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Probing the Internalization and Efficacy of Antibody-Drug Conjugate via Site-Specific Fc-Glycan Labelling of a Homogeneous Antibody Targeting SSEA-4 Bearing Tumors

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Abstract

Antibody drug conjugates (ADC) are an emerging class of pharmaceuticals consisting of cytotoxic agents covalently attached to an antibody designed to target a specific cancer cell surface molecule followed by internalization and intracellular release of payload to exhibit its anticancer activity. Targeted delivery of cytotoxic payload to a variety of specific cells has been demonstrated to have significant enhancement in clinical efficacy and dramatic reduction in off-target toxicity. Site-specific conjugation of payload to the antibody is highly desirable for development of ADC with well-defined antibody-to-drug ratio, enhanced internalization, reduced toxicity, improved stability, desired pharmacological profile and optimal therapeutic index. Here, we reported a site-specific conjugation strategy for evaluation of antibody internalization and efficacy of ADC designed to target SSEA4 on solid tumors. This strategy stems from the azido-fucose tag of a homogeneous antibody Fc-glycan generated via *in vitro* glycoengineering approach for site-specific conjugation and optimization of antibody-drug ratio to exhibit optimal efficacy. The ADC consisting of a chimeric anti-SSEA4 antibody chMC813–70, conjugated to the antineo-plastic agent monomethyl auristatin E via both cleavable and non-cleavable linkers showed excellent cytotoxicity profile towards SSEA4-bearing cancer cells. A clear distinction in cytotoxicity was observed among cancer cells with different SSEA4 expression levels.

Graphical Abstract

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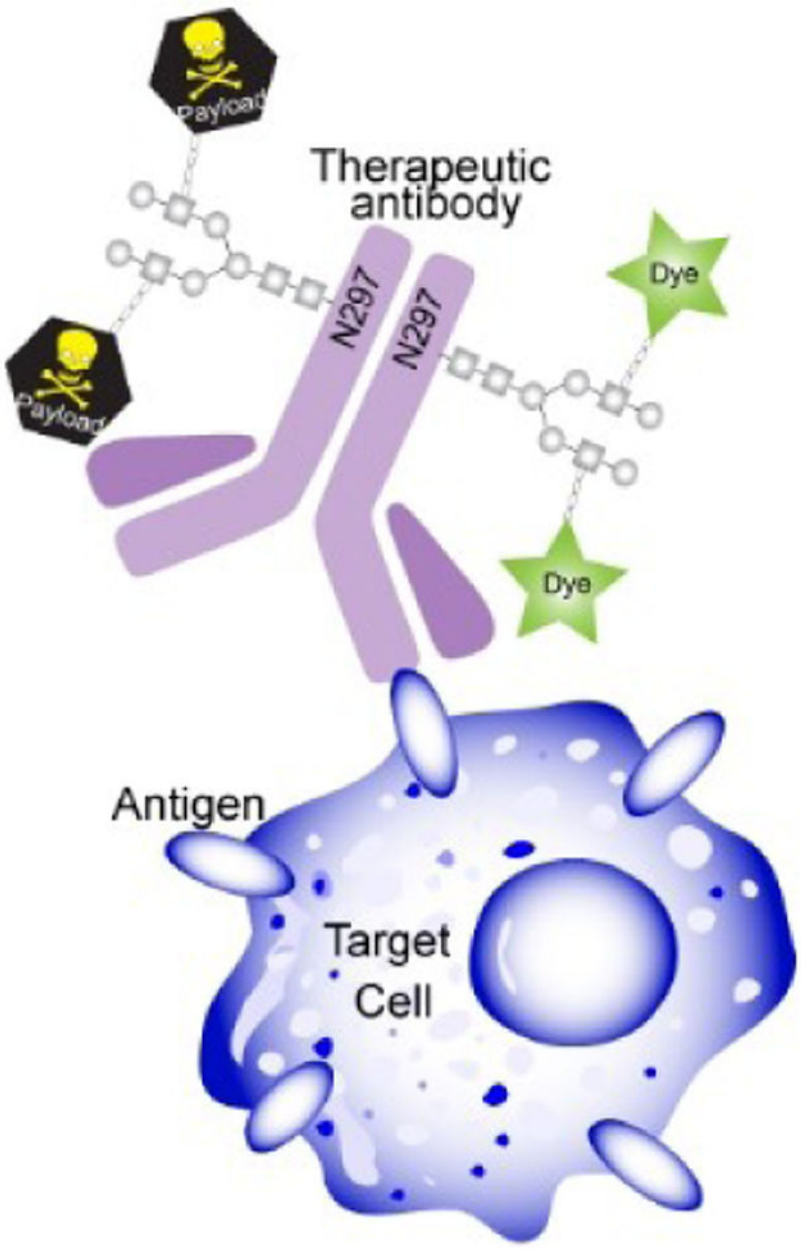

