

“A System Biology Biomarkers Approach to the Differentiation of Recombinant Human Erythropoietin Doping from Confounding Factors”

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Project Overview:

Our previous anti-doping research aimed at improving the detection of recombinant human erythropoietin (rHuEpo) (08C19YP and 12C09YP) has, so far, provided good evidence that “omics” technologies have the potential to significantly strengthen the Athlete Biological Passport (ABP) and contribute to other traditional anti-doping tests. Given the promising results to date, an important next step is to evaluate the effects of major confounding factors on the molecular response to rHuEpo, such as the effects of altitude, strenuous exercise and exercise training. The aim of these experiments were to investigate whether a “transcriptomic” approach can successfully differentiate rHuEpo from major confounders (i.e., altitude, strenuous exercise and training). The transcriptional response to autologous blood transfusion was also investigated and responses compared to rHuEpo and the major confounders investigated. **METHODS:** Altitude training (AT) On the basis of pilot data generated, an altitude study was performed that involved 14 endurance-trained runners living and training at sea level sojourning to a higher altitude (Suluta, Ethiopia, ~2800m above sea level) for 27 days. Strenuous exercise (SE) Two exercise studies were conducted, both involving acute high intensity exercise and one involving 4 weeks high intensity exercise training. Autologous Blood Transfusion (ABT) In this study, 15 healthy males participated in an ABT intervention. Subjects received a saline injection for the control phase, and then donated one bag of blood (500mL) 14 days later, which was stored at 4°C for 36 days before reinfusion (the volume of red blood cells reinfused was approximately 280mL). **RESULTS:** Confounder analysis The marked gene expression response (at 5% false discovery rate) to rHuEpo involving some 15,952 genes was modestly confounded by exercise (1,596 genes), altitude (379 genes) and transfusion (4 genes). Of the remaining 14,016 unique rHuEpo genes, 35 transcripts (involving some 33 genes) were differentially expressed at each time point during the intervention, and 2 and/or 4 weeks post intervention in the previously studied Scottish and Kenyan athletes injected with large doses of rHuEpo. Focusing only on rHuEpo detection post rHuEpo intervention, >300 transcripts were differentially expressed 2 weeks after the last rHuEpo injection and a significant transcriptional response was maintained 4 weeks after the last rHuEpo injection in the Scottish cohort.

Results and Conclusions:

All necessary confounder studies were successfully performed (and biobanked for future use) to address the ambitious objectives of this research. Results confirm the enhanced sensitivity of the blood transcriptomic approach over standard haematological markers to detect

changes in response to rHuEpo and potentially ABT. Importantly, the confounder analysis clearly demonstrates a large and unique transcriptomic response to rHuEpo involving thousands of genes compared to the major confounders that include altitude and strenuous exercise. On the basis of these results, it can be concluded with greater confidence that “omics” technologies will significantly strengthen current anti-doping strategies. In particular, the use of molecular biomarkers with an improved detection window and high sensitivity and specificity to develop the transcriptionally enhanced ABP model for detecting blood doping. Collectively, the findings of the present research, interpreted in the context of the latest “omics” research in biomedical sciences, are most encouraging and confirm claims that a systems biology approach combining various “omics” signatures from genomics, transcriptomics, proteomics and metabolomics will inevitably provide a deeper understanding of the effects of Epo stimulating agents on erythropoiesis with unparalleled potential to improve current drug detection strategies with particular reference to blood doping.