如何改进治疗性抗体的安全性和疗效特征

How to improve safety and efficacy profiles of therapeutic antibodies

来自于Ludger的Dr. Claire Morgan 和 Dr. Daryl Fernandes对治疗性单克隆抗体的聚糖结构如何显著影响产品的安全性和疗效特征进行讨论

Dr. Claire Morgan and Dr. Daryl Fernandes from Ludger discuss how the glycan structures of a therapeutic monoclonal antibody can significantly influence a product's safety and efficacy profiles

自从首次发现单克隆抗体(MAbs)在医药和工业中可能存在的应用价值后，它们已被经常性地用于研究和诊断试验室中，而对其用于治疗的可能性的研究正在以指数级的速度推进。 现在，MAbs成为了发展最快的一类蛋白治疗性抗体，当前主要被研发用于治疗癌症、自身免疫性疾病、感染性疾病和炎性疾病。它们代表了生物技术行业内出现的销量最大的一类制品——治疗性抗体。截止2008年年底，美国治疗性抗体的市场估计价值达40bn美元，预计抗体很快就会超越较大制药市场的销售额。

过去MAb是通过细胞培养生产，由于价格昂贵而难以用于治疗。然而，在过去的几年间，由于MAb的设计和生产采用了新技术，因此成本急剧下降，目前，细胞培养生产的MAb每克价值为200到300美元。在更为有效、更安全和更经济的Mab在生产上存在着严苛的竞争。革新者和同类生物公司对于自身MAb糖酰化过程的优化有助于实现有效、安全和经济的目的。

MAbs 如何发挥作用?

单克隆抗体是高度复杂的糖蛋白，由各种多肽结构域组成，其中一些存在糖基化。这些结构域组织在一起形成两种类型的功能区——Fab和Fc，它们在治疗中发挥着各自不同的功能性作用。泛泛而言，这些结构域的蛋白结构决定着这些功能区的主要功能活性，而其中的聚糖则可以调整或者协调这些活性的强度。

MAbs的各种作用模式包括对靶物质的中和作用，补体依赖的细胞毒作用(CDC)以及抗体依赖的细胞毒作用(ADCC)。

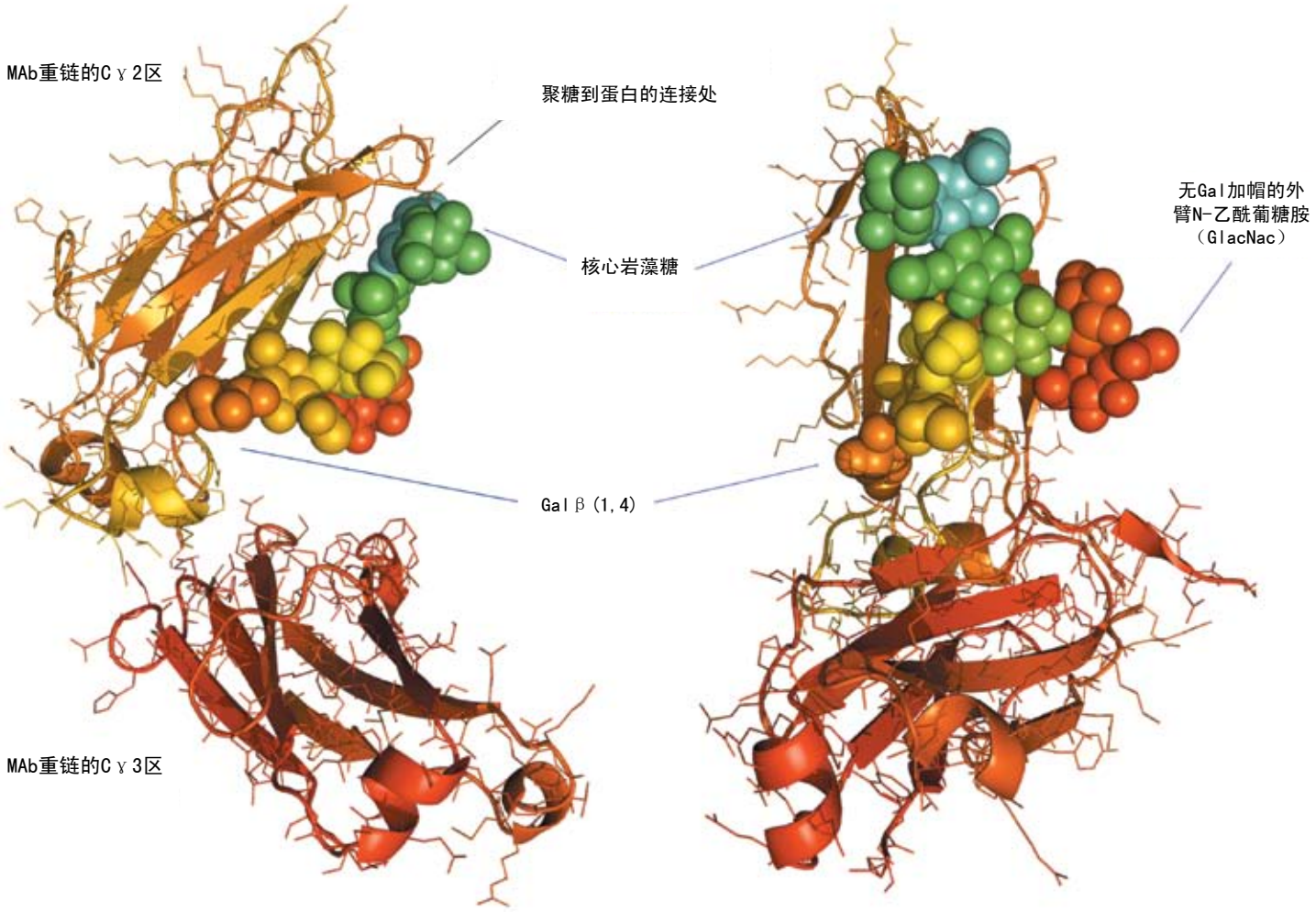
抗体的中和作用依赖于Fab(或者可变区)区与靶物质上的表位相结合，从而阻止因生物分子相互作用而引起的疾病的进展。

