Carbohydrate-dependent protein binding antibodies with superior tumor-specificity

Theresa Neumann, Manon Weis, Stephanie Gurka, Anika Jäkel, Sophie Marinoff, Timo Lischke, Evelyn Hartung, Naomi Kast, Manon Weiske, Marianne Morche, Lisa Weiß, Johanna Gellert, Antje Danielczyk, Patrik Kehler

Theresa.Neumann@glycotope.com; Glycotope, Berlin, Germany

Abstract 316

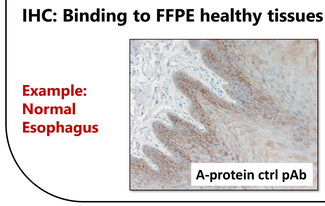
Antibody Discovery: GlycoTarget X

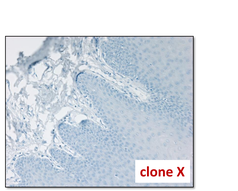
Introduction

- GlycoTarget X is a highly glycosylated cell surface protein that is associated with carcinogenesis in several different cancers like non-small cell lung cancer (NSCLC) and squamous cell carcinoma (SCC) of the head and neck (HNSCC) including esophageal SCC (ESCC) as well as colorectal and breast cancer
- However, under normal physiologic conditions it is expressed in different epithelia. This may cause unwanted side effects in a cancer therapy with an antibody which cannot discriminate between the cancer-associated target and the target on normal tissue.

Strictly O-glycosylation dependent binding to target antigen

ELISA: Antibody binding to glycosylated but not to de-O-glycosylated target protein

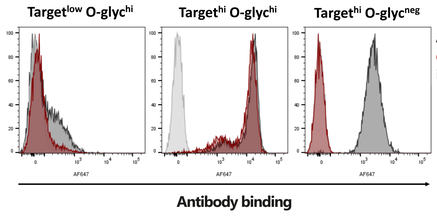




- Discovered antibodies show high affinity and dose-dependent binding with EC50s in the range of 6 to 50 ng/mL
- Binding of newly discovered antibodies is highly restricted to O-glycosylated target protein as there is no binding to de-O-glycosylated GlycoTarget X (shown for clone X as example)
- Protein-specific control pAb is not able to discriminate between the different glycoforms of the target protein

	clone 1	clone 2	clone 3	clone 4	clone 5	clone 6	clone 7	clone X
EC50 [ng/mL]	6.4	50.4	8.3	6.6	5.9	12.2	6.1	6.0

Cellular binding: Binding to cells expressing GlycoTarget X with or without O-glycans

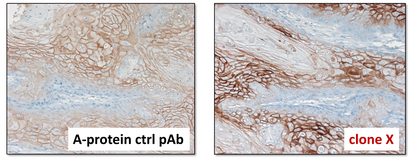


- Clone X specifically binds to cells expressing the O-glycosylated target but not to cells displaying GlycoTarget X without O-glycans
- In contrast, the anti-protein pAb control binds to GlycoTarget X expressing cells irrespective of their glycosylation status

Improvement of tumor specificity

IHC: Binding to FFPE cancer tissues

Example: SCC of mouth floor



- Clone X binds to various GlycoTarget X - positive cancer indications
- Percent of stained tumor cells is comparable to anti-protein pAb control

IHC: Binding to FFPE healthy tissues

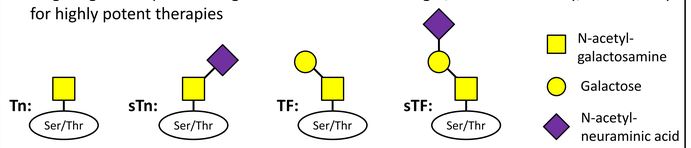
Example: Normal Esophagus

- anti-protein pAb detects target protein expression in epithelium of normal esophagus
- Clone X does not bind to GlycoTarget X expressed in healthy esophagus epithelium demonstrating different glycosylation of the target in healthy tissue compared to tumor tissue

