PROJECT REVIEW

“Development of Ultrasensitive DNA Aptamer-Based Tests for Pharmaceutical Grade Recombinant Human Growth Hormone”

Dr. Bruno, (Operational Technologies Corp, USA)

Operational Technologies Corp. (OpTech) has already demonstrated and published (Bruno J.G., et al. J. Biomolec. Techniques, In Press, 2011) the ability to develop aptamers (surrogate antibodies composed of DNA) which can discriminate synthetic (recombinant) from natural human growth hormone (hGH) with initial funding from WADA. The aptamers developed by OpTech detect minor amino acid modifications and differences added by E. coli host cells as noted by Hepner et al. (2005 and 2006) to research grade synthetic hGH. Under this follow on grant proposal, OpTech will develop a new set of discriminatory aptamers against one or more pharmaceutical grade recombinant hGH targets (Genotropin, Norditropin, etc.). OpTech has recently demonstrated ultrasensitive (sub-picogram/ml) detection of hGH with some of its original hGH aptamers in an aptamer-magnetic bead sandwich electrochemiluminescence (ECL) format. ECL is already used by Roche Diagnostics for ultrasensitive detection of numerous clinical analytes. Therefore, OpTech will develop several combinations of capture and reporter aptamers to detect pharmaceutical hGH at sub-pg levels. Finally, because ECL is a laboratory-bound technique, OpTech will investigate the potential for presumptive field testing for synthetic hGH in aptamer-based lateral flow test strips using blood or urine samples for preliminary screening of athletes. To enhance sensitivity, OpTech will explore the use of quantum dots (QDs) and fluorescent nanoparticles (FNPs) along with a UV penlight to enhance visual detection of synthetic hGH in lateral flow test strips. Disposable low pressure size-exclusion chromatography columns may be needed to remove creatinine and urea in serum and urine prior to aptamer-based assays. These substances appear to denature aptamer secondary and tertiary structures which are critical to proper folding and binding of aptamers to hGH and other targets. This is anticipated to be a two year project.
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Results and Conclusions

Operational Technologies Corp. (OpTech) was unable to obtain Genotropin or Norditropin from Pfizer, Novo Nordisk, or other sources. However, OpTech developed DNA aptamers against the oxidized peptide regions of Genotropin defined by Hepner et al. (2005 and 2006) and thought to be potential sites for discrimination of recombinant vs. natural hGH in ~ 2% of Genotropin molecules. OpTech screened these aptamers against research-grade rhGH and natural pituitary hGH and identified several candidate aptamers (e.g., Hep 5-6R, Hep 6-6R and Hep 6-12R) which may be able to discriminate Genotropin from natural hGH. However, without access to authentic Genotropin, OpTech could not test this hypothesis fully. Therefore, OpTech elected to use the remaining funds to explore new and potentially even better approaches to detecting hGH abuse in athletes with aptamer-based assays. These new approaches were:

1) Development of aptamers to bind human IGF-1 and PIIINP which are both long-lived serum biomarkers of rhGh use. This work resulted in development of a preliminary enzyme-linked IGF-1 aptamer-magnetic bead sandwich assay which functioned in pure human serum with a lower limit of detection of ~ 30 ng/mL and linear detection to ≥ 1,000 ng/mL. The top-ranked IGF-1 aptamer candidates (3F and 25R) may bind a region of IGF-1 that is accessible even when IGF-1 is bound by its binding proteins in serum. Unfortunately, these IGF-1 aptamers also demonstrated significant cross-reactivity with Brain Natriuretic Peptide (BNP), bone collagen Helical Peptide (HP) and rhGH, but much lower affinity for C-telopeptide (CTx) or N-telopeptide (NTx) of bone collagen. Preliminary data also indicated that development of a PIIINP aptamer assay with linear quantification in the high to mid-ng/mL range was possible using at least 3 different aptamer candidates identified during ELISA-like screening (ELASA).

2) Development of aptamers against specific peptide regions of the 20kD and 22kD isoforms of hGH to emulate the current ratiometric immunoassay. OpTech developed aptamers against the 15-amino acid region (EEAYIPKEKYSFLQ) of 22kD hGH which is not present in 20 kD hGH and also developed aptamers against the fused flanking regions (DTYQEFNQFTSL) resulting from alternative splicing to enable discrimination of unique regions on each isoform of hGH. Two lead candidates designated “Splice 20-14R and Splice 22-5F” were identified for potential future ratiometric assay development.
Both approaches appear promising and, if fully developed, would have the advantage of overcoming lot-to-lot variability associated with antibodies and immunoassays, because aptamer DNA synthesis from known sequences is a very high fidelity process.