

## **PROJECT REVIEW**

### **"Development of an ELISA for rapid specific detection of recombinant erythropoietin and ARANESP (Darbepoetin alfa) in biological fluids"**

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A method is proposed which will enable specific detection of administered recombinant erythropoietin(EPO) and ARANESP™(Darbepoetin alfa) in urine, or other biological fluid such as blood, without regard for levels of EPO activity. This method is specific for recombinant erythropoietin and will not be subject to interference by endogenous erythropoietin. This method is based on the immunochemical detection of recombinant erythropoietin and ARANESP™ by specific detection of carbohydrate moieties which are unique to recombinant erythropoietin and ARANESP™ and are absent from the endogenous glycoprotein. This method is feasible due to the fact that the glycosylation of recombinant glycoproteins is dependent on the cell line in which the protein is expressed and the culture conditions. For these reasons, although possible, it is not necessary to determine levels of EPO activity to detect administration of rHuEPO to the athlete. This method will be rapid, cheap and more specific than current methods in use.

Glycozyme, Inc. in discussions with two different companies to produce the ELISA kit which could also be used to monitor the dosage of legitimate patients.

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### **Result and Conclusion**

The immediate goal of this work was to obtain one or more monoclonal antibodies against the carbohydrate moiety of rHuEPO. The clones from the fusion screen are now monoclonal. The initial screen was against EPO and DG-EPO in which they did not bind DG-EPO as well as EPO by an amount that roughly correlates with the sialic acid content. The supernatants from five clones can be almost completely inhibited (at the concentrations tested) from binding EPO by *Maackia amurensis* lectin which specifically binds N-acetylneuraminic acid  $\alpha(2-3)$  galactose which is present on rHuEPO produced in CHO cells. (CHO cells do not have the enzymatic machinery to produce N-acetylneuraminic acid  $\alpha(2-6)$  galactose.) The EPO molecule has been shown to have the carbohydrate chains on one side of the conformation with the antennary chains protruding so it is unlikely that the lectin binding the terminal sugars interfere with the protein chain.

Finally, the supernatants from the twelve clones tested bind the glycopeptides GP-8 and GP-9 which were linked to KLH. This is a very important observation for several reasons. Our original rabbit anti-EPO antibody was obtained from a rabbit immunized with rHuEPO obtained from Amgen. This polyclonal antibody was shown to bind N-acetylneuraminyl  $\alpha(2-3)$  galactose and galactosyl  $\beta(1-4)$  N-acetylglucosamine (lactosamine) (Pazur, et. al., 2000). The rabbit antibody was shown to bind GP-9. The glycopeptides GP-8, GP-9 and GP-10 were obtained by treatment of the rHuEPO with Glu-c. Further, the rHuEPO from which the glycopeptides were isolated was obtained from another company which, according to some, would be considered a biosimilar rHuEPO even though it is still produced in CHO cells. So there then is indirect evidence that the monoclonal antibodies may bind one of the two antigenic determinants of the rabbit antibody.

These results are very encouraging but these are still screening tests so now the antibodies must be produced in sufficient quantities to further characterize the carbohydrate binding specificity.