Keywords

Antibody-drug conjugates; site-specific conjugation; In-vitro glycoengineering; anti-SSEA4 antibody; SPAAC ligation

1. Introduction

Recent advances in the understanding of cancer biology have changed cancer treatment paradigms from traditional chemotherapies to targeted therapies.[1] Many cancer-associated or cancer-specific cell surface antigens have been identified over the years that formed the

basis of cancer immunotherapy using monoclonal antibodies (mAbs).[1f] The therapeutic effect of mAbs usually comes from two distinct steps: the first step is the specific binding of antibody to a target antigen on tumor cells and the second step is the recruiting of immune cells by the antibody to kill the target cell by the released cytokines from the immune cells or by antibody-dependent cellular phagocytosis (ADCP),[2] antibody-dependent cellular cytotoxicity (ADCC),[3] complement-dependent cytotoxicity (CDC).[4] In addition, by taking advantage of the selectivity of mAbs and the potency of cytotoxic small molecules, antibody-drug conjugates (ADC)[5] have been developed to further improve the selectivity and efficacy of targeted therapy.

ADC comprises a mAb that is highly specific for the target antigen and humanized for longer circulatory half-life and reduced immunogenicity, and conjugated to a highly potent cytotoxic agent against cancer cells with low off-target effect with a linker that is stable in circulation to prevent premature release and cleavable to facilitate the release of the payload intracellularly or in the tumor microenvironment.[2a,6] Regarding the internalization of ADC upon administration, the antibody moiety first binds to the tumor-specific antigen, then the whole ADC-antigen complex undergoes receptor-mediated endocytosis.[7] It is then processed in the lysosome or the late endosome to release the active cytotoxin. The cytotoxic agent usually blocks the critical cellular mechanisms for cancer survival, such as DNA or microtubule assembly, leading to tumor cell apoptosis. The first human clinical trial of ADC consisting of an anti-carcinoembryonic antigen antibody conjugated to a vinca alkaloid payload called vindesine was conducted in 1983 to treat advanced-stage metastatic carcinomas;[8] however, this study failed to demonstrate the desired clinical efficacy. Lessons from several clinical studies over the years led to the refinement and improvement of ADCs with desired therapeutic index. Further technological advancements resulted in the approval of thirteen ADC drugs by the USFDA by the end of 2022.[9] Mylotarg™ was the first ADC approved in 2000 for the treatment of CD33-positive acute myelogenous leukemia. However, it was voluntarily withdrawn from the market due to a lack of clinical benefits and fatal toxicity. In 2017, Mylotarg was again approved for patients with CD33-positive acute myeloid leukemia (AML).[10] The most recent addition to the list of approved ADCs is ELAHERE™ from Immunogen Inc. for treatment of folate receptor α (FRα)-positive, platinum-resistant epithelial ovarian cancer.[11]

For an ideal ADC design, selecting an antibody to target a novel antigen with high affinity and specificity is highly desirable. In addition, the target antigen must predominantly be expressed on the surface of the target cell with minimal presence on the healthy cell. It should also undergo rapid internalization to drive the delivery and release of cytotoxic payload inside the tumor cell. Previously, our group reported that the globo-series glycosphingolipids, such as Globo H and stage-specific embryonic antigen-4 (SSEA4) and SSEA3 antigens, are exclusively overexpressed on many solid tumors. SSEA4, a hexasaccharide glycosphingolipid initially identified in human embryonic stem cells, was subsequently shown to be highly expressed on malignant glioma, breast, ovarian, and prostate cancers, and the high expression was linked to poor prognostic outcome. The potential of SSEA4 as a tumor-specific antigen for the development of ADC was demonstrated by the discovery of OBI-998,[12] an ADC comprising a humanized anti-SSEA4 antibody linked to the highly potent microtubule-disrupting agent monomethyl

auristatin E (MMAE), that showed significant SSEA4-dependent antitumor efficacy in a variety of animal models.

