Glycoengineered Lysosome Targeting Constructs (G-LyTAC): A novel class of targeted circulating and membrane protein degraders

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GLYCOERA

Abstract

Targeted protein degradation (TPD) is an emerging therapeutic approach which is attracting significant interest due to its potential to remove proteins directly associated with the pathogenesis of diseases. Most TPD platforms take advantage of the ubiquitin proteasome system and therefore are limited to degrading intracellular proteins.

Here we describe the development of glycoengineered lysosomal-targeting constructs (G-LyTACs), that are capable of targeting either circulating or membrane proteins. G-LyTACs are bifunctional biologics that bind to a specific target protein at one end and selectively engage lysosomal targeting receptors at the other end through specific glycans. One such lysosomal targeting receptor, the asialoglycoprotein receptor (ASGPR), is a glycan sensing, lysosome-trafficking endocytic receptor exclusively expressed on the surface of hepatocytes. GlycoEra's CustomGlycan Platform is a novel and proprietary system for tunable expression of biologics with homogeneous glycans at specific sites on the protein. G-LyTACs generated using GlycoEra's CustomGlycan Platform therefore can bind to specific circulating or membrane proteins and target them for degradation in the lysosomal compartment in the liver.

Different G-LyTACs targeting either a circulating or a membrane protein was designed. *In vitro* experiments, using human hepatocyte cell line HepG2, demonstrated that G-LyTACs is internalized by an ASGPR dependent process in a dose and time dependent manner and subsequently localized in the lysosomes. Additionally, an extracellular target protein was efficiently bound by G-LyTAC, co-internalized and readily degraded following trafficking to the lysosome. Furthermore, *in vivo* studies in rodents confirmed that a single injection of G-LyTAC, rapidly and efficiently bound to the model target protein, and resulted in its near complete removal from circulation by degradation in the liver. Similarly, treatment with a G-LyTAC designed to target a membrane protein on surface of HepG2 cells, resulted in significant reduction of its cell surface levels following lysosomal degradation.

Taken together, we present *in vitro* and *in vivo* evidence that CustomGlycan platform G-LyTACs are potent and efficient degraders of their circulating and membrane targets via the endo-lysosomal pathway mediated by ASGPR. In contrast to classical TPD, G-LyTACs can degrade circulating proteins, which are considered a challenging target for antibodies and small molecules

Drugs & biologics neutralize action of some pathogenic proteins, but many are undruggable

Preventing the activity of disease causing proteins

Unsolved
- Autoantibodies
- Misfolded proteins
- Protein aggregates
- Immune complex
- Complex receptors
- Highly flexible proteins

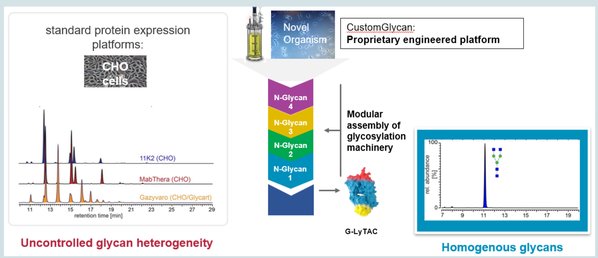
Solved
- Natural products
- Synthetic drugs
- Biologics and Ab

Currently treated with:
- Plasma Exchange
- Surgery
- Radioisotope treatment
- Systemic immune suppression
- Blood Transfusion
- IVIg

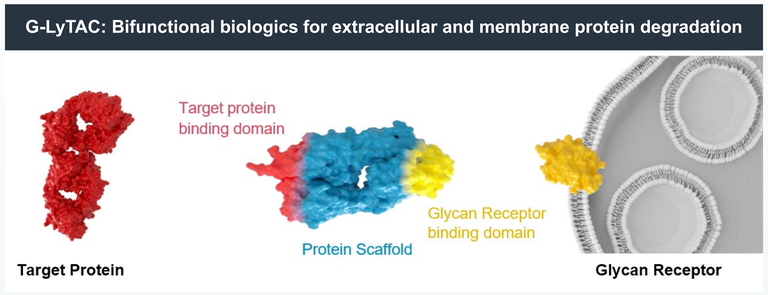
Current treatments are not as effective or safe but costly to healthcare

What if we could selectively degrade disease causing proteins?

