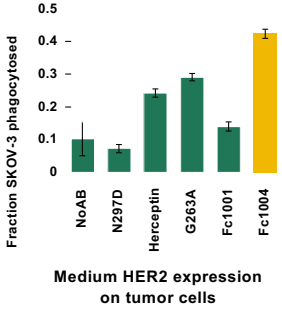
Our immune inhibition Fc domains are the only that bind exclusively to the FcgIIb receptor.
All other Fc domains (Xencor, Chugai) have enhanced binding to FcgIIb but still bind to the FcgIIa receptor as well.

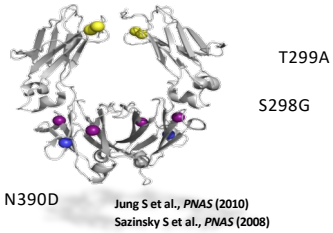
Engineering Antibodies with Exquisite Affinity and Selectivity for FcγRIIa – Increased Potency for Mid to Low Tumor Antigen Expression.

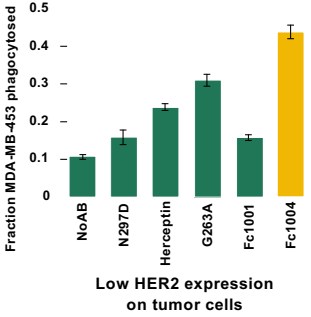


Cytotoxic Domain – Fc1004:

Fc Domain with Increased Binding to FcγRIIa.







- 160x higher affinity for activating FcγRIIa receptor (compared to Herceptin).
- 25x higher ratio of binding to FcγRIIa/ FcγRIIb (compared to Herceptin).
- 50% higher phagocytosis of HER2 medium to low expressing tumors.

Cytotoxic Domain – Fc1004:
Fc Domain with Increased Binding to FcγRIIa.

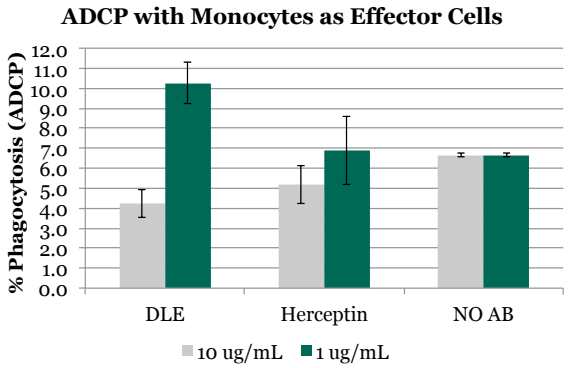
	Changes in K_D relative to Herceptin (fold)			A/I ratio	
	FcgRIIa		FcgRIIb	IIa:IIb ratio	
Fc receptor	FcgRIIa: H131	FcgRIIa: R131	FcgRIIb	IIa H131	IIa R131
Herceptin	1	1	1	1	1
Macrogenics*	3.5	1.4	2.9	1.2	0.5
Xencor	31	70	14	2.3	5.2
Clayton	**5.7**	**163**	**6.5**	**0.88**	**25**

*enhanced for FcgRIIIa binding

- 25 fold A/I ratio increase (to Herceptin)
- Allele R131 is the largest patient population
- Broader patient population available for therapy
- Wider therapeutic window
- Lower injected dose

Cytotoxic Domain – TH408/409:
Fc Domains with Selective Binding to FcγRIII.

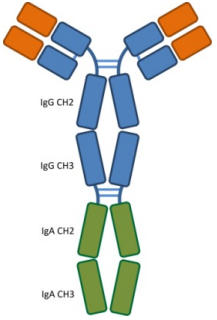
- Fc domains selected for increased binding to the FcγRIII receptor, which is expressed by Natural Killer cells that eliminate cancer cells and pathogens.
- Decreased to no binding to FcγRIII, the immune inhibitory receptor.
- Demonstration that enhanced binding FcγRIII enhances the efficacy of Herceptin and Rituxan, which are antibodies that interact with tumor-specific receptors.



