NASDC
NCI Awardee Skills Development Consortium

Fc Receptors: Drivers of Immunity

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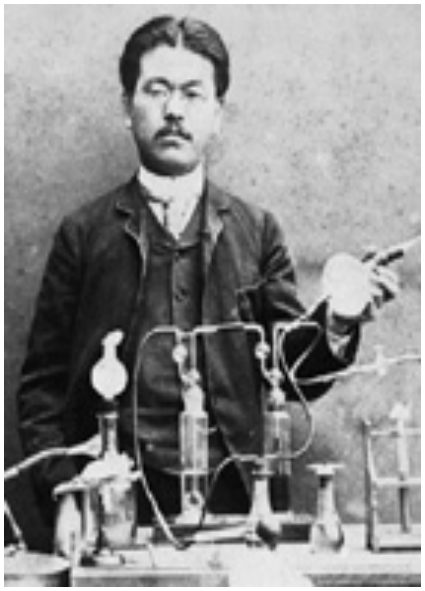
Discovery of antibodies 1890

Emil von Behring
Diphtheria toxin

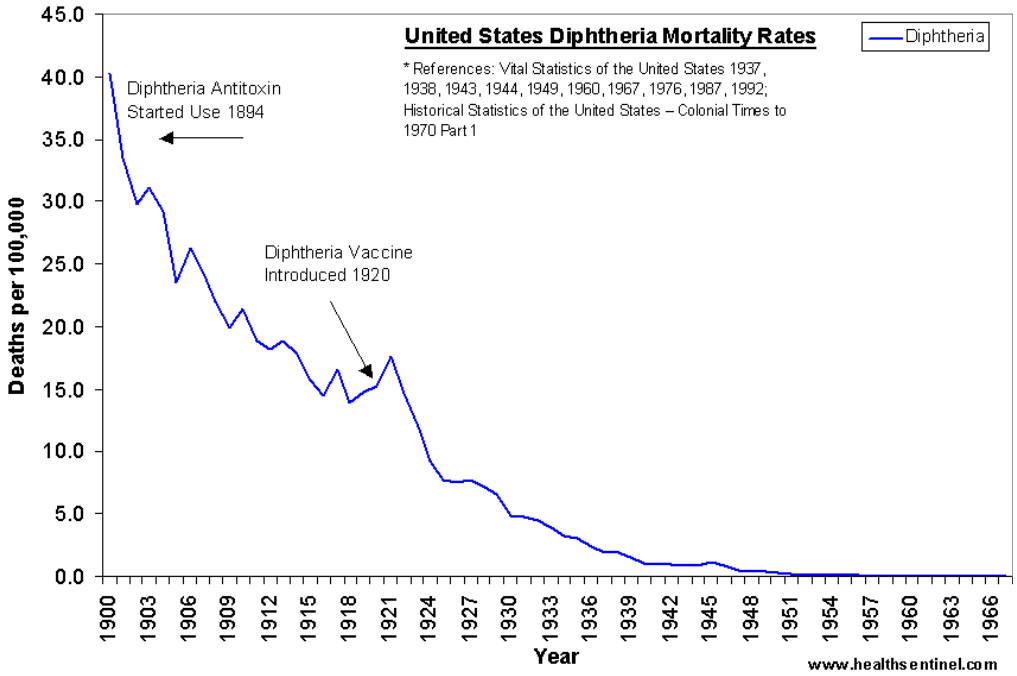


Shibasaburo Kitasato
Tetanus toxin

"The immunity of rabbits and mice depends on the ability of the cell-free blood fluid to render harmless the toxic substance which the bacillus produces"

Antibodies are immunotherapeutics 1894

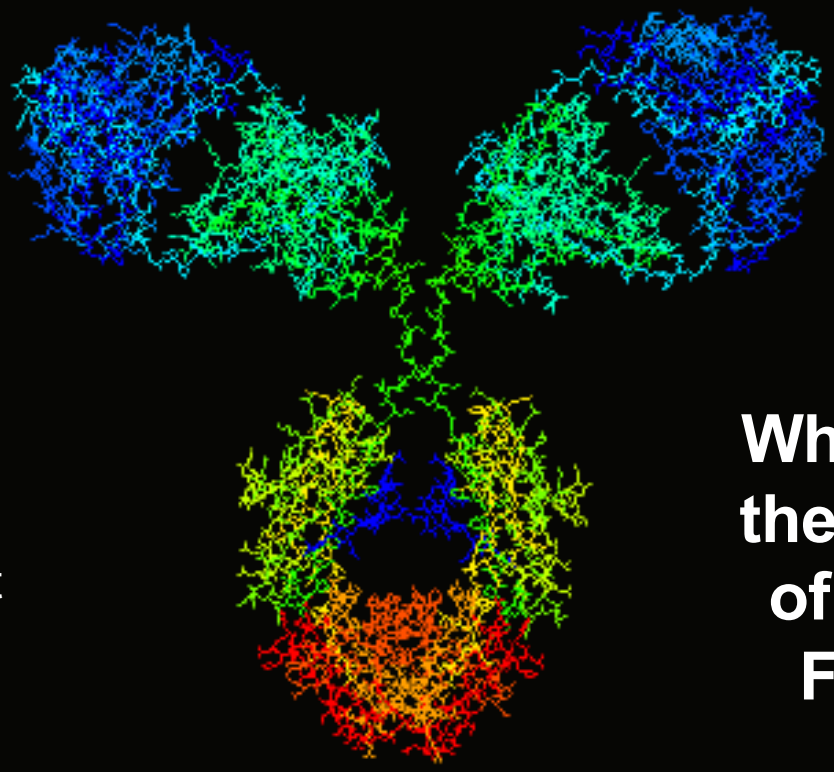
von Behring and Kitasato, 1890



Mortality rate decreased by >70% in a decade after introduction of anti-toxin

The classical view of an antibody

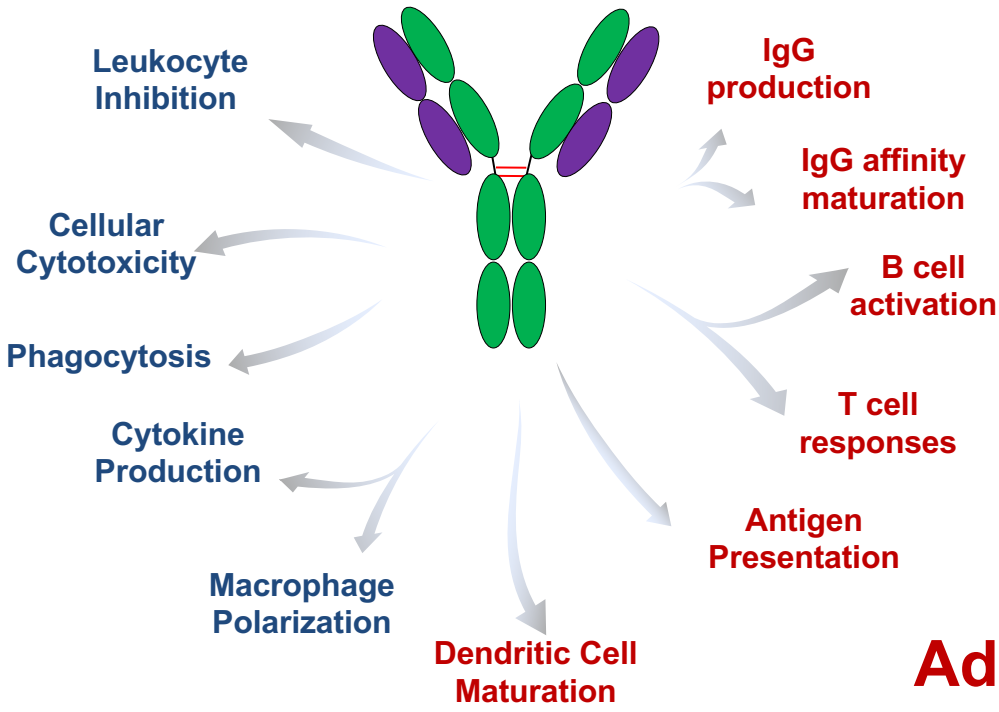
Fab variable



Fc constant

What is the role of the Fc?

The Fc contributes to diverse immune reactions



Innate

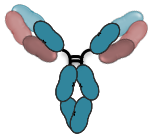
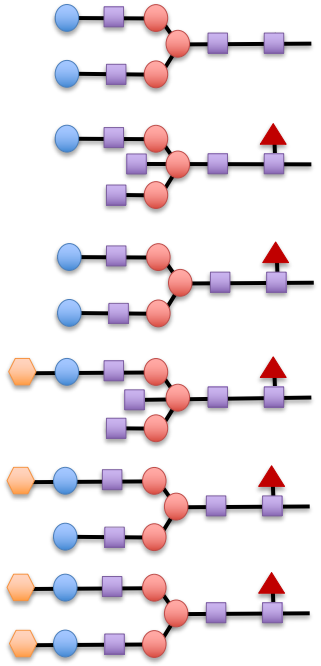
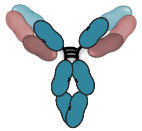
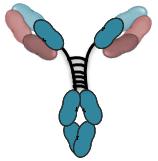
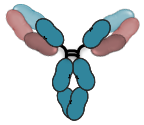
Adaptive

How can an invariant Fc mediate diverse biological functions?

The Fc is structurally diverse

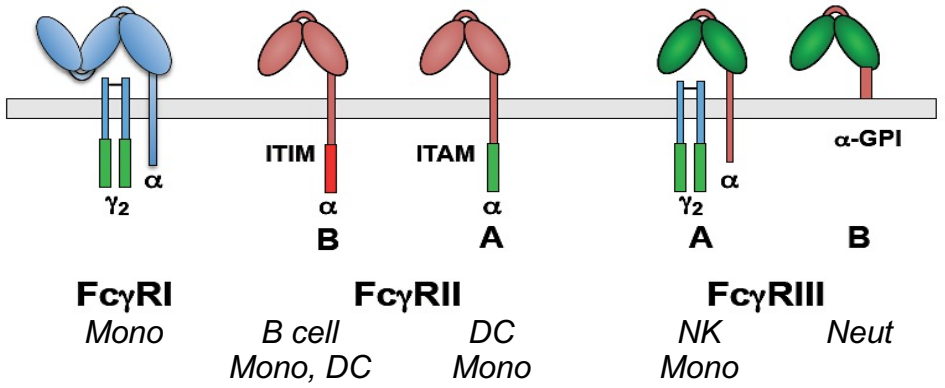
Generating diversity in the Fc

>10^3 different Fc variants for any one V region

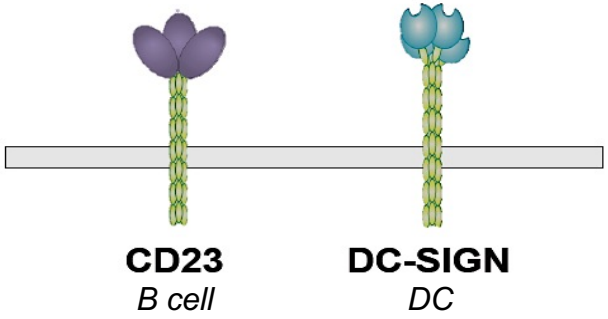
IgG subclasses		Gm Allotypes	Fc glycan
	IgG1	5	
	IgG2	4	
	IgG3	15	
	IgG4	4	
4		30	30

Diversity of Fcγ Receptors

Type I Fc Receptors (IgSF)



Type II Fc Receptors (C-type lectins)



How do Fc and FcR heterogeneity result in functional diversity?

Fc subclass and glycan composition result in selective FcR binding

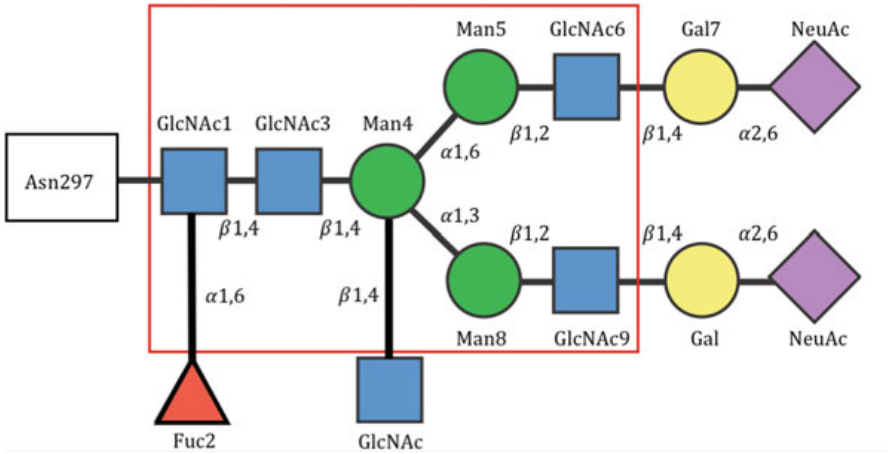
Subclasses display preferential Type I binding

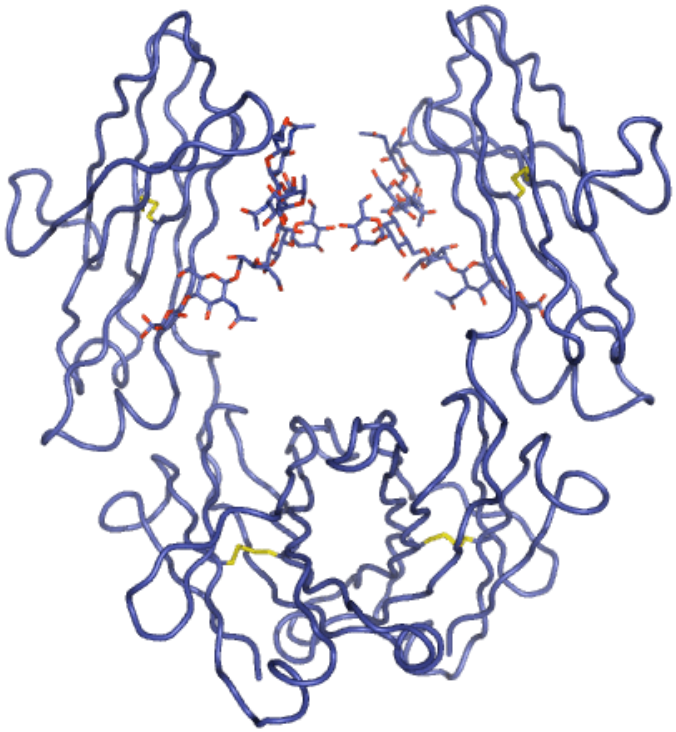
	FcγRI	FcγRIIb	FcγRIII	FcγRIV	A/I	
IgG1	n.b.	3.3	0.3	n.b.	**0.1**	inhibitory
IgG2a	160	0.4	0.7	29	**69**	activation
IgG2b	n.b.	2.2	0.6	17	**7**	
IgG3	n.b.	n.b.	n.b.	n.b.	-	

Ka (x10^6) n.b.: no binding

TA99-IgG1 **TA99-IgG2a** **TA99-IgG2b**

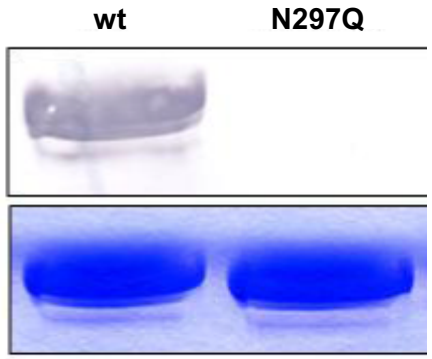
The glycan modulates the Fc structure





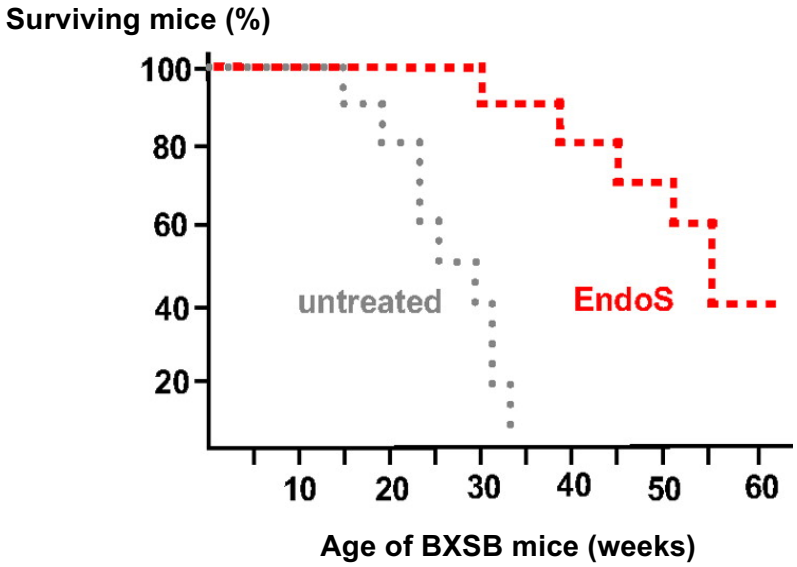
Huber (1976) Nature

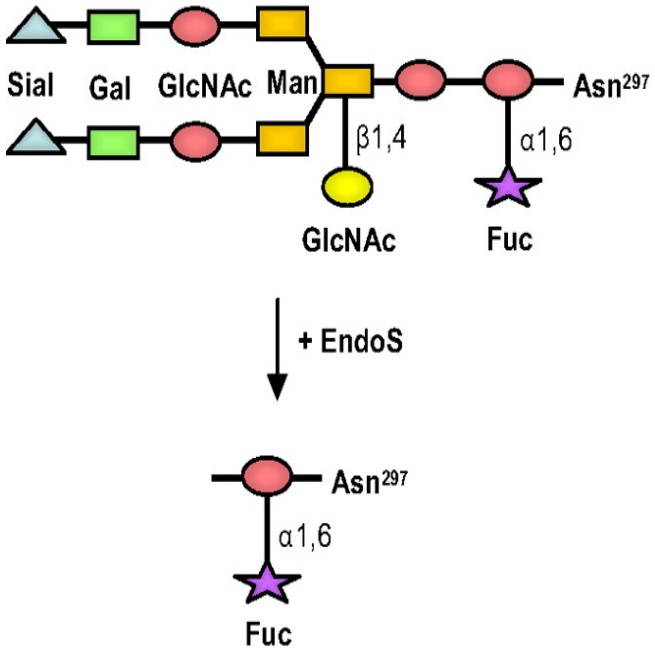
Aglycosylated Fc do not bind Type I FcRs



Variant	RIIA^{131H}	RIIA^{131R}	RIIB	RIIIA^{FF}	RIIIA^{FV}
wt	5.5x10^{-6}	5.0x10^{-6}	9.8x10^{-6}	1.3x10^{-5}	4.6x10^{-6}
N297Q	n.b.	n.b.	n.b.	n.b.	n.b.

