Activities of the European Monitoring Center for Emerging Doping Agents

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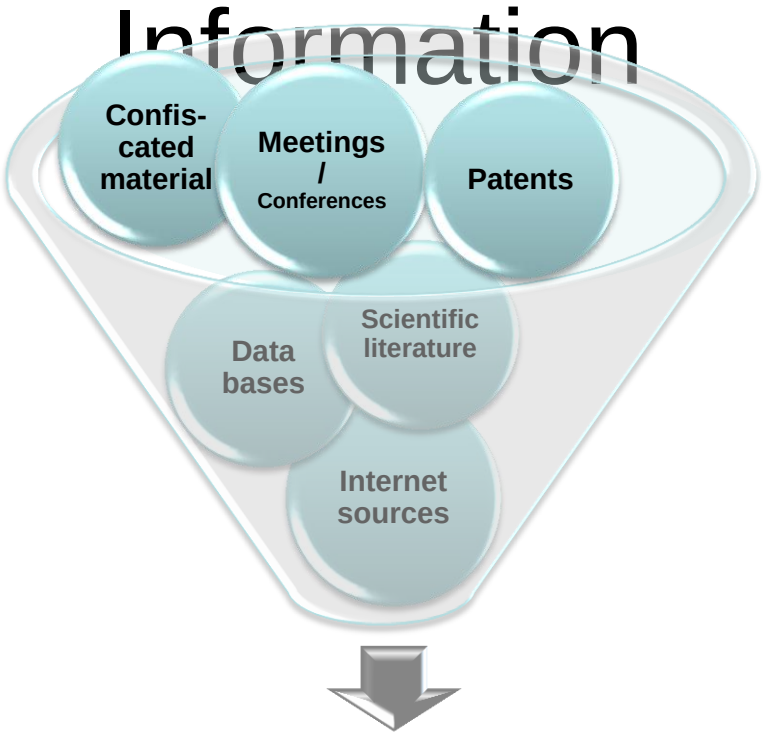
Emerging Doping Agents

Mario Thevis

Institute of Biochemistry / Center for Preventive Doping Research , German Sport University Cologne, Germany
& European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Cologne/Bonn, Germany



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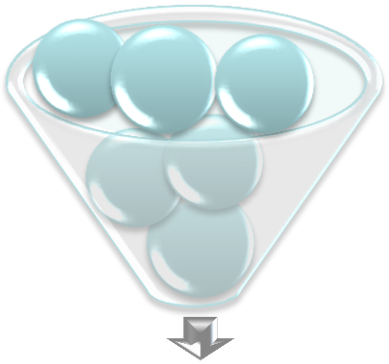
collect information / identify relevant developments / question & summarize



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report

mandate

Expert groups

e.g. WADA List Expert Group

review / evaluate / initiate actions

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Collaboration

- other monitoring centers
- laboratories
- industry
- police / customs control
- scientific community

Literature

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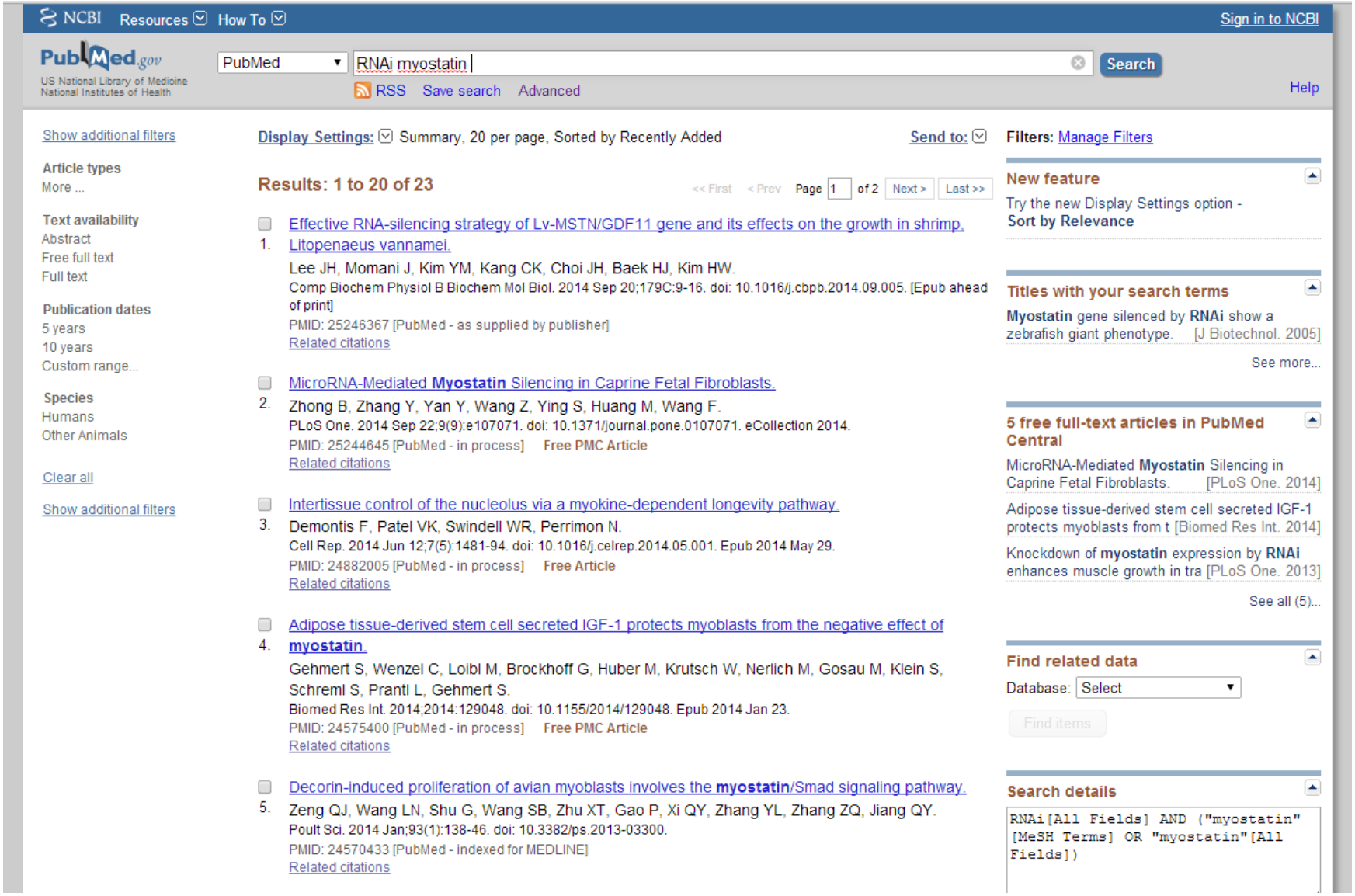

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1. Effective RNA-silencing strategy of Lv-MSTN/GDF11 gene and its effects on the growth in shrimp, Litopenaeus vannamei.
Lee JH, Momani J, Kim YM, Kang CK, Choi JH, Baek HJ, Kim HW.
Comp Biochem Physiol B Biochem Mol Biol. 2014 Sep 20;179C:9-16. doi: 10.1016/j.cbpb.2014.09.005. [Epub ahead of print]
PMID: 25246367 [PubMed - as supplied by publisher]
Related citations

