The Researcher's Perspective

The development of a detection method for **hGH** has been a significant priority for the scientific community devoted to the fight against doping. The widespread implementation of the hGH detection method is expected to occur later this year.

The successful development of one of the strategies to detect hGH is the result of team work involving key contributions from Dr. Zida Wu, Dr. Martin Bidlingmaier and many students and technicians, led by **Prof. Christian Strasburger, chief of clinical endocrinology, Charité Universitätsmedizin Berlin, Campus Mitte**. Prof. Strasburger describes what it was like to take on the challenge of developing the hGH detection method (isoform approach), an often arduous yet in the end fruitful experience.

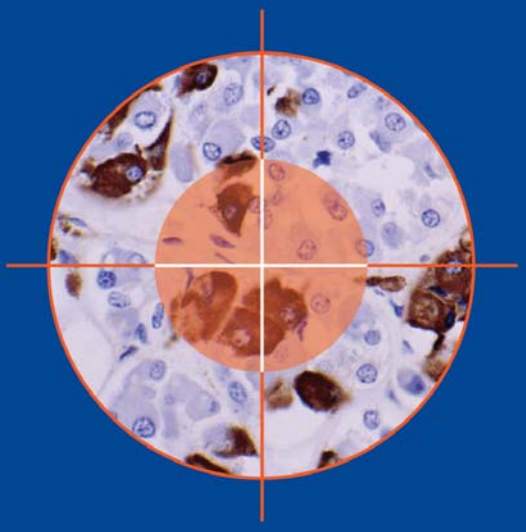
Play True: How did you become involved in research for hGH detection?

Prof. Christian Strasburger: Following completion of my doctoral thesis on chemiluminescent immunoassays in 1984 and two years of clinical training, I had the opportunity to stay for a two-year post-doctoral fellowship at the Weizman Institute of Science in Rehovot (Israel), where I learned how to generate monoclonal antibodies against hGH (1986–1988). After returning to Germany, my team and I started a long series of generating high affinity monoclonal antibodies to hGH.

An eminent problem and dilemma in clinical medicine is that different assay techniques measuring hGH yield very discrepant results, and yet, on these measurements, clinical decisions are based regarding daily injections of short children and nowadays life-long daily injection for adults with growth hormone deficiency. Therefore my colleagues and I developed and validated a method combining a monoclonal antibody and the extracellular part of a growth hormone receptor molecule to report only those GH-forms in circulation which retained the capability of activating GH-receptors. In the validation process of this method we recognized that it had a clear preference for recombinant hGH over pituitary-derived hGH in human serum.

So that was the start. How long has the entire process taken, what have been the major steps involved?

The initial observation was made in 1996 and it took more than 12



About hGH Detection

HGH is a hormone that is synthesized and secreted by cells in the pituitary gland located at the base of the brain. HGH is known to act on many aspects of cellular metabolism and is also necessary for skeletal growth in humans. The major role of hGH in body growth is to stimulate the liver and other tissues to secrete insulin like growth factor (IGF-1). IGF-1 stimulates production of cartilage cells, resulting in bone growth and also plays a key role in muscle and organ growth.

HGH is prohibited both in- and out-of-competition under the List of Prohibited Substances and Methods. Commonly reported side effects for hGH abuse include: diabetes in prone individuals; worsening of cardiovascular diseases; muscle,

months before we obtained initial funding from the German Federal Institute of Sports Sciences to screen the more than 100 monoclonal antibodies to hGH that had previously been generated for their ability to detect the existing structural differences between recombinant hGH as a monomorphic preparation and the mixture of hGH isoforms as secreted by the pituitary gland.

After combining two antibodies each in a sandwich immunoassay format for the measurement of recombinant hGH on one hand and pituitary-derived hGH on the other hand, we approached the consortium GH 2000, led by Prof. Peter Sonksen, and informed them about our potential solution to the problem they were addressing. The consortium collaborated in providing us 40 blinded samples derived from either pituitary stimulation tests for hGH release or from the pharmacokinetic profiles after injection of hGH in GH-deficient adult patients. Our differential immunoassay approach permitted us to differentiate these 40 serum samples without error. This blind test of the differential immunoassay strategy based on hGH isoform differences certainly represented a significant breakthrough and we were able to publish these findings in *The Lancet* in early 1999.

It was tedious to maintain research support and after the 2000 Olympic Games in Sydney, the IOC provided a research grant for three years by which we aimed to further increase the discriminatory potency of our technique and select the most suitable monoclonal antibodies among the large panel of anti-hGH monoclonals that were previously established.

Before the 2004 Summer Olympics, WADA, jointly with USADA, hosted scientific workshops of the most active scientists involved in hGH anti-doping research worldwide and decided to move forward with the isoform approach.

The isoform approach by differential immunoassay strategy for the detection of hGH abuse was applied during the Athens Summer Olympic Games and the Turin Winter Olympic Games. Because it is not feasible that an academic hospital provides immunoassay reagents of the highest quality control standard for a long period of time to all WADA-accredited laboratories, we started to look for collaboration with the diagnostic industry. Unfortunately, our first selected partner, after more than two years of collaboration, decided to discontinue the joint development project for the differential immunoassay kit. We therefore had to identify a new partner in the diagnostic industry and found a highly reputed collaborator in Germany. At the end of 2006, the CMZ-assay company was founded which will be marketing these differential immunoassay kits later in 2007.

What is the status now of the detection method for hGH?

By changing the performance platform of the differential immunoassays from microtiterplate-based fluorescence immunoassays to tube-based chemiluminescent immunoassays and by improving the antibody immobilization technique, the lower detection limit of our

joint and bone pain; hypertension and cardiac deficiency; abnormal growth of organs; accelerated osteoarthritis. In untreated acromegalic individuals (known for pathological over-production of hGH), many of the symptoms described above are observed and life expectancy is known to be significantly reduced.

