Abstract 5896

Platform approach to develop antibodies specifically recognizing cancer-associated glycoforms

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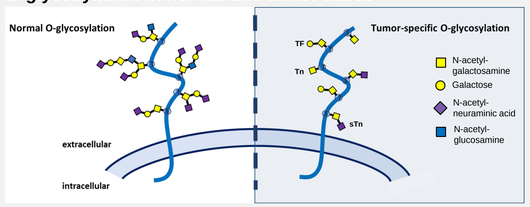
GET IN TOUCH

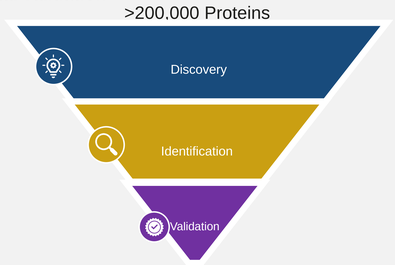
BACKGROUND

- **Glycosylation is strongly altered in cancer** reflecting the drastic changes in tumor metabolism or genetic alterations. Therefore glycans tend to elicit superior tumor specificity compared to proteins.
- Changes in glycosylation give rise to **truncated O-glycans** like the Thomsen-Friedenreich (TF), the Thomsen novelle (Tn) and the sialylated Thomsen nouvelle (sTn) antigen.[1,2]

O-glycosylation in normal and tumor tissue



Glycotope has developed a robust and differentiated approach to discover and evaluate GlycoTargets that involves theoretical and experimental validation



Discovery:

- Identification of plasma membrane expression and extracellular domain (ECD)
- O-glycosylation prediction in ECD

Identification:

- Analysis of protein expression in tumor and normal tissues

Validation:

- Flow cytometry (protein expression on tumor cell lines)
- IHC (tumor and/or normal tissues)
- LC-MS/MS O-glycopeptide analysis of recombinant proteins

Data and output at each step of the workflow is captured in a proprietary database called "GlycoBase"

GLYCOBASE USE CASE EXAMPLE: HEAD & NECK CANCER

This example is intended to give an impression of how GlycoBase can be used to specifically identify new potential antibody targets for certain indications, e.g. head & neck cancer:

1. Step: Select GlycoBase filter to only show targets for which IHC expression data in head & neck cancer was collected
2. Step: Additionally select GlycoBase filter to only include targets for which an O-linked glycosylation is predicted
3. Step: To further limit the number of possible targets and increase the probability of finding a suitable GlycoTarget, the targets proposed after Step 1 and 2 can be further restricted by tightening the requirements for protein expression and predicted O-glycosylation
4. Step: Validation: An internal validation based on FCM, IHC and LC-MS/MS based glycopeptide analysis is conducted to further analyze the potential head & neck cancer GlycoTargets

Use Case Example:
Identifying novel head & neck cancer GlycoTargets

- 3,341 — 3,341 potential antibody targets collected in GlycoBase
- 494 — 1. Step: 494 targets with head & neck cancer expression data available
- 292 — 2. Step: 292 targets with head & neck cancer expression data available **and** predicted O-linked glycosylation
- Validation — 4. Step: Further experimental and manual evaluation to confirm suitability of the selected GlycoTargets for head & neck cancer

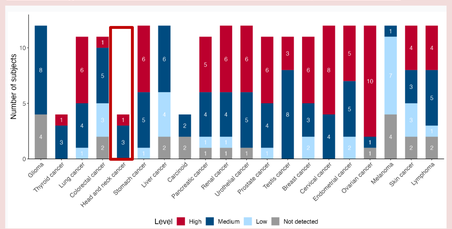
CASE STUDY: GT-008 ANTIBODY DISCOVERY

Summary: GT-008 (Undisclosed Target)

