“Analysis of antibody-based myostatin inhibitors”

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PROJECT OVERVIEW

Key regulator of skeletal muscle mass in developing embryonic and adult skeletal muscle is the growth factor myostatin. In animal models, both myostatin deficiency and inhibition resulted in an increased muscle mass and function due to an elevated volume and/or number of skeletal muscle fibers. Consequently, myostatin inhibitors may not only provide a therapeutic approach for the treatment of muscular diseases but also be misused as performance-enhancing agents in sports.

The aim of the planned project is to establish a detection method for MYO-029 (Stamulumab), a recombinant human myostatin antibody which was developed by Wyeth Pharmaceuticals in 2005. MYO-029 specifically blocks myostatin signaling by preventing the interaction between the growth factor and its target receptor. Mice treated with such a neutralizing antibody showed an enhanced muscularity and a first clinical trial on humans yielded a good tolerance and safety of the drug. Within this project, two different immunological approaches will be used for the detection of MYO-029 misuse in cheating athletes. Both the western blot and ELISA will use recombinant human myostatin as bait to capture MYO-029 potentially present in plasma/serum samples. Antibody specifically bound to the immobilized antigen can then be detected by using an enzyme-coupled secondary antibody direct against its species-specific portion. Additionally, an antibody-based internal standard will be implemented by using different species-specific secondary antibodies conjugated to distinct fluorophores. Following method optimization, both approaches will be thoroughly characterized to ensure their reliability, sensitivity, and transferability. They will serve as proof of principle for the detection of myostatin inhibitors and antibody-based pharmaceuticals in doping control samples

Results and Conclusions:

Myostatin is the key regulator of skeletal muscle mass and inhibition of its signaling pathway can result both in an increased muscle mass and function. Within this project, two complementary detection methods for the recombinant human anti-myostatin antibody MYO-029 were developed by using immunological approaches.

First, a qualitative western blot-based assay was set up and comprehensively characterized. It was found to be highly specific, robust, and linear from 0.1 to 1 µg/mL. The precision was successfully specified at three different concentration levels and the recovery of the affinity purification was 58%. Consequently, in consideration of the World Anti-Doping Code International

Standard for Laboratories (ISL) 2015, paragraph 6.2.4.3.1.3, the assay can be considered fit-for-purpose for an application in routine doping control analysis.

Additionally, an ELISA capable of detecting MYO-029 in human serum was developed as initial testing procedure. The conducted experiments show that a very sensitive and reproducible detection of anti-myostatin antibodies in a microtiter plate is possible. However, further optimization and characterization *e.g.* to reduce non-specific background signals is advisable if such approaches will be considered for routine doping controls, especially when multiplexing for different antibodies relevant for doping controls is desirable.