NAXITAMAB, AN ANTIBODY WITH DISTINCT COMPLEMENTARY DETERMINING REGIONS AND HIGH BINDING AFFINITY TO DISIALOGANGLIOSIDE GD2

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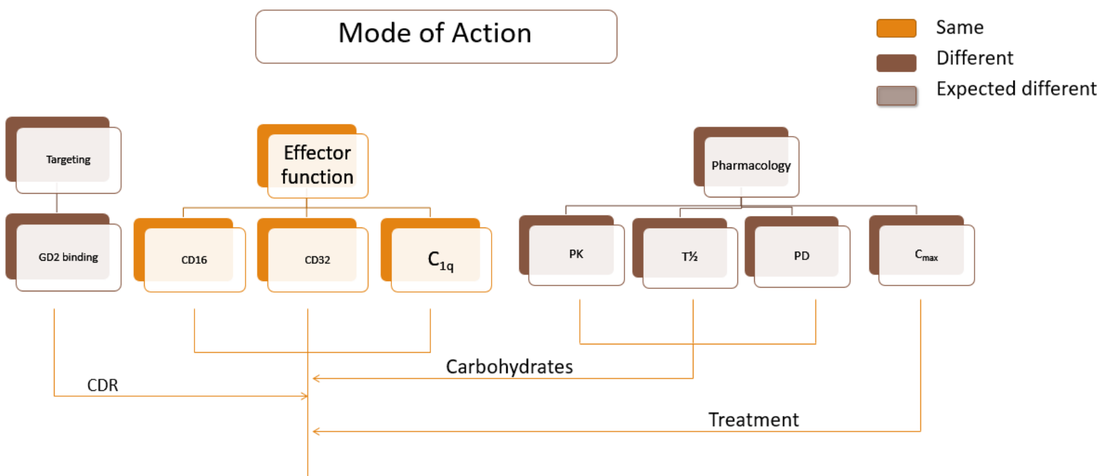
Background/Aim:

- Monoclonal antibodies (mAbs) that target disialoganglioside GD2 have shown clinical efficacy in the treatment of GD2 expressing tumors and are being used as part of the new standard of care for the treatment of high-risk neuroblastoma.
- Two chimeric antibodies already have received regulatory approval for pediatric neuroblastoma: dinutuximab (Unituxin) and dinutuximab beta (Qarziba).
- Here we present detailed structural characterizations of naxitamab, a humanized form of mu3F8 antibody targeting GD2, which has been in clinical development since 2011.

Methods:

The three-dimensional structural models of the GD2-targeting complementary determining regions (CDRs) of dinutuximab beta and naxitamab were compared and their in vitro GD2 binding kinetics and affinity were evaluated side-by-side using surface plasmon resonance (SPR). The fragment crystallizable region (Fc) structure and function of the two mAbs were also compared in terms of their N-glycan profiles using LCMS-FLR and in vitro antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) activities by using cell-based reporter assays.

Structural features with the potential to modify effector antibody function



Results:

- Clear differences in the CDR regions of naxitamab and dinutuximab beta were observed with only 23% similarity (Table 1). The significance of this is substantiated by the structural analysis showing that the three-dimensional structure of the GD2-targeting CDRs of dinutuximab beta and naxitamab are distinct (Figure 1).
- In line with finding above, a difference in GD2 binding properties were detected with SPR namely an approximately ten-fold higher affinity for naxitamab to GD2 compared to dinutuximab beta (Table 2 & Figure 2). The higher affinity was predominantly due to a slower off-rate for naxitamab (Table 2).
- The Fc-mediated functions (ADCP and CDC) for the two antibodies are comparable (Table 3), which is consistent with their similar N-glycan profiles (Figure 3).

