Antibodies specifically recognizing cancer-associated glycoforms of mucin-like proteins

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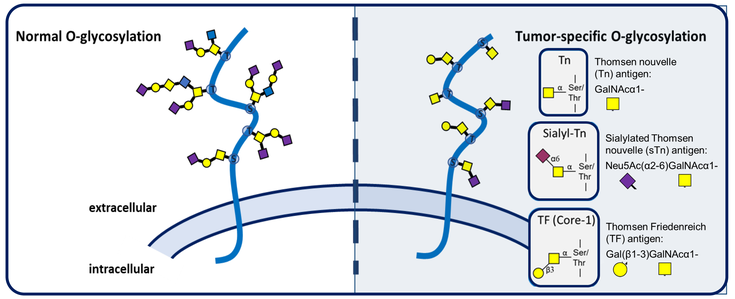
GLYCOTOPE

Abstract 1462

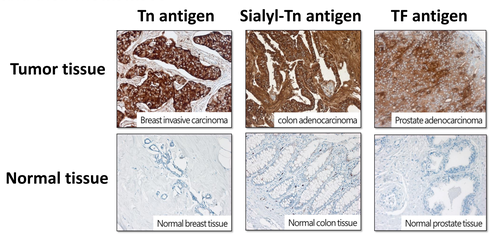
Tumor-specific O-glycans and their potential for highly potent therapies

Introduction

- One of the most drastic changes in cancer is the altered glycosylation of proteins and lipids, giving rise to truncated glycans which are essentially absent on normal cells. Thus, developing antibodies against protein/carbohydrate combined epitopes (GlycoTargets) comprising these tumor-specific glycans enables highly potent therapies with reduced on-target/off-tumor toxicity and allows targeting of otherwise "undruggable" normal-tissue expressed proteins.[1,2]



- 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
- Truncated O-glycans are expressed on many different carcinomas, leukemias, lymphomas and their metastases



➢ Truncated glycans, such as Tn, sTn and TF are hidden by chain elongation in normal tissue

GlycoTargets for Superior Tumor-Specificity

- **GlycoTargets = Tumor-associated protein/carbohydrate combined epitopes**
- **GlycoTargets offer superior tumor specificity** compared to protein targets
- **GlycoTargets exhibit reduced on-target/off-tumor toxicity**, which is key for highly potent therapies

GlycoTarget Platform:

- Bioinformatic predictions or cellular screenings are used to identify proteins carrying truncated O-glycans
- **GlycoCells** are used to produce soluble or membrane bound **GlycoTargets** with defined O-glycosylation pattern
- Due to their glycan dependency, our antibodies show markedly decreased off-tumor binding
- The increased tumor selectivity may improve safety for highly potent therapeutic approaches like ADCs, CARs or radiopharmaceuticals

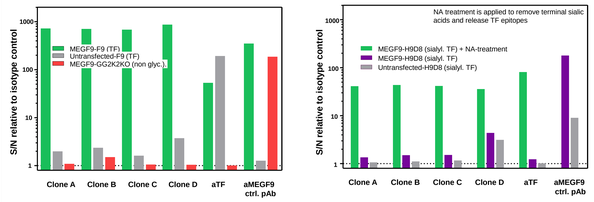
Antibody Discovery against GlycoTargets: MEGF9

Overview

- MEGF9 (Multiple EGF-like domains protein 9)
- Transmembrane protein, large ECD with 5 laminin EGF-like domains, ca. 180 kDa
- Several N- and mucin-like O-glycosylation sites, suitable glycan-clusters for GlycoTargeting approach
- Broad expression in various cancers and normal tissue
- Function unknown (potentially guidance or signaling molecule)
- An Ab discovery campaign, with the aim of identifying glycan-specific MEGF9 Abs, was conducted. Potential clones were compared to a protein-specific Ab

Characterization of anti-MEGF9 mAbs

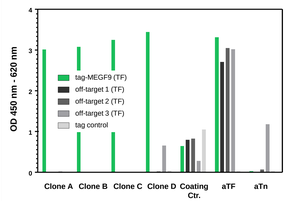
FCM: Binding to GlycoCells



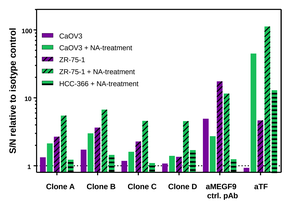
- aMEGF9 mAbs bind only to glycosylated MEGF9
- aMEGF9 mAbs do not bind to sialylated MEGF9

