A new approach on targeting aberrant glycans in cancer cells

Nuno Prego R.[1*], Daniela F. Barreira[2], Rita A. Lourenço[2], Sara Lemos[3], Benedita Pinheiro[2], Filipa Marcelo[2], Leonardo Chicaybam[3], and Paula A.Videira[4*]

1. Faculdade de Ciências da Universidade de Lisboa, Portugal & CellmAbs, Portugal, **2**. UCIBIO, Department of Life Sciences, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal, **3**. CellmAbs, Portugal **4**. UCIBIO, Department of Life Sciences, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal, & CDG Professionals and Patient Associations International Network (CDG & Allies-PPAIN), Lisbon, Portugal

*npregoramos@gmail.com; p.videira@fct.unl.pt

Graphical Abstract

Production and evaluation of murine anti-STn mAbs

murine IgM

- Reformatting from murine to chimeric IgG;
- Humanization process

humanized mAb

Evaluation of humanized mAbs:

- SPR
- Flow cytometry
- Glycan array

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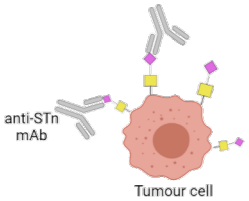
Selection of mAbs with the highest affinity and specificity to STn

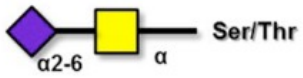


A – Introduction

Overexpression of sialyl-Tn (STn), a glycan antigen in Carcinomas is usually associated with poor prognosis and reduced Overall Survival

Cancer-Specific target
(antigen rarely expressed in normal cells)

Promising target using anti-glycan monoclonal antibodies (mAbs)



(Sialyl-Tn antigen)

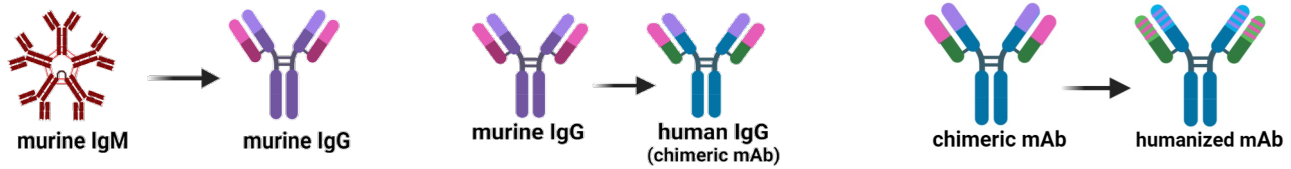
- Major challenges in the generation and characterization of highly specific anti-glycan mAbs:
  - **Glycan recognition by mAbs** → Need to selectively discriminate between different carbohydrate structures
    → Compete with the glycome at the cell surface
  - **Glycan – mAb interaction** → Binding and affinity depend on:
    – antigen presentation
    – type of carrier (Glycoprotein) expressing the glycan antigen
    – amino acid to which the glycan is attached (Ser/Thr)

Objective: to develop humanized anti-STn antibodies with the most suitable affinity range for addressing solid tumors

B – Methods

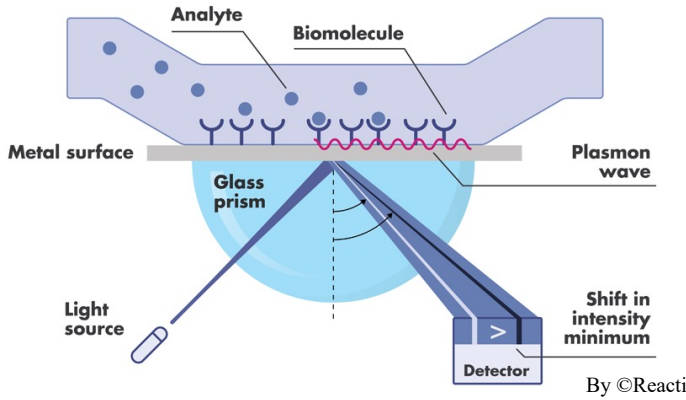
Hybridoma technology ⟶ **murine mAb[1],** that was later reformatted for clinical application:

Isotype change › Species change › Humanization process ➡ **Result:** generation of several humanized variants with different profiles (enabling its use in different therapeutic modalities)