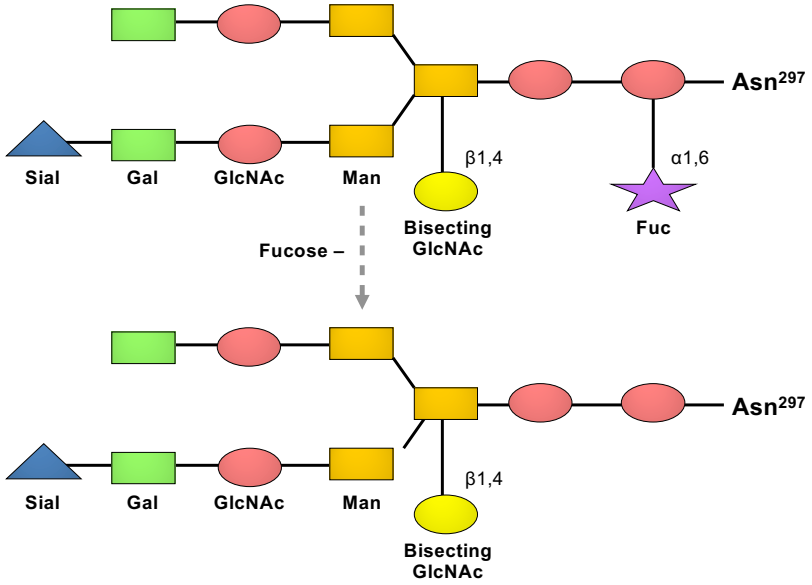
S. Pyogenes EndoS abrogates IgG activity by deglycosylation of the Fc





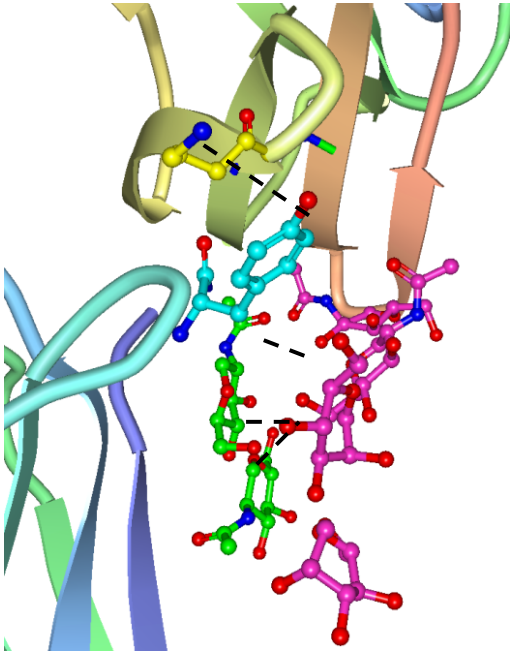
Albert H., et al. 2008

Fucose modulates Fc cytotoxicity by enhancing FcRIIIA – Fc glycan interactions



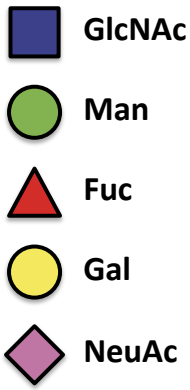
A/I = 7

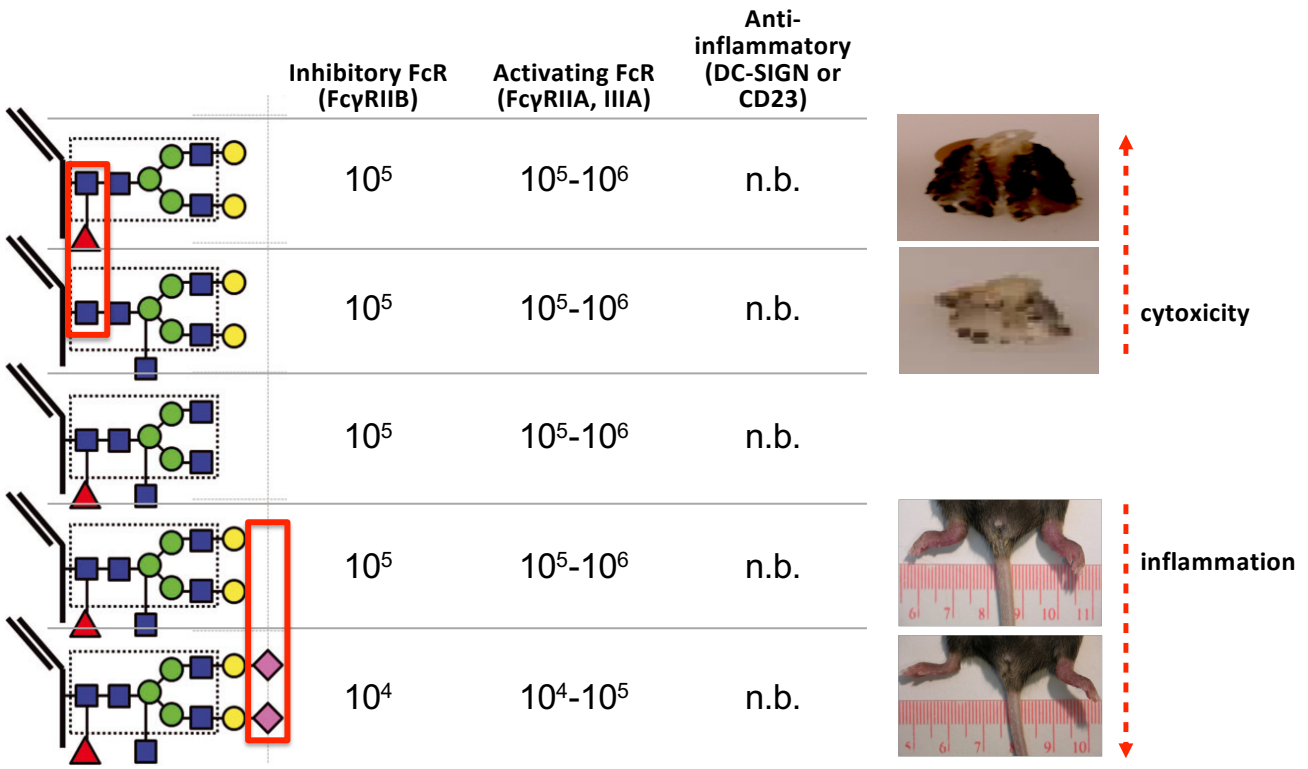
A/I = 20



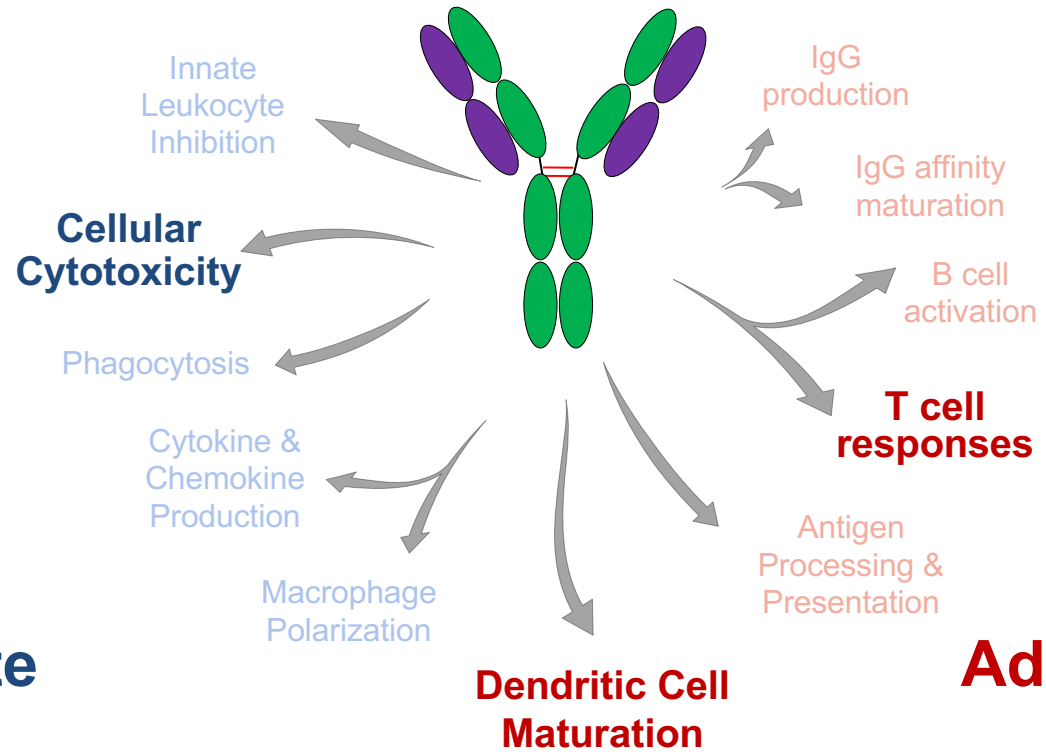
Ferrara (2011) PNAS

Fc glycosylation regulates Fc-FcR interactions



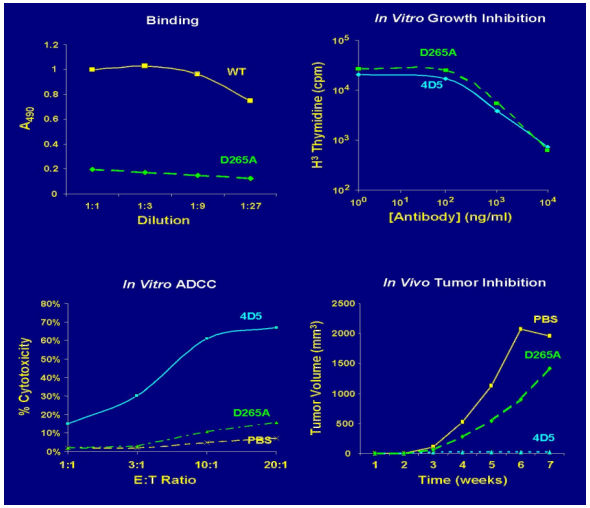


The Fc contributes to diverse immune reactions



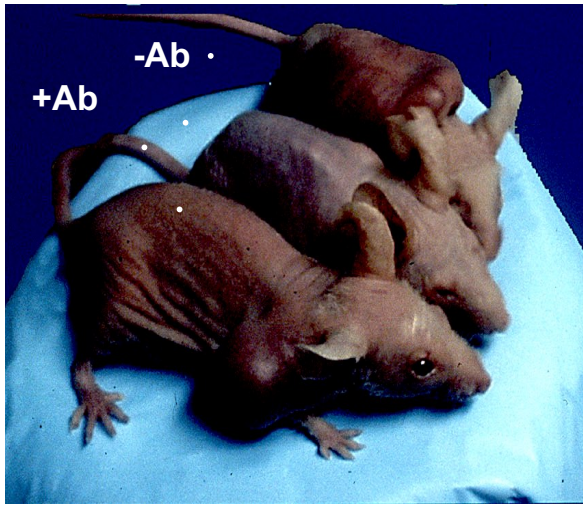
FcγRs are required for the therapeutic effects of anti-tumor antibodies

Breast CA



Herceptin

Lymphoma



WT

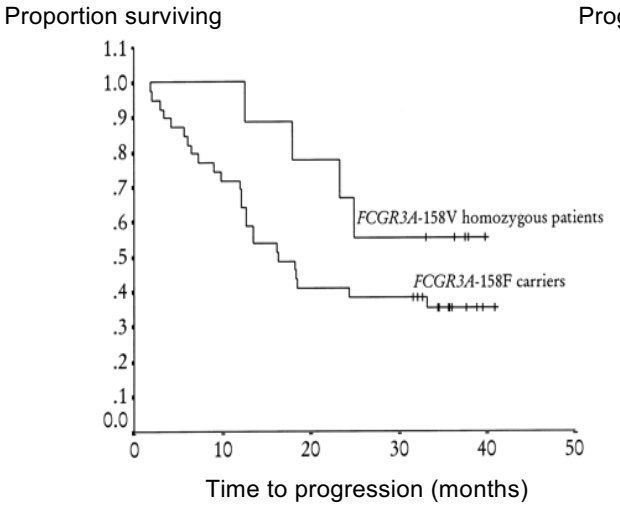
WT

$\gamma^{-/-}$

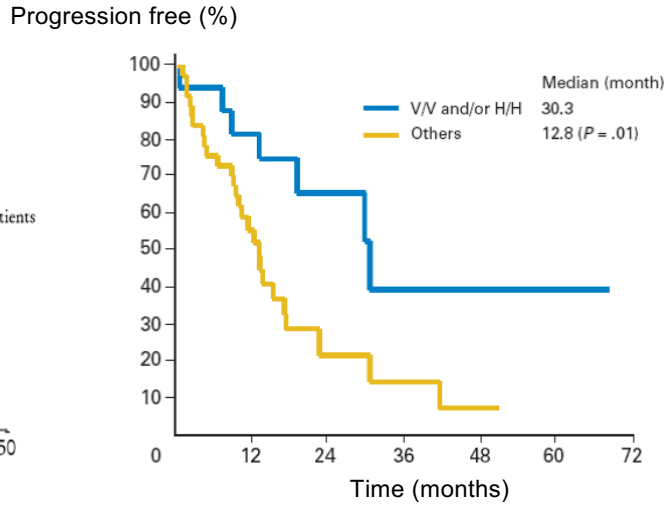
Rituxan

FcRIIIA alleles with increased IgG affinity correlate with improved clinical outcome

Anti-CD20[1]

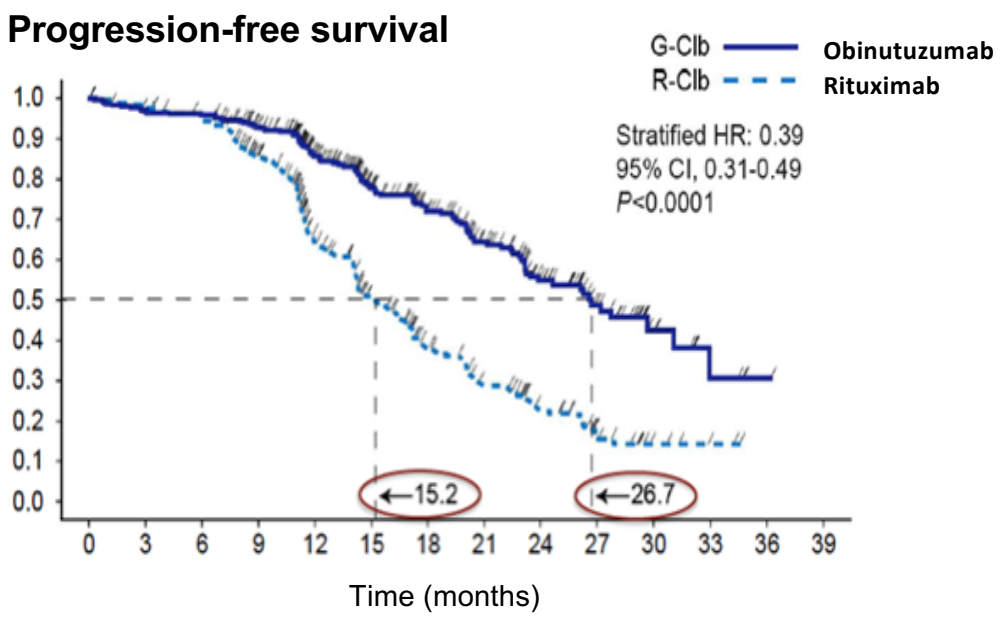


Anti-Her2neu[2]



