What does it take to be a successful bioinnovator in South Africa?

The UCT Biopharming Research Unit's experience with molecular biotechnology and patenting

Ed Rybicki

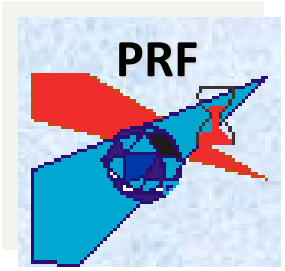
Dept MCB / IIDMM

University of Cape Town

BRU BIOPHARMING RESEARCH UNIT

SPES BONA

Funding / Disclosure



University Research Unit

- Research-intensive
- Student-centred
- Produce valuable recombinant proteins, mainly in plants
- Early-stage products

Plant Virology, 1981 → Human Virology, 1988

Married Anna-Lise

Secrets for success:

Luck, Opportunity, Agility
DIVERSIFYING FUNDING SOURCE!!
And Time

vaccines, enzymes, antibodies

Biopharming Research Unit, 2013

Innovation & IP

WHY?

- You can't **sell** it if you haven't protected it
- **No-one else will want it** if it isn't protected
- You might actually earn some **money** – for you, your group and the institution
- Your invention may actually be **used: UCT is spinning out companies that may help**

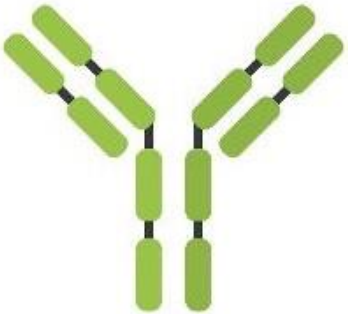
A first for South Africa and a first for the African continent.

The Team.... read more

Hydroponic vertical pharming ... read more

Plant made Proteins

Plants as bio processors



The science behind making proteins in plants

BRU BIOPHARMING RESEARCH UNIT

University of Cape Town • iYunivesithi yaseKapa • Universiteit van Kaapstad

the dti
Department:
Trade and Industry
REPUBLIC OF SOUTH AFRICA

Our Partners

19 November 15:20

Application in Reference style	Granted Regions	Pending Regions
Rybicki, E.P., Varsani, A.D. Chimaeric Human Papillomavirus 16 L1 Virus Like Particles and a Method for Preparing the Particles.	AU, ZA, US, EP: [LU, IT, MC, RO, PT, DE, SE, SI, TR, NL, CY, SP, AT, FI, CH, IE, CZ, DK, SK, FR, GB, GR, HU, BE, EE, BG]	JP, US Reg.
Rybicki, E.P., Varsani, A.D., Williamson, A-L. Pharmaceutical Compositions and a Method of Preparing and Isolating Said Pharmaceutical Compositions, and Use of Said Compositions for Prophylactic Treatment of Lesions and Carcinomas.	IN, ZA, CN	–
Rybicki, E.P., Varsani, A.D., Williamson, A-L. Vectors, Constructs, and Transgenic Plants for HPV-11 and HPV-16 L1 Capsid Protein.	CN, ZA	ARIPO
Mangwende, T., Rybicki, E.P., Shepherd, D.N., Thomson, J.A. An Isolated Nucleotide Sequence and Transgenic Organism Containing Said Sequence.	ZA	–
Meyers, A.E, Rybicki, E.P., Williamson, A-L. A Method for the Production of HIV-1 Gag Virus-Like Particles.	NA, ZA	–
Heath, L., Rybicki, E.P., Williamson, A-L. Beak and Feather Disease Virus Sequences, Compositions and Vaccines and the Use Thereof in Therapy, Diagnosis and Assays.	EP	ZA, AU
Halsey, R.J., Rybicki, E.P., Tanzer, F.L., Williamson, A-L. Chimaeric HIV-1 Subtype C GAG-Virus-Like Particles.	ZA	IN
Rose, R.C., Rybicki, E.P., Williamson, A-L. Oral Immunization with Papillomavirus Virus-Like Particles.	US	EU, JP, CA
Hitzeroth, I.I., Maclean, J.M., Rybicki, E.P., Williamson, A-L. Expression of Proteins in Plants.		CN, ZA, EP, IN, US
Rybicki, E.P., Tanzer, F.L. Expression System Incorporating a Capsid Promoter Sequence as an Enhancer of a Cytomegalovirus Promoter.	ZA	ARIPO, BR, EP, IN, US
Mangwende, T., Rybicki, E.P., Shepherd, D.N., Thomson, J.A. A Transgenic Organism and Method of Producing Same.	ZA	NA
Hitzeroth, I.I., Rybicki, E.P. Method for Enhancing the Expression of HPV L1.	Prov	–

Monday Paper archives

Volume 29.17
8 November 2010

back to archived volume

Patents office will mother your invention

Learning Curve: Prof Ed Rybicki has 44 granted patents.