→ **Glyco**Target specific binding

ELISA: Binding to off-target proteins



FCM: Binding to cancer cells



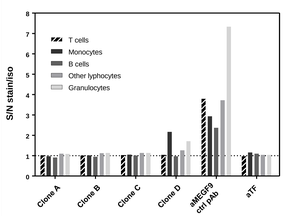
- aMEGF9 mAbs do not bind to other glycosylated proteins

→ Glyco**Target** specific binding

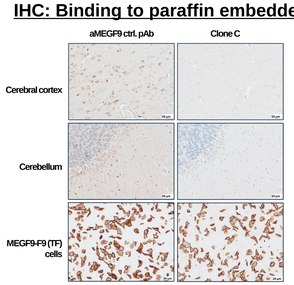
- aMEGF9 mAbs bind to cancer cells after neuraminidase (NA) treatment

→ Cancer cell binding

FCM: Binding to normal cells



IHC: Binding to paraffin embedded tissue and cells



- aMEGF9 mAbs do not bind to MEGF9 expressing PBMC
- aMEGF9 mAbs do not show off-tumor binding on normal tissue

→ Reduced off-tumor toxicity

Summary

- **Anti-MEGF9 clones specifically recognize their target in a glycosylation-dependent manner** as proven by ELISA and FCM
- This glycosylation-dependent binding enables **differentiation between MEGF9 on tumor cell lines and MEGF9 on normal cell lines or healthy donor PBMCs**
- This study **perfectly confirms the technical approach of our GlycoTarget platform to generate glycosylation-dependent antibodies** against a protein of choice to reduce on-target/off-tumor binding

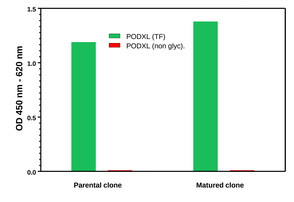
Antibody Discovery against GlycoTargets: PODXL

Overview

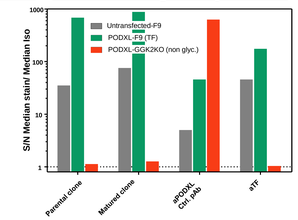
- PODXL (Podocalyxin-like protein 1)
- Sialomucin type I membrane protein of the CD34 family, ca. 60 kDa
- Extensively O- and N-glycosylated, with suitable O-glycosylation clusters for GlycoTargeting approach
- Expressed on solid tumors (e.g. breast, ovarian, lung, prostate) and healthy hematopoietic progenitors
- Regulates cell adhesion and is involved in cancer progression, metastasis and immune suppression
- An Ab discovery campaign, with the aim of identifying glycan-specific PODXL Abs, was conducted. Potential clones were compared to a protein-specific Ab

Characterization of anti-POXDL mAb

ELISA: Binding to different glycoforms



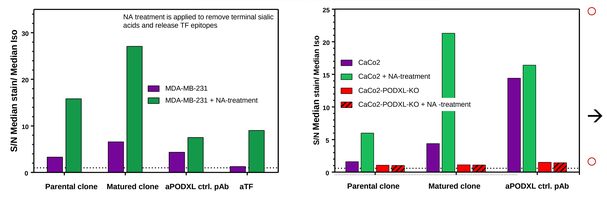
FCM: Binding to GlycoCells



- aPODXL mAbs were generated by phage display, following affinity maturation of the most promising clone
- aPODXL mAbs bind only to glycosylated PODXL

→ **Glyco**Target specific binding

FCM: Binding to cancer cells



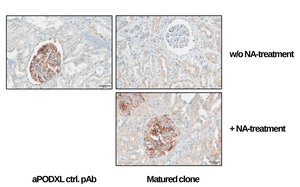
- aPODXL mAbs bind to cancer cells after neuraminidase (NA) treatment

→ Cancer cell binding

- aPODXL mAb do not bind to PODXL knockout (KO) cells

→ Glyco**Target** specific binding

IHC: Binding to paraffin embedded tissue (kidney)



- aPODXL mAbs binds to PODXL-positive glomeruli in healthy kidney tissue only after neuraminidase (NA) treatment

→ Reduced off-tumor toxicity

Summary

- The parental and matured **Anti-PODXL clones specifically recognize their target in a glycosylation-dependent manner** as proven by ELISA and FCM
- This **glycosylation-depending** recognition enables **binding on cancer cells** after their epitopes have been demasked by neuraminidase treatment
- **Affinity maturation lead to an increase in on-target binding**, especially on cancer cells
- The matured clone does **not bind to healthy tissue**, as the **epitopes are masked by sialylation**

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.
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