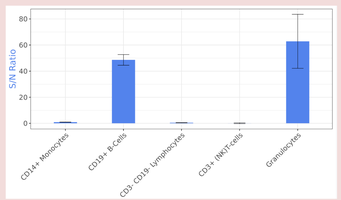
- highly O-glycosylated adhesion molecule and novel immune checkpoint, contributing to cancer progression. Expressed in many solid and hematological tumors, but also on many healthy tissues (e.g. epithelia, immune cells & inflammatory cells)
- Proprietary GlycoCells were used to generate a toolbox of glycoforms for mAb discovery (recombinant soluble proteins and cell lines)
- Several antibodies were generated by an immunzation-based B cell screening approach with rigid screening for glycosylation-dependent binding
- The showcased lead candidate targets a specific glycoform leading to reduced binding to protein-positive healthy immune cells and epithelia, but positive staining of tumors from various indications including head & neck cancer

Exemplary data from GlycoBase:

Staining information per indication (from HPA)



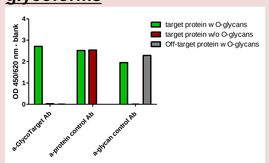
FCM: Expression on healthy donor PBMCs



- Protein is strongly expressed on healthy B cells and granulocytes

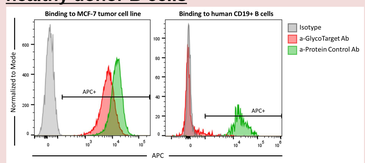
Exemplary data from antibody discovery:

ELISA: Binding to different protein glycoforms



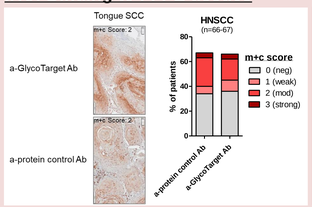
- a-GlycoTarget Ab demonstrates glyco-dependent protein binding

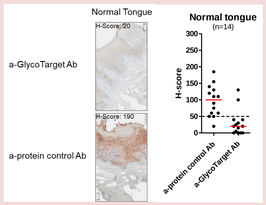
FCM: Binding to tumor cell line and healthy donor B cells



- a-protein control Ab shows strong binding to MCF-7 tumor cells and healthy donor B cells. In contrast a-GlycoTarget Ab only binds to the glycoform expressed on MCF-7 tumor cells

IHC: Binding to FFPE tissues





- a-protein control Ab confirmes protein expression in head and neck SCC and normal tongue tissue
- a-GlycoTarget Ab shows binding to SCC of the head & neck, but reduced binding to healthy tongue epithelium, demonstrating different glycosylation of the target in healthy tissue compared to tumor tissue

GLYCOBASE

- Data and output at each step (discovery, identification, validation) of the workflow is captured in a proprietary database called GlycoBase
- To easily access the compiled data and to allow for faster evaluation and easy listing of GlycoTargets, a corresponding frontend application was developed
- Using the application all information and data corresponding to analyzed targets can be accessed
- Additionally a Target overview table allows to filter the targets based on:
  - Available experimental data
  - Different O-glycosylation properties
  - Publicly available information
  - Tumor Indications with highest protein expression (20 indications included)
- The single target view allows access to all available information related to the particular target including:
  - gene name, gene function, protein isoform information
  - predicted O-linked glycosylation
  - information on protein expression according to The Human Protein Atlas
  - staining information per indication
  - manual analysis of staining localization
  - flow cytometry data from cell lines and/or PBMCs
  - immunohistochemistry data from normal and/or tumor tissues
  - results from LC-MS/MS based glycopeptide analysis
  - and competitor information

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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.
3. Neumann T, Hartung E, Gellert J, Weiß L, Weiske M, Kast N, Gurka S, Marinoff S, Jäkel A, Danielczyk A, Kehler P. Targeting a cancer-specific LYPD3 glycoform for tumor therapy. Front. Drug Discov., 2023. doi:10.3389/fddsv.2023.1298916

We aim to continuously expand our collaborations with industry and academic partners to further exploit the potential of our technology. Please contact Business.Development@glycotope.com