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Increased effector functions of a monoclonal antibody by glycoform engineering

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Introduction

Igeneon is currently testing a humanized monoclonal antibody specific for Lewis Y (IGN311) in clinical trials of passive cancer immunotherapy. The aim of the current study is to enhance the effector function of this antibody by genetically modifying the antibody producing cell line to express the glycosyl transferase GnTIII. The presence of this enzyme leads to glycosylation of the antibody with a bi-secting N-acetyl-glucosamine group and the absence of core fucosylation.

Methods

First, heavy and light chain genes of IGN311 were isolated, cloned into an expression vector and transfected transiently into EBNA cells: Genes for GnTIII tranferase expression were co-transfected resulting in a new expression product; an antibody, now called IGN312, with modified N-glycosylation pattern. A control wild-type antibody IGN311 wt. was expressed using exactly the same expression vectors and the same host but without co-transfection of genes for GnTIII expression. Both expression products were purified to homogeneity using an identical protein-A based down stream process. Expression products were characterized by SDS-PAGE, IEF and a target antigen specific sandwich ELISA.

Results

No degradation products could be detected and target affinity of the glyco-engineered antibody as well as assembling of heavy and light chains was not affected by GnTIII expression. *In vitro* experiments showed an up to 25 fold increased ADCC lysis activity of the glyco-engineered antibody IGN312 in comparison to the wild type expression product using six Lewis Y positive target cancer cell lines (SKBR5, SKBR3, LoVo, MCF7, OVCAR3 and Kato III). However, CDC activity measured on SKBR5 target cell line was 40% reduced. The reduction of CDC activity could be prevented by using a slightly different molecular-biological approach for increased levels of complex N-linked oligosaccharides of bisected, non-fucosylated type. With this approach, it could be shown to increase ADCC activity without reducing CDC activity. In fact, in this particular case the CDC activity was even 2- fold enhanced. Binding activity for this second-generation glyco-engineered antibody measured by specific sandwich ELISA was not affected.

Conclusions

Next steps will be the generation of a stable IGN312 expressing cell line that produces an antibody with enhanced effector functions. The long term medical and economical goal will be that, due to the enhanced potency of IGN312, the minimal effective dose needed for successful treatment of patients with epithelial Lewis Y expressing, cancers can be reduced significantly.