› Humanized mAbs specificity and affinity to STn was assessed by:

SPR



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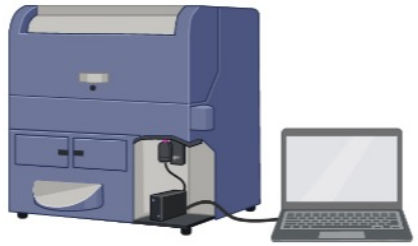
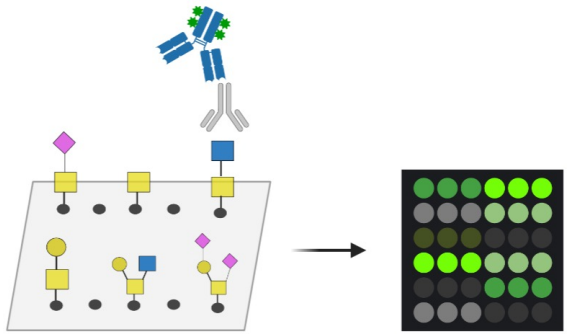
Flow cytometry



Cell lines used:
-MDA-MB-231 WT cell line
- MDA-MB-231 STn⁺ cell line

Glycan array



Microarray platform with sequence-defined neoglycolipid (NGL) probes

Fluorescent results

[1]Loureiro LR, Sousa DP, Ferreira D, Chai W, Lima L, Pereira C, et al. Novel monoclonal antibody L2A5 specifically targeting sialyl-Tn and short glycans terminated by alpha-2–6 sialic acids. Scientific Reports 2018;8:12196. https://doi.org/10.1038/s41598-018-30421-w.

C – Results & Discussion

SPR

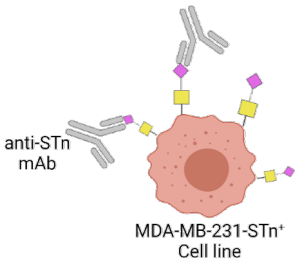
– Immobilized target: BSA-ser-STn conjugated ligand

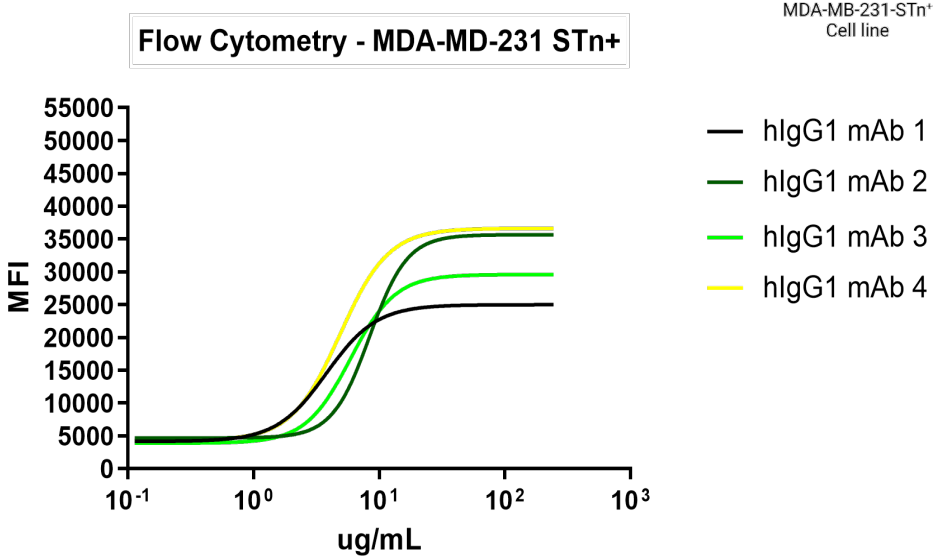
Affinity measurement of antibodies to STn-serine

Ligand	Analyte	Chi² (RU²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
BSA-STn-serine	hIgG1 mAb1	4.13E-01	1.18E+05	3.51E-03	2.97E-08	32.6
BSA-STn-serine	hIgG1 mAb2	2.55E-01	1.74E+05	5.53E-03	3.17E-08	17.8
BSA-STn-serine	hIgG1 mAb3	3.30E-01	1.71E+05	5.68E-03	3.32E-08	21.3
BSA-STn-serine	hIgG1 mAb4	1.35E-01	1.32E+05	5.19E-03	3.94E-08	13.8