2. MicroRNA-Mediated **Myostatin** Silencing in Caprine Fetal Fibroblasts.
Zhong B, Zhang Y, Yan Y, Wang Z, Ying S, Huang M, Wang F.
PLoS One. 2014 Sep 22;9(9):e107071. doi: 10.1371/journal.pone.0107071. eCollection 2014.
PMID: 25244645 [PubMed - in process] Free PMC Article
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3. Intertissue control of the nucleolus via a myokine-dependent longevity pathway.
Demontis F, Patel VK, Swindell WR, Perrimon N.
Cell Rep. 2014 Jun 12;7(5):1481-94. doi: 10.1016/j.celrep.2014.05.001. Epub 2014 May 29.
PMID: 24882005 [PubMed - in process] Free Article
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4. Adipose tissue-derived stem cell secreted IGF-1 protects myoblasts from the negative effect of **myostatin**.
Gehmert S, Wenzel C, Loibl M, Brockhoff G, Huber M, Krutsch W, Nerlich M, Gosau M, Klein S, Schreml S, Prantl L, Gehmert S.
Biomed Res Int. 2014;2014:129048. doi: 10.1155/2014/129048. Epub 2014 Jan 23.
PMID: 24575400 [PubMed - in process] Free PMC Article
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5. Decorin-induced proliferation of avian myoblasts involves the **myostatin**/Smad signaling pathway.
Zeng QJ, Wang LN, Shu G, Wang SB, Zhu XT, Gao P, Xi QY, Zhang YL, Zhang ZQ, Jiang QY.
Poult Sci. 2014 Jan;93(1):138-46. doi: 10.3382/ps.2013-03300.
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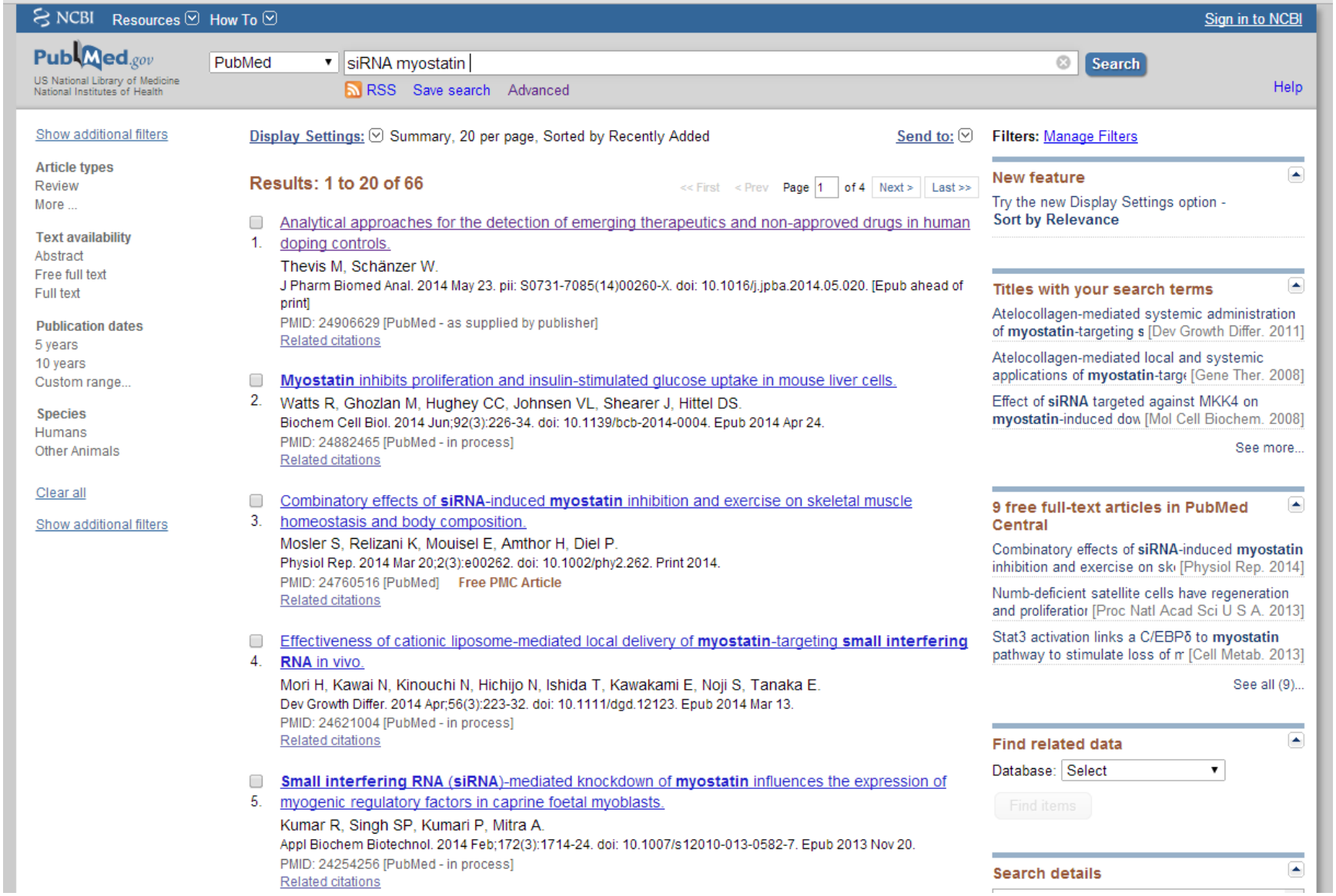

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1. Analytical approaches for the detection of emerging therapeutics and non-approved drugs in human doping controls.
Thevis M, Schänzer W.
J Pharm Biomed Anal. 2014 May 23. pii: S0731-7085(14)00260-X. doi: 10.1016/j.jpba.2014.05.020. [Epub ahead of print]
PMID: 24906629 [PubMed - as supplied by publisher]
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2. **Myostatin** inhibits proliferation and insulin-stimulated glucose uptake in mouse liver cells.
Watts R, Ghozlan M, Hughey CC, Johnsen VL, Shearer J, Hittel DS.
Biochem Cell Biol. 2014 Jun;92(3):226-34. doi: 10.1139/bcb-2014-0004. Epub 2014 Apr 24.
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Related citations

3. Combinatory effects of **siRNA**-induced **myostatin** inhibition and exercise on skeletal muscle homeostasis and body composition.
Mosler S, Relizani K, Mouisel E, Amthor H, Diel P.
Physiol Rep. 2014 Mar 20;2(3):e00262. doi: 10.1002/phy2.262. Print 2014.
PMID: 24760516 [PubMed] Free PMC Article
Related citations

4. Effectiveness of cationic liposome-mediated local delivery of **myostatin**-targeting **small interfering RNA** in vivo.
Mori H, Kawai N, Kinouchi N, Hichijo N, Ishida T, Kawakami E, Noji S, Tanaka E.
Dev Growth Differ. 2014 Apr;56(3):223-32. doi: 10.1111/dgd.12123. Epub 2014 Mar 13.
PMID: 24621004 [PubMed - in process]
Related citations

5. **Small interfering RNA** (**siRNA**)-mediated knockdown of **myostatin** influences the expression of myogenic regulatory factors in caprine foetal myoblasts.
Kumar R, Singh SP, Kumari P, Mitra A.
Appl Biochem Biotechnol. 2014 Feb;172(3):1714-24. doi: 10.1007/s12010-013-0582-7. Epub 2013 Nov 20.
PMID: 24254256 [PubMed - in process]
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Atelocollagen-mediated local and systemic applications of **myostatin**-targ [Gene Ther. 2008]
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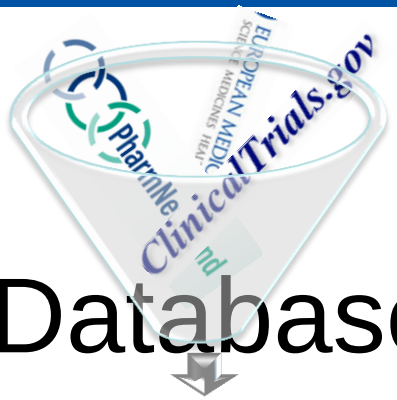