GlycoTargets

Background

- Highly potent therapeutic approaches require very clean targets. However, the majority of antibodies in clinical development or approved for cancer therapy address protein targets that are only overexpressed on cancer cells and yet often show significant expression in healthy organs
- Glycans tend to elicit superior tumor specificity as compared to proteins since glycosylation is strongly altered in cancer reflecting the drastic changes in tumor metabolism
- The changes in glycosylation are mostly due to mutated or mislocated glycosyltransferases and glycosidases giving rise to highly fucosylated, highly sialylated and truncated glycans [1]
- Therefore, proteins expressed in cancer cells can carry tumor-associated carbohydrates like the Thomsen-Friedenreich (TF) and the Thomsen novelle (Tn) antigen as well as their respective sialylated forms sTF and sTn [2]
- To increase the tumor-specificity of protein-targeting antibodies, we have developed antibodies against several protein/carbohydrate combined epitopes (GlycoTargets)
- Targeting these specific antigens offers reduced on-target/off tumor toxicity, which is key for highly potent therapies



Methods

- The specificity and glycosylation dependency of our antibodies was determined using differentially glycosylated proteins in an ELISA format
- Binding to several tumor cell lines and healthy human leukocytes was analyzed via flow cytometry
- Binding to different cancer tissues as well as healthy tissues was analyzed by immunohistochemistry

Summary

- We have generated antibodies that bind to their target protein only if a specific tumor-associated carbohydrate is present; these antibodies do not cross-react with the non-glycosylated protein itself
- Due to their **carbohydrate-dependent binding, our antibodies show markedly decreased on-target/off-tumor binding** as they do not bind to healthy immune cell subsets in comparison to traditional protein binding antibodies in flow cytometric analyses
- IHC studies reveal that our protein/carbohydrate-dependent antibodies stain tumor tissue of different cancer indications but do not stain related normal tissues
- Therefore, our antibodies offer a new approach to increase the tumor specificity and safety for highly potent therapeutic approaches like ADCs or CARs

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.

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/

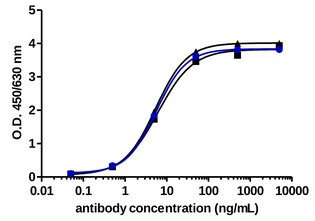
Antibody Discovery: GlycoTarget Y

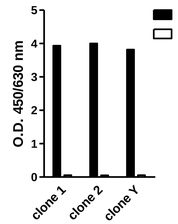
Introduction

- GlycoTarget Y is a highly glycosylated cell surface protein that is associated with tumorigenesis and is broadly expressed in several different 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 expression and expression on healthy tissue

Strictly O-glycosylation dependent binding to target antigen

ELISA: Antibody binding to glycosylated but not to de-O-glycosylated target protein



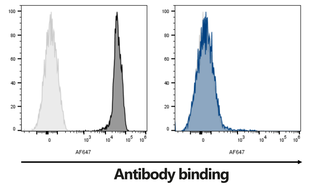


- Antibody clone Y specifically binds its target protein only when decorated with O-glycans as visualized using O-glycosylated vs. de-O-glycosylated protein as coating antigen
- Antibody clone Y shows high-affinity and dose-dependent binding with an EC50 of 5.6 ng/mL

	clone 1	clone 2	clone Y
EC50 [ng/mL]	6.2	5.4	5.6

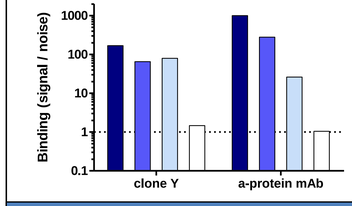
Specific binding to tumor cells but not healthy lymphocytes

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

Flow Cytometry: Binding to tumor cell lines

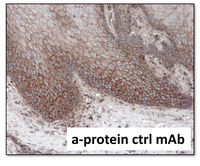


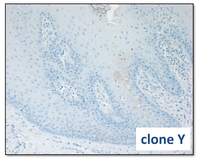
- Clone Y binds to several tumor cell lines of different origins expressing the protein target

Reduced binding to healthy tissues

IHC: Binding to FFPE healthy tissues

Example: Normal Tongue





- The anti-protein control mAb demonstrates high target protein expression in normal tongue
- The glycosylation-dependent binding of clone Y results in drastically reduced binding to normal tissue thus heavily improving off-tumor binding