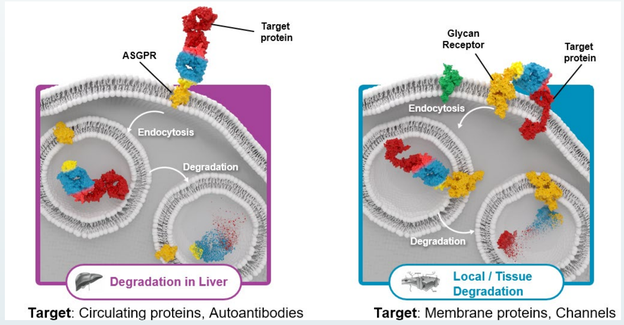
CustomGlycan platform enables making proteins with homogeneous glycans



G-LyTAC: Bifunctional biologics for extracellular and membrane protein degradation



G-LyTAC mediated degradation: soluble or cell membrane proteins



Target: Circulating proteins, Autoantibodies

Target: Membrane proteins, Channels

G-LyTAC (mAb) localizes to liver (*in vivo*) and is rapidly degraded

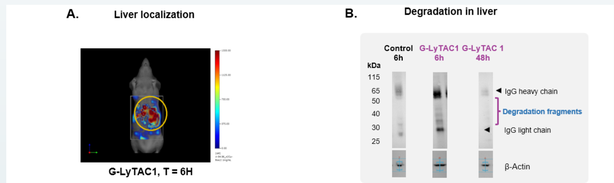


Figure 4 : Mice were injected i.v with CF750-labeled G-LyTAC1 at 5 mg/kg and imaged using fluorescence tomography. **A.** Representative image showing liver localization of G-LyTAC1. **B.** Livers from mice injected i.v. with CF750-labelled antibodies at 5 mg/kg were harvested at indicated time points and protein extracts were obtained for western blot analysis. The CF750 fluorescent signal representing G-LyTAC1 was quantified.

Fast and complete removal of circulating target protein by G-LyTAC (*in vivo*)

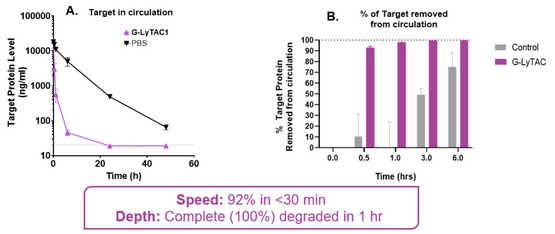


Figure 1 : A. Rats were injected i.v with the Target protein (0.5 mg/kg). 15 minutes later, PBS and G-LyTAC1 (5mg/kg) were injected. Graph shows average ± SD of Target protein serum concentration over time as measured by ELISA. N= 3 or 4 animals /group. **B.** Data obtained in A. were analyzed to represent the percentage of Target protein depleted at each time point for G-LyTAC1.

G-LyTACs (mAb) are rapidly internalized and degraded via ASGPR mechanism

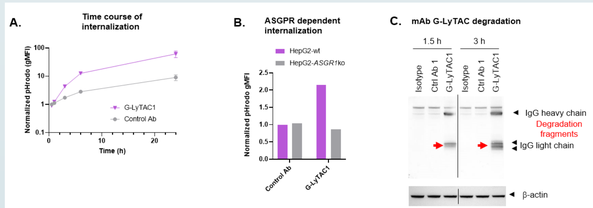


Figure 5 : A. HepG2 cells were treated with 3μg/ml of pHrodo-labeled control antibody or G-LyTAC1 (mAb) for the indicated time. Internalization was monitored by detection of pHrodo fluorescence by flow cytometry. Data were normalized to control antibody. **B.** HepG2-wt or HepG2-*ASGR1*ko cells were treated as in A. for 3 hours. **C.** HepG2 cells were treated with 100μg/ml of the indicated antibodies for 1.5 or 3 hours. Cells were harvested, washed and whole cell extracts prepared for western blot analysis. Internalized antibodies and β-actin were detected using anti-IgG (H+L) and anti-β-actin antibodies respectively.

