Using glyco-engineered cells with flexible expression of tumor-associated carbohydrates for the generation of highly tumor-specific antibodies

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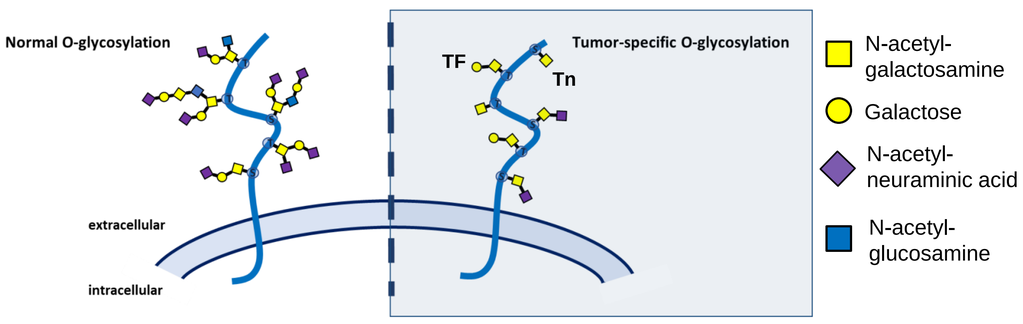
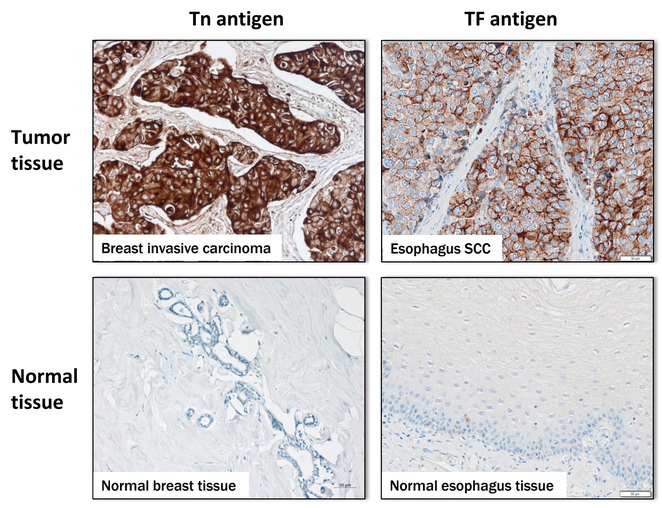
Background

- Potent therapeutic approaches require clean targets. Most antibodies in clinical development or approved for cancer therapy address protein targets that are overexpressed on cancer cells, but often show significant expression in healthy organs.
- Glycans tend to elicit superior tumor specificity compared to proteins since glycosylation is strongly altered in cancer cells, reflecting drastic changes in tumor metabolism.[1]
- Changes in glycosylation arise mostly due to mutated or mislocated glycosyl-transferases and glycosidases, provoking incomplete O-glycan synthesis, giving rise to truncated O-glycans like the Thomsen-Friedenreich (TF) and the Thomsen novelle (Tn) antigen.[1,2]

O-glycosylation in normal and tumor tissue



IHC: Binding of anti-glycan mAbs to FFPE tissue sections



- Truncated O-glycans like Tn and TF are normally hidden by chain prolongation, but become exposed on cancer tissue

- To increase the tumor-specificity of protein-targeting antibodies, Glycotope develops antibodies against tumor-associated protein/carbohydrate combined epitopes (GlycoTargets). This offers reduced on-target/off-tumor toxicity and opens the field for more effective and safe treatment options.
- **A vital tool for achieving specificity and glyco-dependency of our antibodies is Glycotope's engineered cell line platform.**

Methods

- The ability of Glycotope's engineered cell line platform to recombinantly express proteins with distinct carbohydrates was shown by flow cytometry, ELISA and mass spectrometry experiments.
- Tailored screening approach for glyco-dependent antibodies:
  - Multiplex flow cytometry with platform cells
  - ELISA with differentially glycosylated on- and off-target proteins
  - Immunohistochemistry on normal and tumor tissue section

Summary

We have developed a glyco-engineered cell line platform that offers:

- recombinant expression of soluble and membrane-bound proteins carrying defined tumor-associated O-glycans, which can be used for targeted immunization approaches and antibody discovery.
- a versatile tool for target validation and screening of glycosylation-dependent protein binding antibodies.