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e.g. WADA List Expert Group

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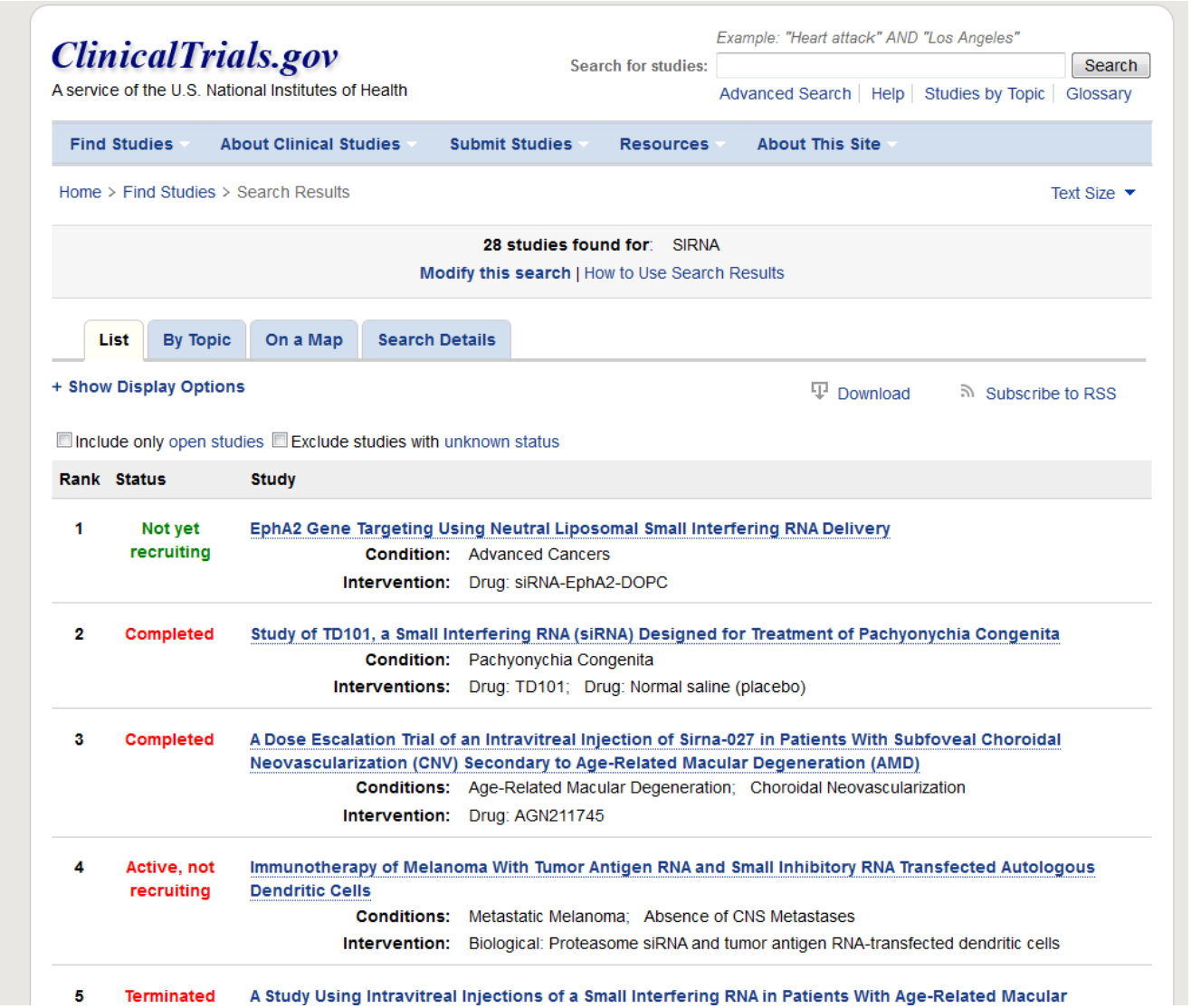

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1	Not yet recruiting	EphA2 Gene Targeting Using Neutral Liposomal Small Interfering RNA Delivery **Condition:** Advanced Cancers **Intervention:** Drug: siRNA-EphA2-DOPC
2	Completed	Study of TD101, a Small Interfering RNA (siRNA) Designed for Treatment of Pachyonychia Congenita **Condition:** Pachyonychia Congenita **Interventions:** Drug: TD101; Drug: Normal saline (placebo)
3	Completed	A Dose Escalation Trial of an Intravitreal Injection of Sirna-027 in Patients With Subfoveal Choroidal Neovascularization (CNV) Secondary to Age-Related Macular Degeneration (AMD) **Conditions:** Age-Related Macular Degeneration; Choroidal Neovascularization **Intervention:** Drug: AGN211745
4	Active, not recruiting	Immunotherapy of Melanoma With Tumor Antigen RNA and Small Inhibitory RNA Transfected Autologous Dendritic Cells **Conditions:** Metastatic Melanoma; Absence of CNS Metastases **Intervention:** Biological: Proteasome siRNA and tumor antigen RNA-transfected dendritic cells
5	Terminated	A Study Using Intravitreal Injections of a Small Interfering RNA in Patients With Age-Related Macular

April 8, 2011

ClinicalTrials.gov
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Combined Single / Multiple Dose Escalation Study in Patients With Renal Anemia Due to CKD (Chronic Kidney Disease)

This study is currently recruiting participants.
Verified on July 2011 by Bayer

First Received on April 8, 2011. Last Updated on July 14, 2011 History of Changes

Sponsor:	
Information provided by:	
ClinicalTrials.gov Identifier:	NCT01332942

Purpose

The drug that is under investigation during this study is BAY85-3934 which is intended to be used as a treatment for patients suffering from renal **anemia** due to chronic kidney disease (stage 3 and 4).

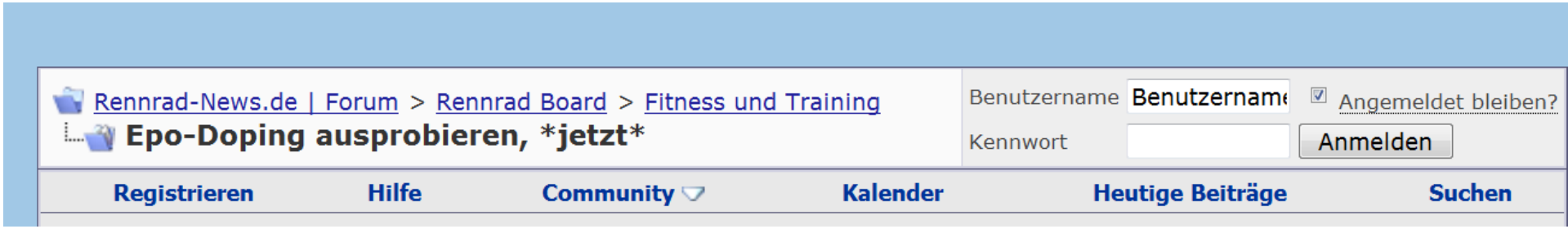
The purpose of this study is to provide safety and tolerability information on the drug. Other objectives of the study are to investigate the effect of the drug on the body (pharmacodynamics) as well as the absorption, breakdown, metabolism, distribution and excretion (pharmacokinetics) by measuring the concentration in blood and urine.

The study will be conducted in one study center in the United Kingdom and several centers in **Germany**. 48 patients who meet the inclusion criteria will participate in the study. BAY 85-3934 will be given following a combined single / multiple dose escalation design in four dose steps.

Condition	Intervention	Phase
Anemia	Drug: BAY85-3934	Phase I

Study Type: Interventional
Study Design: Allocation: Randomized
Endpoint Classification: Safety Study
Intervention Model: Parallel Assignment

April 18, 2011



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Expert groups

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Deutsche Sporthochschule Köln
German Sport University Cologne

Center for Preventive Doping Research
Institute of Biochemistry
European Monitoring Center for Emerging Doping Agents

May 28, 2006

Proceedings of the 54th ASMS Conference on Mass Spectrometry and Allied Topics

High Throughput Quantitative Analysis of a Synthetic Peptide Dimer in Biological Samples by LC/MS/MS

JongSoo Hyun; Xun Cheng; Douglas L. Cole; Jing J. Zhang*
Affymax, Inc., Palo Alto, CA

AFFYMAX

Overview

- **Purpose**: Synthetic peptides and their PEGylated forms are now well recognized as an important drug class. In order to better understand the PK and PD properties of peptide dimers, we have developed a high throughput method to quantitatively determine peptide dimer concentrations in biological samples.
- **Method**: Peptide dimer was extracted from rat plasma and analyzed by LC/MS/MS.
- **Result**: A high throughput quantitative LC/MS/MS method was developed for PK sample analysis. The results agreed with those generated from an ELISA biological method. But the LC/MS/MS method has much lower variability and detection limit.

Introduction

Synthetic peptides are an increasing important drug class and it has been reported that dimeric peptides can exhibit entranced binding affinity at protein receptors. In order to better understand the PK and PD of peptides of this type, we developed a high throughput LC/MS/MS method to quantitatively determine the concentrations of both PEGylated and non-PEGylated such peptides in biological samples. The development of a high throughput quantitative method for a model peptide dimer was particularly challenging due to its relatively high molecular weight.

