PROJECT REVIEW

"Rapid Screening (and Confirmatory) Method for rEPO and NESP Based on Immunorecognition of its Exogenous *N*-Glycolyl-Neuraminic Content (GLEPOLYL)"

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The detection of recombinant EPO (rEPO) and analogues (i.e. NESP) has shown to be very challenging due to different factors. First because they are virtually identical to their endogenous counterparts. The only alleged differences so far seem to be localised in their carbohydrates. Second because they are present in urine in very low concentrations (ca. < 1 pM). Third because, as glycoproteins they are not pure single chemical entities but present as a plethora of so called isoforms. Thus each "detectable" isoform is present in much lower concentrations (i.e. < 10 fM).

The current method (isoelectric focusing, IEF) used is based on the differences observed in the charge of those isoforms. Unfortunately so far those differences do not allow an absolute identification of the recombinant species since endogenous and recombinant species seem to show just differences in the proportion in which each isoform is expressed rather than expressing new ones. Furthermore, the method is not amenable for screening purposes since it is expensive, labour intensive and very time consuming. Our group recently showed both in NESP as well as in rEPO the presence of the non-human carbohydrate *N*-glycolylneuraminic acid (Neu5Gc). It is synthesised by CHO (and BHK) cells where the recombinant molecules are produced. Unfortunately, such molecule will only be present in ca. 10% of the rEPO or NESP molecules which poses additional difficulties for their detection.

As a result, the aim of the present project is to produce a monoclonal antibody able to recognise the *N*-glycolylneuraminic acid present in recombinant glycoproteins and develop an immunoaffinity test (e.g. ELISA) that will result in a fast and cheap screening method to recognise the presence of the Neu5Gc moiety in the EPO or NESP molecules in order to determine absolutely their exogenous origin.