Patents are the currency of the information age and with the introduction of the new Intellectual Property Rights Act, researchers are being encouraged to protect their inventions ahead of publishing. The UCT patent portfolio is growing steadily, and 2010 will see returns from commercial success break the R2 million barrier for the first time.

UCT's Research Contracts & Intellectual Property Services (RCIPS) is a protective buffer standing between the inventor and the wild world of pending patents and property rights RCIPS evaluates all inventors' disclosures for commercial merit or social benefit, protects intellectual property and (in collaboration with inventors) licenses and markets the innovation.

The office has recently released three publications: *Innovation at UCT 2010* is an overview of the university's record in this branch of research; the *UCT Laboratory Notebook* for researchers, which ensures compliance with the requirements for proof of invention and intellectual property rights; and the *Inventors Guide*, which details the step-by-step process inventors must follow in filing a patent

To recognise UCT's innovators, RCIPS held an Innovation Evening on 2 November, an event which also served as the launch for the three new publications.

Licencing income at UCT

YEAR	Licensing (R)	Sale of IP (R)	Profit UCT Companies[1] (R)	Total (R)
2001	0	87,143	0	87,143
2002	0	107,952	0	107,952
2003	0	0	0	0
2004	13,905	0	0	13,905
2005	1,728	0	0	1,728
2006	70,058	0	0	70,058
2007	49,815	0	0	49,815
2008	170,346	150,000	0	320,346
2009	77,310	59,184	693,630	830,124
2010	3,531,989	0	351,021	3,883,010
2011	558,545	0	1,165,597	1,724,142
2012 [2]	997,829	382,003	362,513	1,742,345
2013	1,757,948	0	127,378	1,885,326
TOTAL	**7,229,474**	**786,282**	**2,700,139**	**10,715,895**

HOW?

With some cautionary tales

- Do **applied research**: you can publish good work AND patent it
- **Get funded by industry** or bodies like TIA
- DON'T PUBLISH BEFORE YOU PROTECT!!
- DON'T TALK ABOUT IT IN PUBLIC!
- **REALLY!!**
- Write it up like a paper and submit it with a disclosure form to RC&I at UCT / your IP office
- Commit to a several-years-long project of seeing the same thing again and again and again…

A Real Problem:

Engineering resistance in maize to maize streak virus: a successful end to a 20-year battle

Plant Biotechnology Journal
Open Access

Open Access

Maize streak virus-resistant transgenic maize: a first for Africa

Dionne N. Shepherd, Tichaona Mangwende, Darren P. Martin, Marion Bezuidenhout, Frederik J. Kloppers ... See all authors

First published:15 August 2007 |
https://doi.org/10.1111/j.1467-7652.2007.00279.x | Citations: 41

Patented: Funded by and licenced to Pannar Ltd

Summary

In this article, we report transgene-derived resistance in maize to the severe pathogen maize streak virus (MSV). The mutated MSV replication-associated protein gene that was used to transform maize showed stable expression to the fourth generation.

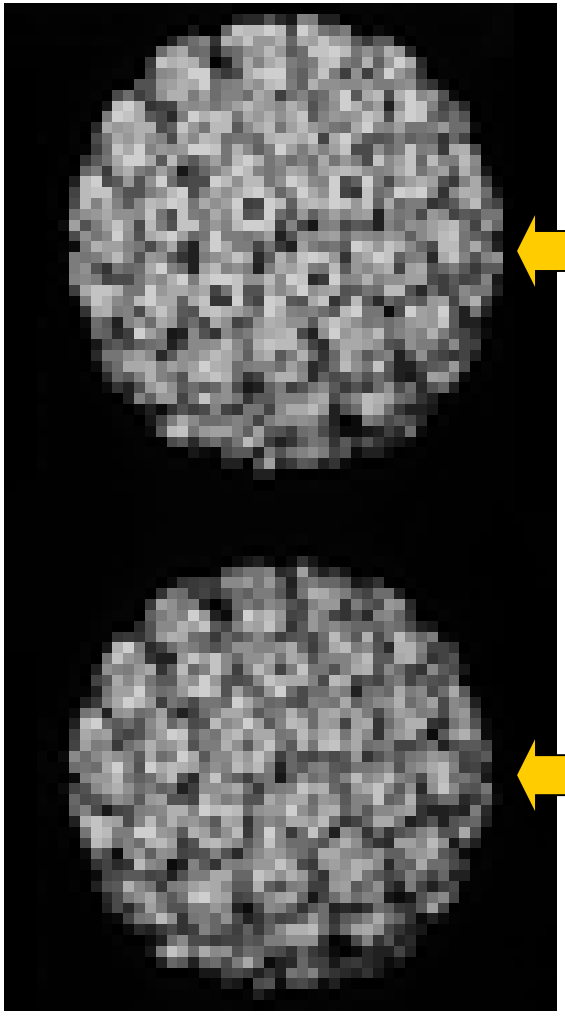
Proteins Expressed at UCT over 20 years:

- Human papillomavirus (HPV) proteins:
  - L1, L2 and E7 and chimaeric L1 and PsVs
- HIV-1C Gag and Env
- Avian influenza (HPAI) H5 + H1 haemagglutinin
- Human rotavirus capsid proteins
- Bluetongue virus capsid proteins and VLPs
- African horse sickness virus proteins and VLPs
- Beak and feather disease virus capsid protein
- SCFv antibodies / Horseradish peroxidase

Properties of the HPV L1 capsid protein

L1 protein can self-assemble into virus-like Particles (VLPs) that are antigenically identical to virions - **and are already released** (Merck's Gardasil, GSK's Cervarix - made via yeast and baculovirus)

COST = US$70-120 x 3



Natural capsid: L1+L2 +DNA

Capsid consisting only of L1

Searching US Patent Collection...

Results of Search in US Patent Collection db for:
IN/Rybicki AND virus: 6 patents.
*Hits **1** through **6** out of **6***

Jump To

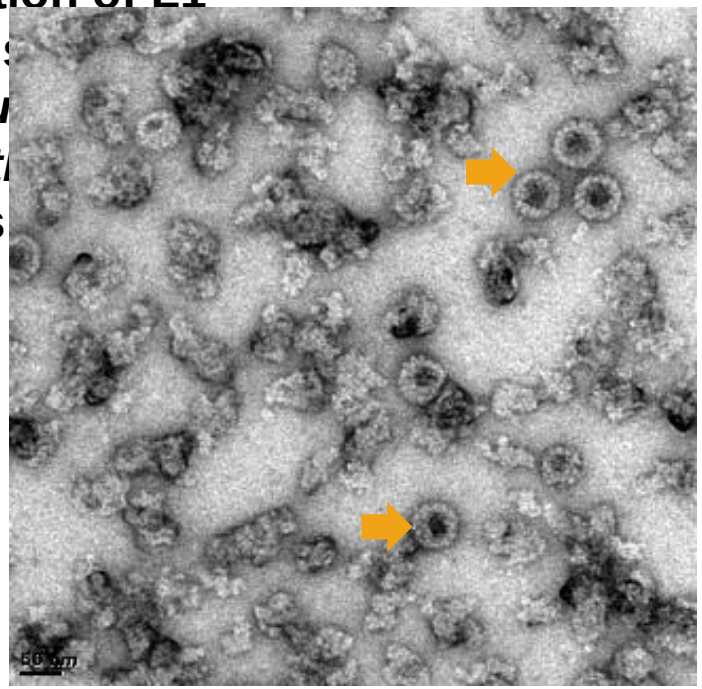
Refine Search IN/Rybicki AND virus

	PAT. NO.	Title
1	9,017,987 T	Expression of proteins in plants
2	8,535,930 T	Expression of proteins in plants
3	8,460,933 T	Expression system incorporating a capsid promoter sequence as an enhancer
4	8,163,557 T	Chimaeric human papillomavirus 16 L1 virus-like particles and a method for preparing the particles
5	7,407,807 T	Chimaeric human papillomavirus 16 I1 virus like particles and a method for preparing the particles
6	6,153,201 T	Oral immunization with papillomavirus virus-like particles

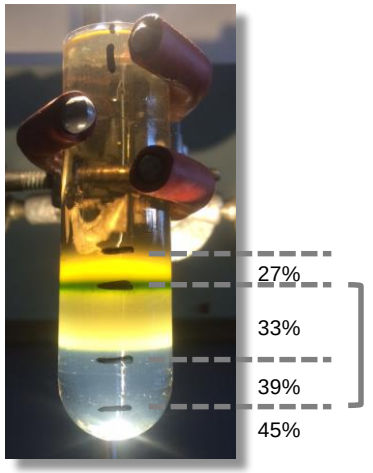
Plant-made HPV L1 VNPs as Vaccines

Expression and purification of L1 proteins of 10 HPV types in plants

Vacuum infiltration of L1-expressing cons
Agrobacteriu
Nicotiana benti
plants



Discontinuous Optiprep™ density gradient purification



Total yields of L1 were 100-500 mg/kg fresh weight

Alta van Zyl*, Paulina Naupu, BRU

Total VLP yields per kg of fresh weight biomass of ~6 mg/kg were obtained from fractions 3-5

nature.com > scientific reports > article

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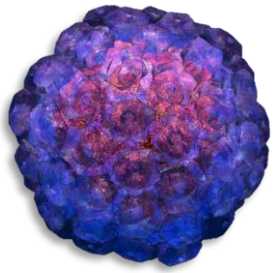
Article | OPEN