Figure 1. Cartoon scheme of a typical peptide dimer

Method

Materials

- Synthetic peptide dimer (**Figure 1**).
- Blank Rat Plasma
- Rat PK plasma samples

Instrument:

- Applied Biosyste
- Agilent 1100 Bina
- Agilent 1100 Auto

LC/MS/MS Condit

- Column: Agilent 2
- Injection volume:
- Mobile Phase A:
- Mobile Phase B:
- Gradient table

Time (min.)	Fl
0.00	
1.00	
6.00	
7.00	
7.10	
9.00	

- Detection mode:
- Q1/Q3 monitoring

Sample Preparatio

- To a 2 mL 96-we
  - 50 µL of plasm
  - 100 µL aceton
- Vortex for 1 min,
- Take 50 µL aliqu

Positive ion electr
most abundance
scan of it shows t
By MRM 975.4 >
as shown in **Figur**

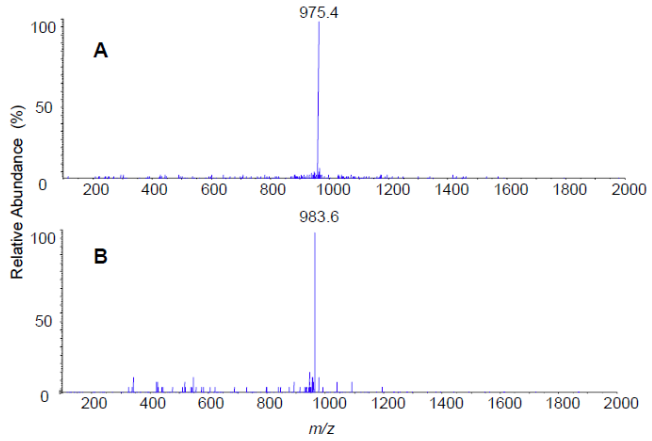


Figure 2. (A) Positive ion electrospray mass spectrum of peptide dimer fragment
(B) Product ion electrospray mass spectrum of peptide dimer fragment

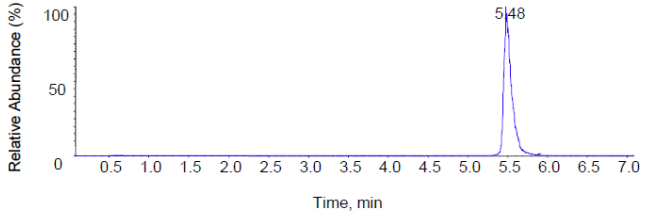


Figure 3. LC/MS/MS chromatogram of peptide dimer

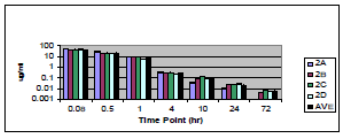


Figure 5. PK sample data generated by ELISA.

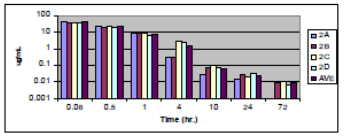


Figure 6. PK sample data generated by LC/MS/MS method.

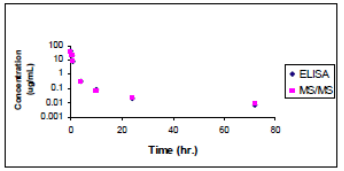


Figure 7. PK data comparison.

Figure 7 shows the excellent similarity of results from the LC/MS/MS and ELISA methods.

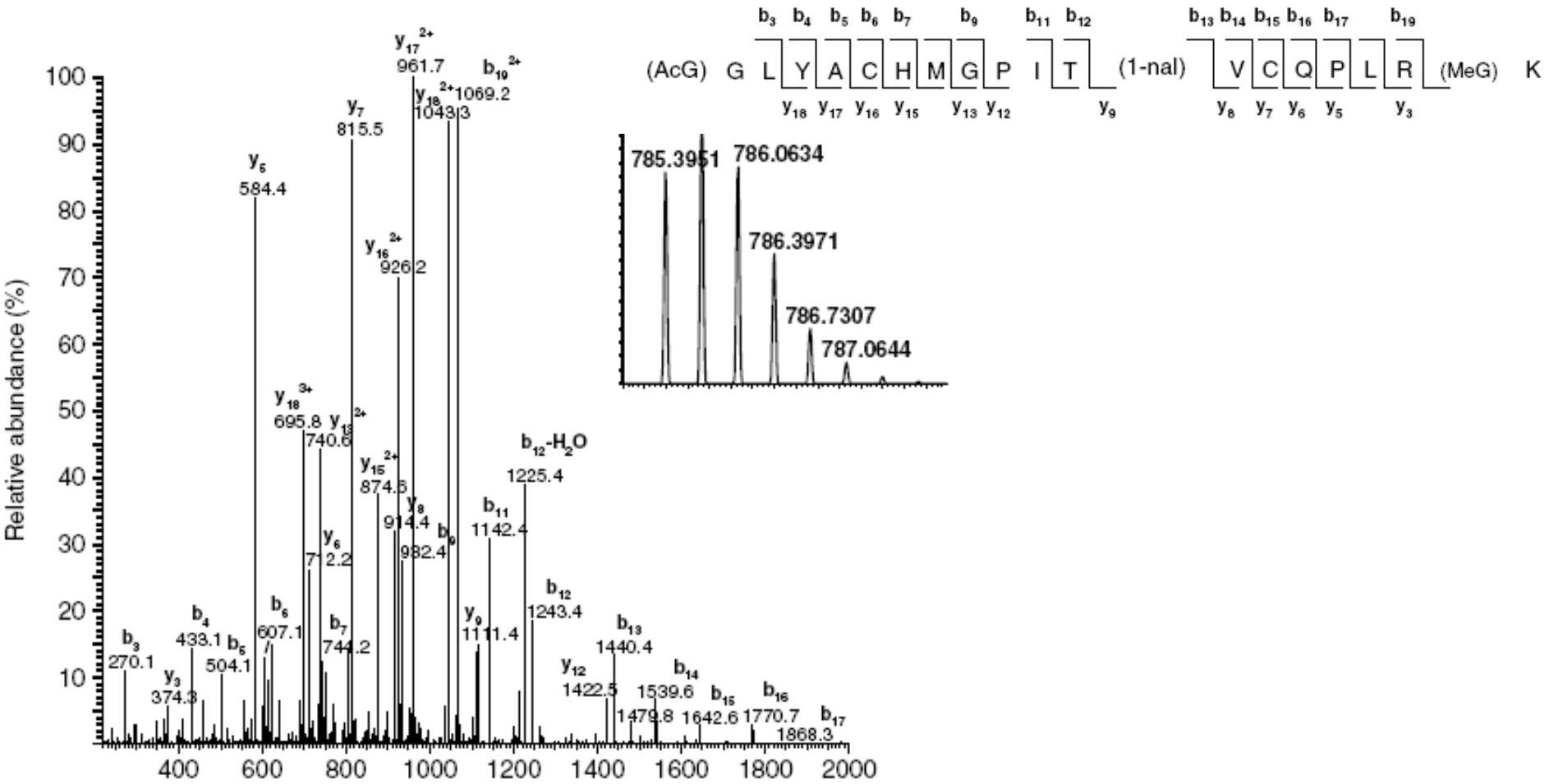
Conclusions

Results from the new LC/MS/MS method and the reference ELISA method are essentially identical, but the LC/MS/MS method has lower variability and higher sensitivity without antibody limitation.

Acknowledgements

We thank H. Jividen, M. Chen and P. Schatz for providing ELISA datas.

February 2009



Police find unlicensed drugs after trawling bins of Tour de France cyclists

BAS CZERWINSKI/AP/PA

One drug that cyclists are thought to be using increased exercise performance in mice by 44%

Paul Benkimoun PARIS

France's antidoping agency has uncovered "a surprising therapeutic arsenal," including two drugs that are not yet licensed, after scrutinising bins in the wake of the 2009 Tour de France.

Michel Rieu, the scientific adviser of the French Agency Against Doping (Agence Française de Lutte Contre le Dopage), said at a press conference on 7 October, "These are incongruous products in a milieu where people are supposed to be in good health."

Professor Rieu said that this "surprising therapeutic arsenal" had been reported to the World Anti-Doping Agency in July.

The agency said it suspected that some cyclists were using blood transfusions and two unlicensed substances.

Pierre Bordry, head of the agency, told the French daily *Le Monde* on 28 July that he was "convinced that two new products have been used during the [2009] tour, two drugs that are not yet on the market."

The first is a "third generation" erythropoietin called Hematide, which helps maintain stable haemoglobin concentrations—fluctuating haemoglobin being a sign that an athlete has taken banned substances. Hematide is still in phase III clinical trials for the treatment of anaemia and is not expected to reach the market before 2011.

The second compound, known as Aicar, increases performance of endurance exercise and decreases adiposity.

Exercise performance in sedentary mice treated with Aicar is 44% better than that in control mice, as if they had undergone training (*Cell* 2008;134:405-15).

