Reversible Chemical Modulation of Antibody Effector Function Maintains Anti-tumor Activity While Mitigating Peripheral Immune Activation

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Background

- Removal of fucose on the antibody core glycan increases binding to FcγRIIIa (CD16a) and drives increased antibody-dependent cellular cytotoxicity (ADCC) and immune agonism.
- Robust antibody-Fcγ engagement of non-fucosylated antibodies on immune cells in the periphery can lead to induction of systemic cytokine release and other dose-limiting infusion-related reactions.
- Example: Difference in immune activation for anti-CD40 antibodies is tied to increased FcγRIIIa binding.

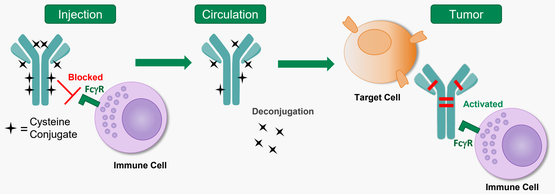
Antibody	FcγRIIIa Affinity (K_D)	RP2D*
Dacetuzumab (hS2C6, SGN-40)	11	8 mg/kg[1]
SEA-CD40 (non-fucosylated hS2C6)	232	10 mcg/kg[2]

*Recommended Phase II dose

- An ongoing challenge in the field of antibody and immuno-oncology therapeutics is identifying a balance between effective engagement of Fcγ receptors that can induce antitumor activity without incurring systemic immune activation.
- A method for the reversible modulation of antibody-Fcγ receptor interactions was designed and applied to several effector-function enhanced antibodies.

Technology Overview

- High concentrations of agonistic antibody levels during infusion can lead to rapid immune activation and cytokine production.
- Goal: Decrease concentration of active species at the time of infusion but restore binding and function over time.
- Strategy:
  - Full conjugation to antibody interchain disulfides impairs FcγR binding at the time of infusion.
  - Reversible linkage of maleimide to mAb cysteines results in deconjugation over time in circulation that then restores binding and function.
  - Use of short, defined polyethylene glycol (PEG) maleimide forms homogeneous conjugates and is inert after deconjugation.

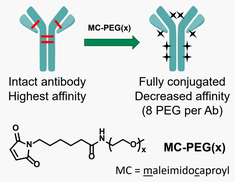


Scheme 1. Chemical conjugation to the antibody Fc prevents unwanted peripheral immune engagement and cross-linking at the time of administration. Deconjugation of the blocking groups over time in circulation results in reformation of antibody interchain disulfides and restoration of Fc binding and immune function.

Results

PEGylation of antibody interchain disulfides impairs Fc-FcγR interactions

Binding of conjugates to FcγRIIIa was assessed using biolayer interferometry (BLI)



- Tunable affinity based upon size of PEG
- Binding to all FcγRs is decreased similarly upon PEGylation
- Binding to FcRn and antigen are not affected
- PEG12 format reduces non-fucosylated binding to wild-type IgG1

SEA-CD40 (non-fucosylated) Conjugate(8)	FcγRIIIa affinity (K_D)
Non-Fucosylated CD40 mAb	**11 nM**
Fucosylated CD40 mAb	**232 nM**
N-ethyl maleimide	53 nM
MC-PEG_4	75 nM
MC-PEG_8	169 nM
MC-PEG_{12}	**197 nM**
MC-PEG_{24}	234 nM
MC-PEG_{36}	665 nM
MC-PEG_{48}	776 nM
MC-PEG_4-$(PEG_8)_3$	913 nM
MC-PEG_4-$(PEG_{24})_3$	> 1 mM

Table 1. Impact of PEGylation on FcγRIIIa binding, assessed by biolayer interferometry

Fc PEGylation is a general approach for modulating Fc-FcγR interactions

- Simple, conjugatable format impact on FcγRIIIa is generally applicable to any non-fucosylated antibody
- Effect of PEGylation was assessed using FcγRIIIa NFAT signaling reporter assays:

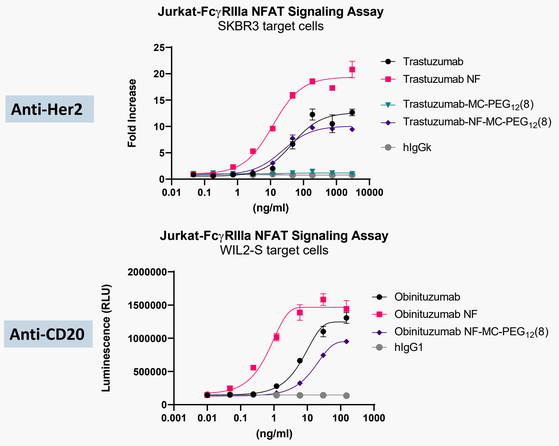


Figure 1. Impact of PEGylation on FcγRIIIa binding for trastuzumab (top) and obinutuzumab (bottom) assessed using a Jurkat FcγRIIIa NFAT signaling assay.