Table 1. Vast differences are observed between the CDR of naxitamab and dinutuximab beta

CDR	Identity (%)	Non-similarity (%)
CDR total	23	77
CDRH1	20	80
CDRH2	35	65
CDRH3	9	91
CDRL1	13	87
CDRL2	57	43
CDRL3	10	90

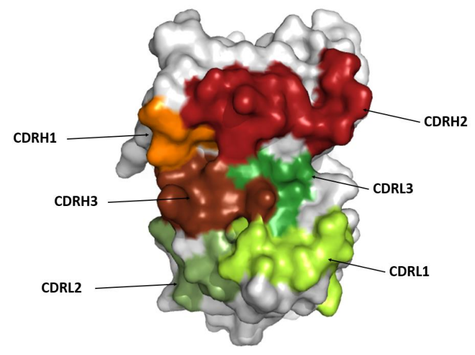


Table 2. GD2 binding kinetics comparison by surface plasmon resonance (SPR)

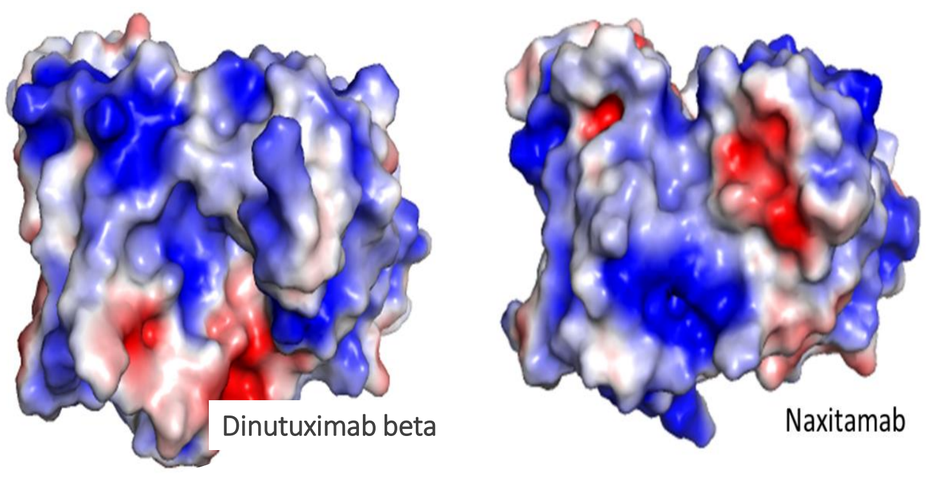
Drug	ka (1/Ms)	kd (1/s)	KD (M)
Naxitamab	3.8×10^{4}	7.6×10^{-4}	2.0×10^{-8}
Dinutuximab beta	1.4×10^{5}	3.5×10^{-2}	2.5×10^{-7}

Observations:
Naxitamab has a slightly lower on-rate (ka) and much slower off-rate (kd) than dinutuximab beta, resulting in an overall higher affinity (lower KD) for naxitamab (approximately 10x).

Table 3. Fc-functional activity comparison

Assay	Relative potency of dinutuximab beta vs naxitamab reference standard
CDC	99%
ADCP	120%

Figure 1. Electrostatic surface representation of naxitamab and dinutuximab beta



A 3-dimentional representation of dinutuximab beta (left figure) and naxitamab (right figure).
A surface representation of the CDR of the two antibodies shown in the same orientation and coloured according to the electrostatic potential.
Positive charged surface areas are coloured blue. Negatively charged surface areas are coloured red.

Figure 2. GD2 binding kinetics comparison by surface plasmon resonance (SPR)