Besides selecting optimal target-specific mAb and the highly potent cytotoxin, conjugation chemistry and the linker design are critical factors that affect ADC efficacy especially with regard to pharmacology and therapeutic window.[13] The most commonly used methods for tethering mAb to a payload and linker are based on chemical and enzymatic conjugation. Traditionally, ADCs (e. g., Mylotarg, Cadcyla and Adcetris) were constructed by utilizing accessible amino acid residues (e. g., lysine or cysteine) on antibodies for controlled chemical conjugation with activated linker moieties.[14] However, these strategies generated heterogeneous ADC species with variable antibody-drug ratios (DARs) and conjugation sites, which can lead to reduced efficacy. In addition, ADCs with high DAR can result in a decrease in potency, risk of aggregation, reduced half-life, and premature systemic release of payload.[15] Nevertheless, these limitations were overcome by the insertion of non-natural amino acids through genetic engineering.[16] Enzymatic conjugation strategies have been used to install a unique handle on native or engineered mAb that allows site-specific conjugation to the payload. For example, bacterial transglutaminase is an enzyme that catalyzes the acyl transfer reaction between specific glutamine (Q295)[17] within the antibody and the linker containing primary amine at one end, resulting in the generation of ADC with a defined DAR of 2, and a DAR of 4 can be reached with N297Q.[18] The N-glycan at N297 in the Fc-domain of antibody provides another attractive site for payload conjugation without the need for genetic engineering.[19] For example, the core fucose, galactose, or sialic acid residues on N-glycan have been modified with the desired tag for site-specific conjugation of payloads.[20] Our development of homogeneous antibodies with *in vitro* glycoengineering allows us to develop a robust approach for site-specific conjugation of payload to the Fc-glycan of antibody.[21]

Here, we reported the development of an ADC consisting of a homogeneous anti-SSEA4 antibody, chMC813–70, conjugated to the anti-neoplastic agent, MMAE, site-specifically through a linker to the Fc-glycan. We utilized *in vitro* glycoengineering to insert an azido-fucose tag in the antibody Fc-glycan using human α−1,3 fucosyltransferase. A bi-antennary complex type glycan was modified with azido-fucose and tranglycosylated to IgG-GlcNAc acceptor to provide a handle for site-specific payload conjugation to give a well-defined DAR of 4. ADCs were constructed by using non-cleavable and cleavable linker containing a cathepsin B cleavage site. The resultant ADCs showed excellent target specificity, rapid internalization, and potent cytotoxicity. Taken together, these results support the proof of principle that anti-SSEA4 ADC is a potential therapeutic agent for treatment of SSEA4-bearing solid tumors. It is noted that conjugation of payload to the antibody-Fc glycan has been reported,[20a] but development of a site-specific labelling platform using homogeneous antibodies to probe antibody internalization and ADC efficacy as demonstrated in this study represents a new efficient and high-yield approach to ADC.

2. Results and Discussion:

2.1. Endocytosis of Chimeric Antibody chMC-813–70 in SSEA4 Positive Cancer Cells

To construct an ADC, the glycan component was prepared by chemo-enzymatic methods according to the methods previously developed by our group.[21b] The glycan synthesis commenced with commercially available sialylglycopeptide (SGP) as a starting material, which is treated with WT Endo-β-N-acetylglucosaminidase S2 (EndoS2) to produce a sialyl-complex type glycan G1 (SCT). Next, terminal sialic acid residues were removed by neuraminidase to obtain the complex-type glycan G2 (CT). Next, the complex type glycan G2 was modified with azido-fucose in the presence of GDP-azido-fucose and human α−1,3 fucosyltransferase (FuT6) to afford the fucosylated complex glycan G3, which was then converted into the oxazoline form G4 before transglycosylation to the IgG-GlcNAc-Fuc acceptor (Figure 1A). The anti-SSEA4 antibody, MC813–70, exhibited CDC in $SSEA4^+$ glioblastoma multiforme cells (GBM) *in vitro* and inhibited the growth of GBM cells in a xenograft mouse model.[22] This commercially available Fab domain of mouse IgG3 MC813–70 was merged to the Fc domain of human IgG to generate chimeric MC813–70 (chMC813–70) and used to construct ADC. The chMC813–70 produced in WT Expi293™ cells was treated with WT EndoS2 to generate core fucosylated chMC813–70-GlcNAc.Transglycosylation of the glycan oxazoline G4 to chMC813–70-GlcNAc (Fuc) acceptor was accomplished using glycosynthase (EndoS2D184 M) to afford an homogeneous antibody glycoform with four azido tags on the Fc-glycan (Figure 1B).

The density of tumor antigen on the cell surface directly correlates with the efficacy of ADC. However, some studies suggest that the correlation of efficacy with antigen density depends on the type of cancer cell, probably due to the varying rate of internalization of the antigen-ADC complex. To study the internalization of ADCs in $SSEA4^+$ cells, we conjugated the azido-fucose containing antibody with Alexa-Fluor 488 by strain-promoted [3 + 2] azide alkyne cycloaddition reaction (SPAAC) to form chMC813–70-AF488 (Figure 2a and supplementary information Figure S2). Next, we incubated $SSEA4^+$ breast cancer cell line MDA-MB-231 with chMC813–70-AF488 for 1 h at 4 °C and washed the cells to remove unbound antibodies. Then the cells were incubated at 37 °C to allow endocytosis to start. At indicated time points as shown in the figure, cells were fixed and permeabilized. The lysosomal marker LAMP-1 was stained by APC-conjugated antibody and nucleus was stained by DAPI followed by confocal microscopy analysis. The chMC-813–70-AF488 was only observed on the cell surface at 0 min. When cells were incubated at 37 °C for 1 h, the internalization of chMC-813–70-AF488 could be observed (Figure 2b, Green panel). After longer incubation, the majority of chMC813–70-AF488 moved to the intracellular space. This result showed that the glycoengineered chMC-813–70 antibody is internalized by MDA-MB-231 cells, which is consistent with a previous report by Daniotti et al.[23]

