

# Cancer cell associated glycans as targets for immunotherapy

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Therapeutic anti-glycan antibodies for cancer treatment are limited, in spite of the differential glyco-phenotype of cancer cells and associated biology. We have generated anti-Le glycan antibodies with clinical potential and multifaceted functionality. Increased understanding of all aspects of glycomics research will ensure the continued development of glycan-targeted immunotherapeutics.

Post-translational glycosylation of proteins and lipids has important functional consequences for mammalian cells.<sup>1</sup> The considerable complexity of the glycome is a direct result of the variety of glycosidic linkages between monosaccharides giving rise to wide-ranging structural diversity. It has been estimated that over 50 % of proteins are glycosylated. In mammalian cells, the glycolipid composition of biomembranes ranges from less than 5% to 20% of the membrane lipids.

Recent advances in cancer cell glycomics have highlighted the differential glycan make-up of tumor cells versus their normal counterparts.<sup>2</sup> These transformation-associated glycosylation changes constitute: (i) increased branching (N-glycans), (ii) higher density (O-glycans), (iii) incomplete synthesis, (iv) neosynthesis, and (v) increases in sialylation and fucosylation, arising mainly from the genetic and epigenetic dysregulation of the biosynthetic enzymes.<sup>3</sup> Importantly, the altered glyco-phenotype is intricately linked to the majority of cancer cell biology hallmarks: proliferative signaling, inducing angiogenesis, activating invasion and metastasis, and resisting cell death.<sup>4</sup> Targeting the tumor glycan signature thus provides an attractive strategy for immunotherapy.

Developing glycan-specific antibodies for cancer immunotherapy has been challenging as glycans are poor immunogens often resulting in weak affinity IgM antibodies with limited clinical value.<sup>5</sup> We have developed a successful method to raise high-affinity antibodies against cancer cell glycans with therapeutic potential.

Through immunizations with cancer cell plasma membrane glycolipid extracts we recently generated two anti-glycan antibodies recognizing a unique subset of lewis (Le) glycans.<sup>6</sup> Le glycans are formed by the sequential addition of fucose onto oligosaccharide precursors on glycoproteins as well as glycolipids through the concerted action of a set of glycosyltransferases. Type I chains (containing Gal $\beta$  (1 $\rightarrow$ 3)GlcNAc) form Le<sup>a</sup> and Le<sup>b</sup>, whereas type II chains (containing Gal $\beta$  (1 $\rightarrow$ 4)GlcNAc) form Le<sup>x</sup> and Le<sup>y</sup>.<sup>7</sup> Our antibodies bound avidly (subnanomolar  $K_d$ ) to Le<sup>c</sup>Le<sup>x</sup>- and Le<sup>a</sup>-containing glycoproteins and glycolipids on a wide range of tumor cells and tissues, the combination of which led to multimodal functionality such as: (i) atypical direct cell killing, (ii) glycoepitope-density dependent cellular internalization with lysosomal delivery of cargo (ADC), and (iii) sound immune effector functions (ADCC and CDC)

(Fig. 1), culminating in significant survival benefit in a colorectal cancer xenograft model.

The direct cell killing in particular is appealing as it was associated with rapid cellular aggregation coinciding with propidium iodide uptake (reflecting cellular permeability), and the formation of membrane pores of heterogeneous sizes, ultimately leading to cell death via a mechanism resembling oncotic necrosis. Although the finer details of the molecular mechanisms underlying this membrane-centered cytotoxicity remain to be elucidated, it bore no resemblance to classical apoptosis in that there was little evidence of caspase activation or DNA fragmentation. A limited subset of other anti-glycolipid antibodies has been shown to induce a similar mode of cell killing, notably a number of ganglioside antibodies that are currently undergoing clinical evaluation such as racotumomab (anti-NeuGcGM3) for the treatment of non-small cell lung cancer (NCT01460472, NCT01240447) and a number of pediatric tumors (NCT01598454) and KW2871 (anti-GD3) for the treatment of metastatic melanoma (NCT00679289). In the context of B-cell lymphoma, type II anti-CD20 antibodies such as tositumomab, are