1. Cartron, et al., 2002; Weng and Levy, 2003
2. Musolino, et al., 2008

Enhancing FcRIIIA binding of an anti-CD20 antibody improves survival in CLL



Goede (2014), NEJM

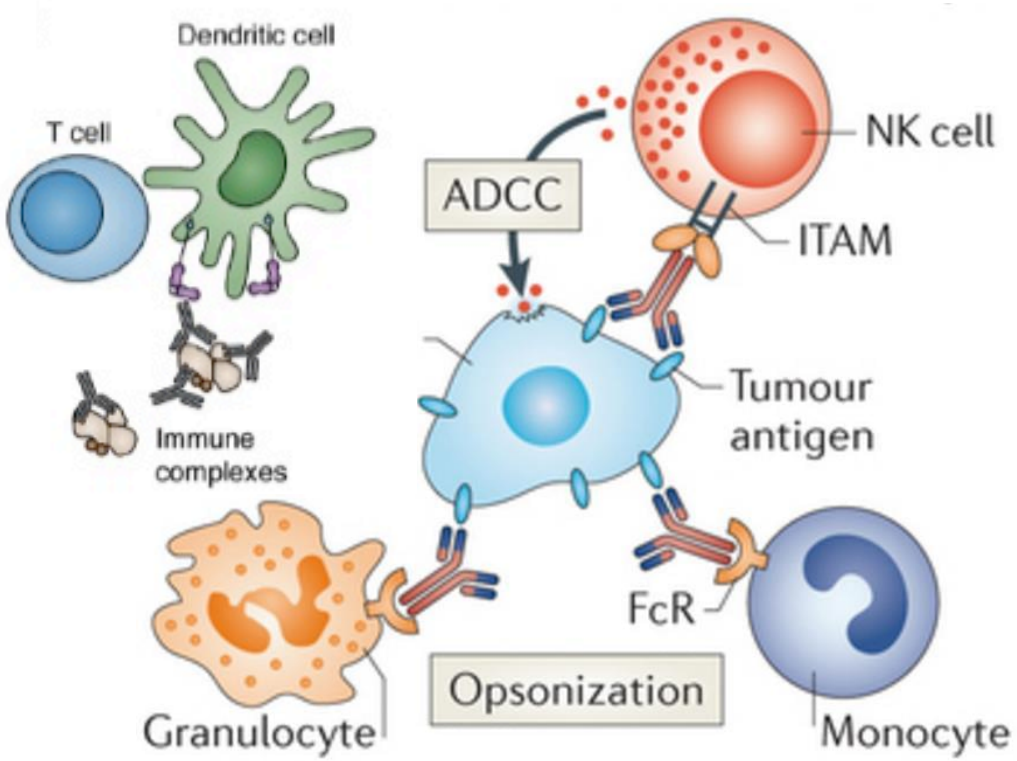
Fc-optimized anti-tumor antibodies

Optimized for cytotoxicity by enhancing FcRIIIA binding

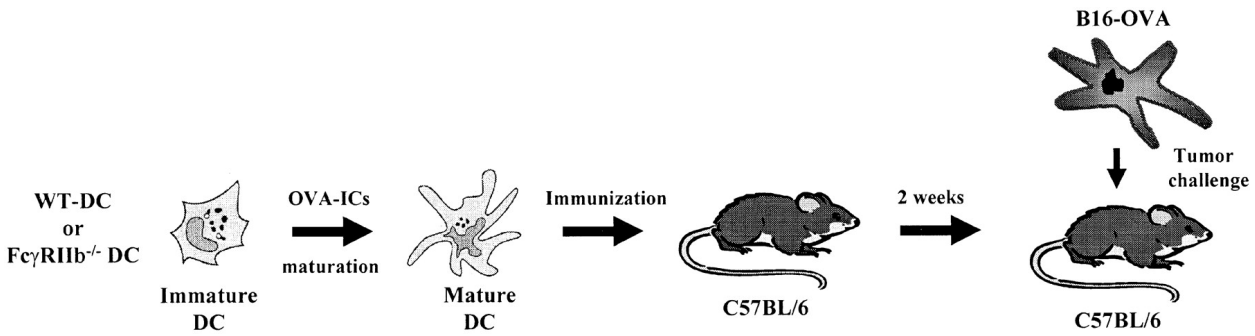
Antibody (company)	Antigen	Engineering Strategy	Indication	Status	ClinicalTrials.gov Identifier
Ublituximab (TG Theraputics)	CD20	Low fucose (host: YB2/0; EMABling© technology)	CLL, DLBCL	phase III	NCT02612311, NCT02793583
Margetuximab (MacroGenics)	HER2	AA substitutions: L235V/F243L/R292P/Y300L/P396L	breast cancer	phase III	NCT02492711
Talacotuzumab, JNJ-56022473 (CSL, Janssen Biotech)	CD123	AA substitutions: 239D/I332E (Xmab© technology)	AML	phase II/III	NCT02472145
MOR208, Xmab-5574 (Xencor, MorphoSys)	CD19	AA substitutions: S239D/I332E (Xmab© technology)	DLBCL	phase II/III	NCT02763319
Inebilizumab, MEDI-551 (MedImmune LLC)	CD19	Non-fucosylated (host: FUT8-deficient CHO; Potelligent© technology)	DLBCL, CLL	phase II	NCT01453205, NCT01466153
BI 836826 (Boehringer Ingelheim)	CD37	AA substitutions: S239D/I332E (Xmab© technology)	CLL	phase II	NCT02624492
CetuGEX (Glycotope GmbH)	EGFR	Related fucosylation, optimized galactosylation and degree of breaching; GEX™ platform)	squamous cell carcinoma of the neck	phase II	NCT02052960
PankoMab-GEX (Glycotope GmbH)	TA-MUCI	Related fucosylation, optimized galactosylation and degree of breaching; GEX™ platform	Ovarian cancer	phase II	NCT01899599
Lumretuzumab (Roche)	HER3	Low fucose (GNT III over-expression; GlycoMab© technology)	NSCLC	phase II	NCT02204345

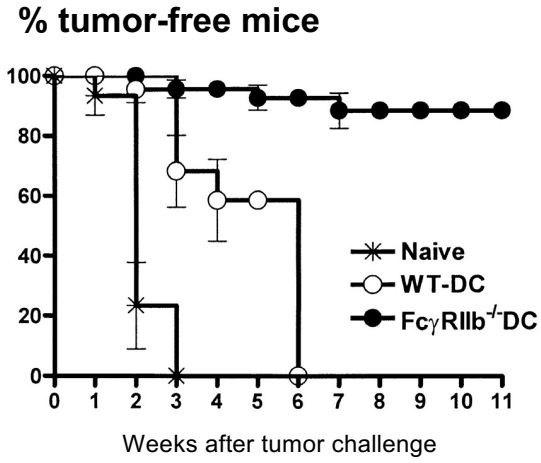
Tumor killing by Fc Receptors

Can antibodies also prime an adaptive response?



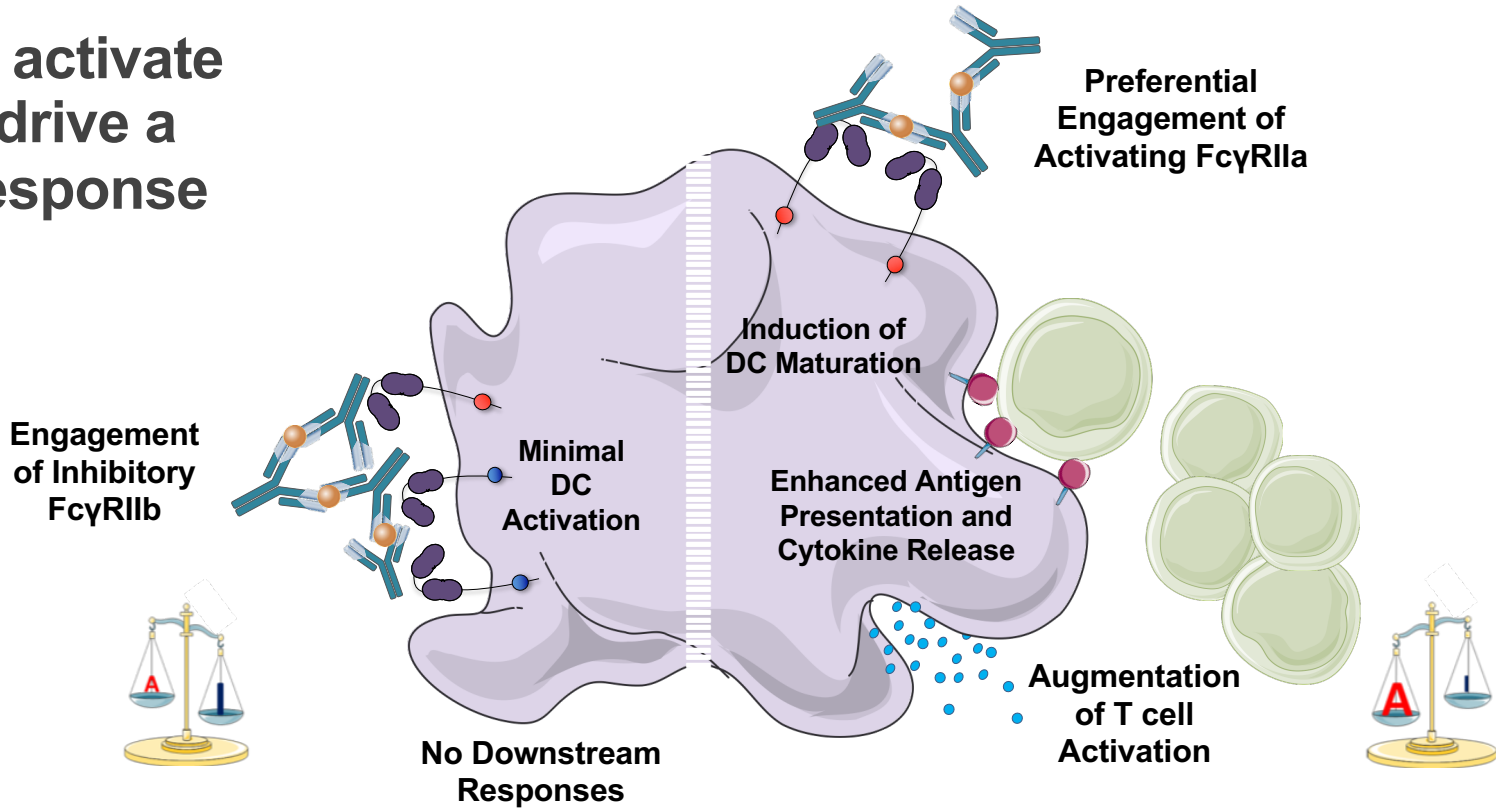
Immune complexes engage FcγRs on APCs driving cellular immune responses





Kalergis et al. JEM, 2002

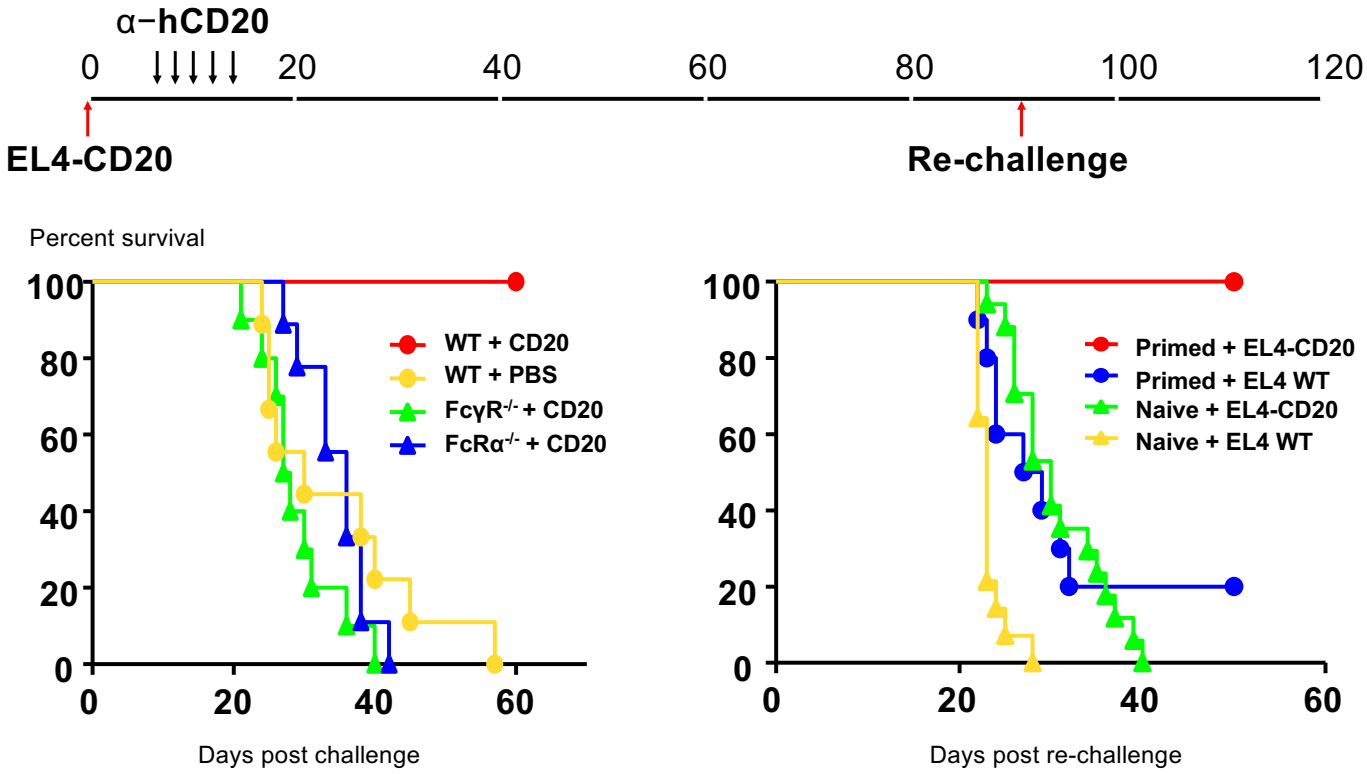
ICs can activate DCs to drive a T cell response



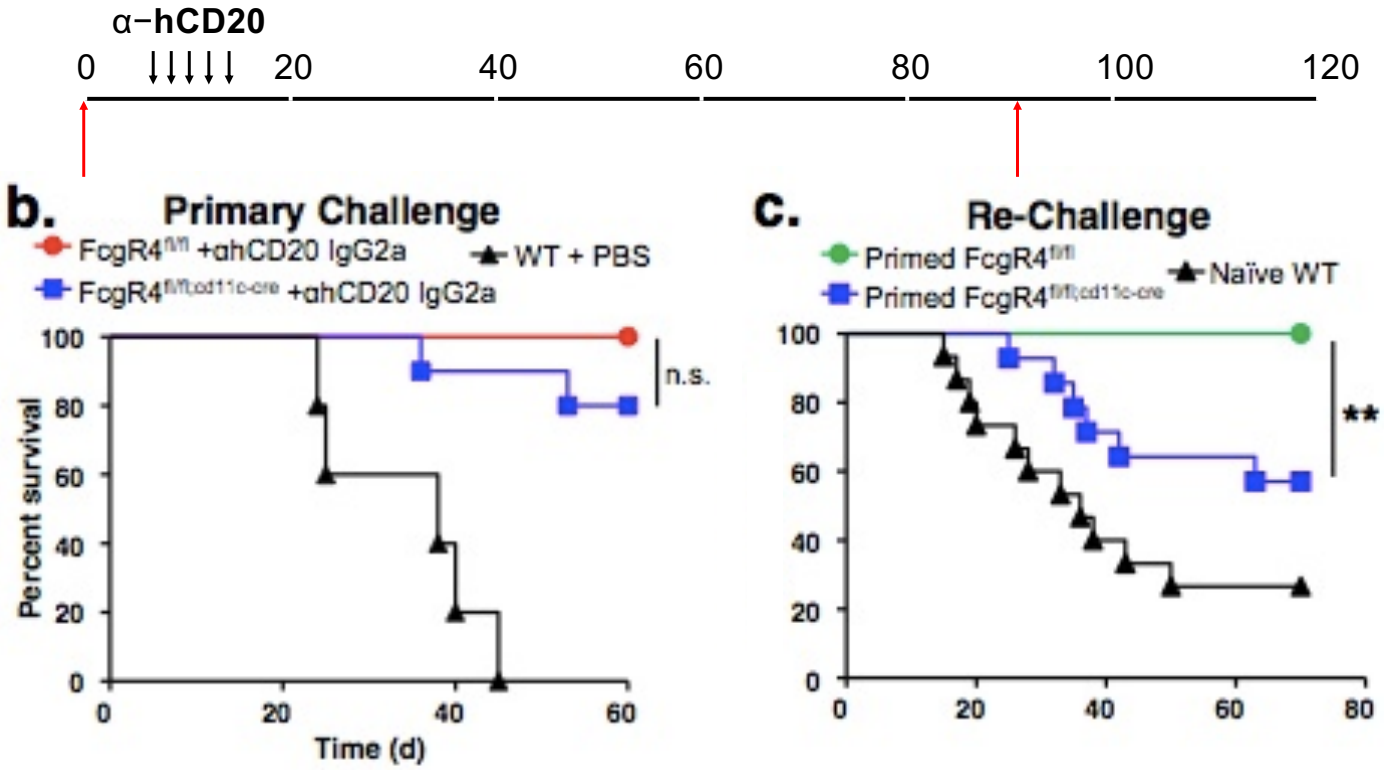
Can long-term survival be improved?