2.2. Conjugation of chMC-813–70 to MMAE via Cleavable and Non-Cleavable Linkers

Given the rapid internalization ability of chMC-813–70, we sought to test if the antibody could deliver MMAE to SSEA4-expressing cancer cells. MMAE is a highly potent microtubule-targeting agent which blocks tubulin polymerization to arrest cancer cell division and has been used in at least three approved ADCs and multiple ADCs in clinical

studies. Once the ADC is internalized, MMAE is released, typically by lysosomal cleavage, and leads to the efficient killing of the cancer cell. In some instances, MMAE also diffuses into the surrounding cells to exhibit a bystander effect.

The design and properties of the linker that connects MMAE to chMC-813–70 play a critical role in determining the therapeutic efficacy of ADC. The linkers lacking sufficient plasma stability tend to release toxic payload in circulation before reaching the target site and cause undesired toxicity. In addition to its stability, the linker must be cleaved in the tumor cell to release the payload once ADC is internalized. Cleavable linkers are designed to take advantage of the extra- and intracellular tumor microenvironments, such as differences in pH or overexpression of specific lysosomal enzymes (e. g., cathepsin B is overexpressed in various cancers). The cathepsin B-specific sequences like valine-citrulline (Val-Cit) have been coupled with *p*-aminobenzyloxycarbonyl (Val-Cit-PABS) and is one of the most commonly used cleavable linker for ADC development.[24]

To test the impact of linker design and the conjugation site on antibody, we constructed chMC-813–70-ADCs using commercially available DBCO-PEG4-Val-Cit-PAB-MMAE as cleavable and DBCO-PEG4-PAB-MMAE as non-cleavable linkers. The chMC-813–70 with four azido tags on Fc-glycan was conjugated to the linkers using SPAAC ligation to generate chMC-813–70-VC-PAB-MMAE (ADC-1) and chMC813–70-PAB-MMAE (ADC-2) with DAR of 4 (Figure 3 and supplementary information Figure S3). Conjugation protocols were optimized to achieve maximum DAR of 4 and the ADC was purified using a proteinA gel column, and the purity was confirmed by SDS-PAGE and characterized by mass spectrometry (Supporting information Figure S4). The ADC consisting of mAb Herceptin targeting HER2 receptor was also constructed using the same strategy and used for comparison study (Supplementary information Figure S4 and Table S1).

2.3. In Vitro Cytotoxicity Studies of chMC-813–70-VC-PAB-MMAE and chMC813–70-PAB-MMAE

Having ADCs with cleavable and non-cleavable linkers in place, we next assessed there *in vitro* cytotoxicity activities using SSEA4-expressing ovarian SKVO3 cells and breast cancer cells SKBR3 with a negligible expression of SSEA4 and 293T cells as a control. SK-OV3, SKBR3, and 293T cells (2×10^3 cells/well) were incubated with ADC-1, ADC-2, and chMC-813–70 for 72 h and then treated with MTS at 37 °C. The conversion of the tetrazolium salt MTS (3-(4,5-dimeth-ylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) to a purple formazan, as a measure of the percentage of viable cells, was measured by absorbance at 490 nm after 4 h. The untreated cells' absorbance was considered 100 % survival (Figure 4).

ADC-1 containing cathepsin B labile cleavable linker showed excellent cytotoxicity with median IC_{50} = 0.211 nM against high SSEA4-expressing SKVO3 cells compared to chMC-813–70. ADC-2 with a non-cleavable linker showed reduced cytotoxicity activity against SKVO3. However, SKBR3 treated with ADC-1 also showed some cytotoxicity (IC_{50} = 4.16 nM), probably due to the low expression of SSEA-4 on SKBR3 cells. The Herceptin ADC with a cleavable linker, when treated with HER-expressing SKBR3 and SKOV3 cells, showed improved cytotoxicity (IC_{50} = 29 pM and IC_{50} = 0.70 nM respectively) compared

to the parent antibody. Whereas no cytotoxicity was observed for HEK293T cells used as a negative control.