A spokeswoman for Affymax, which makes Hematide, said it was unaware of the drug being used to enhance athletic performance and that it was working with the World Anti-Doping Agency to ensure that the drug is used only for its intended purpose.

She said, "We share its [the agency's] founding principle that doping endangers the health of athletes and undermines the integrity of sports. We place a top priority on patient safety."

The French antidoping agency wanted to run another round of tests on some samples collected from cyclists during the 2009 Tour de France, but the samples "belong" to the International Cycling Union, which did not grant it authorisation. Mr Bordry expressed his frustration with the International Cycling Union. "We can have questions, but we can't go beyond that," he said.

Cite this as: *BMJ* 2009;339:b4201

May 2011, Test methods

Research Article

Rapid Communications in Mass Spectrometry

Received: 24 May 2011 Revised: 27 May 2011 Accepted: 27 May 2011 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2011**, *25*, 2115–2123
(wileyonlinelibrary.com) DOI: 10.1002/rcm.5109

Synthesis, characterisation, and mass spectrometric detection of a pegylated EPO-mimetic peptide for sports drug testing purposes

Ines Möller, Andreas Thomas, Hans Geyer, Wilhelm Schänzer and Mario Thevis*
Institute of Biochemistry – Center for Preventive Doping Research, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany

Forensic Science International 213 (2011) 15–19

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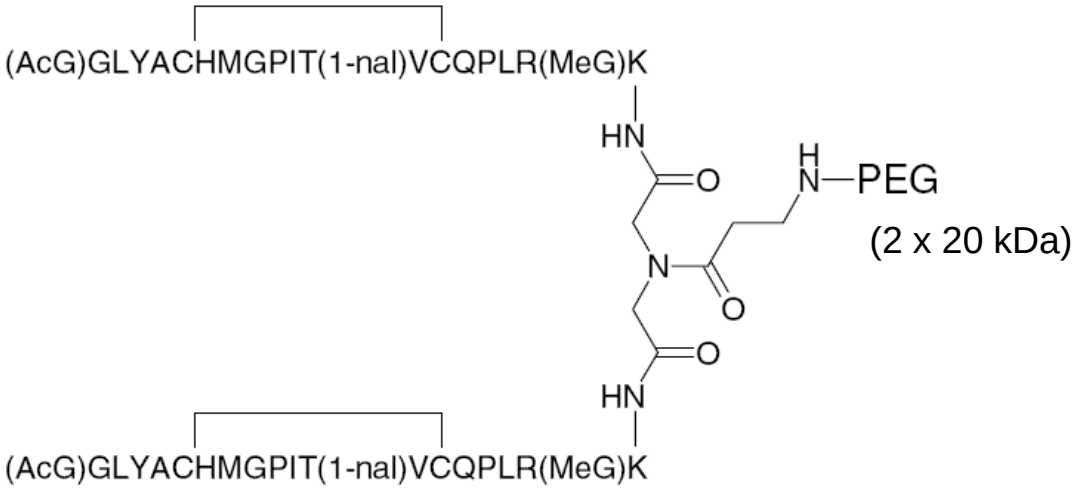
Methods for detection and confirmation of Hematide™/peginesatide in anti-doping samples☆

Nicolas Leuenberger[a], Jérémy Saugy[a], Richard B. Mortensen[b], Peter J. Schatz[b], Sylvain Giraud[a,*], Martial Saugy[a]

[a] *Swiss Laboratory for Doping Analyses, University Center of Legal Medecine, Geneva and Lausanne, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Ch. des Croisettes 22, CH-1066 Epalinges, Switzerland*
[b] *Biology Department, Affymax Inc., 4001 Miranda Avenue, Palo Alto, CA 94304, USA*

March 2012, FDA Approval



(2 x 20 kDa)

Omontys®
peginesatide

March 2013, FDA Recall

Omontys®
peginesatide

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

Omontys (peginesatide) Injection by Affymax and Takeda: Recall of All Lots - Serious Hypersensitivity Reactions

[Posted 02/23/2013]

AUDIENCE: Patients, Healthcare Professionals, Nephrology, Hematology

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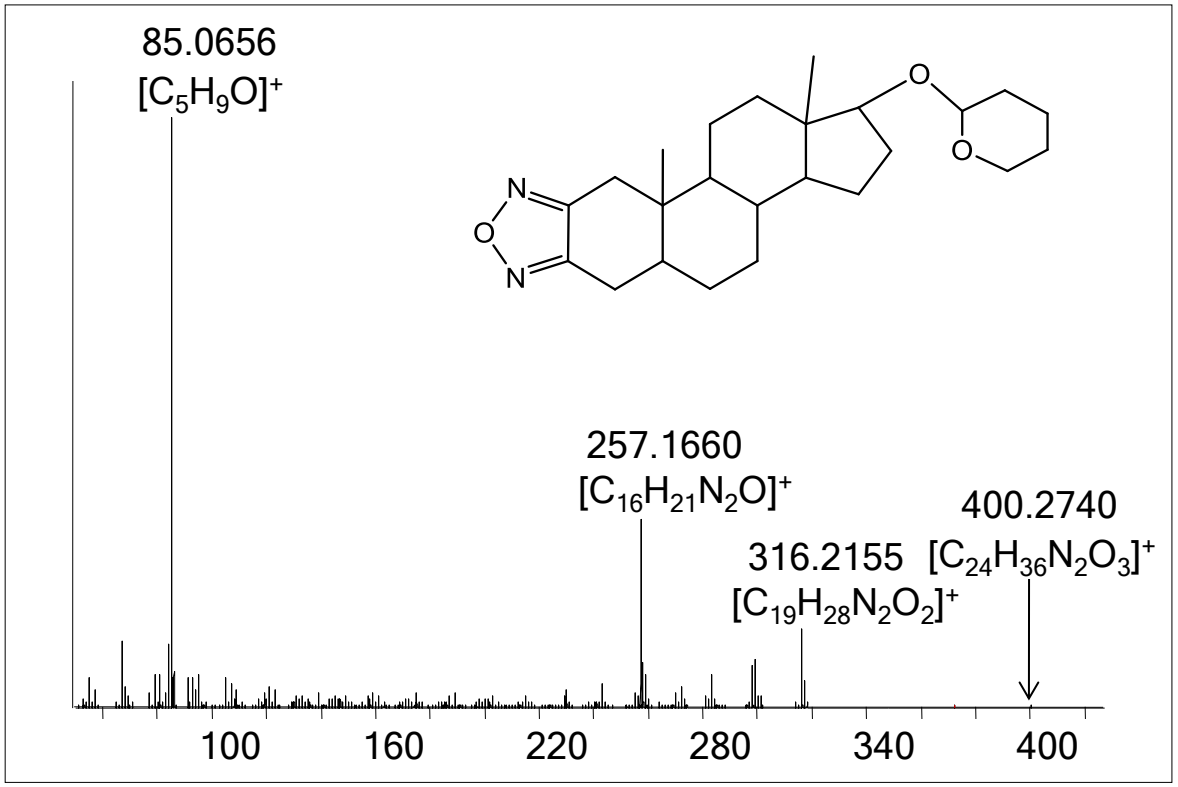

modified furazabol conjugate

(home-made)
testosterone gel dispenser

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November 2005

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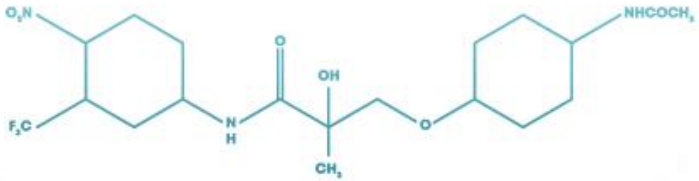

