Abstract 1588

GET IN TOUCH

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

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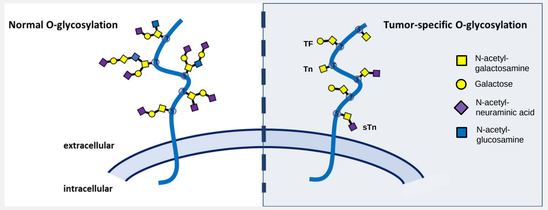
GLYCOTOPE

BACKGROUND

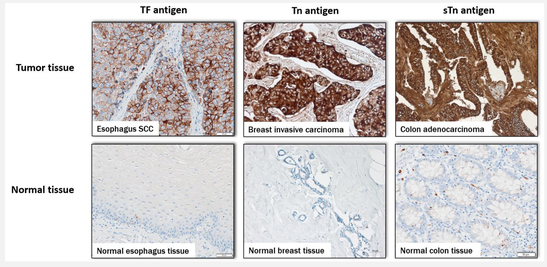
- **Glycosylation is strongly altered in cancer** reflecting the drastic changes in tumor metabolism or genetic alterations. Therefore glycans tend to elicit superior tumor specificity compared to proteins.
- Changes in glycosylation give rise to **truncated O-glycans** like the Thomsen-Friedenreich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

O-glycosylation in normal and tumor tissue



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

Internal IHC data using glycan-specific mAbs on FFPE tissue



- To increase the tumor-specificity of protein-targeting antibodies, Glycotope develops antibodies against tumor-associated protein/carbohydrate combined epitopes (GlycoTargets).
- GlycoTargets offer superior tumor specificity and **reduced on-target/ off-tumor toxicity,** opening the field for more effective and safer treatment options.

- **An essential tool for achieving specificity and glyco-dependency of our antibodies is Glycotope's engineered cell line platform.**

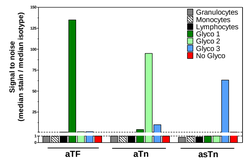
GLYCO-ENGINEERED CELL LINE PLATFORM

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

- Platform cells express proteins carrying the following glycan expression profile:

Cell surface glycosylation pattern

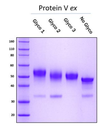
Flow Cytometry: Binding of glycan-specific mAbs to platform cell lines and PBMCs



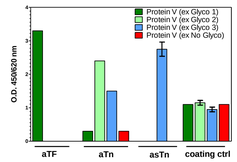
- Specific glycosylation of surface proteins on platform cells was verified.
- No binding of anti-TF, anti-Tn and anti-sTn mAbs to PBMC subsets.

Expression of target proteins - showcase

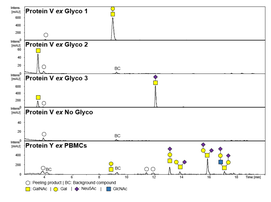
- Protein V was recombinantly produced in platform cell lines and successfully purified.
- Size of purified glycoproteins differs depending on the expression line and corresponding glycosylation.



ELISA: binding of glycan-specific mAbs to purified proteins



O-glycan profiling: of purified proteins



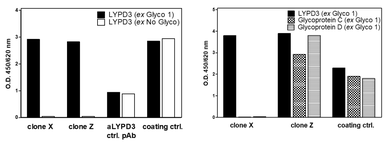
- ELISA and LC-MS O-glycan profiling consistently show that protein V carries a different O-glycan pattern, depending on the expression cell line.
- Protein Y purified from PBMCs by IP carries larger, mainly sialylated glycan structures (right panel, bottom chromatogram), demonstrating differences in glycosylation between normal and tumor cells.

SCREENING OF GLYCO-DEPENDENT ANTIBODIES

Showcase: LYPD3

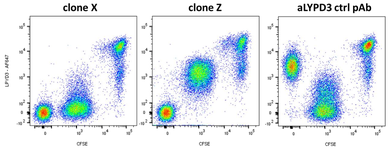
LYPD3 is a highly glycosylated cell surface protein linked to carcinogenesis but also highly expressed in healthy epithelia. Glycotope's platform cells were used for tailored immunization and screening approaches to produce glyco-dependent anti-LYPD3 antibodies that show binding to LYPD3 with tumor-associated glycosylation (patent submitted).[3]

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



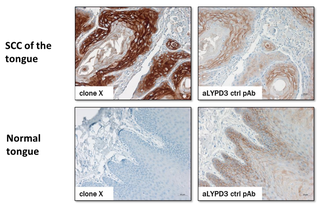
- Clone X demonstrates glyco-dependent protein binding. Clone Z demonstrates solely glyco-specific binding.

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.

IHC: Binding 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.

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

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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. *Manuscript in preparation*

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