The test to detect hGH abuse is a blood test and is reliable. It was introduced at the Athens Olympic Games in 2004 and other major sport events. However, because hGH is often taken by doping athletes in the off-season to optimize performance, the test is most effective when implemented in a no-advance-notice out-of-competition strategy. Widespread implementation of the test, once produced on a commercial basis, will allow routine testing.

Another test, in its final research stage, will be combined with the current test to further enhance the detection window for hGH abuse. The concepts and development of both hGH tests have been systematically reviewed by international independent experts in such fields as hGH, endocrinology, immunoassay, analytical chemistry, etc. These tests are the outcome of nearly US$10 million in research over the course of more than 11 years, first initiated by the IOC and the European Union, and then taken over by WADA when it was created and had adopted scientific research as one of its priority activities.

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method could be dramatically improved. The method is robust and stable and is expected to pass industrial validation and certification tests in the forthcoming months to then become generally available.

Why is the detection of hGH so important?

HGH has apparently been used widespread because it was considered undetectable and the known physiological effects of growth hormone are muscle building as well as lipolytic and therefore providing energy substrates which cheating athletes obviously fancy. Growth hormone has been discovered in the luggage of an elite athlete and several athletes have meanwhile confessed having abused hGH to enhance their performance. If clean athletes are to compete on a level playing field, then hGH detection must be implemented.

How confident are you in the detection method's reliability and validity?

We are extremely confident in this method because it is a very direct approach. If an athlete abuses hGH, then by the direct measurement of hGH, doping analysts have the best opportunity to prove the abuse. Our method's underlying principle is that these structural differences between recombinant hGH and hGH in human sera exist regardless of age, gender, ethnic background or sports discipline. With the improved lower detection limit as achieved during the presently completed commercialization, the usefulness of the method is further significantly improved.

What have been the most significant challenges you have encountered along the way?

After the idea was there, the major challenge was to obtain and maintain research funding for this activity at a university teaching hospital. We also had to learn that the transfer from an academic setting to commercial rollout of such a differential immunoassay involved tedious work, but also provided opportunities of methodological improvement. Finally, when we started working on this project, WADA was not yet founded and the message in support of anti-doping was not as unequivocal as it is today. Even our colleagues were sceptical about patenting such an idea due to the limited number of interested organizations at the time.

What advice would you give young scientists interested in anti-doping research?

I would advise young scientists who have begun to enjoy research in one area to take a step back and take a broader look at different approaches to the solution of the problems supposed. While traditional anti-doping laboratories are using gas chromatography / mass-spectromaty (GC-MS) methods to detect small substances like anabolic steroids, amphetamines and so on, recombinant proteins approximately 100 times larger such as EPO or growth hormone cannot be detected from serum samples by these techniques and if the GC-MS methodology is to be applied, then an immuno-recognition and affinity chromatography step has to precede the analytical step to allow removal of the bulk of other proteins in the sample otherwise interfering. Therefore, immunoassays traditionally used in the neighboring discipline of endocrinology have their place and in turn endocrinologists are learning from doping analytics and are bringing GC-MS for steroid metabolites to the clinical routine. Young scientists should broaden their horizons and experience and know that there is "more than one way to Rome."

What do you see for the future of anti-doping research?

I believe anti-doping research has to use a dual strategy. First, there needs to be more funding of anti-doping analytical research to narrow the gap between the also science-driven doping versus the anti-doping movement. Secondly the phenomenon of doping in my opinion requires sociological and psychological research looking at sports as a mirror image of human society and we have to strive to better understand what goes wrong in society leading to the use of performance enhancing drugs in sports. We need to convince young athletes that they should compete by fair means, even if it means not winning.

BRIEF BIO:

Prof. Christian Strasburger is chief of clinical endocrinology at Charité Universitätsmedizin Berlin, Campus Mitte. He received post-doctoral training at the medical faculties of the universities in Lübeck and Munich (Germany). He also held a post-doctoral fellowship at the Weizmann Institute of Science in Revohot (Israel).

Prof. Strasburger has published more than 140 scientific articles and serves on the editorial board of *Pituitary, GH- and IGF-research, Journal of Endocrinological Investigation.* He was recently appointed editor-in-chief of the *European Journal of Endocrinology*. In addition, Prof. Strasburger serves on the councils of the Growth Hormone Research Society and the European Neuroendocrine Association, and served formerly on the German Endocrine Society Council.

Prior to university, he was a rower on the German junior national team and competed at the world championships.

GLOSSARY:

Affinity chromatography: Chromatographic method of separating biochemical mixtures, based on a highly specific biologic interaction such as that between antigen and antibody.

Chemunilescence: Emission of light (luminescence) without emission of heat as the result of a chemical reaction.

Chromatography: Family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a “stationary phase,” which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.

Immunoassay: Biochemical test that measures the concentration of a substance in a biological liquid, typically serum or urine, using the reaction of an antibody or antibodies to its antigen. The assay takes advantage of the specific binding of an antibody to its antigen.

Isoform: Version of a protein with only small differences to another isoform of the same protein.

Mass spectrometry: Analytical technique used to measure the mass-to-charge ratio of ions. It is used in anti-doping to find the composition of a physical sample by generating a mass spectrum representing the masses of sample components.

Microtiterplate: a flat plate with multiple "wells" used as small test tubes.

Monoclonal antibodies: Antibodies that are identical because they were produced by one type of immune cell and are all clones of a single parent cell.

Pharmacokinetics: Discipline of pharmacology dedicated to the determination of the distribution and elimination of substances administered to a living organism.