Can immune complexes prime a long-term anti-tumor cellular immune response?

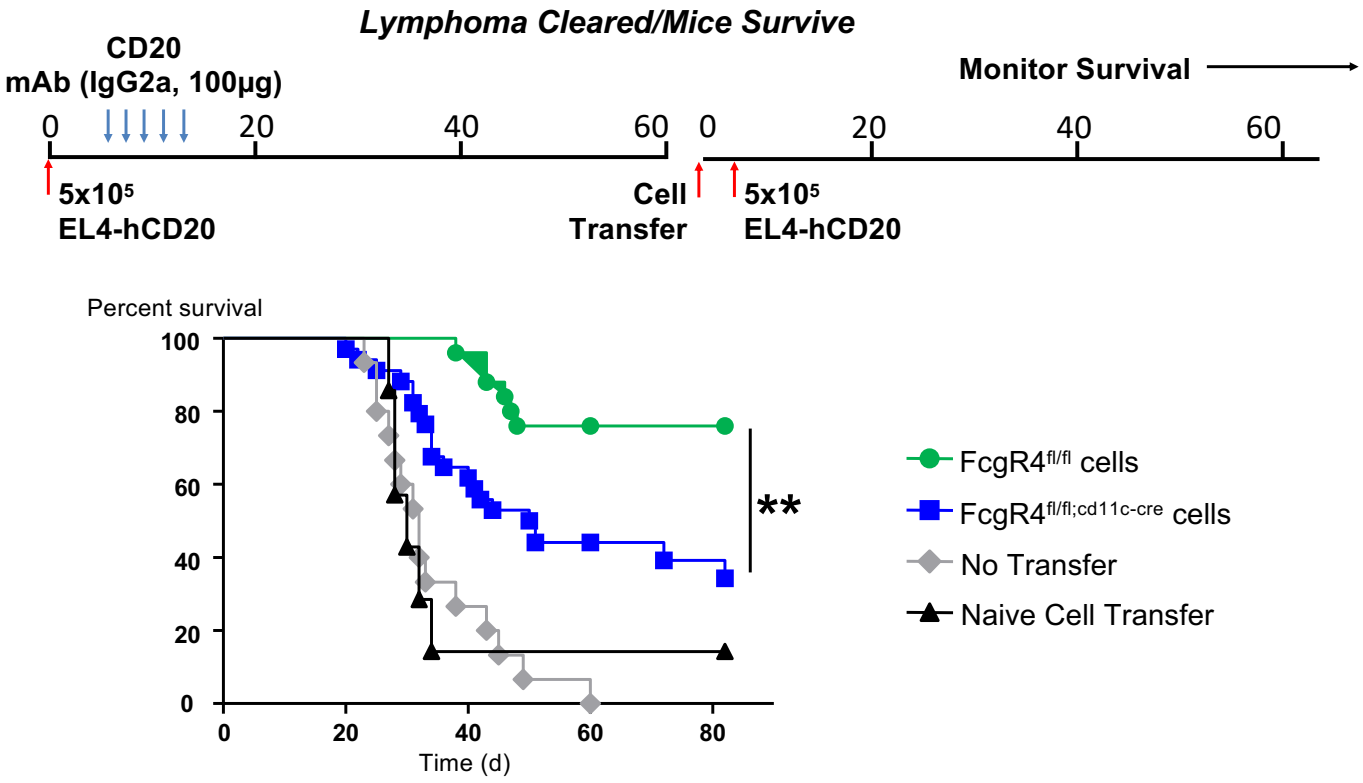
Anti-hCD20 induces a vaccinal response



DC expression of FcR is required for vaccinal response



Adoptive transfer of T cells from primed mice protects against re-challenge



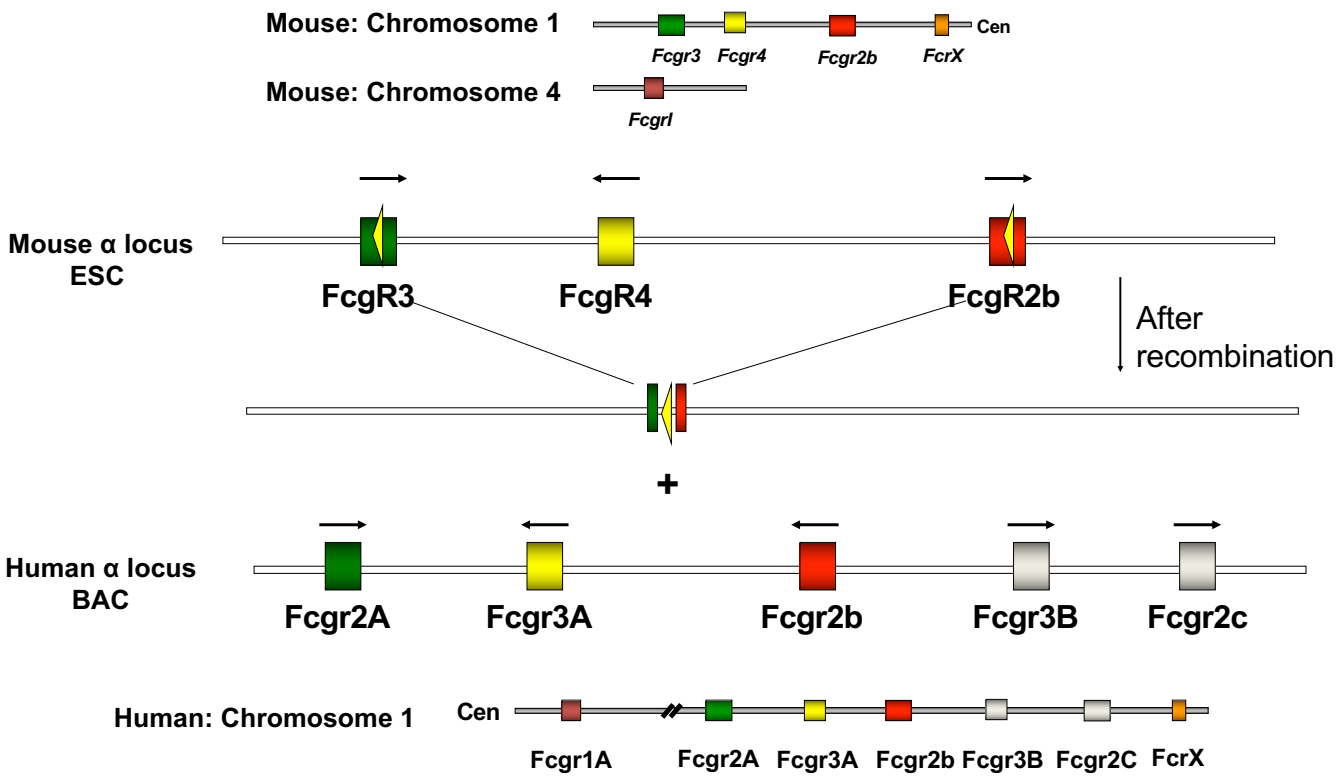
Comparison of hFcR expression in mice and man

	Human					Mouse				
	RI	RIIA	RIIB	RIIIA	RIIIB	RI	RIIB	RIII	RIV	RIIA IIB RI
B cells	-	-	+	-	-	-	+	-	-	-
T cells	-	-	-	-	-	-	-	-	-	-
MΦ	+	+	+	+	-	+	+	+	+	-
Neutrophils	+	+	+	-	+	+	+	+	+	-
DCs	-	+	+	-	-	+	+	+	+	-
NK cells	-	-	-	+	-	-	-	+	-	-

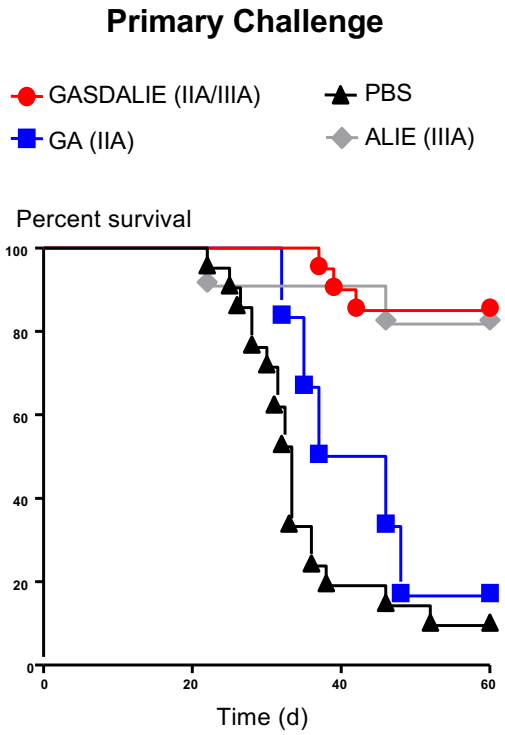
vaccinal

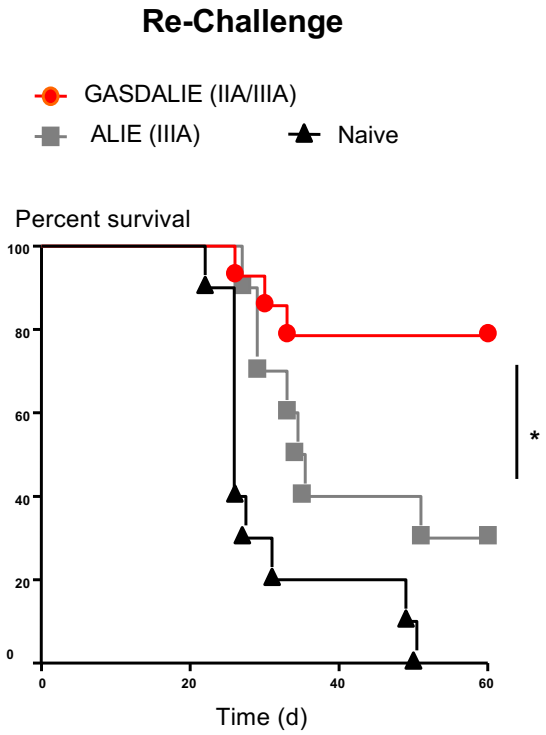
cytotoxicity

FcR Humanization

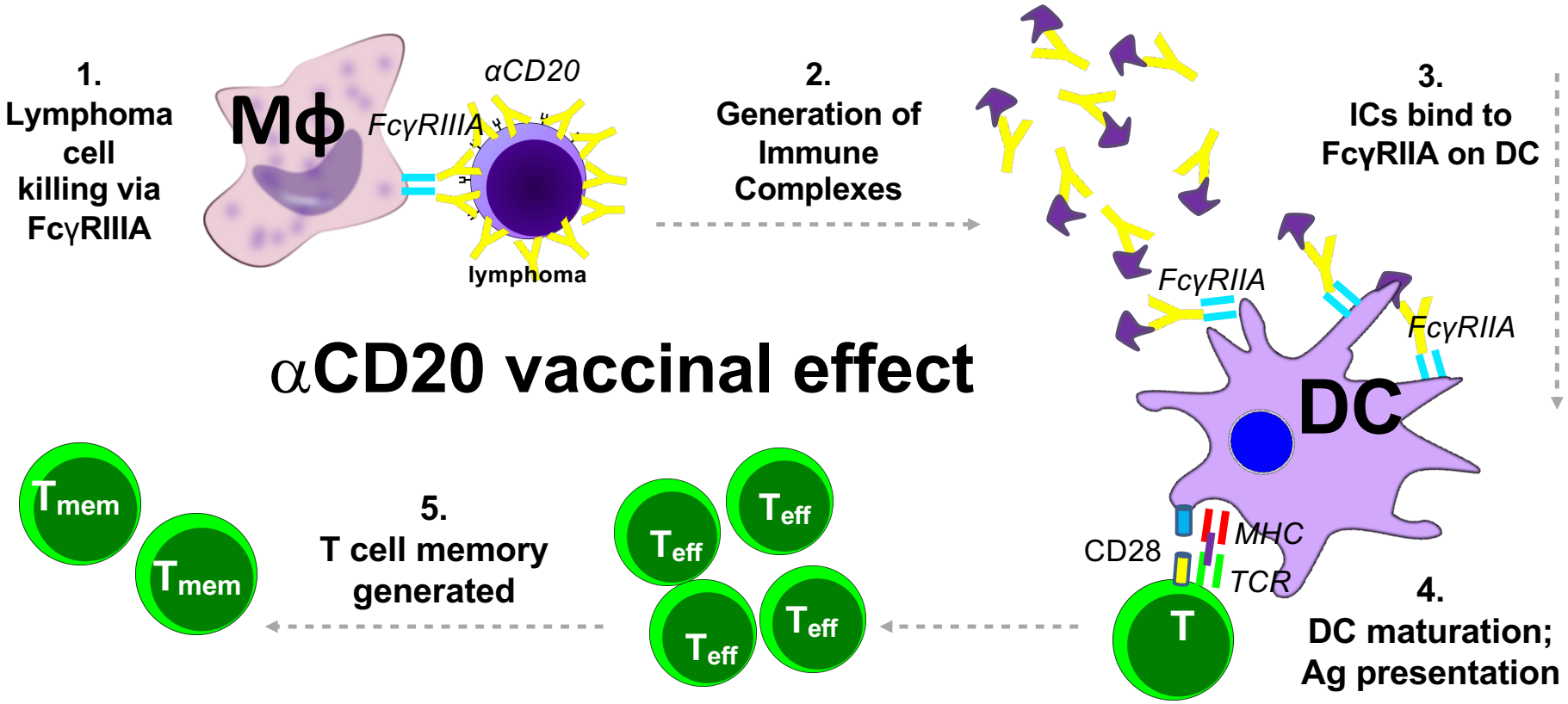


Differential hFcR engagement is required for a vaccinal effect





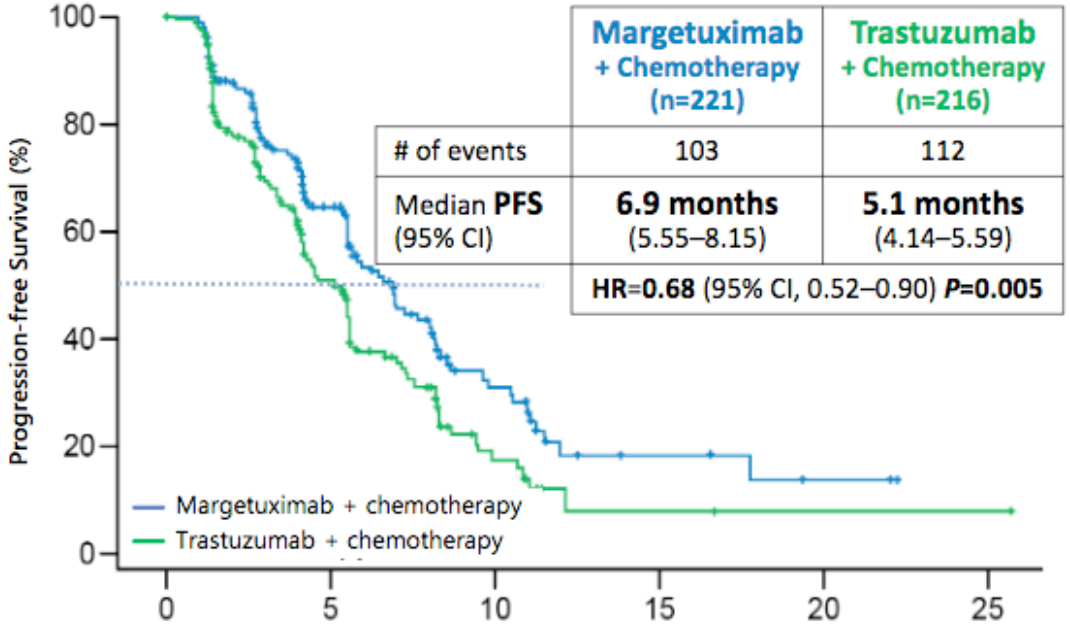
αCD20 vaccinal effect



Margetuximab, Herceptin with Fc engineered for enhanced FcR A/I (Approved 12/20/20)

Induction of Her2+ CD8 T cells in treated patients

32% Risk Reduction of Disease Progression



	Margetuximab + Chemotherapy (n=221)	Trastuzumab + Chemotherapy (n=216)
# of events	103	112
Median **PFS** (95% CI)	**6.9 months** (5.55–8.15)	**5.1 months** (4.14–5.59)
HR=0.68 (95% CI, 0.52–0.90) ***P*=0.005**		

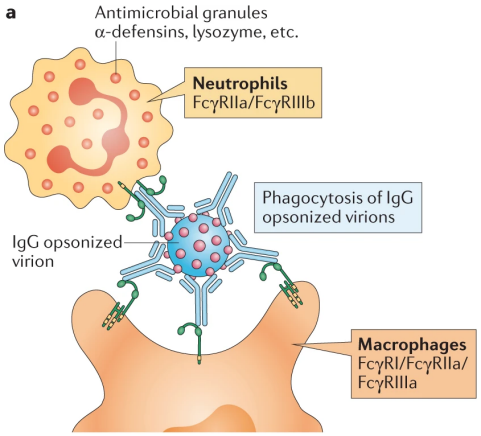
Macrogenics (Presented ASCO 2019)

How general is the mechanism?

