GENOVIS PRESENTS

Site-specific Antibody Drug Conjugation with 2-step Cleavable Linker-payloads Displays Potent *In Vivo* Efficacy

AUTHORS

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INTRODUCTION

Site-specific antibody conjugation using the GlyCLICK technology has proven a promising platform for obtaining homogenous antibody drug conjugates (ADCs). We have previously demonstrated GlyCLICK conjugated antibodies to stay longer in circulation and increase tumor uptake compared to randomly conjugated antibodies. In this work we combine enzymatic conjugation of antibodies at the Fc glycans with two-step cleavable glycopeptide linker-payloads and characterize the conjugation process as well as study the functional properties of the ADCs *in vitro* and *in vivo*.

The results demonstrate site-specific conjugation and generation of unique ADCs using a glycopeptide linker and potent tumor killing activities *in vitro* and *in vivo*.

SUMMARY

- Aim: Characterize site-specific ADCs generated using GlyCLICK conjugation and two-step cleavable linker-payloads using *in vitro* and *in vivo* assays
- GlyCLICK is a conjugation technology that enables site-specific attachment of linker-payloads to Fc glycans
- ADCs displayed DAR 2.0 with homogenous conjugation at the Fc glycans as shown by middle-level LC-MS characterization
- In vitro cytotoxic effects were observed for MMAE and PNU conjugated linker-payloads with IC50 values in the pM range
- A custom ADC with site-specific conjugation and a glycopeptide linker-payload showed potent in vivo efficacy in a mouse melanoma tumor model using a single dose

RESULTS

Characterization of Site-specific ADC Conjugates

For antibody drug conjugates, the site of conjugation and the number of conjugated payloads may impact the efficacy of the therapeutic molecule. The glycans has emerged as a desired spot for attachment of payloads and the enzymatic conjugation strategy GlyCLICK enables site-specific conjugation using the conserved Fc glycans of a native IgG (Fig 1).

In this work, a novel glycopeptide linker with a two-step digestion for release, was conjugated to panitumumab and trastuzumab with MMAE and PNU as toxins (Fig 2). The conjugation process was characterized using middle-level LC-MS and confirmed DAR 2.0 and site-specific conjugation at the Fc glycans. The data shows the mass shifts from native antibody (top) to complete conjugation with two linker-payloads per antibody (Fig 3).

Figure 1. The GlyCLICK technology is a two-step enzymatic modification of the IgG Fc-glycans adding an azide group to the inner GlcNAc. The azide-activated antibody is then conjugated using click-chemistry to an alkyne-carrying probe with a payload of choice.

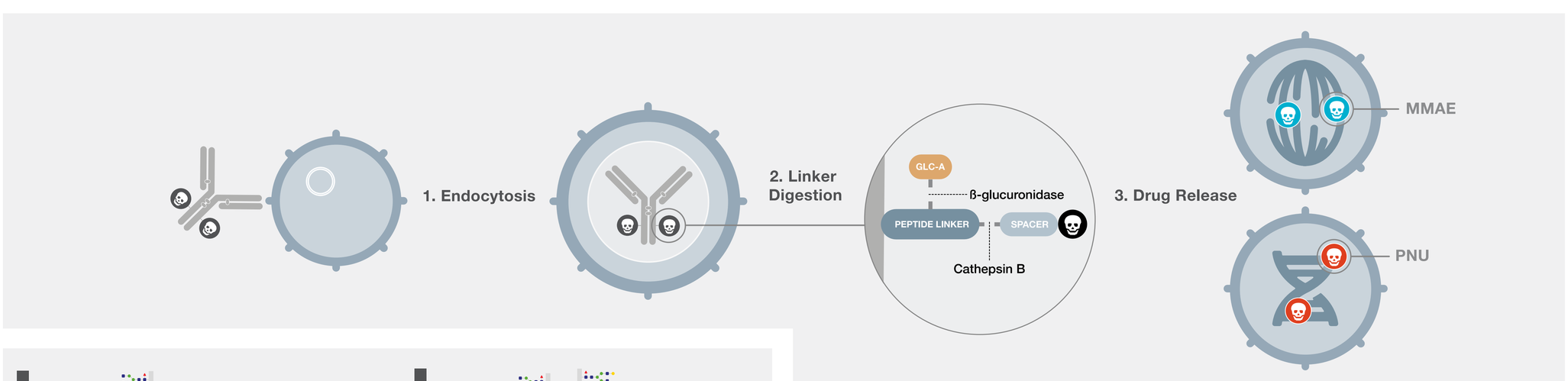

Figure 2. Toxin release mechanism of the ADC using a glycopeptide cleavable linker with two-step hydrolysis required for payload release within the target cell. The glycopeptide cleavable linker relies on both β-glucuronidase and cathepsin B for release.