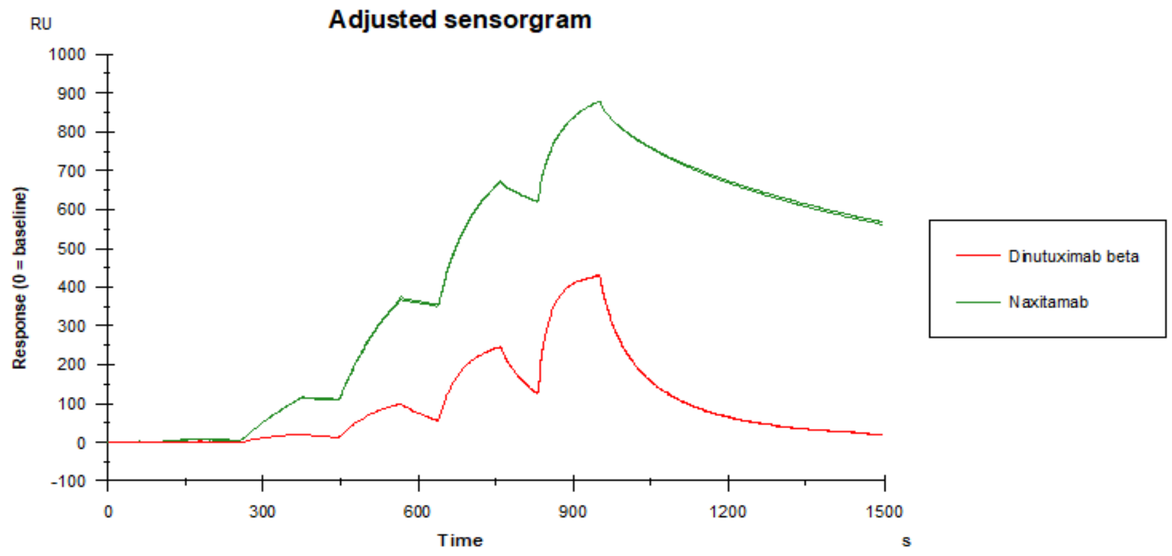
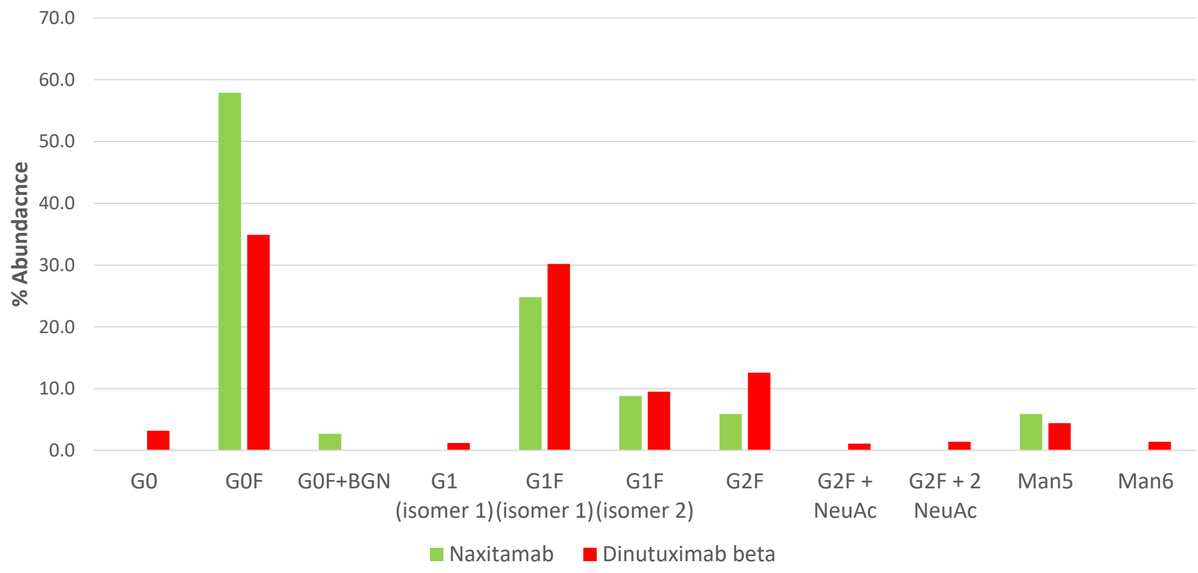


Figure 3. N-glycan profile comparison



Conclusions:

- Naxitamab, a humanized anti-GD2 antibody which has been in clinical development for more than 8 years, and dinutuximab beta demonstrate clear differences in GD2 targeting properties
- The prominent differences in the three-dimensional structure of the GD2-targeting CDRs indicate a differentiated binding capability
- This is supported by an approximate 10-fold higher affinity for GD2 by naxitamab, predominantly due to its slower off-rate

Future Directions for Research:

The above properties together with optimized clinical dosing schedules suggest that naxitamab could represent a new approach to target GD2 in pediatric oncology.

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