Is it relevant to pathogen clearance?

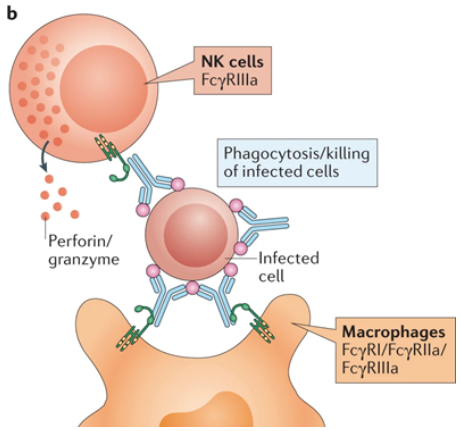
Innate and adaptive antiviral immune mechanisms modulated by Fc-FcR interactions

Clearance of IgG-opsonized virions from circulation



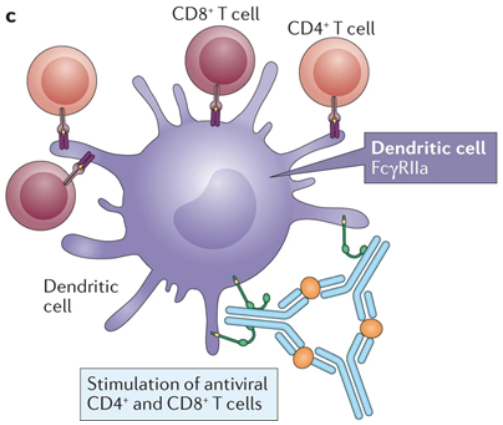
Bournazos 2014 Cell

Elimination of infected cells



Lu 2016 Science

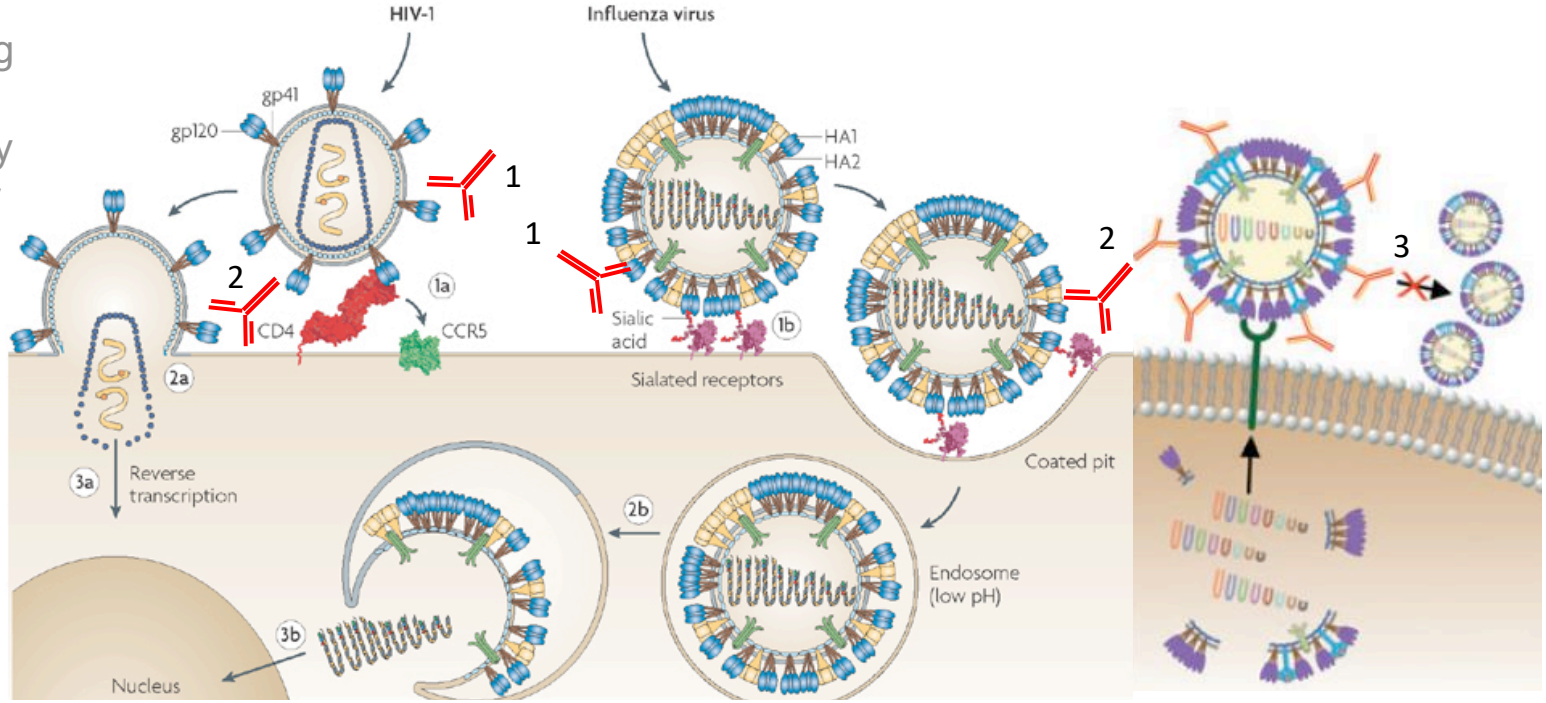
DC maturation and induction of antiviral CD8 T cell immunity



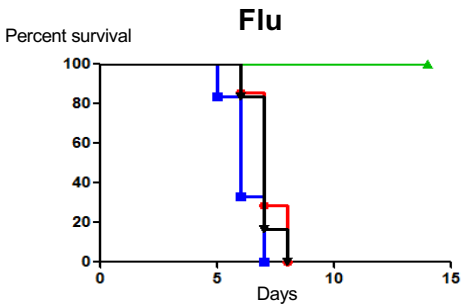
Bournazos 2020 Nature

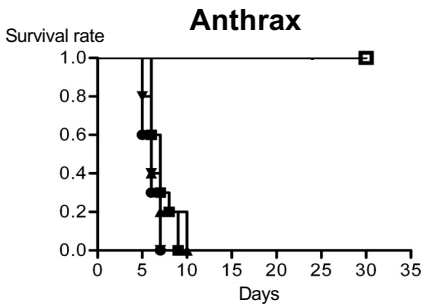
Mechanisms of antibody Fab neutralization

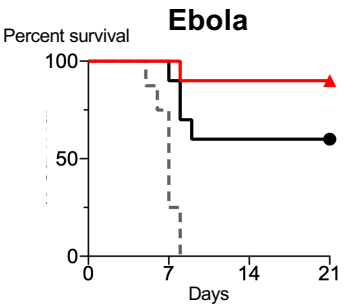
Antibody binding to viral antigens can disrupt entry (1), fusion (2) or release (3)



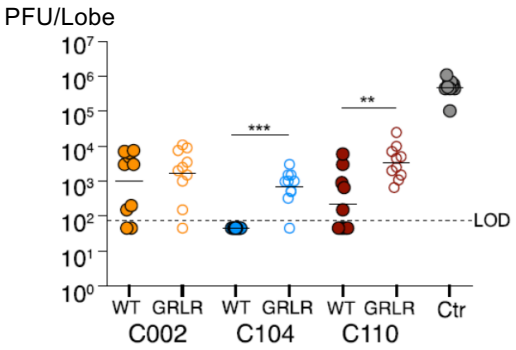
Neutralization of viruses and toxins in vivo requiresFc-FcR engagement