Since it was first noted that monoclonal antibodies (MAbs) could be valuable for medicine and industrial use they have become routinely used in research and diagnostic laboratories and their potential as therapeutics has continued to be explored at an exponential rate. MAbs now comprise the fastest growing class of protein therapeutics and are currently being used and developed primarily to treat cancers, autoimmune diseases, infectious diseases and inflammatory diseases. They represent some of the largest selling products to emerge from the biotechnology industry – by 2008 the US therapeutic antibody market was estimated to be worth around USD 40bn – and antibodies are soon expected to outpace the sales of the larger pharmaceutical markets.

MAbs have traditionally been produced in cell culture and have been prohibitively expensive for most therapeutic uses. Over the years, however, the cost has dropped and, as a result of new technologies for their design and production, MAbs are now being produced in cell culture for USD 200 to USD 1,000 per gram. There is fierce competition to produce more effective, safer and less expensive MAbs. Innovator and biosimilars companies can help achieve these aims by optimising the glycosylation of their MAbs.

How do MAbs work?

Monoclonal antibodies are highly complex glycoproteins composed of various polypeptide domains, some of which are



单个抗体重链的Fc功能区显示N-聚糖链接到Asn-297。这种聚糖为二分支N-链接到单个半乳糖（1，4）残基和一个核心岩藻糖。 如果存在唾液酸，它将会链接到半乳糖残基。 在完整的抗体中，这种片断可以和相当的Fc片断配对，而这种Fc可能有两个Fab臂连接在上面的铰链区
Fc region of a single antibody heavy chain showing the N-glycan attached to Asn-297. The glycan is a biantennary N-linked with a single galactose (1,4) residue and a core fucose. If a sialic acid were present it would be linked to the galactose residue. In the intact antibody this fragment would be paired with an equivalent Fc fragment and this Fc would have two Fab arms attached at the hinge region above

通过CDC和ADCC的MAbs（所谓的“Fc效应功能”）依赖于两阶段的作用：第一阶段——抗原结合，涉及到抗体Fab区与靶细胞上特异的表位结合（例如：癌细胞表面上的CD20受体）。 在下列效应因子阶段期间，各种类型的免疫系统受体可以结合到抗原结合抗体的Fc（或者固定）区，从而引发免疫系统中“效应”细胞的募集。这些细胞引发一系列的事件最终导致抗体结合细胞的破坏。CDC和ADCC涉及到不同的受体、效应细胞和靶细胞死亡模式，但是均受到抗体Fc功能区中聚糖类型的显著影响。

glycosylated. These domains are grouped into two types of region – Fab and Fc – which play different functional roles in the therapeutic. Broadly speaking, the protein structures of the domains determine the primary functional activities of these regions while the glycans tune or modify the intensity of these activities.