3. Conclusions

In summary, this study reports the synthesis, characterization, endocytosis, and *in vitro* cytotoxicity assessment of ADC targeting the cancer specific SSEA4 antigen on solid tumors. Using the probing method developed in this study, we demonstrated that the chimeric chMC-813–70 antibody specific for SSEA4 is rapidly internalized by the SSEA4-expressing cells. In addition, the *in vitro* glycoengineering of antibody Fc-glycan has been used to incorporate four azido fucose tags to the homogeneous antibody glycoform for covalent attachment of highly potent microtubule-targeting agent MMAE through cleavable and non-cleavable linkers to achieve a DAR of 4. The anti-SSEA4 ADCs showed excellent cytotoxic activities towards SSEA4-expressing ovarian SKVO3 cells but not the SSEA4 negative HEK293T cells. The ADCs with cathepsin B labile cleavable linker showed better cytotoxicity than the non-cleavable counterpart, indicating the importance of lysosomal release for therapeutic efficacy. This study offered an effective platform and strategy for site-specific conjugation of a payload to a homogeneous antibody Fc-glycan with desired therapeutic outcome to develop optimal antibody-drug conjugates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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This paper is dedicated to the memory of Professor Richard Lerner, for his monumental contributions to science and human health and his transformational leadership at Scripps Research.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

[1]. a)Lerner RA, Nat. Rev. Immunol 2016, 16, 498–508; [PubMed: 27374636] b) Lin CW, Lerner RA, Int. J. Mol. Sci 2021, 22(8), 4123 ; [PubMed: 33923551] c) Sinha SC, Li LS, Miller GP, Dutta S, Rader C, Lerner RA, Proc. Natl. Acad. Sci. USA 2004, 101, 3095–3099; [PubMed: 14981258] d) Fu Z, Li S, Han S, Shi C, Zhang Y, Signal Transduct Target Ther 2022, 7, 93; [PubMed: 35318309] e) Falzone L, Salomone S, Libra M, Front. Pharmacol 2018, 9, 1300; [PubMed: 30483135] f) Patel A, Unni N, Peng Y, Cancers (Basel) 2020, 12, 2081. [PubMed: 32731409]

[2]. a)Tay MZ, Wiehe K, Pollara J, Front. Immunol 2019, 10, 332; [PubMed: 30873178] b) Cao X, Chen J, Li B, Dang J, Zhang W, Zhong X, Wang C, Raoof M, Sun Z, Yu J, Fakih MG, Feng M, Sci. Adv 2022, 8, eabl9171. [PubMed: 35302839]

[3]. a)Pereira NA, Chan KF, Lin PC, Song Z, mAbs 2018, 10, 693–711; [PubMed: 29733746] b) Thomann M, Reckermann K, Reusch D, Prasser J, Tejada ML, Mol. Immunol 2016, 73, 69–75. [PubMed: 27058641]

[4]. Zhang Q, Joubert MK, Polozova A, De Guzman R, Lakamsani K, Kinderman F, Xiang D, Shami A, Miscalichi N, Flynn GC, Kuhns S, Biotechnol. Prog 2020, 36, e3045. [PubMed: 32627435]

[5]. Joubert N, Beck A, Dumontet C, Denevault-Sabourin C, Pharmaceuticals (Basel) 2020, 13, 245. [PubMed: 32937862]

[6]. Sheyi R, de la Torre BG, Albericio F, Pharmaceutica 2022, 14, 396.

[7]. Maass KF, Kulkarni C, Betts AM, Wittrup KD, AAPS J. 2016, 18, 635–646.

[8]. Ford CH, Newman CE, Johnson JR, Woodhouse CS, Reeder TA, Rowland GF, Simmonds RG, Br. J. Cancer 1983, 47, 35–42. [PubMed: 6821632]

[9]. a)Tong JTW, Harris PWR, Brimble MA, Kavianinia I, Molecules 2021, 26, 5847; [PubMed: 34641391] b) Kantarjian HM, Kadia TM, DiNardo CD, Welch MA, Ravandi F, Cancer 2021, 127, 1186–1207. [PubMed: 33734442]

[10]. Norsworthy KJ, Ko CW, Lee JE, Liu J, John CS, Przepiorka D, Farrell AT, Pazdur R, Oncologist 2018, 23, 1103–1108. [PubMed: 29650683]

[11]. Heo YA, Drugs 2023, 83(4), 347–352. [PubMed: 36802324]

[12]. Chen I-J, Wang C-C, Shia C-S, Su C-C, Lu C-H, Chang H-W, Chiu P-T, Wu Y-C, Lai M-T, Tang W-C, Tung H-Y, Hsu R-Y, Cancer Res. 2021, 81, 955–955.