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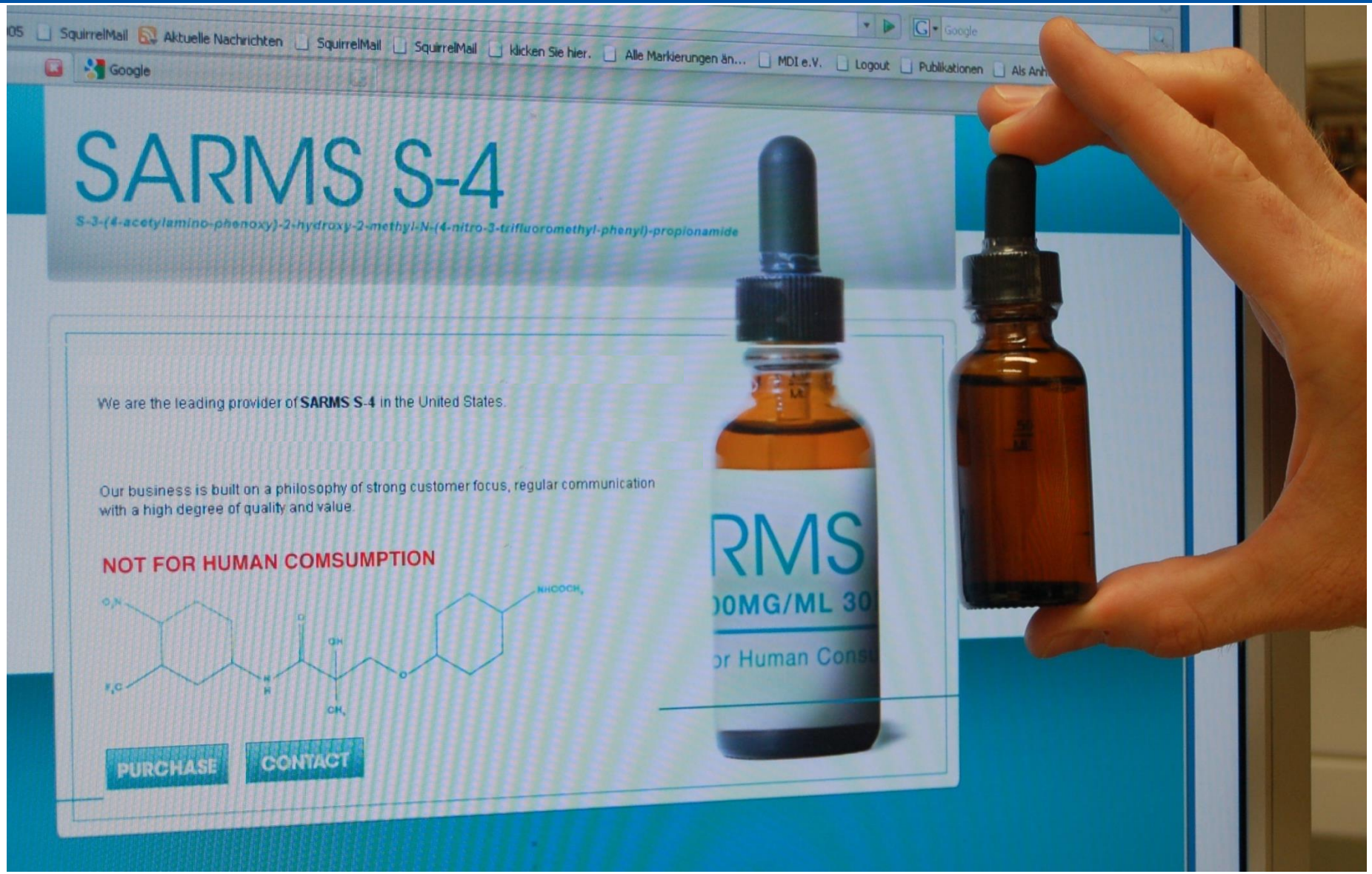
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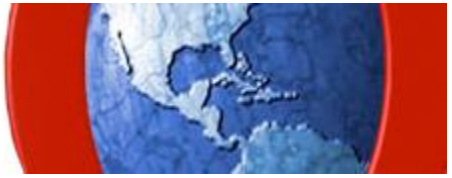
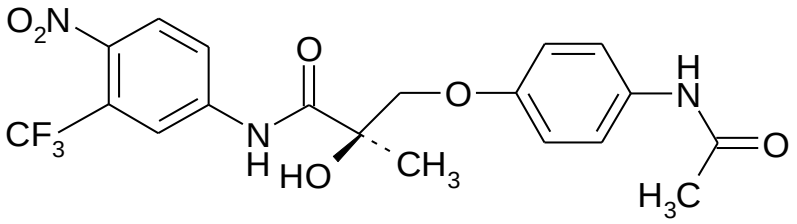


SPORT

JAAA awaits result of Wilkins drug hearing

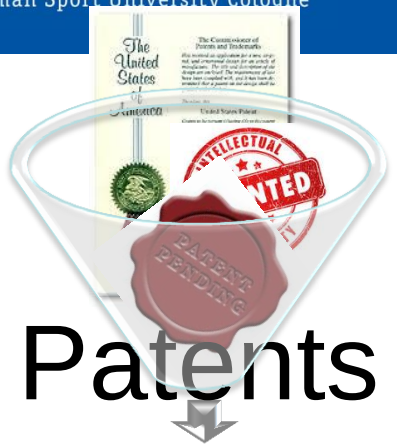


BY DANIA BOGLE Observer staff reporter
Wednesday, July 14, 2010

SHARE

THE Jamaica Amateur Athletic Association (JAAA) is expecting a result soon from the disciplinary hearings being held into the positive drugs test result returned by quarter-miler Bobby-Gaye Wilkins, Dr Warren Blake told the Observer.

Wilkins tested positive for the Selective Androgen Receptor Modulator (SARM) Andarine, which is listed by the World Anti-Doping Agency (WADA) as an anabolic agent, at the 13th IAAF World Indoor Championships (WIC) in Doha, Qatar in March.



Patents

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for
Emerging Doping Agents

Contact
Collaboration

- other monitoring centers
- laboratories
- industry
- police / customs control
- scientific community

report

mandate

Expert groups

e.g. WADA List Expert Group

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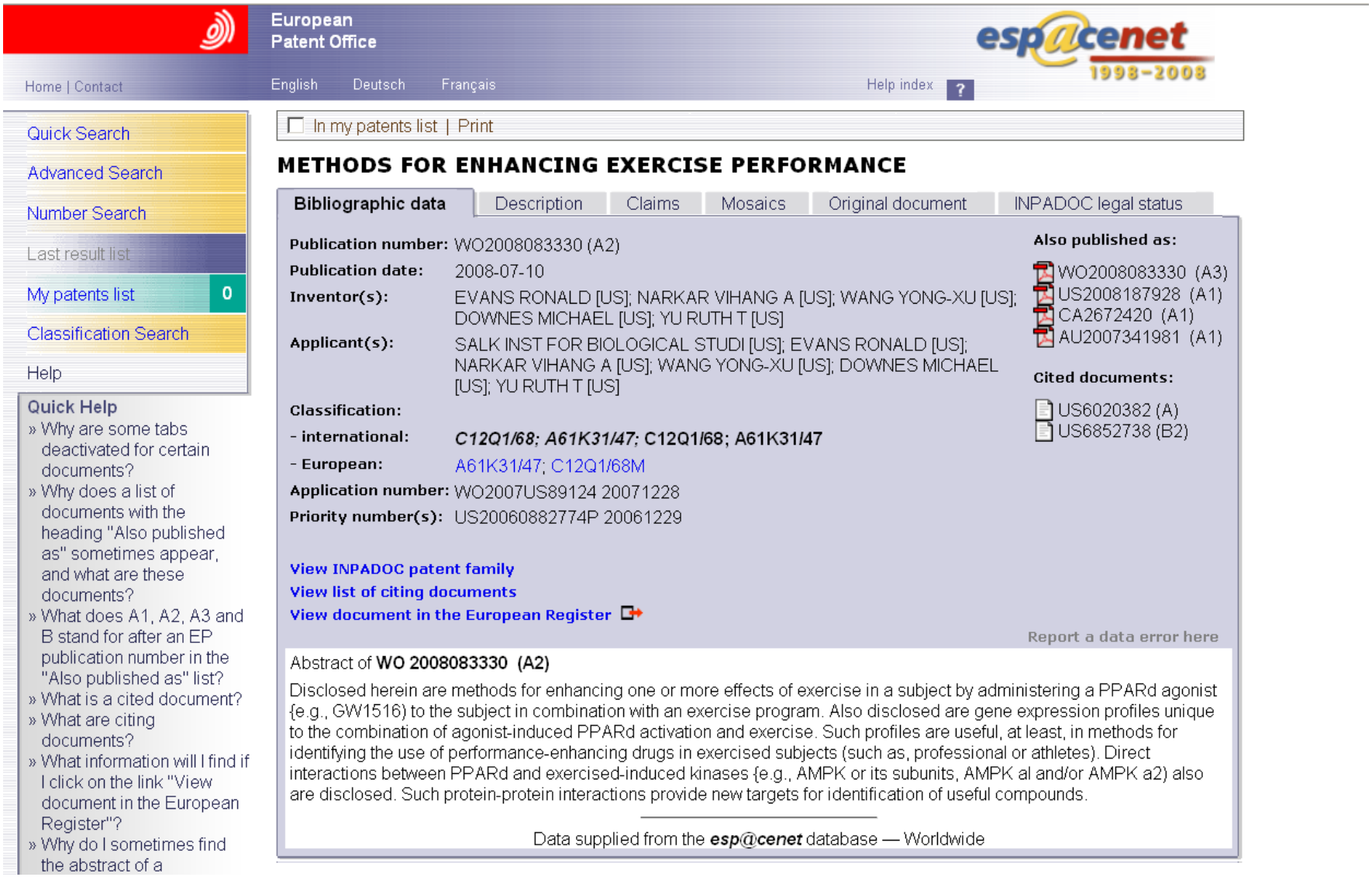

