

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

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GLYCOERA

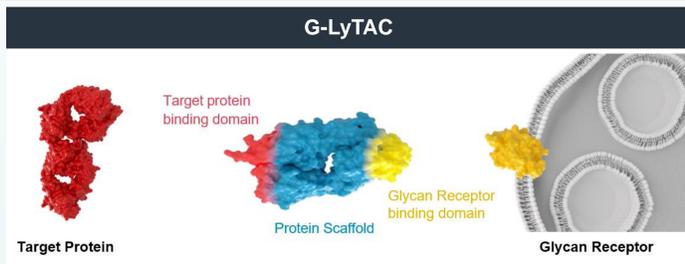
## Abstract

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.

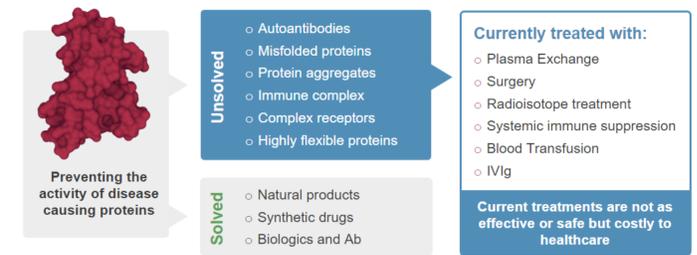
Here we describe the development of glycoengineered lysosomal-targeting constructs (G-LyTACs), that are capable of degrading either circulating or membrane proteins. G-LyTACs are bifunctional biologics that bind to a specific target protein at one end and selectively engage lysosomal targeting receptors at the other end through specific glycans. One such lysosomal targeting receptor, the asialoglycoprotein receptor (ASGPR), is a glycan-binding, lysosome-trafficking endocytic receptor expressed on the surface of hepatocytes. GlycoEra's CustomGlycan Platform (CGP) is a novel and proprietary system for tunable expression of biologics with homogeneous glycans at specific sites on the protein. CGP-expressed G-LyTACs bind to specific circulating and 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 were designed.
- *In vitro* experiments, using human hepatocyte cell line HepG2, demonstrated that G-LyTACs were 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-LyTACs, 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 G-LyTACs 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 CGP expressed 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

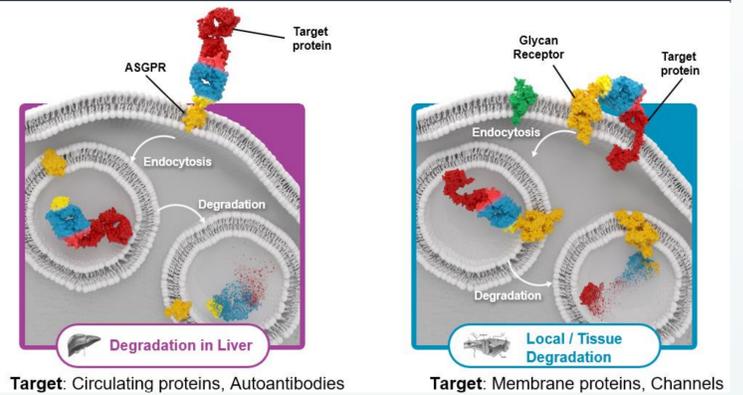


## Drugs & biologics neutralize action of some pathogenic proteins, but many are undruggable

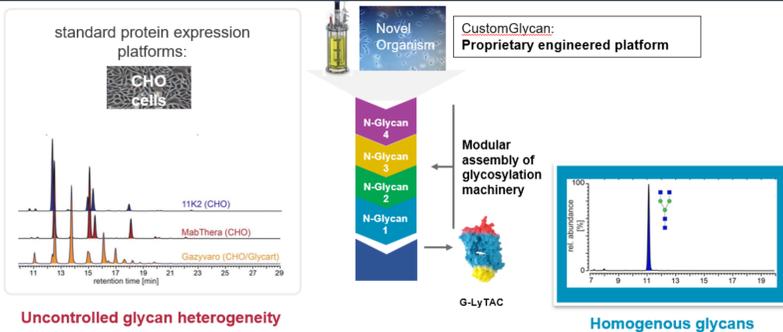


## What if we could selectively degrade disease causing proteins?

## G-LyTAC mediated degradation: soluble or cell membrane proteins



## CustomGlycan platform enables making proteins with homogeneous glycans



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

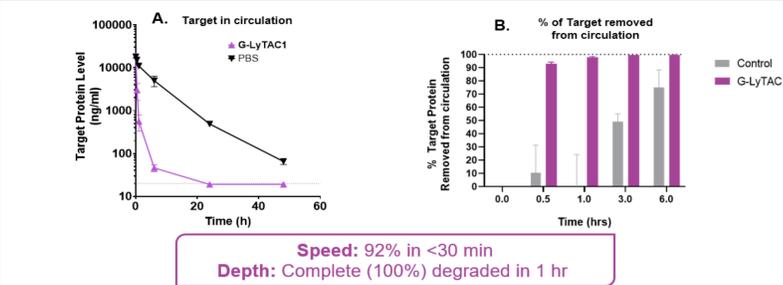


Figure 5 : A. Rats were injected i.v. with the Target protein (0.5 mg/kg). 15 minutes later, PBS and G-LyTAC1 (5mg/kg) were injected. Graph shows average  $\pm$  SD of Target protein serum concentration over time as measured by ELISA. N= 3 or 4 animals /group. B. Data obtained in A. were analyzed to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

