"*Development of Reliable Blood Tests for the Detection of Gene Doping after Intramuscular Injection of Recombinant Adeno-Associated Viral Vectors*"

Dr. Snyder (University of Florida, USA)**, Dr. Mouillier** (University of Nantes, France)**, Dr. Simon,** (Johannes Gutenberg-University, Germany)

Project overview

Specific Aims

Aim 1: Evaluate a combined assay format that is based on real-time PCR and ITC readout with a nested PCR pre-step to improve sensitivity.

Aim 2: Assay analytical validation for specificity, sensitivity, and ruggedness (multiple labs, multiple operators, repeats and replicates) and preliminary pre-clinical performance validation.

Results and Conclusions

It is crucial to develop reliable test methods for the detection of gene doping, given that some members of the sports community are seeking for gene transfer technology to improve exercise performance. Based on previous work we established a ´nested real-time PCR´ assay. This is an assay based on the PCR technology which is used in criminology and well-known in the court of law for the identification of genetic material in samples from sites of crime. We adapted this technique to detect single erythropoietin (EPO) DNA molecules that had been introduced into the body by means of gene transfer. Due to its high sensitivity, our technique is principally able to detect genetic modifications of certain parts of the body in conventional blood samples. Here we aimed at providing evidence of the high sensitivity and specificity of our refined procedure that now enables quantification of the artificial DNA in the blood stream.

Through an inter-laboratory study between Nantes (France), Gainesville (Florida, USA), and Mainz (Germany) two nested real-time assays were validated and utilized for an in vitro blinded study. Both assays showed unique sensitivity and specificity in a large dynamic range. According to the nested assay protocol the detection of ~1 copy of circular transgene molecule in a background of 500 ng gDNA is possible reliably. In a blinded in-vitro study the reliability of the assays to detect between 1000 – 2 copies of transgene molecules and non-template control was proven.

To validate the suitability of the nested real-time assays for the detection of gene doping a non-human primate study was conducted. Two macaques were injected intramuscularly with a recombinant adeno-associated virus serotype 8 (rAAV8) vector harboring human EPO cDNA sequence. One macaque served as non-injected control. The vector was promoterless to

avoid transgene expression. Following the injection of 2.5 x 10^{11} viral genomes/kg, the transgene molecules were detectable 8-14 weeks.

The high sensitivity of the nested qPCR assay along with the specificity for transgene detection is essential for gene doping surveillance. The assay can be adapted to other gene doping candidate genes.