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METHODS FOR ENHANCING EXERCISE PERFORMANCE

Bibliographic data | Description | Claims | Mosaics | Original document | INPADOC legal status

Publication number: WO2008083330 (A2)
Publication date: 2008-07-10
Inventor(s): EVANS RONALD [US]; NARKAR VIHANG A [US]; WANG YONG-XU [US]; DOWNES MICHAEL [US]; YU RUTH T [US]
Applicant(s): SALK INST FOR BIOLOGICAL STUDI [US]; EVANS RONALD [US]; NARKAR VIHANG A [US]; WANG YONG-XU [US]; DOWNES MICHAEL [US]; YU RUTH T [US]
Classification:
- international: *C12Q1/68; A61K31/47;* C12Q1/68; A61K31/47
- European: A61K31/47; C12Q1/68M
Application number: WO2007US89124 20071228
Priority number(s): US20060882774P 20061229

Also published as:
WO2008083330 (A3)
US2008187928 (A1)
CA2672420 (A1)
AU2007341981 (A1)

Cited documents:
US6020382 (A)
US6852738 (B2)

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Abstract of **WO 2008083330 (A2)**

Disclosed herein are methods for enhancing one or more effects of exercise in a subject by administering a PPARd agonist {e.g., GW1516) to the subject in combination with an exercise program. Also disclosed are gene expression profiles unique to the combination of agonist-induced PPARd activation and exercise. Such profiles are useful, at least, in methods for identifying the use of performance-enhancing drugs in exercised subjects (such as, professional or athletes). Direct interactions between PPARd and exercised-induced kinases {e.g., AMPK or its subunits, AMPK al and/or AMPK a2) also are disclosed. Such protein-protein interactions provide new targets for identification of useful compounds.

Data supplied from the ***esp@cenet*** database — Worldwide

GW501516 positives confirmed, three out of 4 riders are from the same BCR Pizza Hut team

By Shane Stokes @ 3:10 PM Monday, April 15, 2013

UCI confirms Vargas Barrantes, Mudarra Segura, Morales Castillo, Villalobos Azofeifa, and Miguel Ubeto provisionally suspended

Sergej Lisin arguably first athlete tested positive with GW1516 (2012)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2007 (12.04.2007)

PCT

(10) International Publication Number
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PCT/GB2006/003678

(22) International Filing Date: 3 October 2006 (03.10.2006)

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(26) Publication Language: English

(30) Priority Data:
0520176.9 4 October 2005 (04.10.2005) GB

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(10) International Publication Number
WO 2007/039731 A1

WO 2007/039731 A1

(54) Title: USE

(57) Abstract: Use of xenon is described. Xenon is used as an organ and/or tissue and/or cell protectant in the manufacture of a pharmaceutical for the protection from injury of organs and/or tissue and/or cells that express HIF.

February 8, 2014

Science and Technology

Athletic enhancement

Breathe it in

An obscure gas improves athletes' performance

НОВЫЕ МЕДИЦИНСКИЕ ТЕХНОЛОГИИ
ЗАО "АТОМ-МЕД ЦЕНТР"
МОСКВА

МЕТОДИКА В ДЕЙСТВИИ.

MEDXENON®

Проведение процедуры на гребной базе
УТС Кальдос-де-Арегос, Португалия.

Исследование биоэлектрической активности коры головного мозга.

The amended 2014 Prohibited List (Section S2.), which now includes Hypoxia-Inducible Factor (HIF) activators Xenon and Argon, will be in force as of September 1.

Research Article

Rapid Communications in Mass Spectrometry

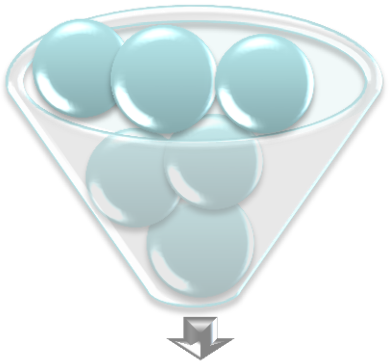
Received: 14 April 2014 Revised: 16 April 2014 Accepted: 17 April 2014 Published online in Wiley Online Library

Rapid Commun. Mass Spectrom. **2014**, *28*, 1501–1506
(wileyonlinelibrary.com) DOI: 10.1002/rcm.6926

Measuring xenon in human plasma and blood by gas chromatography/mass spectrometry

Mario Thevis[1,2]*, Thomas Piper[1], Hans Geyer[1], Andreas Thomas[1], Maximilian S. Schaefer[3], Peter Kienbaum[3] and Wilhelm Schänzer[1]

[1]Center for Preventive Doping Research – Institute of Biochemistry, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
[2]European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Cologne/Bonn, Germany
[3]University Hospital Düsseldorf, Department of Anaesthesiology, Moorenstr. 5, 40225 Düsseldorf, Germany



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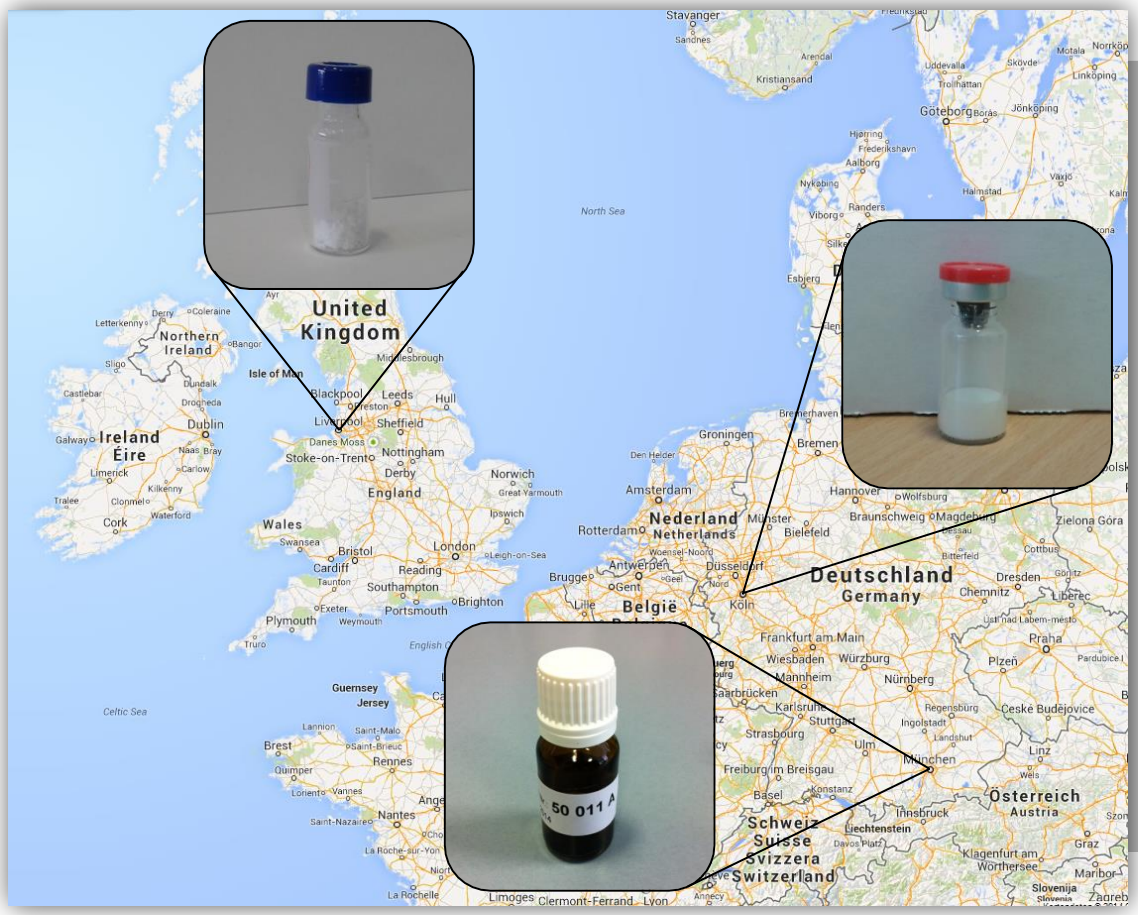
European Monitoring Centre for Drugs and Drug Addiction