Cytotoxic Domain – IgGA.3

Fcγ-Fcα fusion with binding to FcαRI and FcγRs.

- FcaRI is expressed on neutrophils (most abundant innate immune cells), monocytes, macrophages, eosinophils, dendritic cells and binds to IgA (K_D 300 nM).
- FcaRI:IgA immune complexes mediate ADCC by neutrophils and ADCP by macrophages much more potently than IgG.
- IgA difficult to manufacture.
- IgA heavily glycosylated hinge mediates rapid clearance via ASGP-R.
- Glycan heterogeneity.
- **No CDC or Fcg receptor mediated effector functions.**
- Short circulation half-life ($t_{1/2}$ 4-6 days in humans).

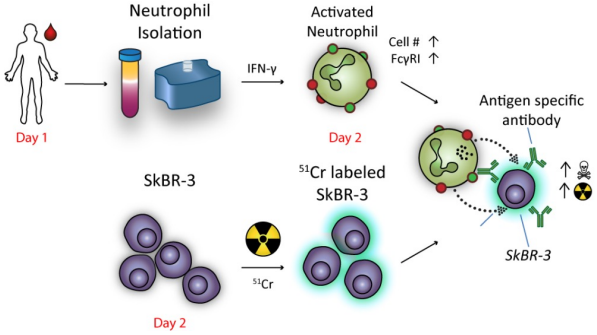


Why not an Fcγ-Fcα fusion?

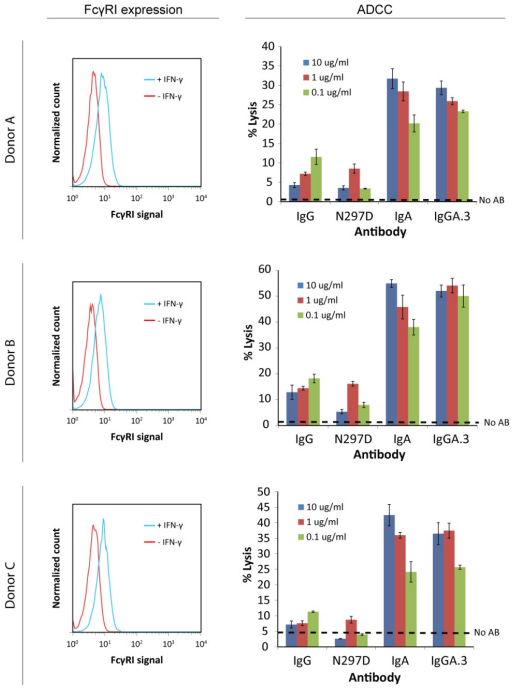
Cytotoxic Domain – IgGA.3

IgGA.3Trastuzumab Mediates Very Effective ADCC with Human Neutrophils as Effectors.

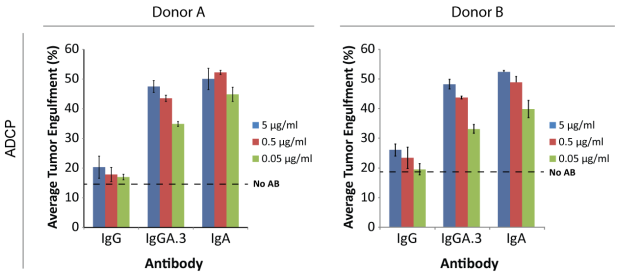
Target Cells:
Her2 High (SkBr3) breast cancer



IgG: Clinical grade Trastuzumab
N297D: Aglycosylated Trastuzumab

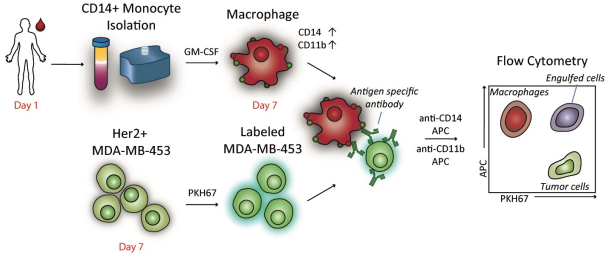


Cytotoxic Domain – IgGA.3-Trastuzumab Mediates Highly Potent ADCP of Human Macrophages.

