Antibodies against GlycoTargets as novel platform approach to address unmet needs in cancer therapy

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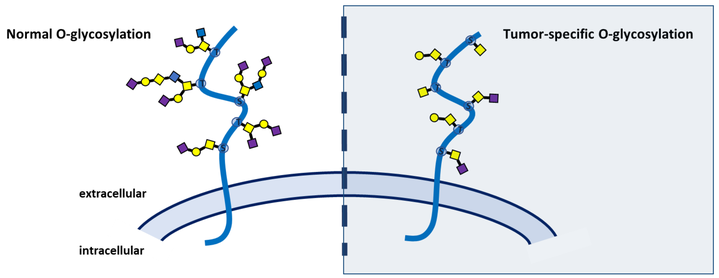
GLYCOTOPE

Abstract 1346

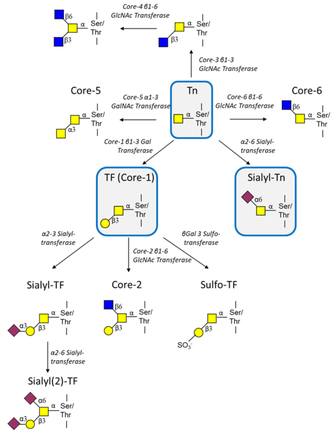
Tumor-Associated O-Glycans

Introduction

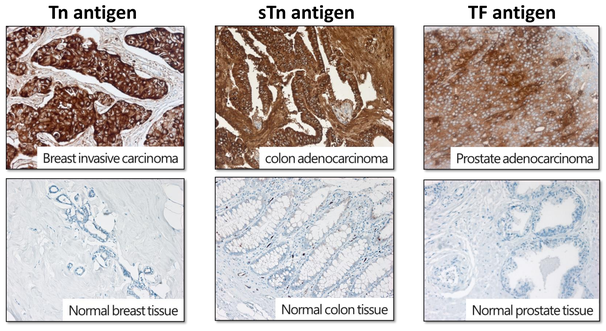
- **Glycosylation is strongly altered in cancer**[1] reflecting the drastic changes in tumor metabolism or genetic alterations
- **Proteins** expressed on cancer cells can **carry tumor-associated carbohydrates** like **TF, Tn and sTn antigen**[2]



- These **truncated O-glycans** are early intermediates of the O-glycan biosynthesis, are **normally hidden** by chain prolongation and become **de novo exposed on cancer cells** due to aberrant O-glycosylation of cell membrane proteins:
  - Thomsen nouvelle (Tn) antigen: GalNAcα1-
  - Sialylated Thomsen nouvelle (sTn) antigen: Neu5Ac(α2-6)GalNAcα1-
  - Thomsen-Friedenreich (TF) antigen: Gal(β1-3)GalNAcα1-
- Truncated O-glycans are expressed on many different carcinomas, leukemias, lymphomas and their metastases



Exemplary internal IHC data using glycan-specific mAbs:



GlycoTargets

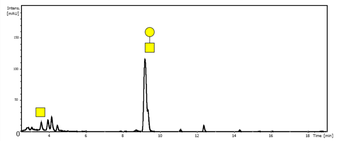
GlycoTargets for Superior Tumor-Specificity

- **GlycoTargets = Tumor-associated protein/carbohydrate combined epitopes**
- **GlycoTargets offer superior tumor specificity** compared to protein targets, which often show significant expression in healthy organs
- **GlycoTargets exhibit reduced on-target/off tumor toxicity**, which is key for highly potent therapies
- Suitable GlycoTargets for antibody development were either identified using a cellular screening approach or via RNA sequencing and bioinformatic prediction

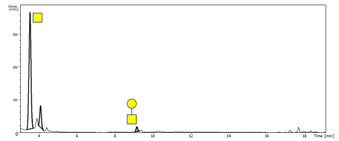
Structured Approach to Antibody Generation

- A **proprietary toolbox of cell lines** is used to produce fully characterized and highly pure cancer-specific GlycoTargets for targeted immunization approaches

O-glycan profile of GlycoTarget Y produced to carry tumor-specific TF glycans



O-glycan profile of GlycoTarget Y produced to carry tumor-specific Tn glycans



- Antibody candidates are screened by binding analysis to differently glycosylated and non-glycosylated target antigens by ELISA as well as multiplex flow cytometry (FCM)