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

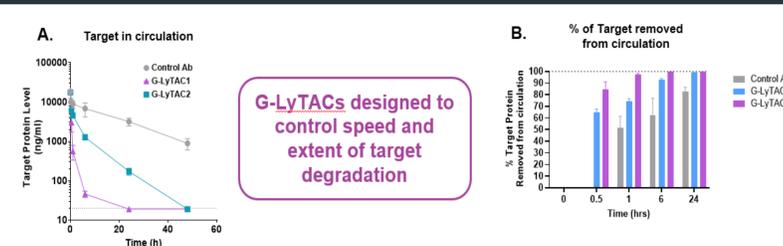


Figure 6 : A. Rats were injected i.v. with the Target protein (0.5 mg/kg). 15 minutes later, control Ab, G-LyTAC1 or G-LyTAC2 (5mg/kg) rationally designed to explore glycan requirements for level of ASGPR engagement and degradation were also injected. Graph shows average  $\pm$  SD of Target protein 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.

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

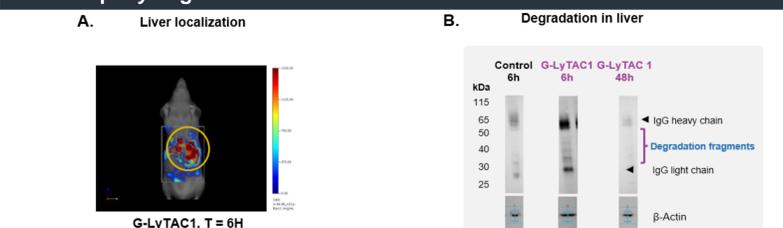


Figure 7 : Mice were injected i.v. with CF750-labeled G-LyTAC1 at 5 mg/kg and imaged using fluorescence tomography. A. Representative image showing liver localization of G-LyTAC1. B. Livers from mice injected i.v. with CF750-labelled antibodies at 5 mg/kg were harvested at indicated time points and protein extracts were obtained for western blot analysis. The CF750 fluorescent signal representing G-LyTAC1 was quantified.

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

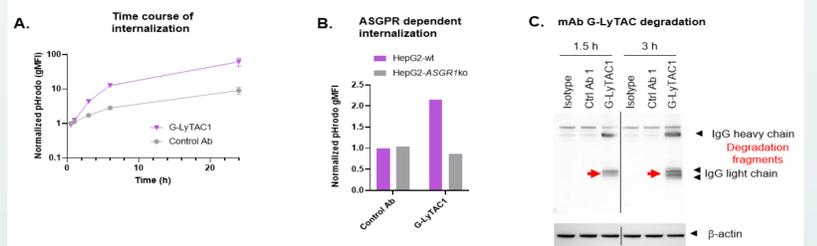


Figure 8 : A. HepG2 cells were treated with 3 $\mu$ g/ml of pHrodo-labeled control antibody or G-LyTAC1 (mAb) for the indicated time. Internalization was monitored by detection of pHrodo fluorescence by flow cytometry. Data were normalized to control antibody. B. HepG2-wt or HepG2-ASGR1ko cells were treated as in A. for 3 hours. C. HepG2 cells were treated with 100 $\mu$ g/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  $\beta$ -actin were detected using anti-IgG (H+L) and anti- $\beta$ -actin antibodies respectively.

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

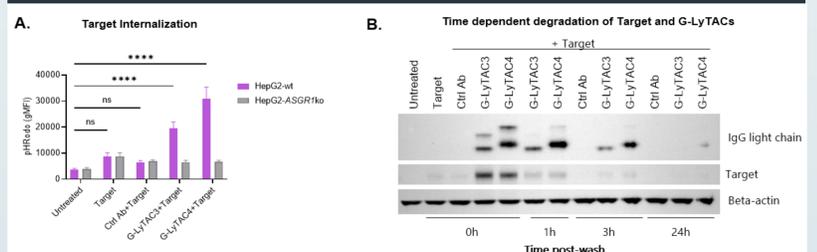


Figure 9 : A. Different G-LyTACs (Fabs) were allowed to complex with a pHrodo-labeled soluble target for 30 minutes. HepG2-wt or HepG2-ASGR1ko cells were treated with the target alone or with complexes for 4 hours. Internalization of the target was monitored by detection of pHrodo fluorescence by flow 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  $\beta$ -actin were detected using anti-target, anti-IgG k light chain and anti- $\beta$ -actin antibodies respectively.

## ASGPR-dependent membrane protein degradation

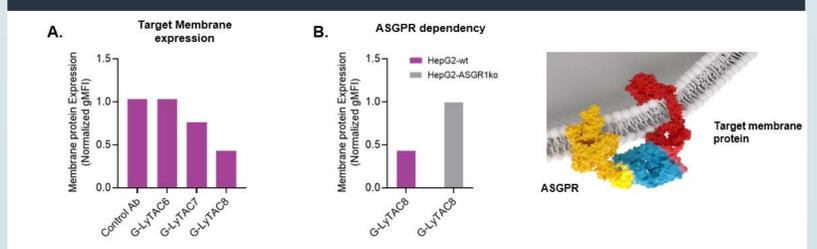


Figure 10 : A. HepG2 cells were treated with 1 $\mu$ g/ml of control antibody or G-LyTACs for 4 hours. Cells were then harvested, and membrane target expression detected using a non-competitive fluorescent anti-target antibody. Data were acquired by flow cytometry. Data were normalized to control antibody. B. HepG2-wt or HepG2-ASGR1ko cells were treated as in A. Data were acquired by flow cytometry as described in A.



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## Conclusion

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