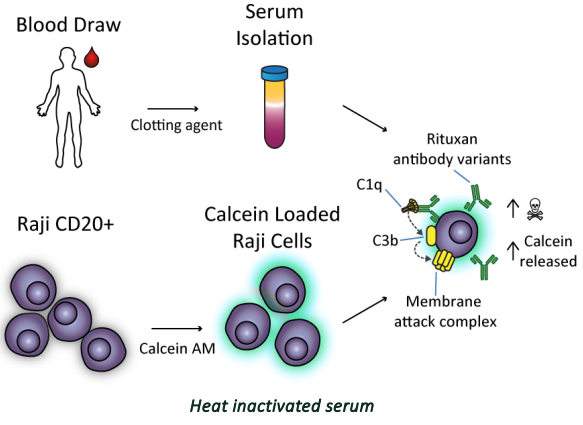


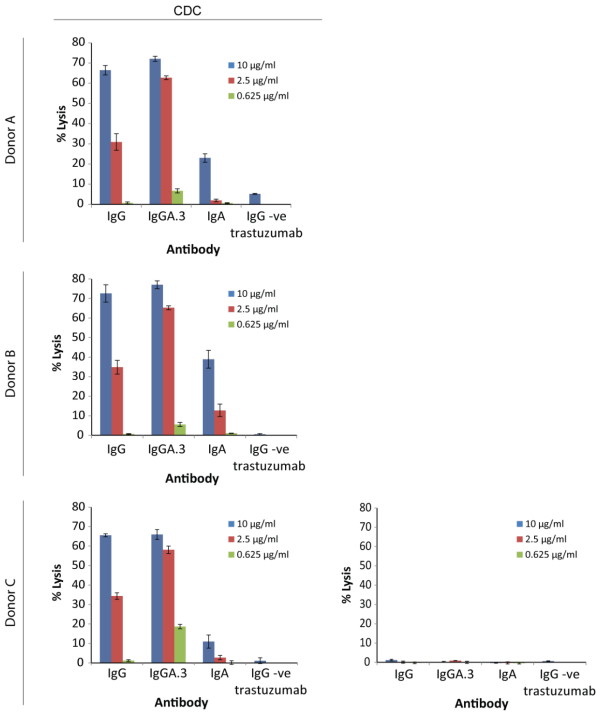
Target cells:
Her2 low/medium
(MDA-MB-453) breast cancer

IgG: Clinical Grade Trastuzumab



Cytotoxic Domain – IgGA.3 CDC of CD20+ cells by IgGA.3-Rituxumab.





Engineering Antibodies with Exquisite Affinity and Selectivity for FcγRI - Potential New Mechanism of Action.

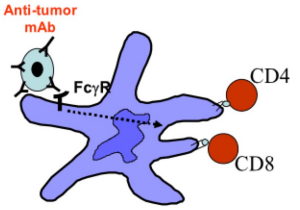
Mode of Action of IgG Antibody therapeutics:

- Direct effect (ligand/receptor binding blockade).
- Innate effect (cytotoxic activation, ADCC, CDC).
- Adaptive effect (adaptive immunity / T cell immunity).

Adaptive immunity by IgG antibodies is mediated by binding to Fcγ receptors on Dendritic Cells (DC) and is enhanced with:

- High binding to FcγRI.
- Low binding to FcγRIIb.

Targeting tumor antigens to Fcγ receptors of dendritic cells via anti-tumor monoclonal Ab enhances anti-tumor immunity



•The enhanced presentation requires presence of Fcγ receptors on DCs.

