Project Review

"A Pilot Study to Develop a Reliable Blood Test for the Detection of Gene Doping after Intramuscular Injection of Naked Plasmid DNA"
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With the development of potent gene transfer technology, the availability for illegal gene doping is becoming more likely. To stay ahead of this eventuality, we strive to empower WADA/IOC with the diagnostic capability to detect genetically modified athletes (GMA). We will conduct a pilot study to determine the feasibility of detecting plasmid gene transfer vector sequences and cDNA sequences that may be used for illegal performance-enhancing gene doping from blood samples.

Using appropriate reporting systems with naked plasmid DNA, we will determine if the DNA sequences can be detected in blood cells using PCR-based DNA analysis. Eventually, this work may lead to the development, validation, and manufacture of a kit for routine use by WADA/IOC to screen athletes for illicit gene doping-based performance enhancements.

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Results and Conclusions

Real-time PCR assays were developed to detect a performance enhancing transgene (Epo) or plasmid backbone sequences (SV40 polyA, kanamycin resistance), and endogenous cellular sequences. In addition, the steps involved in DNA extraction, DNA storage and DNA transport were validated. By PCR, the vector transgene is distinguishable from the genomic DNA sequence because of the absence of introns and other unique features. After the performance of the assays (sensitivity and specificity parameters) was optimized, two macaques received IM, one single dose of a plasmid carrying a PGK-macaque Epo-SV40 polyA cassette. Since parameters such as plasmid quality, concentration, excipient, electro-transfer, etc…, interfere with muscle transduction, the macaques received a high plasmid dose intended to achieve a significant, but not life-threatening, raise in hematocrit. DNA extracted from blood was tested by the real-time PCR assay to support the hypothesis that blood can be collaterally transduced in this context and can be used as a surrogate marker for gene doping. We demonstrate that IM injection of a conventional plasmid results in the transient presence of DNA that can be detected at high levels in blood prior to rapid elimination.