GlycoTarget Platform Summary

- Bioinformatic predictions or cellular screenings are used to identify proteins carrying truncated O-glycans
- Using our proprietary cell lines as toolbox for antigen production, we were able to generate cancer-specific GlycoTargets for tailored immunization approaches and antibody screenings
- Two case studies (please also see poster 1347) show that our approach is suitable to target tumor-associated protein/carbohydrate combined epitopes with specific antibodies
- Due to their glycan dependency, our antibodies show markedly decreased off-tumor binding. They lack unwanted binding to healthy immune cells in contrast to conventional anti-protein antibodies and stain several cancer indications but not related normal tissues
- The increased tumor selectivity may improve safety for highly potent therapeutic approaches like ADCs, CARs or radiopharmaceuticals

References

1. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer. 2015 Sep;15(9):540-55. doi: 10.1038/nrc3982. Epub 2015 Aug 20. PMID: 26289314.
2. Kudelka MR, Ju T, Heimburg-Molinaro J, Cummings RD. Simple sugars to complex disease--mucin-type O-glycans in cancer. Adv Cancer Res. 2015;126:53-135. doi: 10.1016/bs.acr.2014.11.002. Epub 2015 Feb 7. PMID: 25727146; PMCID: PMC5812724.

Case Study: Antibody Discovery against GlycoTarget Y

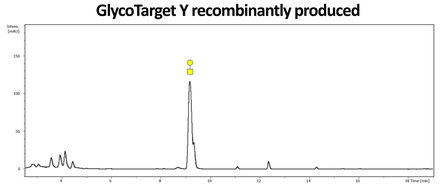
Introduction

- GlycoTarget Y is a highly glycosylated cell surface protein that is associated with tumorigenesis and is broadly expressed in several cancer indications
- However, it is also expressed in many healthy epithelial and lymphoid tissues which for therapeutic use requires an antibody that can distinguish between cancer-associated and healthy tissue expression

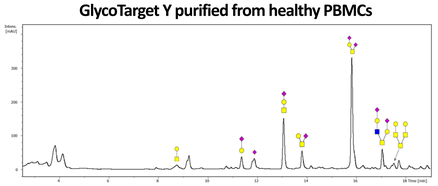
O-Glycosylation of Tumor- and Healthy GlycoTarget Y

O-glycan profiling

GlycoTarget Y recombinantly produced



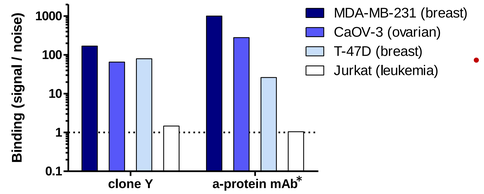
GlycoTarget Y purified from healthy PBMCs



- Recombinantly produced GlycoTarget Y carries mainly TF glycans
- GlycoTarget Y purified from healthy PBMCs carries larger and mainly sialylated glycan structures

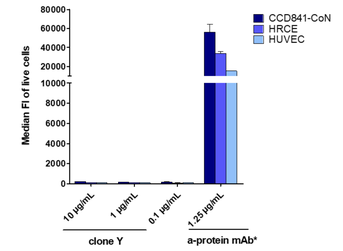
Unique Binding Profile Compared to Competitors

Flow Cytometry: Binding to tumor cell lines



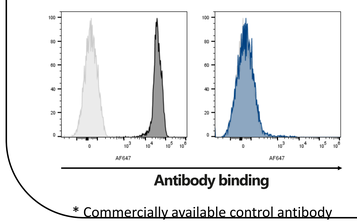
- Clone Y binds to several tumor cell lines of different origins expressing GlycoTarget Y

Flow Cytometry: Binding to normal cell lines



- The anti-protein control mAb strongly binds to various normal cell lines expressing the target protein
- Clone Y does not bind to GlycoTarget Y expressed in normal cell lines demonstrating different glycosylation of GlycoTarget Y in normal compared to tumor cell lines

Flow Cytometry: Binding to healthy human lymphocytes



- The anti-protein control mAb strongly binds to healthy human lymphocytes expressing the target protein
- The O-glycosylation-dependent protein binder clone Y does not bind to healthy human lymphocytes

* Commercially available control antibody

We aim to continuously expand our collaborations with industry partners and academic centers to further exploit the unique potential of our technology.
For further discussion please contact business.development@glycotope.com or visit our webpage https://www.glycotope.com/contact/