J Exp Med 195:125, 2002

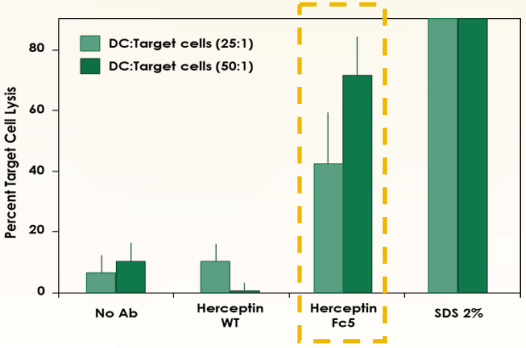
Dendritic Cell Activating Domains

- Fc5: Selective binding to FcγRI.

Antibody	FcγRI (nM)	FcγRIIa$^{R13}_{1}$ (μM)	FcγRIIa$^{H13}_{1}$ (μM)	FcγRIIb (μM)	FcγRIIIa ^{V158} (μM)	FcRn
Herceptin WT	1.5	0.31	0.12	1.30	0.20	+
Herceptin Fc5	5.7	-	-	Negligible	-	+

Only Herceptin-Fc5, not Herceptin WT can stimulate cytotoxicity with monocyte derived DCs as effector cells.

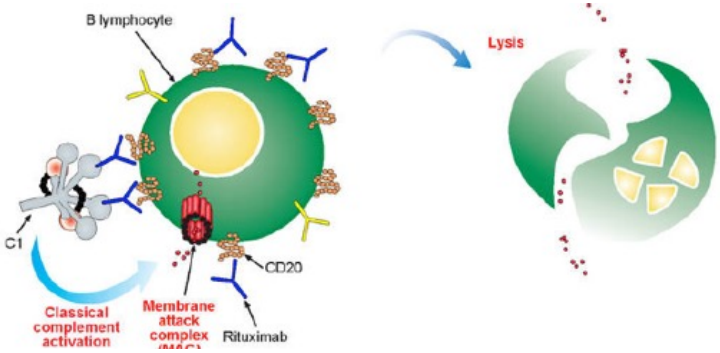
Herceptin-Fc5 Killing of Her2-Overexpressing Cells (SkBr3) by Monocyte-Derived DCs.



The selective binding to FcγRI elicits specific dendritic cell activation, and thereby induces adaptive immunity.

Engineered Antibodies with Exquisite Affinity and Selectivity for C1q.

- **Initiated by the binding of the Fc to the complement protein C1q.**
- In complement-dependent cytotoxicity, antigen-bound antibodies activate the complement system to induce the formation of membrane attack complexes on the surface of the target cell. These complexes disrupt the integrity of the cell membrane, resulting in lysis.
- Complement deposition also mediates cell cytotoxicity and phagocytosis (CDCC, CDCP).



Complement Activation Domains: Fc801, 802, 805:
Selective and Increased Binding to C1q.

	N-Gly	C1q	Monomeric FcγRIa	Dimeric FcγRIIa 131H	Dimeric FcγRIIa131R	Dimeric FcγRIIb	Dimeric FcγRIIIa158V
Native IgG1	Yes	23.0 nM	1.5 nM	120 nM	310 nM	1300	195 nM
Class I (802)	No	0.145 nM 158 fold	N.B.	N.B.	N.B.	N.B.	N.B.
	Yes	N.B.	N.B.	N.B.	N.B.	N.B.	N.B.
Class II (801)	No	0.108 nM 213 fold	N.B.	N.B.	N.B.	N.B.	N.B.
	Yes	0.385 60 fold	N.B.	N.B.	N.B.	N.B.	N.B.
Class III (805)	No	1.6 14.3 fold	13.4 0.11 fold	102 0.81 fold	127 2.4 fold	N.B.	80 3 fold

Engineered Antibodies with Exquisite Affinity and Selectivity for C1q.