↓ KD ↑ Binding affinity

Flow cytometry

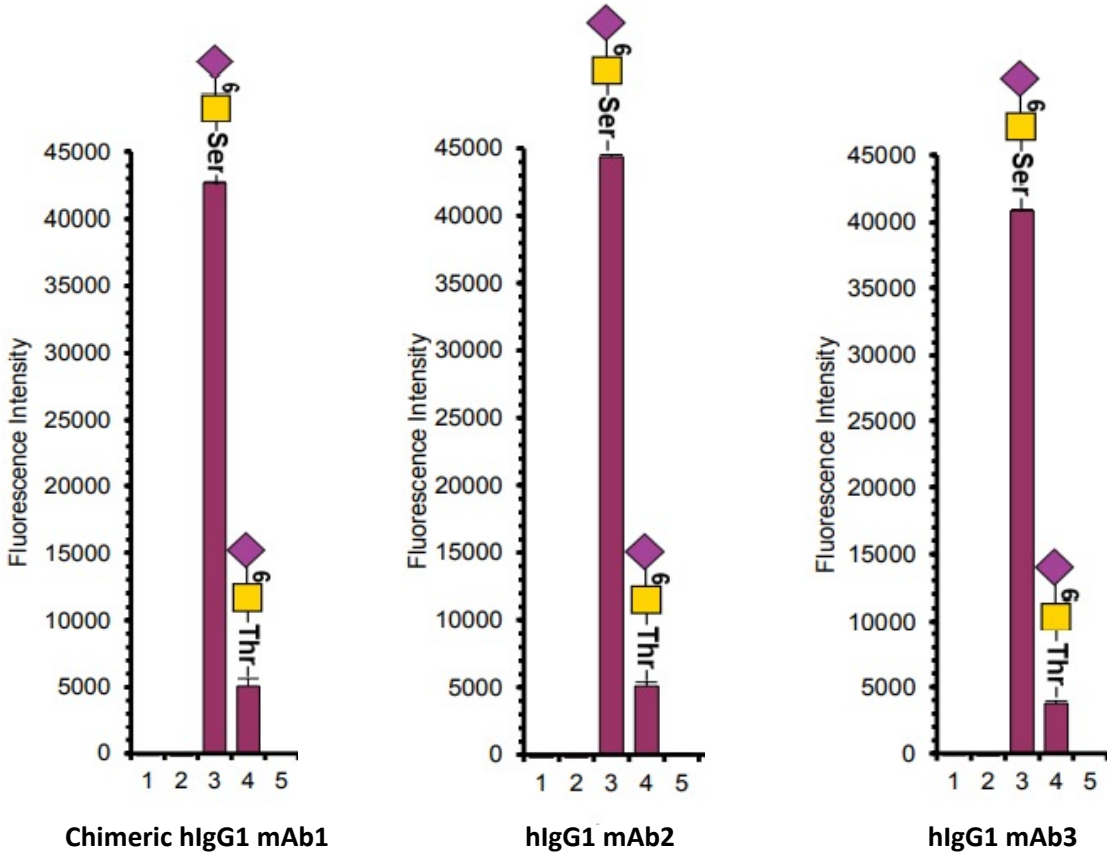




We screened and characterized some of the different mAbs, evaluating glycan affinity and cellular binding to STn^+ cell lines.

C – Results & Discussion

Glycan array



STn(Ac)	NeuAcα-6GalNAcα-	O-Ser-NH-DA	3
		O-Thr-NH-DA	4

NGL-DHPA microarray platform

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32 sialylated and core *O*-glycan related probes

- Reformatted antibodies improved selectivity and STn specificity when compared to the murine;
- Showed high binding intensity to STn.

DHPA – N-(4-formylbenzamide)-1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine

D – Impact

- Several anti-STn mAb variants, were generated, having a broad range of STn affinity;
- By developing mAbs with different profiles, several tumour types at different stages can be targeted by using various therapeutic modalities;
- Due to similarities in the glycosylation pattern of cancer cells and viruses, the antiviral potential of anti-STn mAbs is being evaluated and explored further.

Acknowledgments

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