

Project Review

"Development of tests for detecting myostatin-based doping to enhance athletic performance"

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Numerous genetic and/or pharmacological strategies exist that can increase muscle mass and strength. Many were developed to treat muscle diseases such as Duchenne's muscular dystrophy (DMD). Importantly, these strategies have great potential to be abused by elite athletes seeking to gain a (unfair) competitive advantage, since they are, a) currently available and b) not detectable by current anti-doping protocols.

Prime amongst these are strategies for modulating the growth/development factor myostatin (GDF8); a negative regulator of muscle mass. We described an antibody-based blockade strategy to increase muscle strength and reduce muscle damage in the mouse model of DMD (Bogdanovich et al. 2002 *Nature*). Other blocking strategies have been described since our initial report, including a propeptide-based strategy (Bogdanovich et al. 2005 *FASEB J*). Of immediate concern regarding doping is the development of the myo-29 "humanized" myostatin-blocking antibodies by Wyeth, that are currently in human Phase II trials

Currently, tests do not exist to detect myostatin blockade based 'doping' with antibodies, propeptides, or other reagents. The fact that a number of web-based resources currently offer 'myostatin-blockade' reagents, underscores the need for rapidly developing a test to detect 'doping' not just the currently described reagents but also those that would be generated in the near future, including the use of gene doping approaches such as RNAi.

Thus, the challenge is to develop a standardized detection test that would be specific, sensitive and standardized enough to hold up to legal challenges that anti-doping agencies would almost certainly face by athletes caught using these tests. These challenges are not insurmountable; we propose to develop a robust and standardized assay for 'total myostatin activity' to serve this need.

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Results and Conclusions

The overall aims of this project are to develop tests to detect myostatin-based 'doping' (blockade) and precluding abuse by elite athletes seeking to gain a (unfair) competitive advantage using myostatin-based doping. We have made excellent progress toward completion of this project and have cloned and developed the WADA-myostatin CAGA assay to serve as doping detection tests for this purpose. This luciferase-based assay emits light proportional to total myostatin activity and is used widely in research and industrial labs. We have developed two assays one in the C2C12 muscle cell line and one in the easier to grow HEK cells, hence it would be easy and efficient to transfer to WADA testing laboratories worldwide.

It is important to point out that in myostatin-blockade strategies relevant for doping myostatin levels themselves are not altered rather the ability to activate the receptor or 'activity' is altered. Hence for detection of myostatin blockade in athletes the activity-based assay we developed i.e. the "WADA-myostatin CAGA assay" will detect doping, however, assays based on detecting the molecule itself will not be useful, irrespective of their sensitivity. Technical analyses of the WADA-myostatin CAGA assay have been completed in our laboratory and the test is robust and valid based on the Z' scores we obtained. We have also treated wild type mice and *mdx* mice with different myostatin inhibitors to obtain serum for validation of the myostatin-doping test if needed. A set of standards has been generated that are suitable for distribution to WADA laboratories worldwide.

Given that our results demonstrate that the WADA-myostatin CAGA assay is sensitive, reproducible and robust we believe that the next logical steps would entail single-and double-blind testing of the assay with serum samples from athletes to develop normograms and developing standard operating procedures (SOP's) for incorporation into current WADA testing protocols.