Confiscated Enobosarm

- Seized 2012 by German Customs Control
- 1 kg of pure Enobosarm (MK-2866)


United Kingdom
Ireland
Éire
England
Wales
Nederland
Netherlands
Deutschland
Germany
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Schweiz
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Österreich
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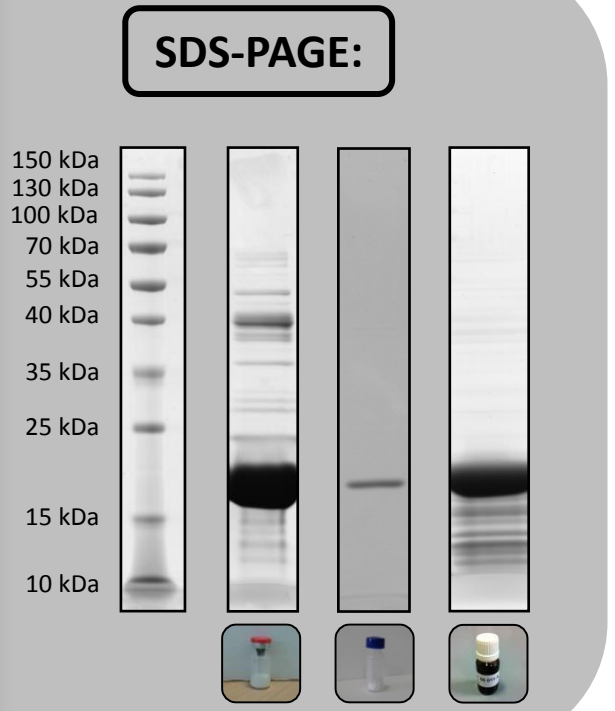
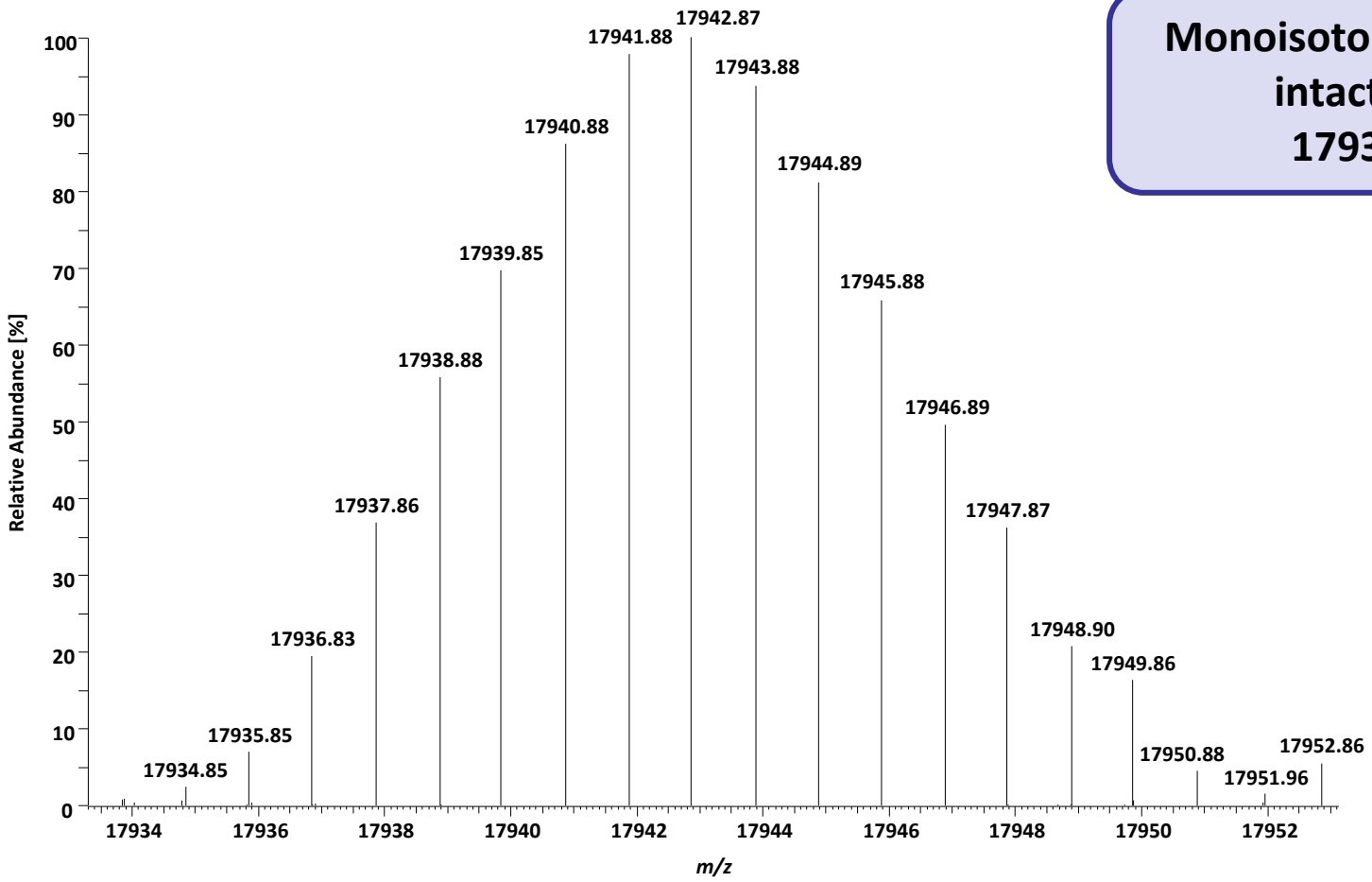

SDS-PAGE:
150 kDa
130 kDa
100 kDa
70 kDa
55 kDa
40 kDa
35 kDa
25 kDa
15 kDa
10 kDa

Unknown black market sample



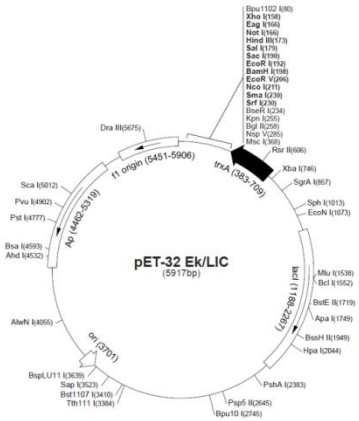
Monoisotopic mass of the intact protein: 17931.84 Da

pET-32 Ek/LIC Vektor (Novagen):

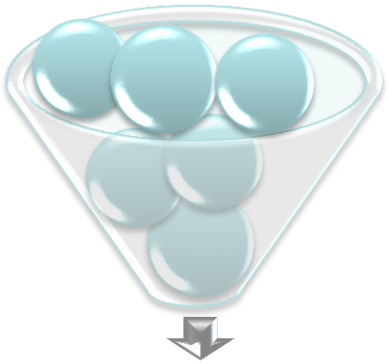
- Hypothetical peptide including protein tags and MCS:

  → ~~183 AS, 19.5 kDa~~

Black market product = „empty“ vector



pET-32 Ek/LIC cloning/expression region



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World Anti-Doping Agency

Council of Europe (Advisory Group on Science)

World Association of Anti Doping Scientists

DFG Senate Commission on Food Safety

Network of (European) NADOs

Industry partners

BAYER

AFFYMAX

NOVARTIS

MERCK

IBA
INSTYTUT BIOTECHNOLOGII I ANTYBIOTYKÓW

Roche

gsk GlaxoSmithKline

MERCK SERONO

Takeda

GTx
New Science. Established Pathways. Better Medicines®

BASF
The Chemical Company

Thera technologies

Acknowledgments

- German Federal Ministry of the Interior

Federal Ministry of the Interior

- World Anti-Doping Agency

WORLD ANTI-DOPING AGENCY