The various modes of action of MAbs include neutralisation of the target, complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC).

Neutralising antibodies rely on binding of the Fab (or variable) region to an epitope on the target to block biomolecular interactions involved in disease development.

MAbs working via CDC and ADCC (so called “Fc effector functions”) rely on two stages of action. The first stage – antigen binding – involves binding of the antibody Fab region to a specific epitope on the target cell (e.g. the CD20 receptor on the surface of cancer cells). During the following effector

改进安全性和有效性特征

各家公司得益于对Fc聚糖与效应功能之间结构功能关系的了解（例如ADCC和CDC），从而能够设计新的治疗性抗体，通过优化糖基化作用来显著改善已有药物的效能。糖基化作用还会影响MAbs的安全性特征。特别是非人源聚糖，具有潜在的免疫原性。而且，存在Fab功能区的聚糖可以同时影响此类药物的安全性和有效性特征。

对于Mab安全性和有效性而言特别重要的糖基化作用的特征包括：

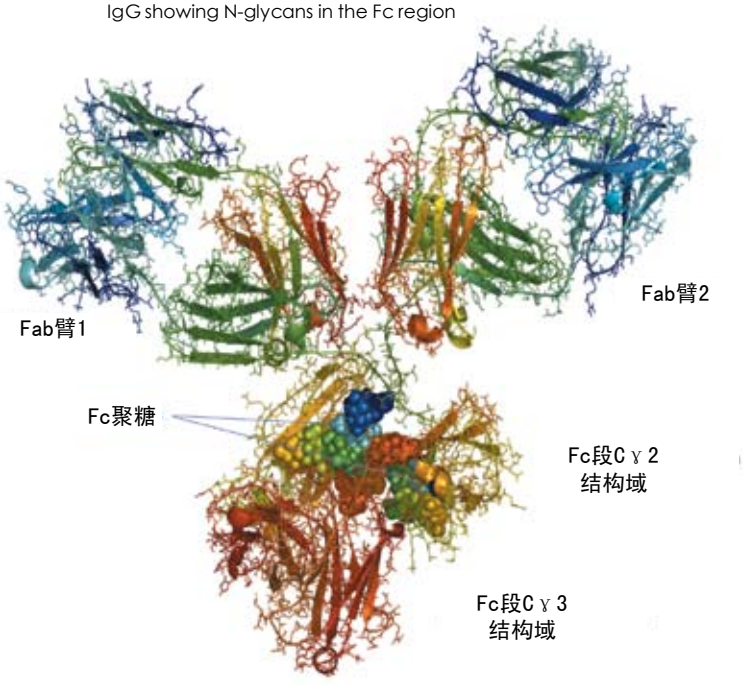
1. 核心岩藻糖基化作用和半乳糖基化作用

人类IgG1同型Mabs，因其能够介导ADCC、CDC以及直接诱导凋亡作用在内的多种效应功能而常被用于治疗当中。Fc功能区通过与白细胞受体(FcγRs)或者补体成分相互作用，进而触发ADCC和CDC作用。这些抗体的每一个Fc功能区重链上在Asn-297位存在两个N-连的二分支复合型寡糖。在人类IgG1中，这些聚糖的很大一部分都储存在所有的“核心岩藻糖”（一个岩藻糖链接到距离天冬酰胺最近的GlcNAc残基上）上。现已明确，通过降低岩藻糖基化水平，可大大地增加治疗性IgG1型MAbs的ADCC活性。其机制是改善了与这些低岩藻糖MAb糖形的FcγRIIIa的结合能力。目前，这种低岩藻糖的工程化抗体已经投入生产，通过改善与FcγRIIIa的结合能力，使它们能够避开对血浆IgG（由于岩藻糖基化而与FcγRIII低强度结合）ADCC活性的抑制作用。其它糖基化作用也会改变Fc效应功能，而对于抗-CD20 Rituximab等某些治疗性抗体，据报告显示，Fc末端上的Galβ(1,4)残基可以影响与C1q的结合并改变CDC活性。

2. 唾液酸化作用

在哺乳动物表达系统中产生的MAb中存在两种主要类型的唾液酸残基，分别是N-乙酰神经氨(糖)酸(NeuAc)和N-羟乙基神经氨酸(NeuGc)。对于治疗性糖蛋白，NeuAc是所需要的正常人类中的唾液

IgG显示Fc功能区中的N-聚糖
IgG showing N-glycans in the Fc region



stage, various types of immune system receptors can attach to the Fc (or constant) region of the antigen-bound antibody to initiate recruitment of "effector" cells from the immune system. These start a series of events that lead to destruction of the target cell to which the antibody is bound. CDC and ADCC involve different receptors, effector cells and mode of target killing but both are greatly affected by the type of glycans in the Fc region of the antibody.