	EC50 Serum only (CDC, nM)	EC50 Serum + PBMC (nM)	EC50 Serum + PMNs (nM)
Isotype IgG	-	-	-
Rituximab	17.7 ± 0.4	0.41 ± 0.04	0.21 ± 0.02
Rituximab-802	5.7 ± 0.2	1.12 ± 0.12	0.95 ± 0.03
Rituximab-801	2.9 ± 0.1	0.18 ± 0.01	0.05 ± 0.01

} Without FcgR engagement and hence no ADCC/ADCP

Killing of CD20+ Raji cells

+ 25% Serum
+ 25% Serum + PBMCs
+ 25% Serum + PMN

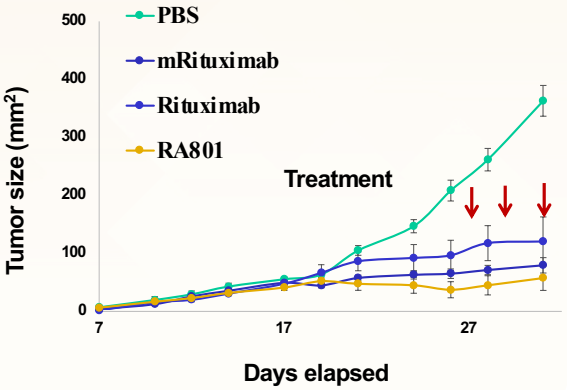
(after o/n incubation with GM-CSF)

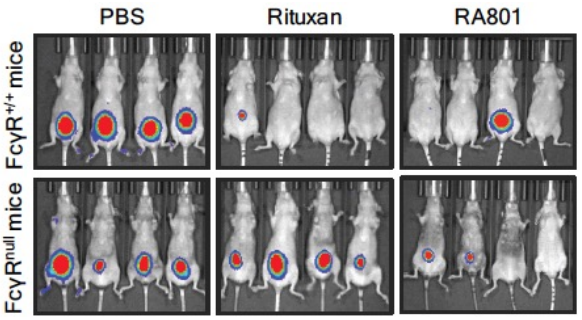
Complement activation through selective high affinity C1q binding results in markedly increased target cell clearance (also in the mouse model).

Complement Activation Domain: Fc801, 802, 805:
Selective and Increased binding to C1q.

		K_D (nM) / Increasing fold (comparing with glycosylated IgG1)						
	N-Gly	C1q	Monomeric FcγRIa	Dimeric FcγRIIa 131H	Dimeric FcγRIIa 131R	Dimeric FcγRIIb	Dimeric FcγRIIIa 158V	Dimeric FcγRIIIa 158F
IgG1	No	n.b.	382	n.b.	n.b.	n.b.	n.b.	n.b.
	Yes	23.0	1.5	120	310	1300	195	390
Class I RA801	No	0.108 (213x)	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
	Yes	0.385 (60x)	648 (0.002x)	n.b.	n.b.	n.b.	n.b.	n.b.
Class II RA802	No	0.145 (158x)	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
	Yes	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.

RA801 – Rituximab engineered with high selectivity for C1q and no binding to FcγRs shows increased tumor killing.





RA805: Aglycosylated Antibody that recapitulates the killing mechanisms exhibited by "authentic" glycosylated human therapeutics with no binding to the inhibitory receptor.

	K_D (nM)						
	C1q	FcγRIa	Dimeric FcγRIIa $_{H131}$	Dimeric FcγRIIa $_{R131}$	Dimeric FcγRIIb	Dimeric FcγRIIIa $_{V158}$	Dimeric FcγRIIIa $_{F158}$
IgG1	23.0	1.5	120	310	1300	195	390
RA805	1.60	13.4	102	127	No Binding	79.8	390

Advantages: Homogeneity, avoidance of side effects due to glycan interactions.