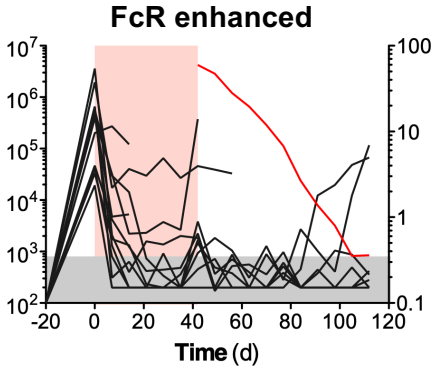


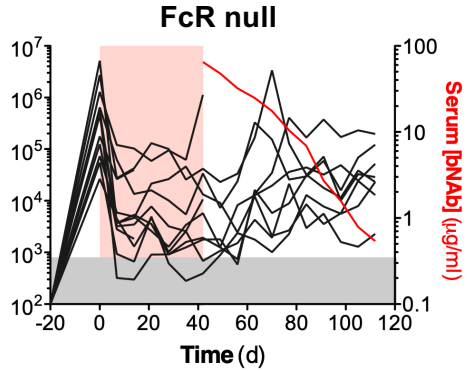


SARS-CoV2

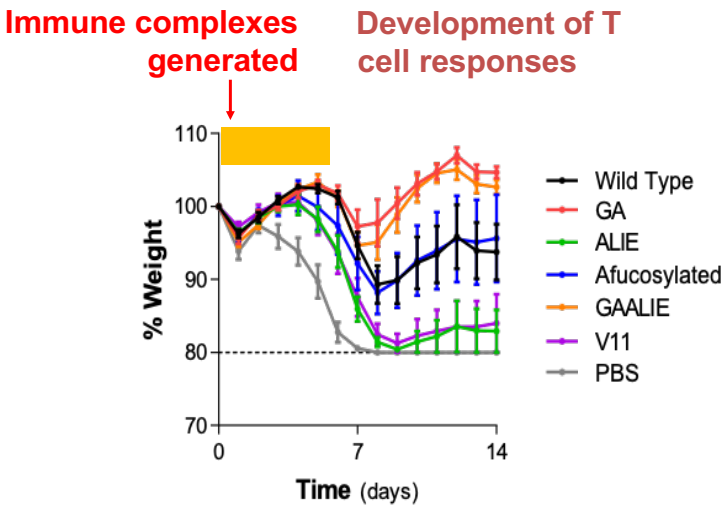


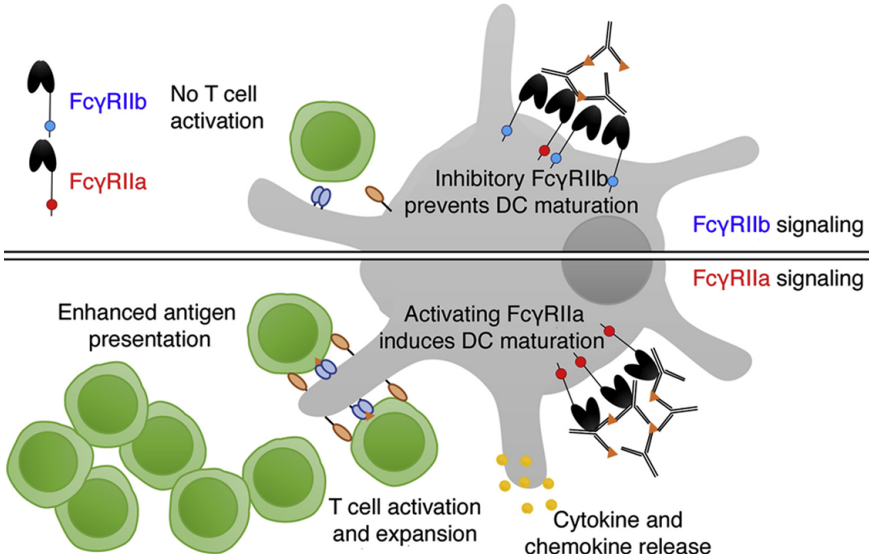
HIV





Antibody protection from flu is driven by anti-HA optimized for DC engagement

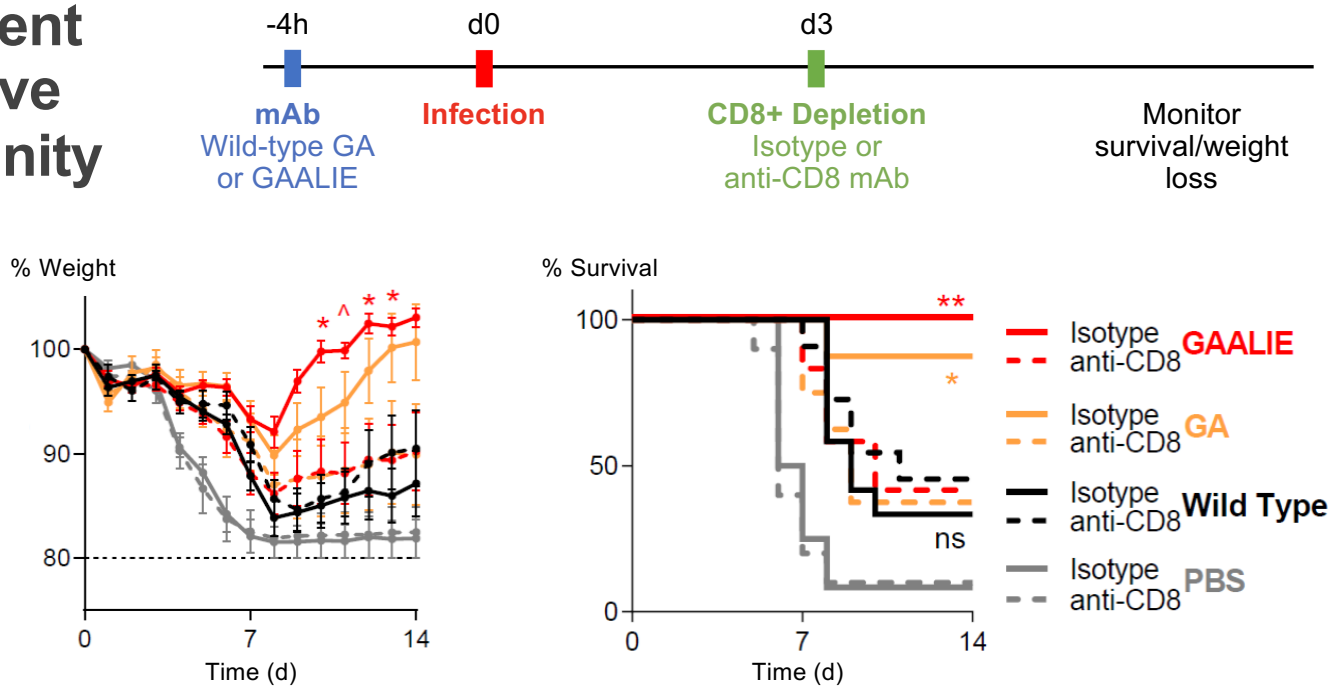




Induction of CD8 anti-flu response

FcRIIa engagement induces protective CD8 T cell immunity

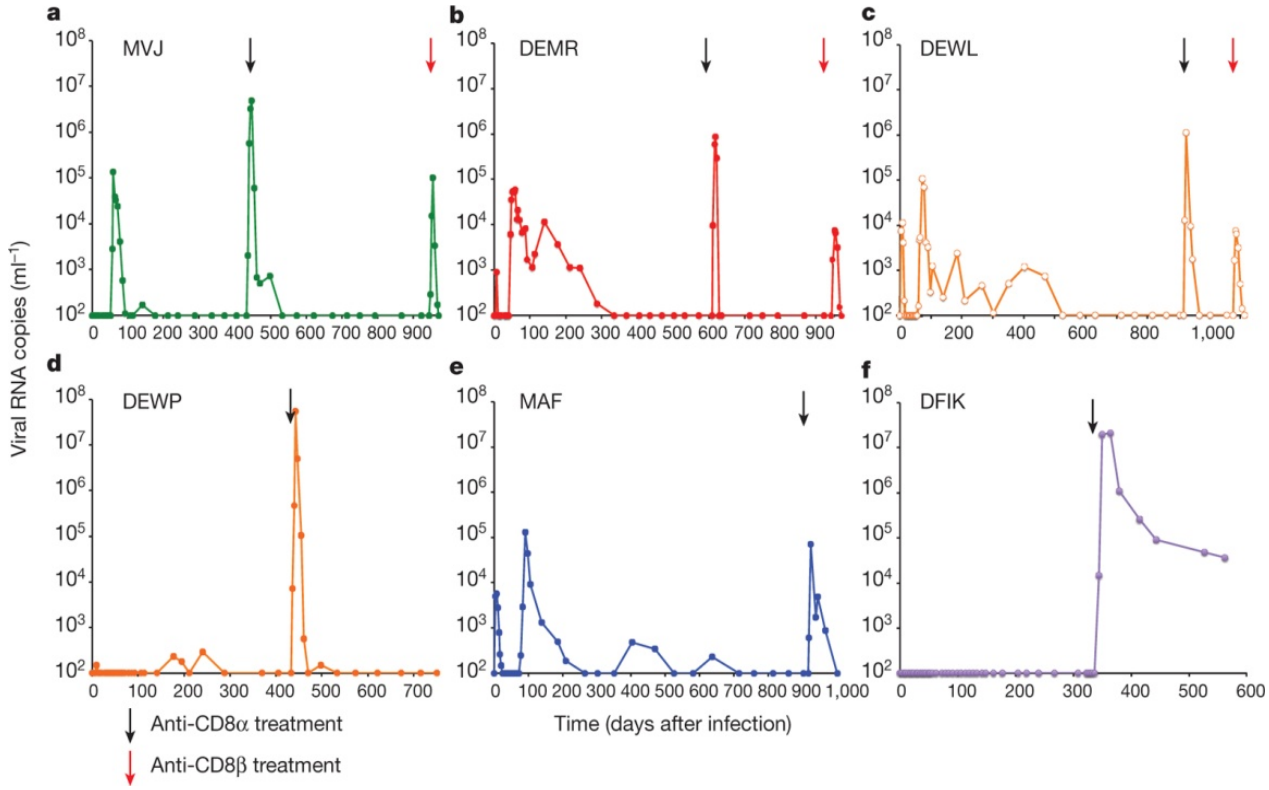
Depletion of CD8 T cells abrogates the improved protective activity of Fc variants with enhanced FcRIIa affinity



2 mg/kg i.p. , 4h prior challenge with 5mLD50 PR8 • Anti-CD8 (150 µg) administered i.v. on 3 d.p.i.

Antibody treatment of SHIV infected macaques can result in a controller phenotype

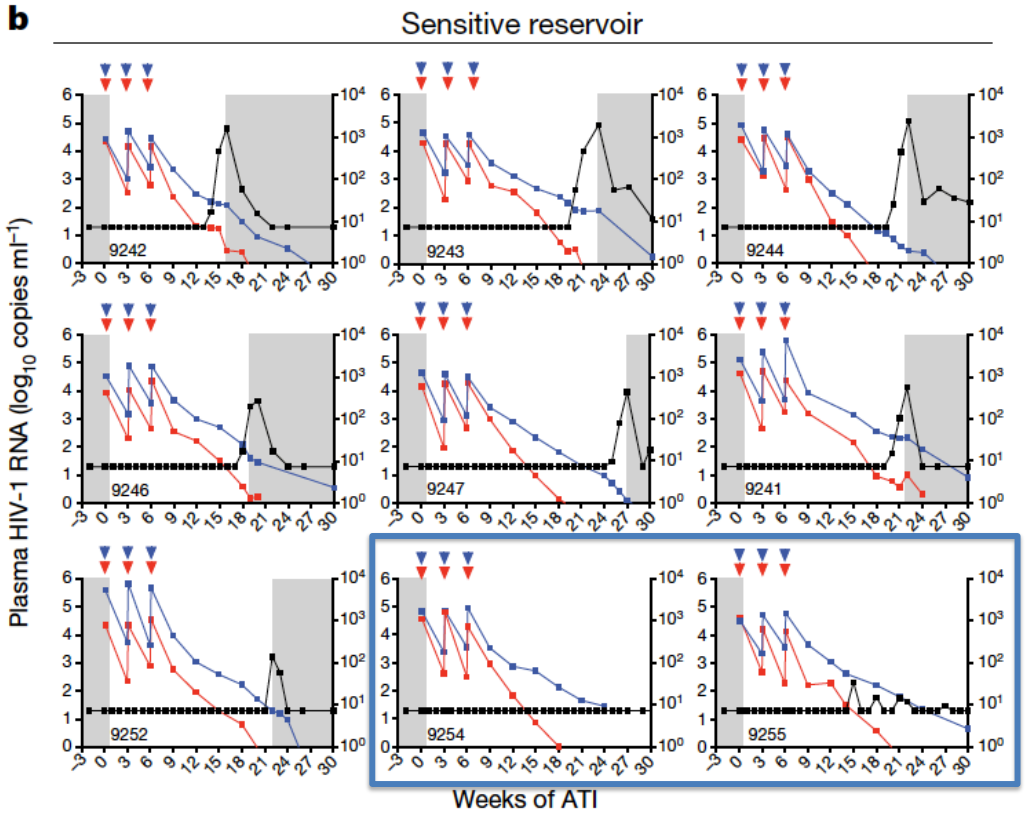
CD8 cells maintain virus control



Y Nishimura et al. Nature (2017)

Combination bNAB treatment of HIV infected individuals can trigger long-term virus control

Induction of a vaccinal effect?



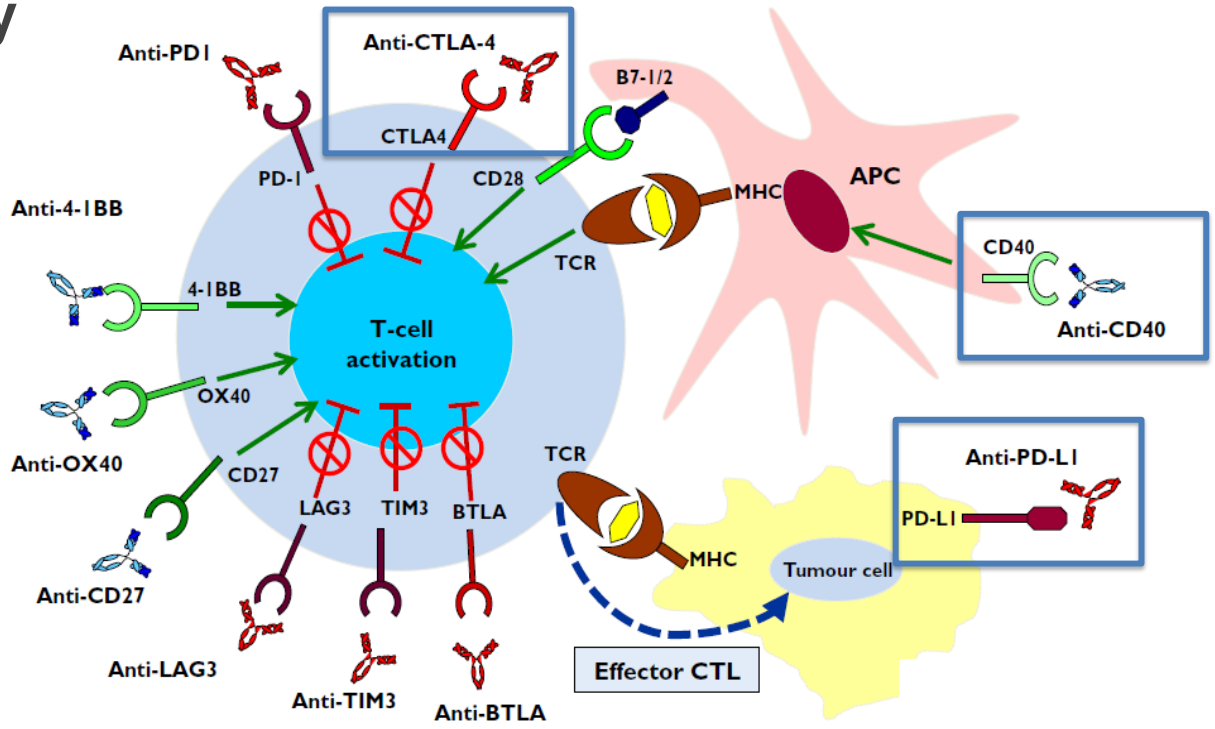
Mendoza, et al. (2018) Nature

Inducing cytotoxic T cells

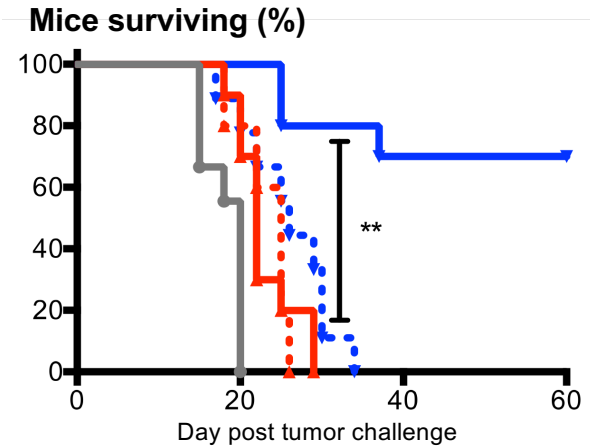
- Immune complex activation of DCs
- Block inhibitory signaling (PD-1, CTLA-4)
- Stimulate agonistic signals (CD40, OX-40)

Immunomodulatory Abs promote immunity

Are Fc-FcR interactions required?

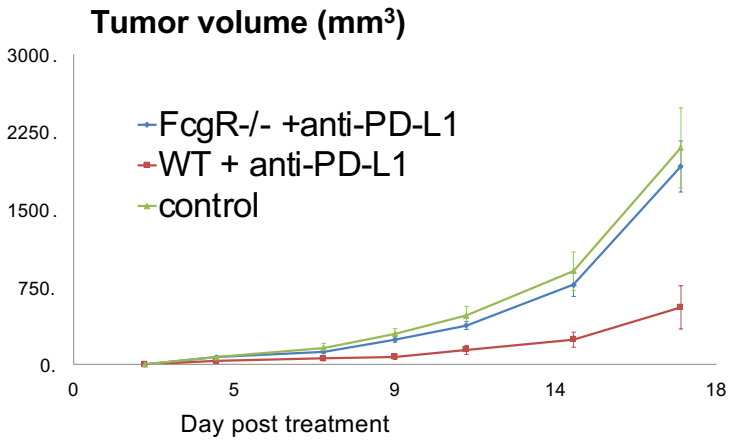


FcRs contribute to checkpoint blocking antibodies for anti-tumor activity



Anti-CTLA4 depletes Tregs through ADCC

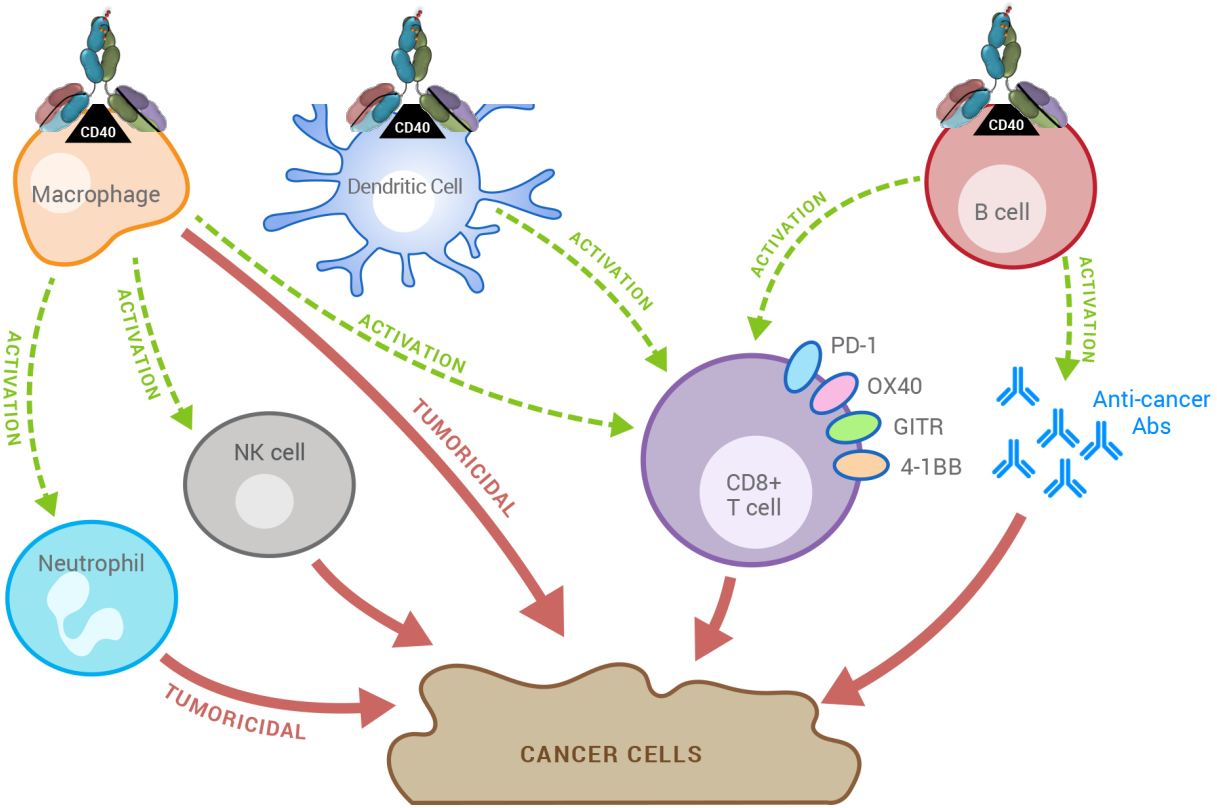
1. Simpson, et al., 2013 JEM



Anti-PDL1 depletes intratumoral myeloid suppressor cells

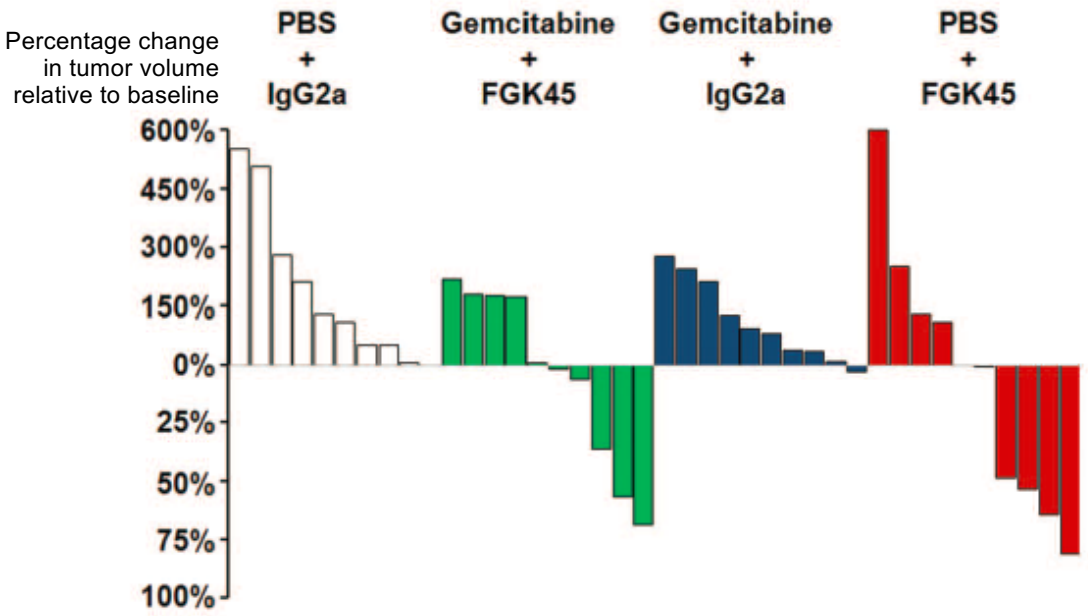
Dahan, et al. 2015 Cancer Cell

CD40 activates innate and adaptive immunity



Agonistic CD40 antibody modulates tumor growth in the KPC tumor model

KPC mice with pancreatic tumors were treated with PBS or Gemcitabine with control or agonistic anti-CD40 antibody (clone FGK45, rat IgG2a).