Improving safety and efficacy profiles

Knowledge of the structure-function relationships between Fc glycans and effector functions such as ADCC and CDC allows companies to design new antibody therapeutics with optimised glycosylation to greatly improve the potency over existing drugs. Glycosylation can also influence the safety profile of MAbs. In particular, non-human glycans are potentially immunogenic. Furthermore, glycans found on the Fab region can influence both safety and efficacy profiles of the drug.

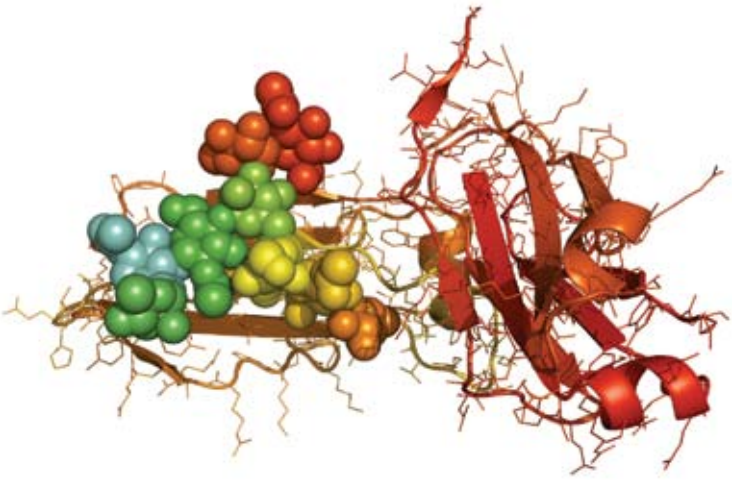
Glycosyation features that are particularly important for MAb safety and efficacy include:

1. Core fucosylation and galactosylation

Human IgG1 isotype MAbs are commonly used as therapeutics because of their ability to mediate multiple effector functions including ADCC, CDC and direct apoptosis induction – ADCC and CDC being triggered following the interaction of the Fc region with either leukocyte receptors (FcγRs) or complement components. These antibodies bear two N-linked biantennary complex-type oligosaccharides at Asn-297 on each of the Fc region heavy chains. In natural human IgG1 a high proportion of these glycans bear so-called 'core fucose' (a fucose linked to the GlcNAc residue nearest the asparagine). It is now well established that the ADCC activity of therapeutic IgG1 type MAbs can be greatly increased by reducing the levels of fucosylation. The mechanism is improved binding to FcγRIIIa of the low fucose MAb glycoforms. Engineered antibodies with low fucose are now being produced; their improved binding to FcγRIIIa allows them to evade the inhibitory effect on ADCC of plasma IgG (which is fucosylated and binds to FcγRIIIa with lower strength). Other glycosylation features can also alter Fc effector functions and for some therapeutic antibodies such as the anti-CD20 Rituximab, Galβ(1,4) residues at the termini of the Fc glycans have been reported to influence C1q binding and alter CDC activity.

2. Sialylation

The two main types of sialic acid residues found in MAbs produced in mammalian expression systems are N-acetyl-neuraminic acid (NeuAc) and N-glycolylneuraminic acid (NeuGc). NeuAc is the desired, normal human-type sialylation, while NeuGc is found in non-human glycoproteins and is considered an undesired, aberrant form of sialylation for therapeutic



酸化作用，而NeuGc被发现存在于非人类糖蛋白中，因而它被认为是一种不良的、异常的唾液酸化作用形式。由于(a)NeuGc可能通过某些患者血清中存在的抗-NeuGc抗体中和作用而使药效降低；而且(b)还认为NeuGc与某些个体中的慢性炎症有关联，因此，对于生物制品生产商而言，控制NeuAC相对于NeuGc的比率非常重要。