[13]. Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, Jonas M, Anderson ME, Setter JR, Senter PD, Nat. Biotechnol 2015, 33, 733–735. [PubMed: 26076429]

[14]. Teicher BA, Morris J, Curr. Cancer Drug Targets 2022, 22, 463–529. [PubMed: 35209819]

[15]. McKertish CM, Kayser V, Biomedicine 2021, 9, 872.

[16]. a)Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, Chen Y, Simpson M, Tsai SP, Dennis MS, Lu Y, Meng YG, Ng C, Yang J, Lee CC, Duenas E, Gorrell J, Katta V, Kim A, McDorman K, Flagella K, Venook R, Ross S, Spencer SD, Lee Wong W, Lowman HB, Vandlen R, Sliwkowski MX, Scheller RH, Polakis P, Mallet W, Nat Biotechnol 2008, 26, 925–932; [PubMed: 18641636] b) Axup JY, Bajjuri KM, Ritland M, Hutchins BM, Kim CH, Kazane SA, Halder R, Forsyth JS, Santidrian AF, Stafin K, Lu Y, Tran H, Seller AJ, Biroc SL, Szydlik A, Pinkstaff JK, Tian F, Sinha SC, Felding-Habermann B, Smider VV, Schultz PG, Proc. Natl. Acad. Sci. USA 2012, 109, 16101–16106; [PubMed: 22988081] c) Kang MS, Kong TWS, Khoo JYX, Loh TP, Chem. Sci 2021, 12, 13613–13647. [PubMed: 34760149]

[17]. Anami Y, Xiong W, Gui X, Deng M, Zhang CC, Zhang N, An Z, Tsuchikama K, Org. Biomol. Chem 2017, 15, 5635–5642. [PubMed: 28649690]

[18]. Ma ZX, Xu Y, Zhao H, Sun FM, Zhang XR, Wang M, Zhang J, Yao Xue Xue Bao 2017, 52, 403–408. [PubMed: 29979846]

[19]. Chon H, Kanamori S, Hibino K, Nagahara T, Suzuki T, Ohara K, Narumi H, Bioorg. Med. Chem. Lett 2022, 80, 129117. [PubMed: 36584791]

[20]. a)Walsh SJ, Bargh JD, Dannheim FM, Hanby AR, Seki H, Counsell AJ, Ou X, Fowler E, Ashman N, Takada Y, Isidro-Llobet A, Parker JS, Carroll JS, Spring DR, Chem. Soc. Rev 2021, 50, 1305–1353; [PubMed: 33290462] b) Agarwal P, Bertozzi CR, Bioconjugate Chem. 2015, 26, 176–192;c) Yang Y, Song Z, Tian T, Zhao Z, Chen J, Hu J, Jiang X, Yang G, Xue Q, Zhao X, Sha W, Yang Y, Li JP, bioRxiv 2022, 2022.2009.2004.506510;d) Tang F, Wang L-X, Huang W, Nature Protocols 2017, 12, 1702–1721. [PubMed: 28749929]

[21]. a)Shivatare SS, Huang LY, Zeng YF, Liao JY, You TH, Wang SY, Cheng T, Chiu CW, Chao P, Chen LT, Tsai TI, Huang CC, Wu CY, Lin NH, Wong CH, Chem. Commun. (Camb.) 2018, 54, 6161–6164; [PubMed: 29809215] b) Lin CW, Tsai MH, Li ST, Tsai TI, Chu KC, Liu YC, Lai MY, Wu CY, Tseng YC, Shivatare SS, Wang CH, Chao P, Wang SY, Shih HW, Zeng YF, You TH, Liao JY, Tu YC, Lin YS, Chuang HY, Chen CL, Tsai CS, Huang CC, Lin NH, Ma C, Wu CY, Wong C-H, Proc. Natl. Acad. Sci. USA 2015, 112, 10611–10616. [PubMed: 26253764]

[22]. Lin CW, Wang YJ, Lai TY, Hsu TL, Han SY, Wu HC, Shen CN, Dang V, Chen MW, Chen LB, Wong C-H, Proc. Natl. Acad. Sci. USA 2021, 118(50), e2114774118.

[23]. Ruggiero FM, Rodríguez-Walker M, Daniotti JL, Immunol. Cell Biol 2020, 98, 187–202. [PubMed: 31916611]

[24]. Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan S, Zhou X, Li S, Acta Pharm. Sin. B 2021, 11, 3889–3907. [PubMed: 35024314]

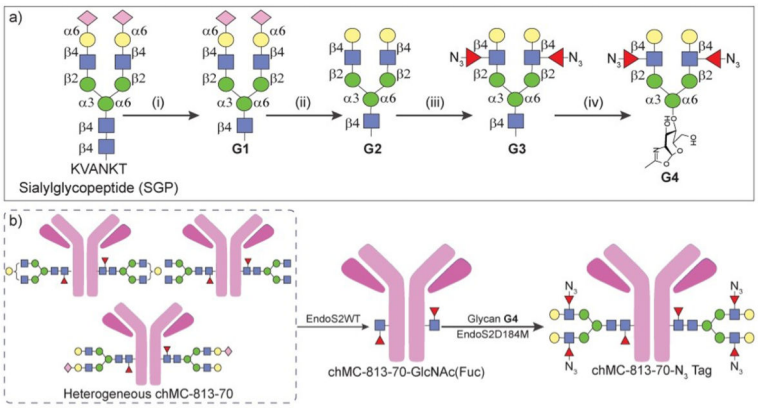

Figure 1.