Our cell line platform provides the basis for generation of therapeutic antibodies with increased tumor specificity and safety for highly potent therapeutic approaches like ADCs, CARs and radiotherapeutics.

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.

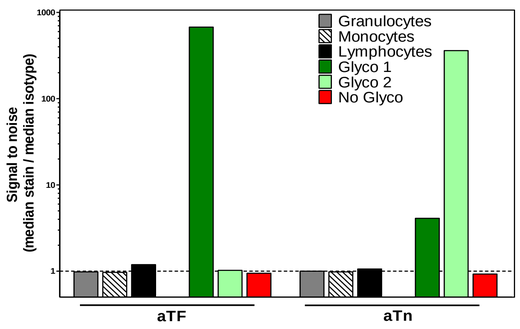
Glyco-engineered cell line platform

Glycotope's cell line platform is engineered to express proteins carrying distinct tumor-associated O-glycosylation. The platform comprises several cell lines each expressing a defined glycosylation pattern, from which a selection of our portfolio is presented.

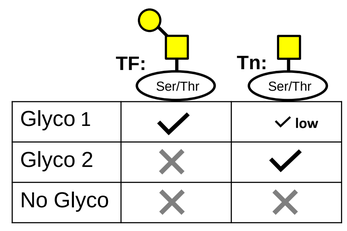
Cell surface glycosylation pattern

FCM: Binding of anti-glycan antibodies to platform cell lines compared to human blood cells

PBMCs were isolated from whole blood of healthy donors (results are representative of three independent experiments)



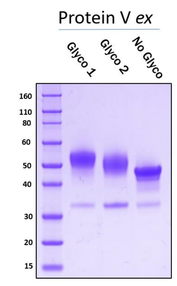
- No binding of anti-TF and anti-Tn mAbs to PBMC subsets
- Platform cells express proteins carrying the following glycan expression profile:

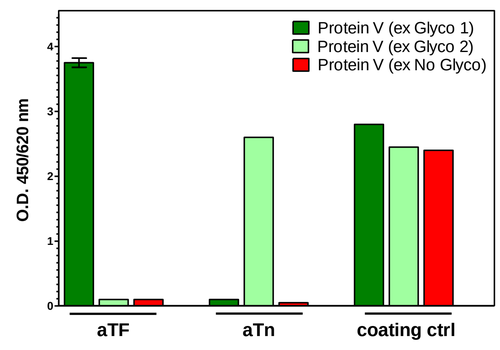


Glyco 1	✓	✓ low
Glyco 2	✗	✓
No Glyco	✗	✗

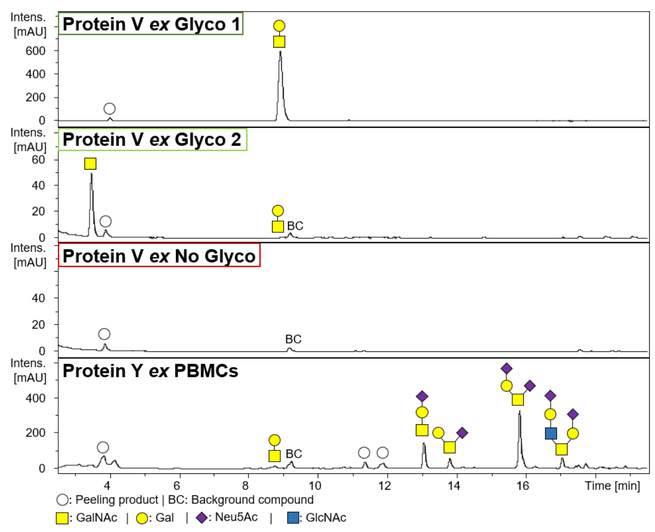
Expression of differentially glycosylated target proteins – showcase

- Protein V was recombinantly produced in three platform cell lines and successfully purified. Expected mass differences due to different glycosylation are visible in SDS-PAGE (top right).
- ELISA (left) and LC-MS O-glycan profiling (bottom right) show consistently that protein V carries a different O-glycan pattern, depending on the expression cell line.