CLFR:430	Title: ENGINEERED IMMUNOGLOBULIN FC POLYPEPTIDES DISPLAYING IMPROVED COMPLEMENT ACTIVATION
	Summary: Provided are Fc domains that bind C1q with an increased affinity relative to its glycosylated Fc counterparts. Data supports the idea that these Fc domains are quite specific for C1q. Normally, the IgG Fc domain binds in addition to C1q to a variety of Fc receptor proteins on the surface of leukocytes. The binding of Fc receptors with the Fc domain has a variety of immunological effects that can aid the clearance of pathogenic cells. However, there are instances in which engagement of Fc receptors is detrimental, for example when the binding of antibody molecules on the surface of cancer cells promotes Fc receptors on a neighboring cell mediate the clustering of the cancer cell surface molecules, in turn signaling to the cancer cell to proliferate. Fc domains that can promote cancer cell killing by CDC with reduced binding to Fc receptors may significantly enhance the therapeutic potency of certain therapeutic antibodies.

Client Reference No.:

Chan Han LEE

George GEORGIOU

Country	Case	Status	Application No.	Filing Date	Patent No.	Granted
United States of America	Utility - ORG	Issued	15/019,395	Feb 9, 2016	10457737	Oct 29, 2019
Australia	Utility - NSPCT	Issued	2016219511	Feb 9, 2016	2016219511	Feb 25, 2021
Canada	Utility - NSPCT	Pending	2,976,236	Feb 9, 2016		
European Patent Office	Utility - NSPCT	Published	16706710.7	Feb 9, 2016		
Japan	Utility - NSPCT	Issued	2017-541808	Feb 9, 2016	6724023	Jun 26, 2020
Hong Kong	Utility - ORG	Published	18106356.6	May 16, 2018		
United States of America	Utility - CON	Issued	16/573,655	Sep 17, 2019	11332538	May 17, 2022
Japan	Utility - DIV	Issued	2020-108886	Feb 9, 2016	6926285	Aug 6, 2021

Competition: Fc Engineered / Fc Platform Landscape.

Fc engineered products

Company name	Product	Target
Eli Lilly/Mentrik	AME-133c/Ocaratuzumab	CD20
Macrogenics	MGAH22	HER2
Xencor/Morphosys	MOR208	CD19
Xencor/Pfizer	PF-04605412	a5b1 integrin

Fc engineering technology platforms

Company name	Platform	Composition of matter
Xencor	Antibody Fc's by Design (in silico)	Glycosylated antibody
Macrogenics	Antibody Fc's by Design/Yeast display	Glycosylated antibody

Our technology is differentiated from the competition:

- Fc domains have absolute selectivity and and/or enhanced binding to one or more desired FcRgs as required for optimal therapeutic function.
- Our antibodies are aglycosylated and therefore are in a unique IP position.
- Advantageous and extensive IP portfolio.

Summary.

Menu of engineered aglcyosylated Fc domains that augment specific antibody function:

Fc Domain	Biochemical Feature
Immune Inhibitor	1. Selective binding to **FcγRIIb** with ITIM activity 2. Selective binding to **FcγRIIb** with no ITIM activity
Cytotoxic	1. Increased binding ratio **FcγRIIa / FcγRIIb** 2. Selective binding to **FcγRIIIa** 3. Binding to **FcaRI** as well as the **FcγRs**
Dendritic Cell Activating	1. Selective binding **FcγRI**
Complement Activating	1. Selective binding to **C1q** 2. Increased binding to **C1q**; Binding to **activating FcRs**; no binding to inhibitory **FcγRIIB**
Hal-life extension	1. Binding to **FcRn** 2. Glycosylated or agylcosylated

Advantages

- Aglycosylated antibodies – simple bioprocessing.
- Advantageous and distinct IP position.

Applications

- Biobetters with a safer and more potent therapeutic profile.
- Rescue of preclinical and clinical mAb drug assets.

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Thank you for your attention