而且研究还发现，唾液酸化作用还负责来源于异源静脉内免疫球蛋白(IVIG)的治疗性IgG的抗炎作用，这一点可以解释治疗自身免疫性疾病时需要高剂量IVIG的原因。

3. Gal–α(1,3)–Gal

Gal-α(1,3)-Gal是一种不良的非人类二糖，发现存在于某些MAb的聚糖上，特别是那些鼠源细胞系内表达的那些Mab中。针对Gal-α(1,3)-Gal的免疫反应会导致异体移植中的组织排斥作用，而且研究人员已经发现这种二糖可被NK细胞直接识别。他们发现某些个体中存在高水平的抗Gal-α(1,3)Gal IgE抗体，如果使用聚糖中含有Gal-α(1,3)-Gal 单元的MAb进行治疗，就会出现严重的超敏反应。 我们在一些使用Cetuximab这种形式抗癌药物治疗的患者中发现了这些过敏性反应，这种抗体药物是在小鼠细胞中生产，在抗体重链的Fab部分的Asn-88上聚糖中含有高水平的Gal-α(1,3)-Gal。

4. 其它聚糖特征

其它聚糖特征也可能会影响治疗性抗体的临床效应，其中包括寡甘露糖结构，这种结构可能会改变药物的药代动力学特征；还包括等分GlcNAc残基，它们与Fc效应功能有关。

质量设计和MAb糖分析

考虑到聚糖对于抗体治疗性特征来说具有的重要意义，各家药物生产商必须能够在生产中测量并控制糖基化过程。这就需要一整套糖分析模块系统来确认各批次药物在糖基化关键性治疗属性上的一致性。而且，药物涉及和开发过程中优化糖基化作用可有助于显著增强药物性能，并减少临床应用中的安全风险。

MAb糖基化非常复杂，建议采用质量设计(QbD)原则来辅助药物聚糖数据解释，并简化整个药物使用周期中糖基化可比性的研究。与适当糖分析方案偶联的良好QbD系统可允许各家公司设计并生产治疗性抗体，该抗体具有一致的、优化的糖基化过程以及可靠的安全性和疗效特征。

glycoproteins. Controlling the ratio of NeuAc to NeuGc is important for biomanufacturers as (a) NeuGc may reduce drug efficacy through neutralisation by anti-NeuGc antibodies found in the serum of some patients and (b) NeuGc is thought to be linked to chronic inflammation in some individuals.

Sialylation has also been shown to be responsible for the anti-inflammatory effect of therapeutic IgG obtained from the heterogeneous intravenous immunoglobulin (IVIG) which may explain why high doses of IVIG are required to treat autoimmune diseases.

3. Gal- α(1,3)-Gal

Gal-α(1,3)-Gal is an undesirable non-human disaccharide found on the glycans of some MAbs, particularly those expressed in mouse-derived cell lines. Immune reactions to Gal-α(1,3)-Gal are responsible for tissue rejection in xenotransplantation and the disaccharide has been shown to be directly recognised by NK cells. Anti-Gal-α(1,3)Gal IgE antibodies are found in high levels in some individuals who can show severe hypersensitivity reactions if treated with MAbs containing Gal-α(1,3)-Gal units on their glycans. Such anaphylactic reactions have been found in some patients treated with a form of the anti-cancer drug Cetuximab that was produced in a mouse cell and which contained high levels of Gal-α(1,3)-Gal in the glycans on Asn-88 of the Fab portion of the antibody heavy chain.

4. Other Glycan Features

Other glycan features may also affect the clinical performance of a therapeutic antibody. These include oligomannose structures which may alter the pharmacokinetics of the drug and bisecting GlcNAc residues that correlate with Fc effector functions.

Quality by design and glycoprofiling for MAbs

Given the importance of glycans for the therapeutic profiles of antibodies it is essential for drug manufacturers to measure and control glycosylation during production. This requires an integrated system of glycoprofiling modules to determine the batch-to-batch consistency of glycosylation critical quality attributes for the drug. Furthermore, optimisation of glycosylation during drug design and development can help to increase drug potency significantly and reduce the risk of safety issues in the clinic.

MAb glycosylation is complex and the use of quality by design (QbD) principles is advised to aid interpretation of data on drug glycans and simplify comparability studies of glycosylation throughout the drug life cycle. A sound QbD system coupled with a suitable glycoprofiling scheme will allow companies to design and manufacture therapeutic antibodies with consistent, optimised glycosylation and reliable safety and efficacy profiles. ■