Fc binding and function can be restored upon maleimide deconjugation

Evaluation of maleimide reversibility ex vivo and in vivo

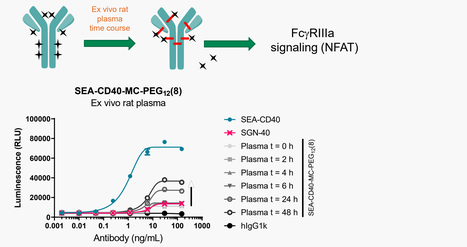


Figure 2. The reversibility of maleimide linkage and antibody effector function was assessed by Jurkat FcγRIIIa NFAT reporter assay following incubation ex vivo in rat plasma at 37 ºC

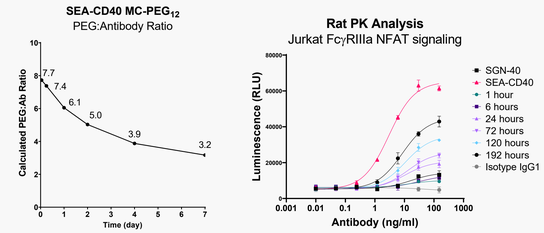


Figure 3. The rate of maleimide-PEG deconjugation was assessed in vivo in rats (15 mg/kg dose). The PEG:Ab ratio was measure by intact SEC-MS, and the extent of binding and effector function measured using a Jurkat FcγRIIIa NFAT reporter assay. Maleimide deconjugation resulted in a PEG:Ab ratio of 3.2 after 7 days, which restored FcgRIIIa binding increased over time.

Unstable PEGylated anti-CD40 antibody has improved efficacy in a syngeneic tumor model in huCD40 mice

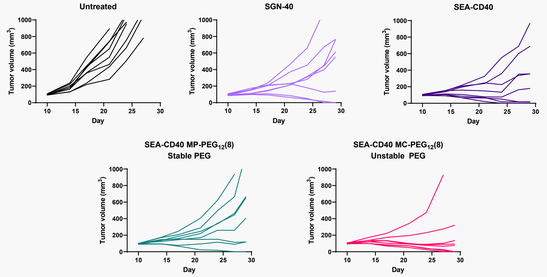
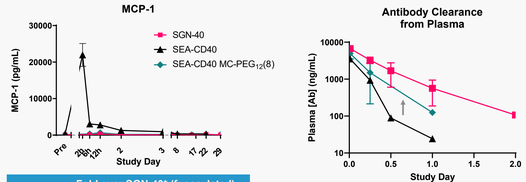


Figure 4. Antitumor activity of CD40 antibodies and PEG conjugates in humanized CD40 mice with A20 tumors expressing human CD40. The conjugate bearing an unstable maleimide, SEA-CD40-MC-PEG12(8) had increased activity over a conjugate bearing a stable maleimide linkage, indicating that Fc impairment is reversible.(MP = maleimidopropyl)

Fc PEGylation dramatically reduces peripheral cytokines despite increased exposure and similar PD effects

Fc PEGylation results in dramatic reductions in peripheral cytokine production with SEA-CD40 with increased exposure



	Fold over SGN-40* (fucosylated), 0.3 mg/kg	
Cytokine	SEA-CD40	SEA-CD40-MC-PEG12
IL-1RA	119	8.9
MIP-1b	71	2.2
MCP-1	49	1.3

*At peak concentration

Figure 5. Cytokine levels and total antibody concentration at 0.3 mg/kg dose of test article in cynomolgus macacques. SEA-CD40 (N=10), SGN-40 & SEA-CD40-MC-PEG_{12}(8) (N=2 each).

PEGylated conjugate drives delayed, but maximal B cell depletion

- Delayed but maximal effect is consistent with reversible attenuation of Fc function

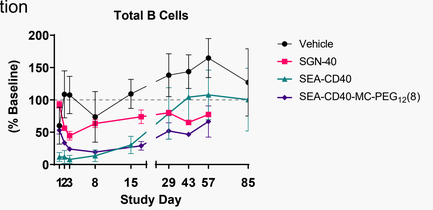


Figure 6. ADCC-mediated B cell depletion in non-human primates after administration of 0.3 mg/kg of test article in cynomolgus macacques. SEA-CD40 (N=10), SGN-40 and SEA-CD40-MC-PEG_{12}(8) (N=2 each).

Conclusions

- A simple and tunable conjugation-based method to reversibly modulate Fc-FcγR interactions was developed.
- Technology is modular and widely applicable to other effector-function enhanced antibodies.
- Application to a CD40 agonist mitigates systemic cytokines while increasing exposure and maintaining efficacy.
- Fully reversible methodology has also been developed and may be preferred for certain antibodies/targets.

References
1. Advani, R., et al. Phase I study of the humanized anti-CD40 monoclonal antibody Dacetuzumab in refractory or recurrent non-Hodgkin's lymphoma. *J. Clin. Onc.* **2009**, 27, 4371. DOI: 10.1200/JCO.2008.21.3017
2. Bajor, D.L., et al. Preliminary results of a Phase 1 study of SEA-CD40, gemcitabine, Nab-paclitaxel, and pembrolizumab in patients with metastatic pancreatic ductal adenocarcinoma (PDAC). *J. Clin. Onc.* **2022**, *40*, no. 4_suppl. DOI: 10.1200/JCO.2022.40.4_suppl.559

DISCLOSURES: All authors are employees of and/or hold stock in Seagen Inc.

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