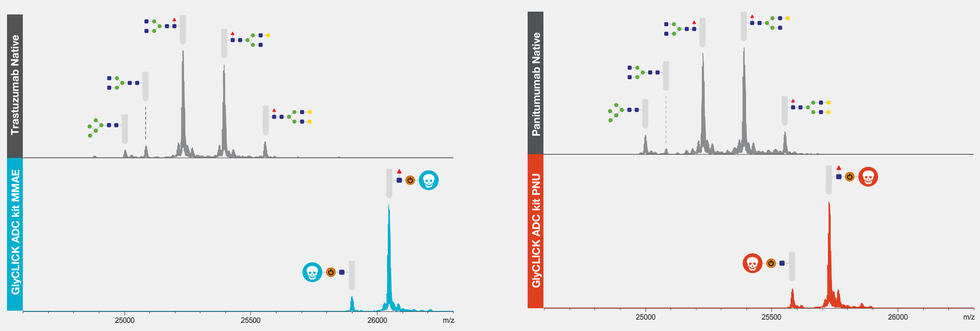

Figure 3. A) LC-MS analysis of trastuzumab conjugated with the MMAE (monomethyl auristatin E) linker-payload using GlyCLICK. The antibody was analyzed after FabRICATOR digestion to determine the native Fc/2 glycan profile (top) and site-specific labeling after conjugation using GlyCLICK ADC kit MMAE (bottom).
B) LC-MS analysis of panitumumab conjugated with the PNU linker-payload using GlyCLICK. The antibody was analyzed after FabRICATOR digestion to determine the native Fc/2 glycan profile (top) and site-specific labeling after conjugation using GlyCLICK ADC kit PNU (bottom).

Potent Cell Killing with Site-specific Glycopeptide Linker Payloads

The ADCs used in this study were further tested for functional killing activity using a HER2+ cell line and trastuzumab conjugated with either MMAE or PNU. The observed cytotoxic effects on the cell lines resulted in IC50 values of 120 pM for the MMAE conjugate and 75 pM for the PNU conjugate (Fig 4). This data demonstrates the functionality of the glycopeptide linker conjugated at the Fc glycans of trastuzumab with two different payloads. This approach could easily be expanded to study the impact of other linkers or payloads of choice.

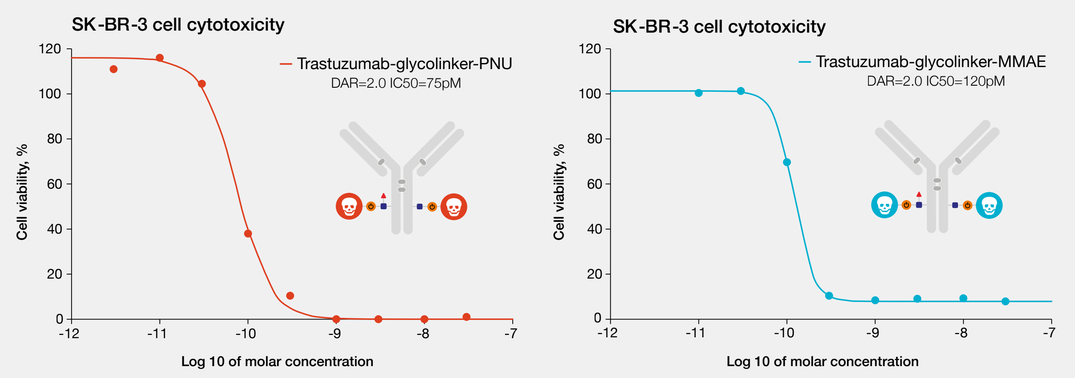

Figure 4. *In vitro* cytotoxicity analysis of trastuzumab conjugated with the **A)** MMAE or the **B)** PNU linker-payload using GlyCLICK ADC kit. HER2+ SK-BR-3 cells were incubated with the ADC and cell viability measured with PrestoBlue after 5-6 days. Curves represent the average result from three replicates.

In vivo Efficacy of Site-specific Conjugated Glycopeptide Linker ADC

To test this novel combination of glycan conjugation with a glycopeptide linker in vivo, a nondisclosed antibody was conjugated with the PNU-linker using GlyCLICK ADC kit. The obtained ADC was evaluated in a mouse B16-F10 melanoma model. Complete and durable responses were observed after a single 5 mg/kg dose of PNU ADC in this difficult-to-treat cold tumor model (Fig 5).

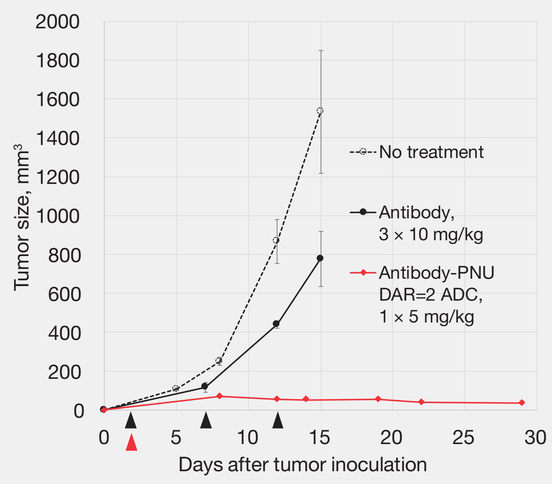

Figure 5. The in vivo study was performed according to ethical committee approval at the TCDM/Central Animal Laboratory, University of Turku, Finland. B16-F10 murine syngeneic melanoma tumors were subcutaneously inoculated to one flank of C57BL/6J mice, 0.5 million cells/mouse. Mice were randomly divided to groups of no treatment (n=5), treatment with mouse IgG2a antibody against a tumor antigen (n=7, 3 intravenous doses of 10 mg/kg) and glycoconjugated DAR=2 ADC: the same antibody conjugated with DBCO-Val-Ser(GlcA)-EDA-PNU payload (n=7, single intravenous dose of 5 mg/kg). First dosings were administered 2 days after inoculation and subsequent doses (antibody group only) at 5 days' intervals. Figure below shows the average tumor volumes of each group until the first death of a mouse due to tumor growth. Error bars show the standard error of the mean (SEM). All mice in the other groups died during the study due to tumor growth, while all tumors in the ADC group shrank in size and showed no regrowth to the end of the study.

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