Reversible chemical modification of antibodies: a complementary approach to tuning FcγR binding that 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 and immune cell binding of non-fucosylated antibodies in the periphery can lead to unwanted 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

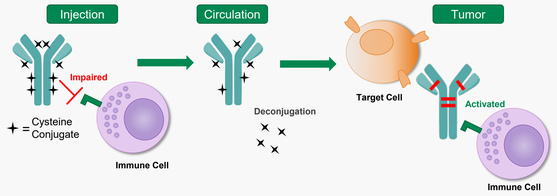
Antibody	FcγRIIIa Affinity (K_D)	RP2D[&]
Dacetuzumab (hS2C6, SGN-40)	232	8 mg/kg[1]
SEA-CD40 (non-fucosylated hS2C6)	11	10 mcg/kg[2]

[&]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 active antibody during infusion can lead to rapid immune activation and cytokine production
- Goal: Decrease concentration of active species at the time of infusion then restore binding and function over time
- Strategy:
  - Complete reduction and conjugation to antibody interchain disulfides impairs FcγR binding at the time of infusion
  - Reversible cysteine-maleimide linkage deconjugation over time in circulation to restore binding and function
  - Short, defined polyethylene glycol (PEG) maleimide forms homogeneous conjugates and is inert after deconjugation

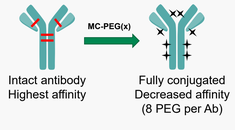


Scheme 1. Chemical conjugation to the antibody Fc prevents unwanted peripheral immune engagement and cross-linking at the time of administration. Deconjugation of the blocking groups over time in circulation results in reformation of antibody interchain disulfides and restoration of Fc binding and immune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

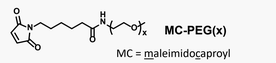
Results

PEGylation of antibody interchain disulfides impairs Fc-FcγR interactions

Binding affinity of PEG conjugates to FcγRs



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_{12}	197 nM
MC-PEG_{48}	776 nM



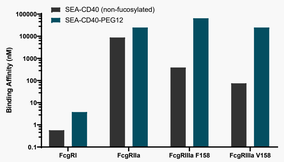


Figure 1. Impact of PEGylation on FcγR binding as assessed by biolayer interferometry. Conjugates showed decrease in binding with larger PEG units. FcRn and antigen binding are unaffected by conjuation.

- PEG12 format reduces non-fucosylated binding to wild-type IgG1 levels

Fc binding and function can be restored upon maleimide deconjugation

Evaluation of maleimide reversibility ex vivo and in vivo

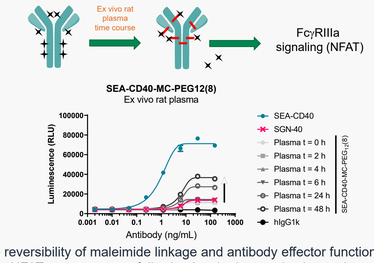


Figure 2. The reversibility of maleimide linkage and antibody effector function was assessed by Jurkat FcγRIIIa NFAT reporter assay following incubation ex vivo in rat plasma at 37 °C

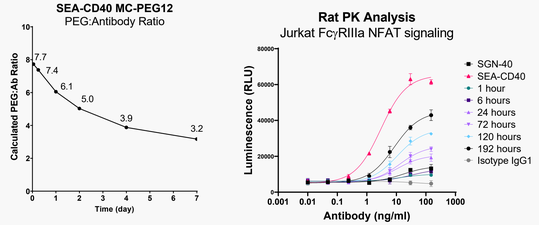


Figure 3. The rate of maleimide-PEG deconjugation was assessed in vivo in rats (15 mg/kg dose). The PEG:Ab ratio was measure by intact SEC-MS, and the extent of binding and effector function measured using a Jurkat FcγRIIIa NFAT reporter assay.

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

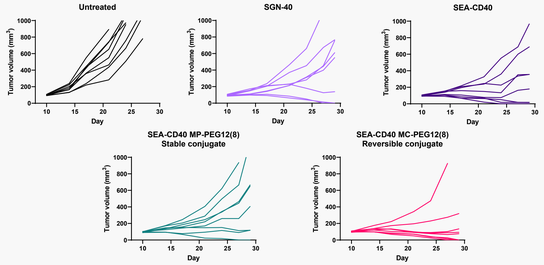
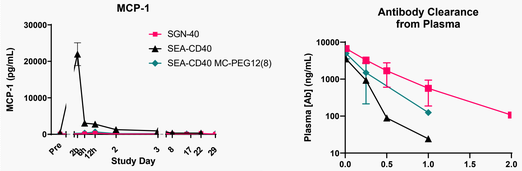


Figure 4. Antitumor activity of CD40 antibodies and PEG conjugates in humanized CD40 mice with A20 tumors expressing human CD40. The conjugate bearing an unstable maleimide, SEA-CD40-MC-PEG12(8) had increased activity over a conjugate bearing a stable maleimide linkage, indicating that Fc impairment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

Fc PEGylation reduces peripheral cytokines despite increased exposure and similar pharmacodynamic effects



	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. Quantitation of cytokine levels and measurement of the total antibody concentration at 0.3 mg/kg dose of test article in cynomolgus macaques. SEA-CD40 (N=10), SGN-40 and 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

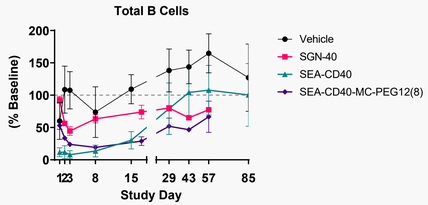
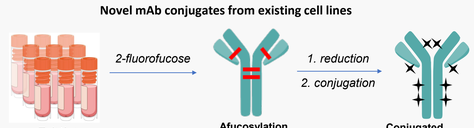


Figure 6. ADCC-mediated B cell depletion in non-human primates after administration of 0.3 mg/kg of test article in cynomolgus macacques. SEA-CD40 (N=10), SGN-40 and SEA-CD40-MC-PEG_{12}(8) (N=2 each).

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

- Simple, conjugatable format is generally applicable to any non-fucosylated antibody
  - Equally applicable to Fc enhancing point mutations S239D I332E (DE)
- Effect of PEGylation was assessed using FcγRIIIa NFAT signaling reporter assays:



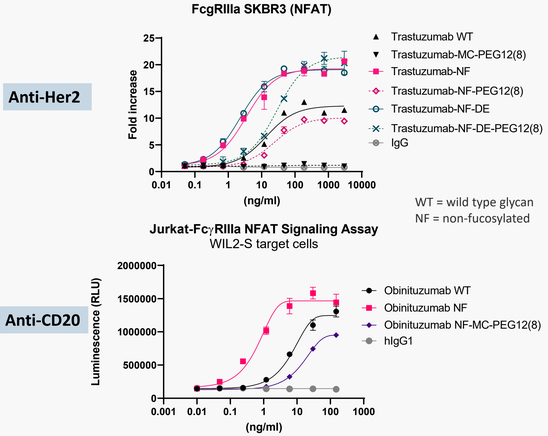


Figure 7. Impact of PEGylation on FcγRIIIa binding for trastuzumab (top) and obinutuzumab (bottom) assessed using a Jurkat FcγRIIIa NFAT signaling assay.

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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