- Protein Y purified from PBMCs by immuno-precipitation carries larger mainly sialylated glycan structures (bottom panel), demonstrating differences in glycosylation between normal and tumor cells.

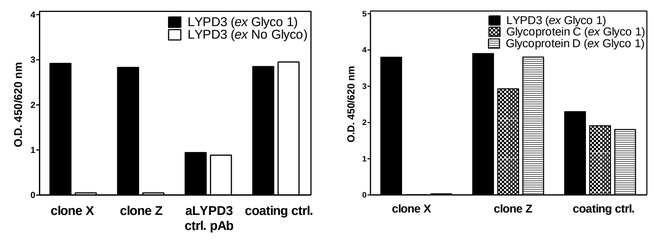


Screening of glyco-dependent antibodies

Showcase: LYPD3

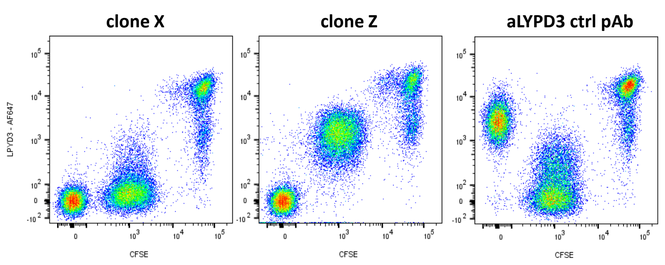
LYPD3 is a highly glycosylated cell surface protein linked with carcinogenesis but also highly expressed in various healthy epithelia. Our platform cells were used to express proteins for tailored immunization to produce glyco-dependent anti-LYPD3 antibodies and for screening of the generated antibodies specifically binding to LYPD3 with tumor-associated glycosylation.

ELISA: Binding of antibody clones to selected on/off target proteins



- Clone X only binds to glycosylated LYPD3 and not to glycosylated off-targets
  → protein/glycospecific binding.
- Clone Z binds to glycosylated LYPD3 but also binds to other proteins with same glycan pattern
  → only glycospecific binding.

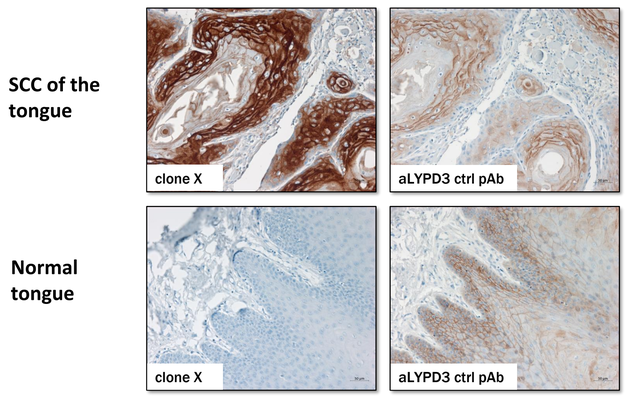
Multiplex FCM: Specific binding of antibody clones to glycosylated LYPD3



- Clone X and Z show binding to glycosylated but not to non-glycosylated LYPD3.
- aLYPD3 control pAb binds to LYPD3 independently of the glycosylation state.

$CFSE^{neg}$ = No Glyco-LYPD3
$CFSE^{low}$ = Glyco 1
$CFSE^{hi}$ = Glyco 1-LYPD3

IHC: Binding of antibody to FFPE tissue sections



- Anti-LYPD3 control pAb demonstrates LYPD3 expression in tissue of normal tongue.
- Clone X does not bind to LYPD3 expressed in healthy tongue epithelium, demonstrating different glycosylation of the target in healthy tissue compared to tumor tissue.
- Clone X strongly binds to several SCCs.

We aim to continuously expand our collaborations with industry and academic partners 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/