Beatty et al., 2011

VOLUME 25 · NUMBER 7 · MARCH 1 2007

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Clinical Activity and Immune Modulation in Cancer Patients Treated With CP-870,893, a Novel CD40 Agonist Monoclonal Antibody

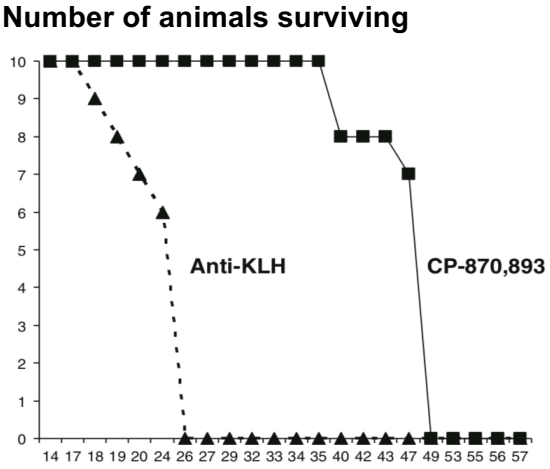
Robert H. Vonderheide, Keith T. Flaherty, Magi Khalil, Molly S. Stumacher, David L. Bajor, Natalie A. Hutnick, Patricia Sullivan, J. Joseph Mahany, Maryann Gallagher, Amy Kramer, Stephanie J. Green, Peter J. O'Dwyer, Kelli L. Running, Richard D. Huhn, and Scott J. Antonia

CP-870,893: Human IgG2 agonistic anti-CD40 mAb

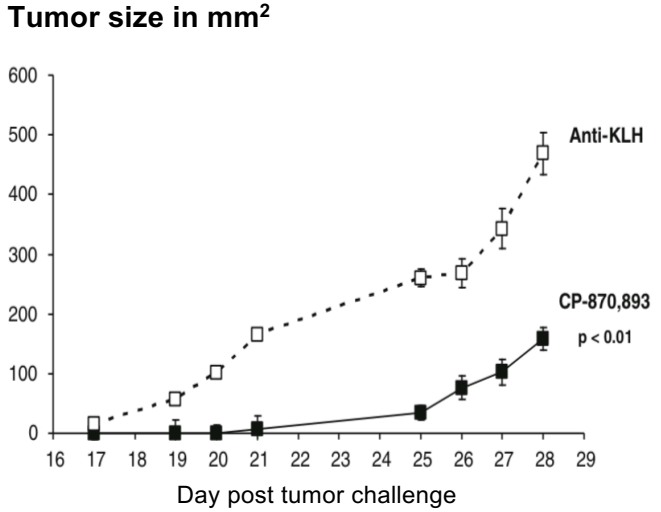
CP-870,893 is active in tumor xenografts

Daudi B cell lymphoma, CD40+, SCID mice wiith murine FcRs

IV tumor challenge



SQ tumor challenge



Gladue et al, 2011

CD40 agonists in clinical trials

Stable disease or partial responses only

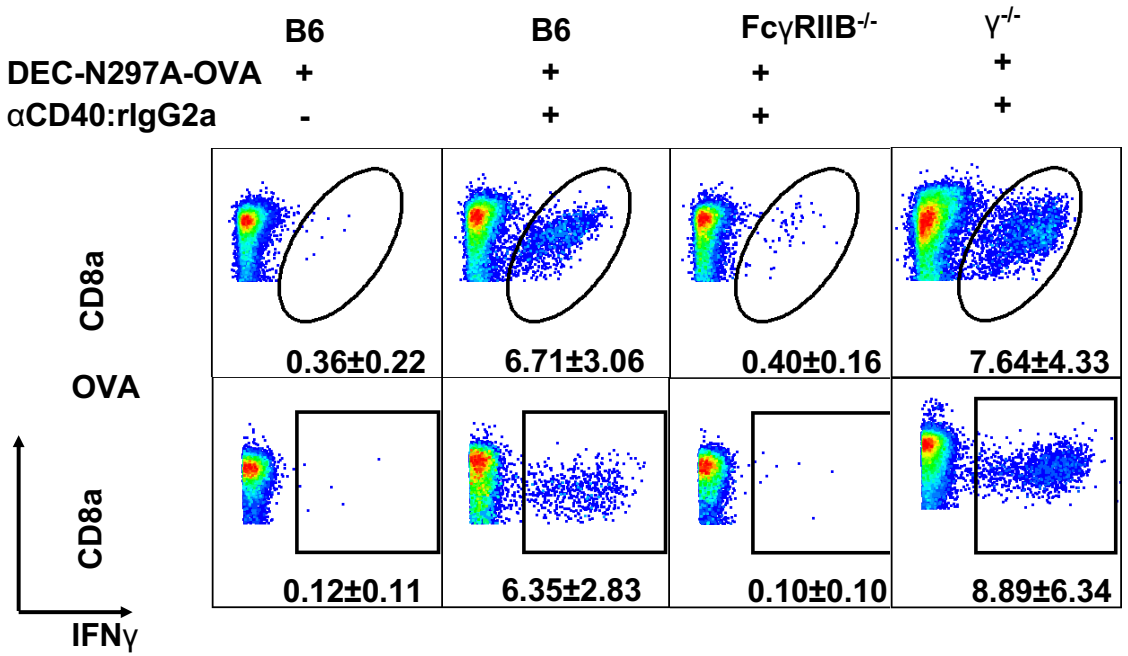
What went wrong?

Drugs	Phase	# of patients	Tumor type	Efficacy	ClinicalTrials.gov Identifier
CP-870,893 (single dose)	I	29	Advanced solid tumors	BORR: 14%	NCT02225002
CP-870,893 (weekly dosing)	I	27	Advanced solid tumors	BORR: 0%	
CP-870,893 + Carboplatin/Paclitaxel	I	30	Advanced solid tumors	BORR 20%	NCT00607048
CP-870,893 + Cisplatin/Pemetrexed	I	15	Mesothelioma	BORR 40%	
CP-870,893 + Gemcitabine	I	21	Pancreas cancer	BORR 24%	NCT00711191
CP-870,893 + Tremelimumab	I	24	Melanoma	BORR 27.3%	NCT01103635
CP-870,893 + Poly IC:LC + peptide vaccine	I		Melanoma	Not reported	NCT01008527
ChiLob 7/4 (weekly dosing)	I	21	Advanced solid tumors	BORR: 0%	NCT01561911

Beatty et al, 2016

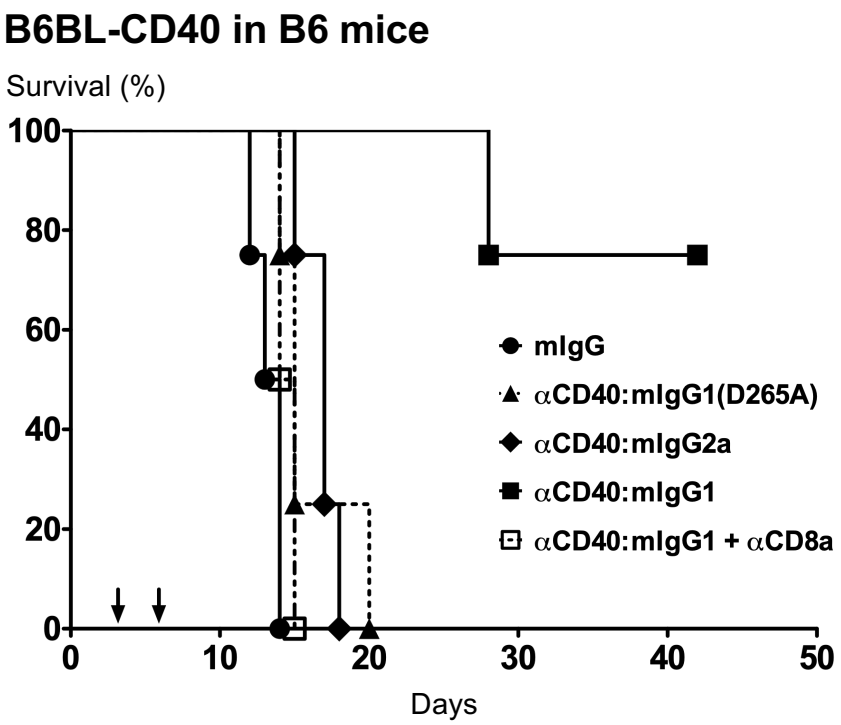
Could it be the Fc?

FcRIIB is required for anti-CD40 agonistic activity



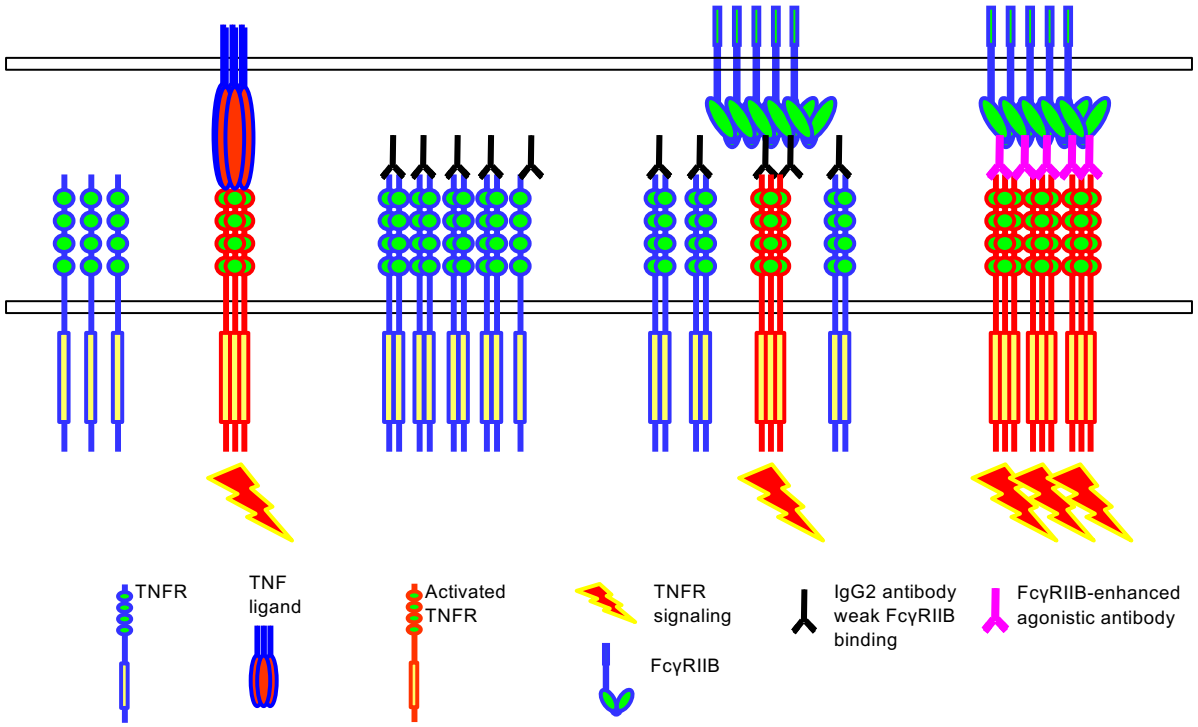
Anti-tumor activity of agonistic anti-CD40 is Fc dependent

mG1 binds
mFcRIIB, mG2a
binds RIV, D265 is
FcR null

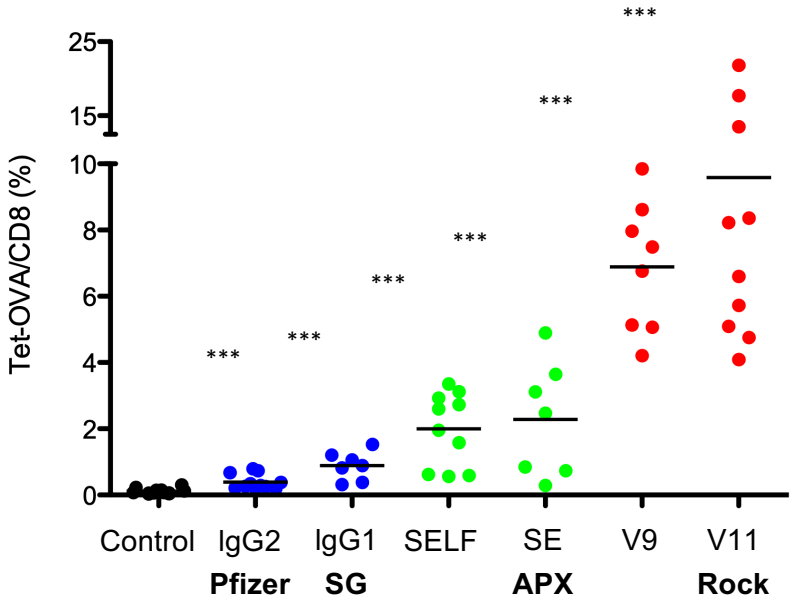


FcγRIIB-dependent activation of agonistic anti-TNFR antibodies

Agonism requires crosslinking provided by Fc



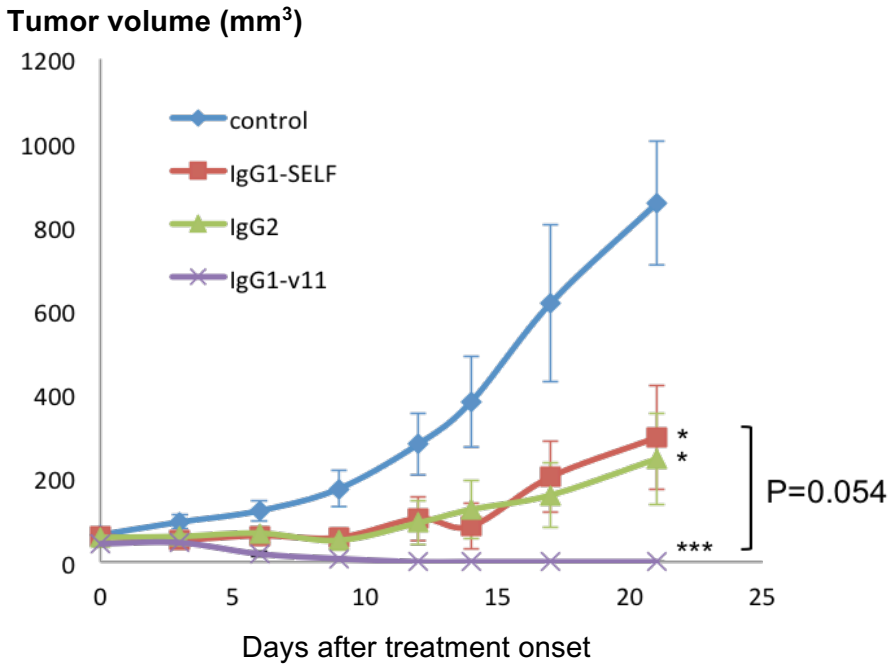
Enhancing FcγRIIB binding improves human anti-CD40 agonistic activity



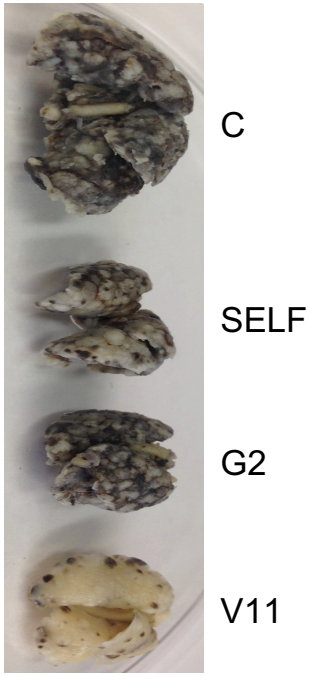
Variant	FcγRIIB-fold	IIA/IIB
IgG1	1	2.7
SELF (S267E/L328F)	70	2.4
SE (S267E)	30	0.9
V9 (G237D/P238D/P271G/ A330R)	32	0.02
V11 (G237D/P238D/H268D/ P271G/A330R)	96	0.01