Removal of circulating target protein is customizable by G-LyTAC (*in vivo*)

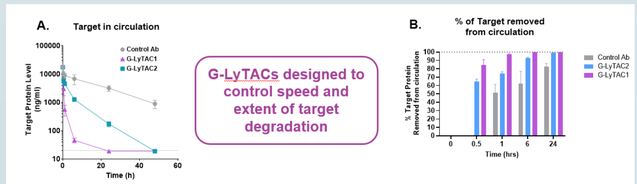


Figure 2 : A. Rats were injected i.v with the Target protein (0.5 mg/kg). 15 minutes later, control Ab, G-LyTAC1 or G-LyTAC2 (5mg/kg) rationally designed to explore glycan requirements for level of ASGPR engagement and degradation were also injected. Graph shows average ± SD of Target protein serum concentration over time as measured by ELISA. N= 3 or 4 animals /group. **B.** Data obtained in A. were analyzed to represent the percentage of Target protein depleted at each time point.

Time and dose dependent G-LyTACs localization within the lysosome

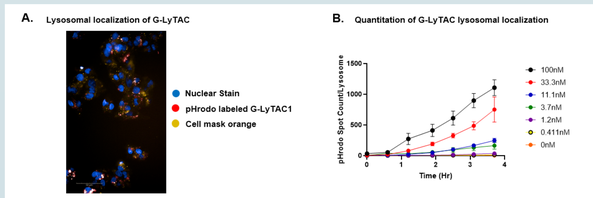


Figure 6 : A. Representative image of the internalization assay of G-LyTAC1 hrs in HepG2 cells. Briefly, the nuclei and cell edges where identified, cells at the edge of the image were removed. The pHrodo signal which represents G-LyTAC1 is represented in red. **B.** Time and dose dependent internalization and localization of G-LyTAC1 in lysosome. Quantification of the colocalized spots normalized to the number of cells in the ROI.

G-LyTAC1 efficiently bind extracellular targets and co-internalize them for degradation

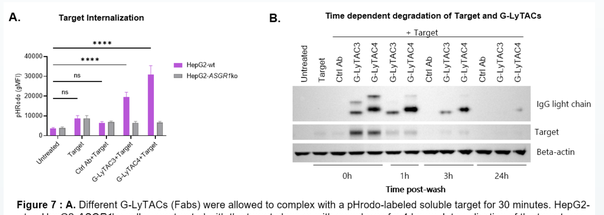


Figure 7 : A. Different G-LyTACs (Fabs) were allowed to complex with a pHrodo-labeled soluble target for 30 minutes. HepG2-wt or HepG2-*ASGR1*ko cells were treated with the target alone or with complexes for 4 hours. Internalization of the target was monitored by detection of pHrodo fluorescence by flow cytometry. **B.** HepG2 cells were treated as in A. for 1, 3 and 24 hours. Cells were harvested, washed and whole cell extracts prepared for western blot analysis. Internalized target, antibodies and β-actin were detected using anti-target, anti-IgG k light chain and anti-β-actin antibodies respectively.

G-LyTACs (Fabs) efficiently bind extracellular targets and co-internalize them for degradation

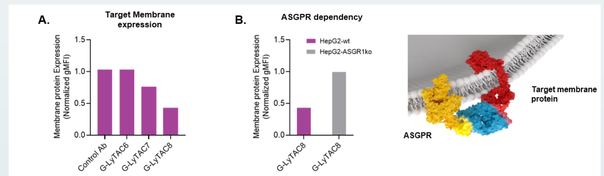


Figure 8 : A. HepG2 cells were treated with 1μg/ml of control antibody or G-LyTACs for 4 hours. Cells were then harvested, and membrane target expression detected using a non-competitive fluorescent anti-target antibody. Data were acquired by flow cytometry. Data were normalized to control antibody. **B.** HepG2-wt or HepG2-*ASGR1*ko cells were treated as in A. Data were acquired by flow cytometry as described in A.

Conclusion

- **GlycoEra's CustomGlycan Platform** is a novel and proprietary system for tunable expression of bifunctional biologics with homogeneous glycans.
- **G-LyTAC** produced using the CustomGlycan Platform –
  - Display selective ASGPR-engaging glycans
  - Selectively target both circulating and membrane protein targets
  - Rapidly directs proteins for complete degradation via the ASGPR-mediated endo-lysosomal pathway
  - Custom designed to exhibit desired time and extent of degradation
- **Novel therapeutic approach** for targeting protein degradation previously considered challenging