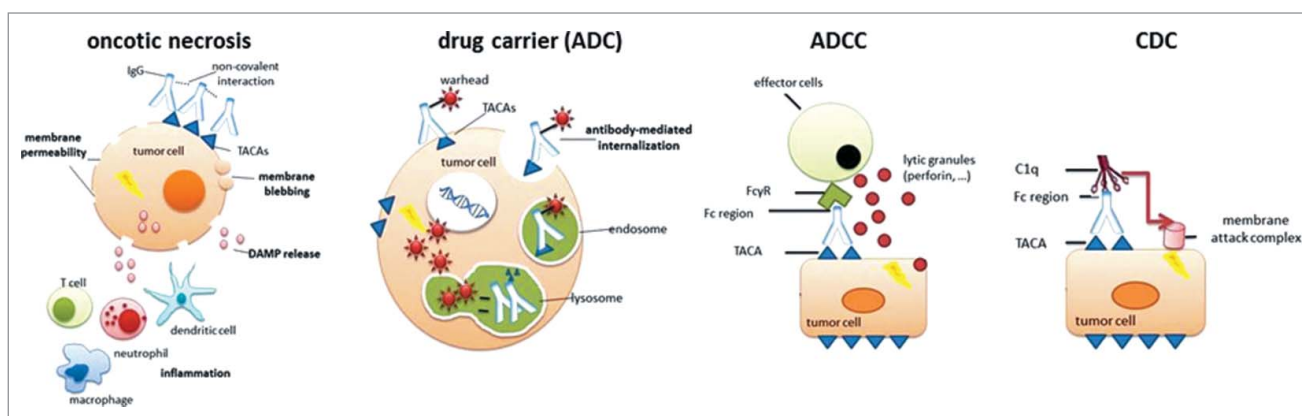
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**Figure 1.** Cell killing modalities of anti-glycan antibodies.

equally effective at mediating homotypic cell adhesion-associated direct cell killing in a caspase-independent manner.<sup>8</sup> This antibody-induced ‘programmed cell death’ was dependent on actin remodeling and involved lysosomal destabilization.

The cell death resulting from antibody-induced pore formation may constitute a form of immunogenic cancer cell death (ICD) through the release of cellular content, including damage-associated molecular patterns (DAMPs), into the extracellular space.<sup>9,10</sup> This may amplify the antibody-mediated effects, counteracting the immunosuppressive microenvironment, potentially resulting in long-lasting antitumor immunity. It remains to be seen whether this will translate into improved efficacy of anti-glycan antibodies *in vivo*.

Anti-glycan antibodies can conceivably be used in a number of alternative settings for instance as carriers for drug conjugates or in a bispecific format to redirect T-cells. In all these settings absence of cross-reactivity with normal healthy tissues is warranted in order to avoid off-target toxicity. The immunohistochemical analysis of our Le antibodies revealed strong staining of a large percentage of tumor tissues covering

colorectal, gastric, pancreatic, non-small cell lung, and ovarian cancer tissues, combined with no cross-reactivity with most normal tissues including lung, liver (parenchyma), heart, brain, and kidney. We observed some (low to moderate) normal cross-reactivity (mainly with a subset of gastrointestinal tissues), but it is unclear whether this represents the same target as the tumor tissues.

As our antibodies bind glycoproteins as well as glycolipids, we have initiated studies using genetically engineered cell lines that express Le<sup>a</sup> glycan either on proteins or on lipids in order to delineate which target recognition drives the observed direct cytotoxic effects. Preliminary results suggest that lipid binding instigates the direct cell killing, as cells expressing the glycoepitope predominantly on glycoproteins are refractory. Interference with ‘glycosynapse’ formation, for instance through glycolipid internalization or via a direct physical effect on the membrane could be one way in which glycolipid-binding antibodies initiate the events culminating in cell death. Ongoing studies also encompass the immunogenic potential of this form of cell death through the combination of syngeneic mouse models

in immunocompetent mice using a vaccination strategy.

In spite of the many advantages of targeting glycans in cancer and recent advances in cancer cell glycomics, the number of cancer glycan-specific antibodies with clinical potential is limited. The glycomics research field is nonetheless expanding, with ongoing efforts encompassing: (i) biomarker discovery and validation through improving the methodology for quantitative glycan profiling of tumor tissues, (ii) increased understanding of glycosylation dysregulation during oncogenesis, and (iii) development of novel glycan immunization strategies, all of which will aid the development of glycan targeting cancer therapeutics.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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