Enhancing hFcRIIB binding improves α-CD40 anti-tumor activity

MC38

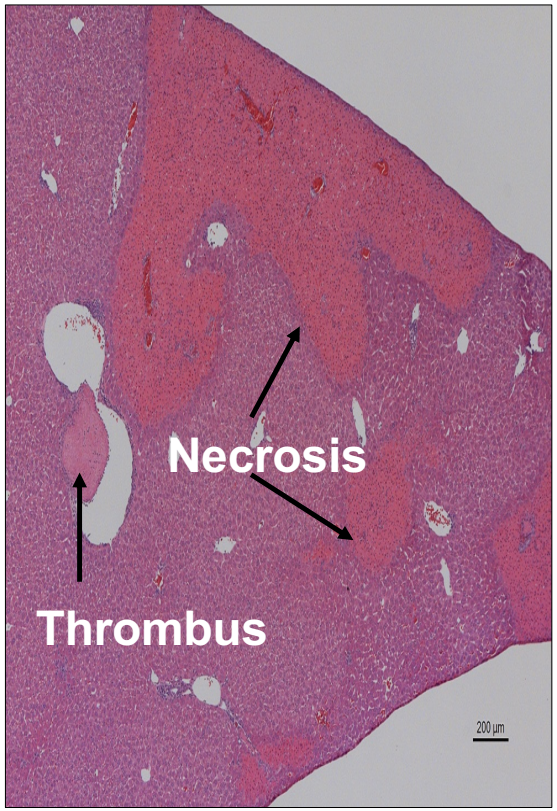


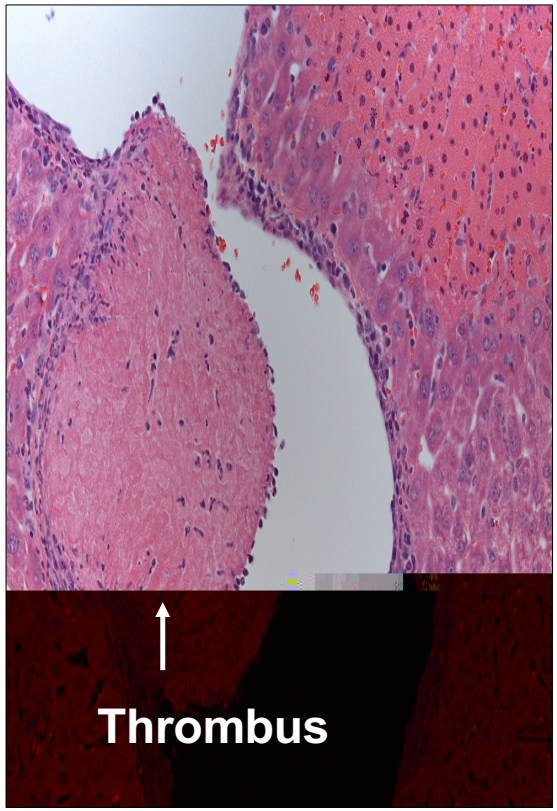
B16



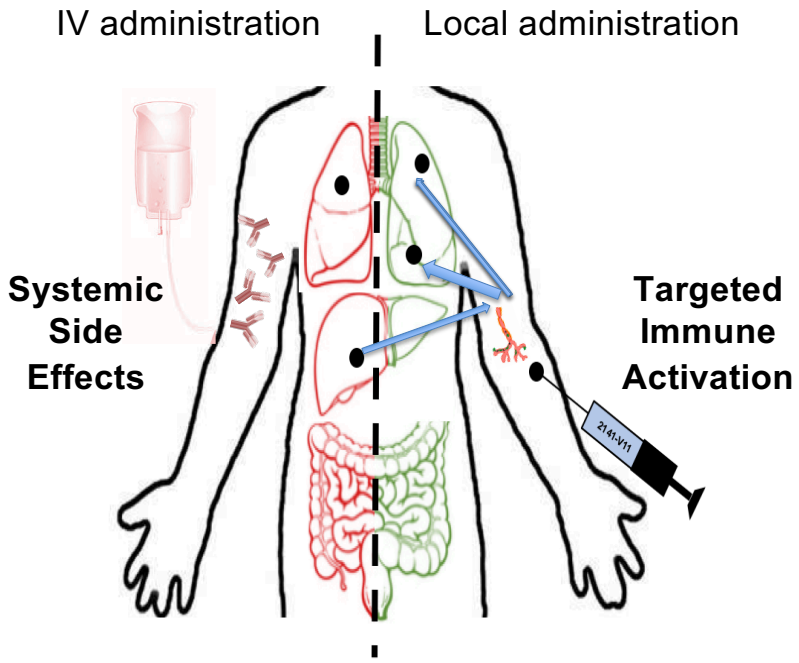
Enhanced efficacy comes with increased toxicity

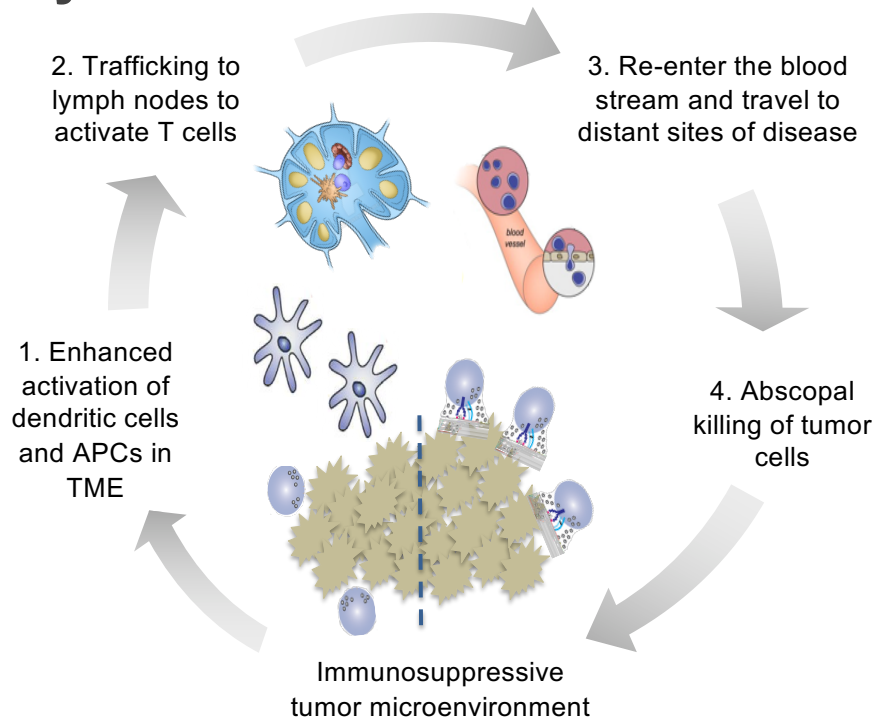
Can we achieve anti-tumor activity without systemic toxicity?



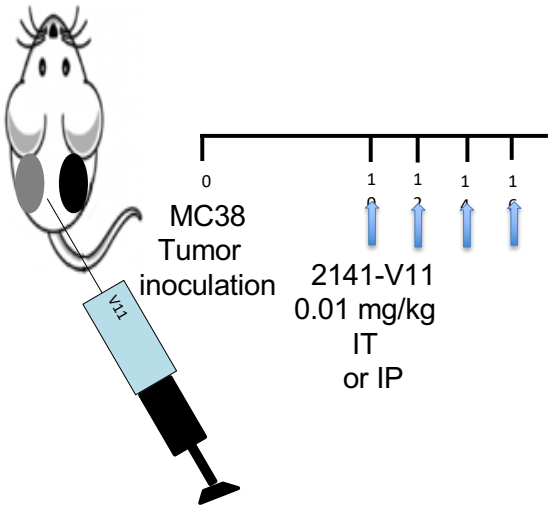


Jumpstarting the cancer-immunity cycle

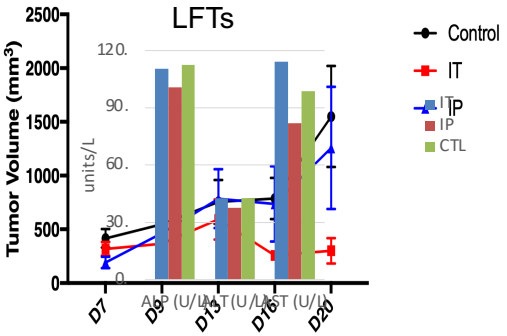




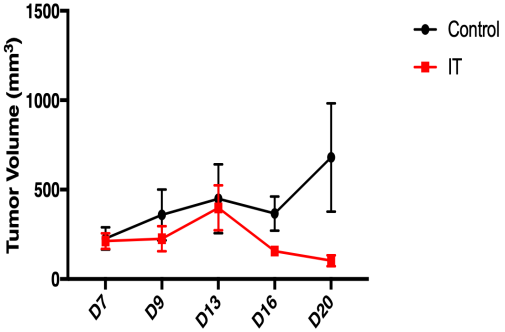
Intratumoral immunization



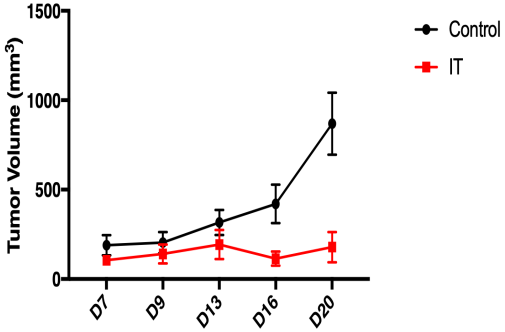
Total Tumor Size



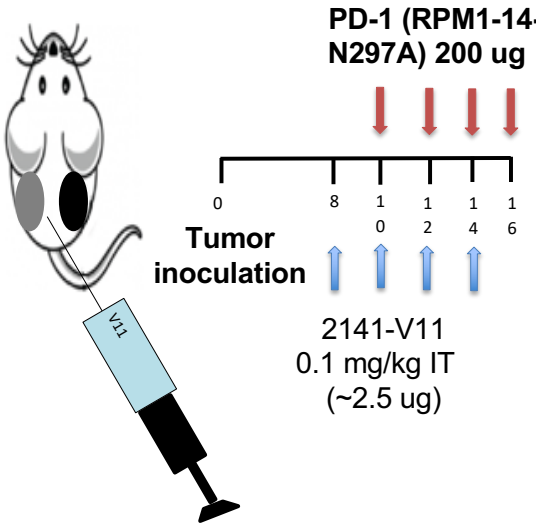
Injected Tumor

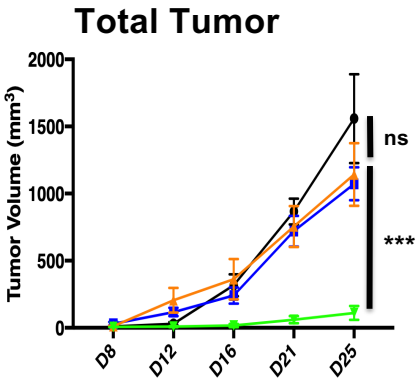


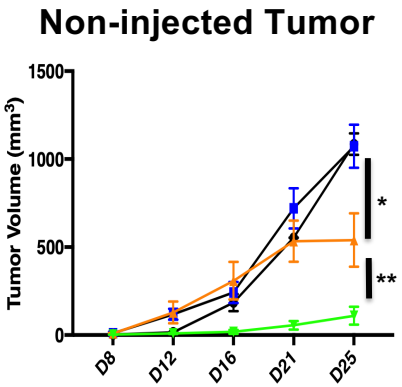
Non-injected Tumor

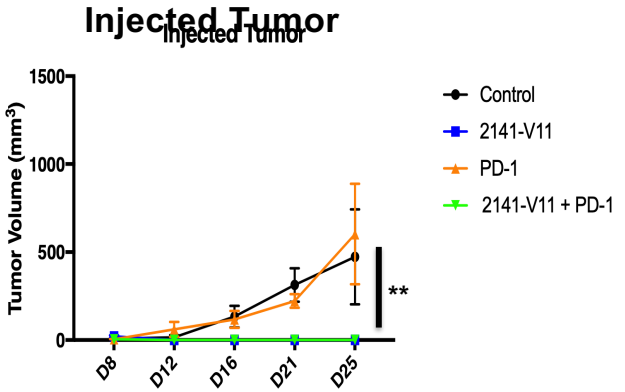


Synergy with anti-PD1 for B16 tumors









Phase I Clinical Trial of 2141-V11

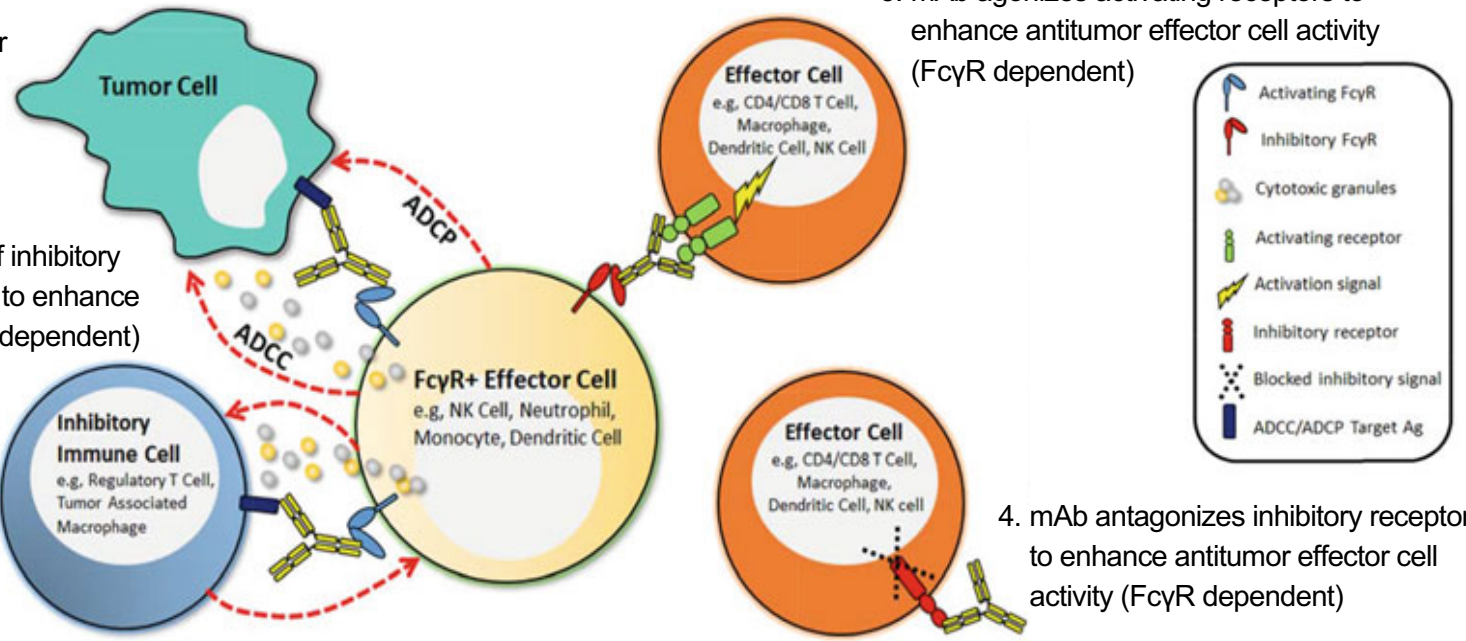
- » GMP clinical product made and vialed
- » Macaque toxicity completed
- » IND approved Q1 of 2019
- » Phase I dose escalation trial in metastatic tumor amenable to IT injection Q2 2019
- » Expansion to other established clinical settings (NMIBC,GBM)

FcRs are critical to the activity of therapeutic Abs

1. mAb mediates direct tumor cell killing by ADCC and ADCP (FcγR dependent)

2. mAb mediates depletion of inhibitory cells by ADCC and ADCP to enhance antitumor immunity (FcγR dependent)

3. mAb agonizes activating receptors to enhance antitumor effector cell activity (FcγR dependent)



4. mAb antagonizes inhibitory receptors to enhance antitumor effector cell activity (FcγR dependent)

Knowledge of FcR function has resulted in the development of new therapeutics

FcR signaling inhibitors for autoimmune diseases

- Fostamatinib, approved 2017

Fc engineered antibodies for enhanced cytotoxicity

- Obinutuzumab, approved 2013 CLL
- Benralizumab, approved 2017 asthma
- Mogamulizumab, approved 2018 CTCL
- Margetuximab, approved 2020 breast carcinoma

Large number of late stage clinical trails in progress for Fc engineered antibodies, FcR inhibitors and modulators

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