Glyco-engineering of chMC813-70 Fc-glycan to incorporate the azido tag. A) Synthesis of glycan oxazoline for transglycosylation. i) EndoS2WT, overnight; ii) Neuraminidase, overnight; ii) GDP-azido-Fucose, α-1,3 FucT; iv) DMC, H_2O: TEA; B) Deglycosylation of heterogeneous IgG using WTEndoS2, followed by transglycosylation in the presence of glycan oxazoline G4 and EndoS2D184 M.

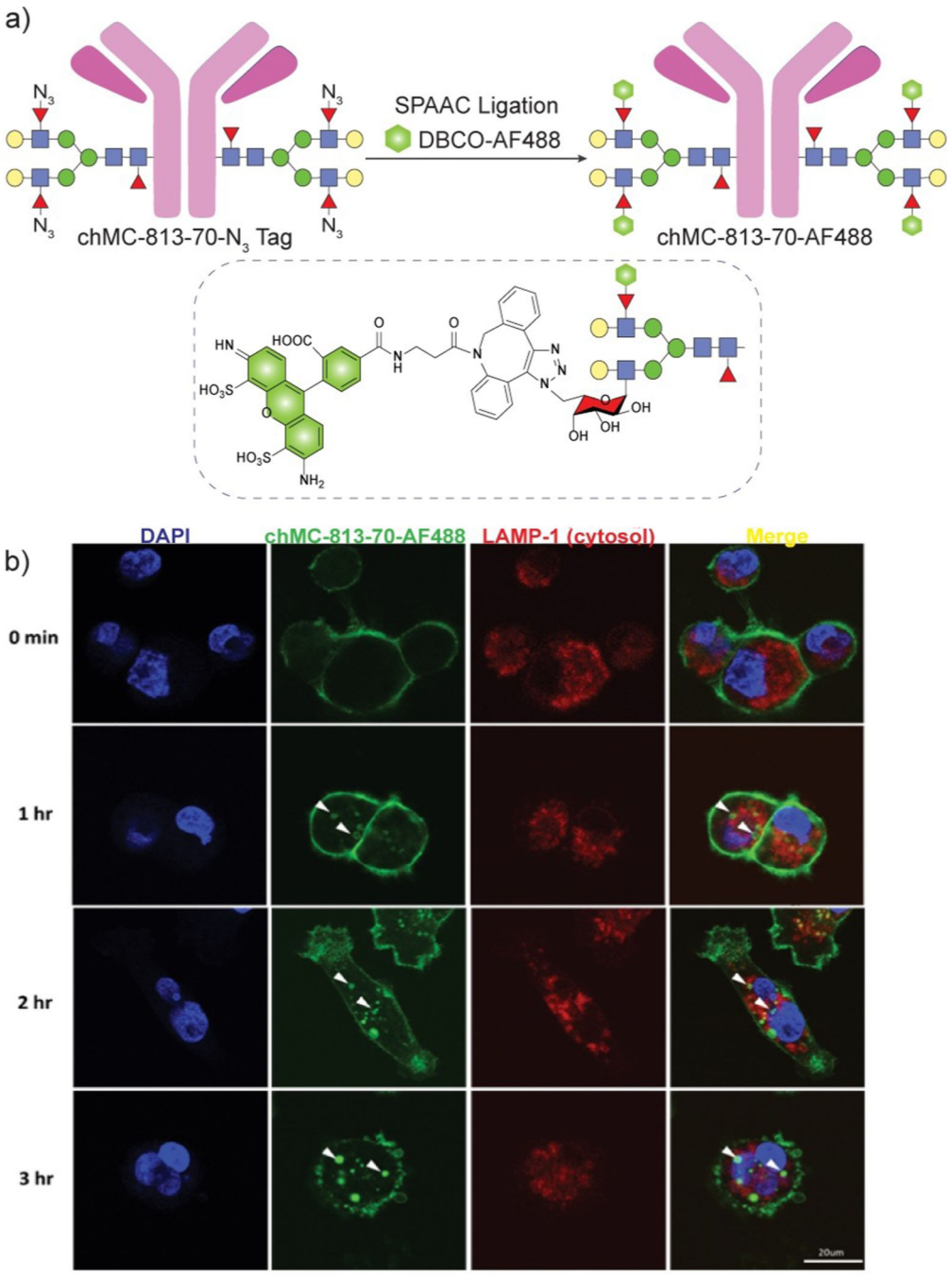

Figure 2.

