particularly when formulating IgG therapeutics at concentrations of 100–150 mg/mL (Spencer et al., 2012). Such high concentration formulations allow the development of self-administration protocols and can reduce dosing intervals, resulting in reduced CoT. Controlling glycoform fidelity at two sites offers a further challenge to the biopharmaceutical industry.

The licensed antibody therapeutic Erbitux (cetuximab), bears an *N*-linked oligosaccharide at Asn 88 of the V_H region; interestingly, there is also a glycosylation consensus sequence at Asn 41 of the V_L but it is not occupied (Qian et al., 2007; Wiegandt and Meyer, 2014). Analysis of the IgG-Fc and IgG-Fab oligosaccharides of Erbitux, produced from Sp2/0 cells, reveal highly significant differences in composition. While the IgG-Fc oligosaccharides are typical, that is, comprised predominantly of diantennary G0F oligosaccharides, the IgG-Fab oligosaccharides are extremely heterogeneous and include complex diantennary, triantennary, and hybrid oligosaccharides; nonhuman oligosaccharides were also present (e.g., galactose in α(1–3) linkage to galactose and *N*-glycylneuraminic acid residues).

Severe adverse reaction to cetuximab therapy have been reported, and in a study of 76 patients treated with Erbitux, 25 experienced hypersensitivity reactions; this was shown to be due to the presence of IgE anti-gal α(1,3)gal antibodies. Interestingly, environmental factors appeared to influence the development of IgE anti-gal α(1,3)gal responses, and IgE antibodies were detected in pretreatment samples from 17 of the patients (Chung et al., 2008; Daguet and Watier, 2011; Lammerts van Bueren et al., 2011). The incidence varied significantly between treatment centers and may be linked to differences in predominant infectious agents present in local environments. Subsequently, it has been demonstrated that many individuals who consume meat (beef, lamb, pork, etc.) have IgG anti-Gal α(1–3)Gal antibodies and a minority IgE anti-Gal α(1–3)Gal antibodies. It is becoming routine, therefore, to monitor patients for the presence of IgE anti-Gal α(1–3)Gal antibodies prior to exposure to Erbitux (Berg et al., 2014; Daguet and Watier, 2011; Mullins et al., 2012; Pointreau et al., 2012).

A detailed analysis of the glycoforms of a humanized IgG rMAb bearing oligosaccharides at Asn 56 of the V_H and Asn 297, also produced in Sp2/0 cells, reveals the expected IgG-Fc glycoform profile of predominantly G0F oligosaccharides. However, eleven oligosaccharides were released from the IgG-Fab, including diantennary and triantennary oligosaccharides bearing gal α(1,3)gal, *N*-glycylneuraminic acid, and *N*-acetylgalactosamine residues (Huang et al., 2006). The consistent observation of higher levels of galactosylation and sialylation for IgG-Fab *N*-linked oligosaccharides, in comparison to IgG-Fc, is thought to reflect increased exposure and/or accessibility. In view of these experiences, it would seem that the perceived virtues of the NS0 and Sp2/0 cells might best be pursued by engineering to inactivate the gal α(1–3) and *N*-glycylneuraminic acid transferases.

The double challenge to produce rMAbs having appropriately glycosylated IgG-Fc and IgG-Fab has led some companies to engineer out V_H or V_L glycosylation motifs when present in candidate rMAbs (Carter et al., 1992). However, present reports suggest that CHO cells can glycosylate V_H and/or V_L motifs in a similar manner to that observed for normal polyclonal IgG (Lim et al., 2008). Since oligosaccharides are hydrophilic, the addition of glycans within V_H and/or V_L regions may impact the