In silico and *in vivo* analysis of erythropoietin using zebrafish embryos as animal model

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Erythropoietin is a glycoprotein hormone that acts principally on erythroid progenitors, stimulating their survival, proliferation and differentiation. Recombinant human erythropoietin (rhEPO) has been widely used in medicine to treat anemia and it is one of the best-selling biotherapeutics worldwide. Hematopoiesis has been extensively studied in zebrafish: genes and molecular pathways are very well conserved; moreover, morphology and function of blood cells are comparable to those of higher vertebrates.

Due to these findings, we employed the zebrafish embryo as a vertebrate animal model to perform *in vivo* pharmacological assays with three rhEPOs. We conducted a functional analysis of rhEPO alpha Eprex® and two biosimilars: the erythropoietin alpha Binocrit® and zeta Retacrit®. These recombinant molecules, industrially produced in CHO cells, have the same amino acid sequence of endogenous human erythropoietin, but differ in the glycosylation pattern. This may influence efficacy and safety, particularly immunogenicity, of the final product.

By *in silico* analysis and 3D modeling we proved the possible interaction between recombinant human erythropoietin and zebrafish endogenous erythropoietin receptor. Then we treated zebrafish embryos with the three rhEPOs and we investigated their effect on erythrocytes production with different assays, analyzing changes both in quality and in quantity. Live imaging in tg (kdrl:EGFP; gata1:ds-red) embryos, o-dianisidine positive area quantification and cyanomethemoglobin content quantification revealed an increase of erythrocytes in embryos treated with rhEPOs when compared with the negative controls. Moreover, we verified that recombinant human erythropoietins did not cause any inflammatory response in the treated embryos.

Our data showed that zebrafish embryo can be a valuable tool to study in vivo effects of complex pharmacological compounds, such as recombinant human glycoproteins, allowing to perform fast and reproducible pharmacological assays with excellent results.