Synthesis and endocytosis of labelled chMC-813-70 in SSEA4 + cancel cells. a) Synthesis of AF488 conjugated antibody using SPAAC ligation; b) Endocytosis of chMC-813-70 at different time points.

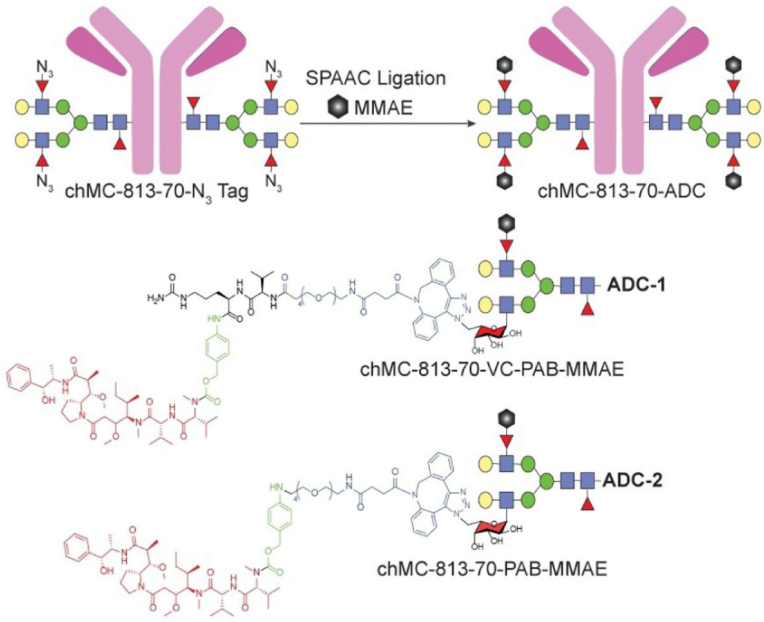

Figure 3.

Synthesis of chMC-813-70 ADCs consisting of cleavable (ADC-1) and non-cleavable (ADC-2) linkers.

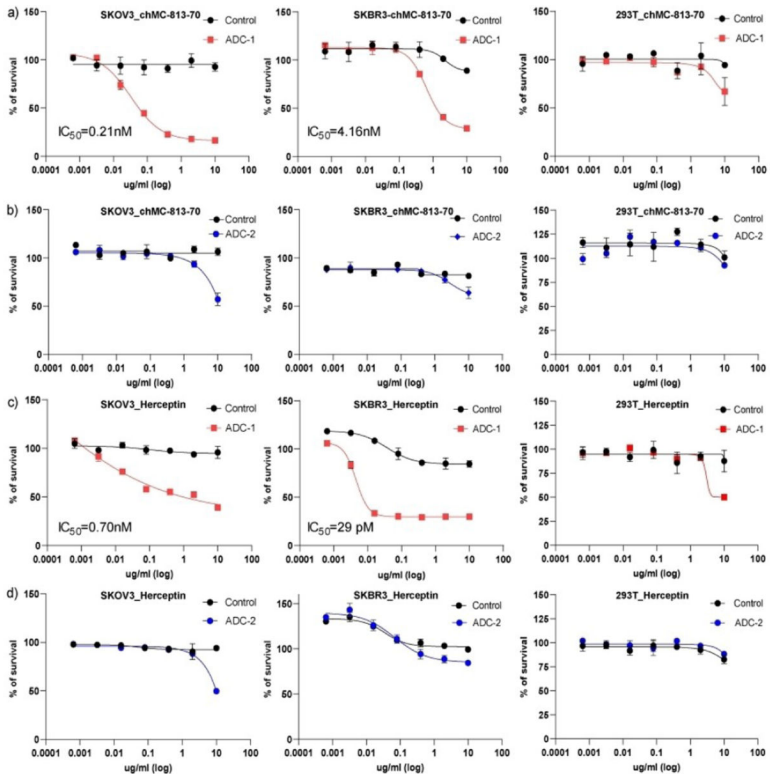

Figure 4.

In vitro cytotoxicity of chMC-813-70 ADCs consisting of a) cleavable (ADC-1) and b) non-cleavable (ADC-2) linkers to the SSEA4-expressing tumor cells.; and Herceptin ADCs consisting of c) cleavable (ADC-1) and d) non-cleavable (ADC-2) linkers to the HER2-expressing tumor cells.