Production of Human papillomavirus pseudovirions in plants and their use in pseudovirion-based neutralisation assays in mammalian cells

Renate L Lamprecht, Paul Kennedy, Suzanne M Huddy, Susanne Bethke, Megan Hendrikse, Inga I Hitzeroth & Edward P Rybicki

Scientific Reports **6**,
Article number: 20431 (2016)
doi:10.1038/srep20431

Received: 21 August 2015
Accepted: 04 January 2016
Published online: 08 February 2016



This is the first demonstration of the production of **ANY** mammalian DNA pseudovirion in plants

It is also the first evidence that DNA vaccines can be made in plants

Influenza Vaccines / Reagents

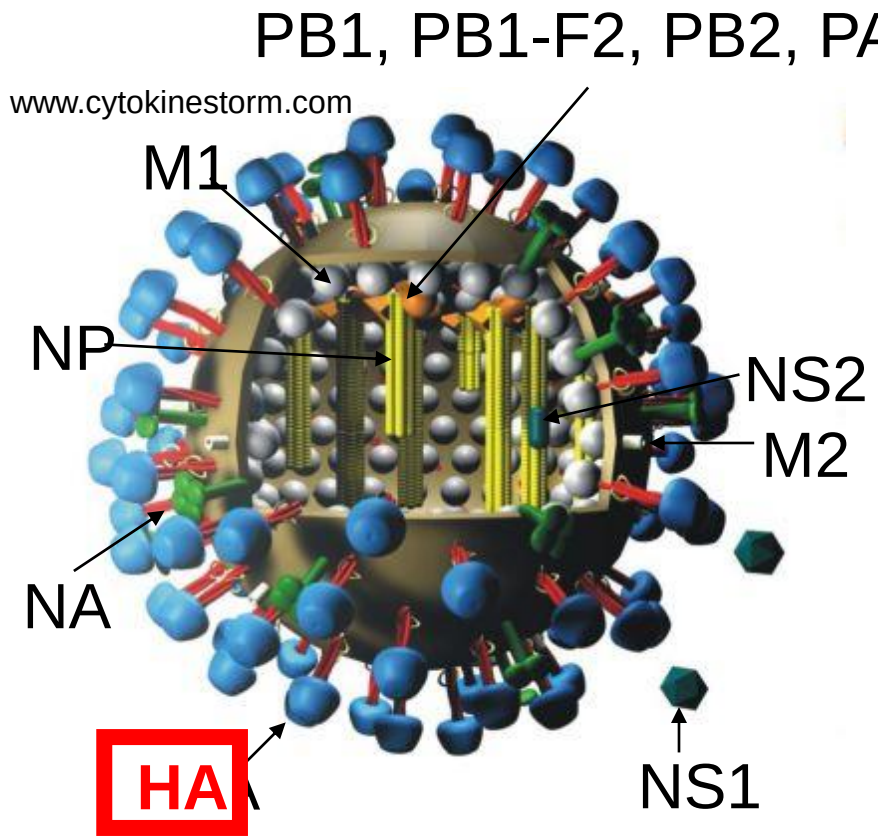
"At a conference in Cape Town in 2005 [1st Virology Africa], a WHO influenza expert warned us

"**When the pandemic comes, you in the developing countries will be on your own**"

We applied for extraordinary funding from the PRF in SA to explore the possibility of making a pandemic flu virus vaccine in South Africa."

E Rybicki (2015). From plant virology to vaccinology: The road less travelled. Human vaccines & immunotherapeutics 11 (11), 2517-2521, 2015

UCT Work: Influenza A virus



Structural proteins

- **HA – glycoprotein, major antigen**
- NA – glycoprotein, virus release (defines subtype)
- M1 – matrix 1 protein, viral budding
- M2 – matrix 2 protein, viral uncoating
- NP – nucleoprotein, nuclear import
- PB1, PB2, PA – polymerase subunits

(Bouvier & Palese 2008, Suri 2007)

BMC Biotechnology
IMPACT FACTOR 2.86

Research article

Open access

Setting up a platform for plant-based influenza virus vaccine production in South Africa

Elizabeth Mortimer, James M Maclean, Sandiswa Mbewana, Amelia Buys, Anna-Lise Williamson, Inga I Hitzeroth and **Edward P Rybicki**

For all author emails, please log on.

BMC Biotechnology 2012, **12**:14 doi:10.1186/1472-6750-12-14
Published: 26 April 2012

We managed, after synthesis of two versions of a single gene, to produce by transient and transgenic expression in plants, two variants of a highly pathogenic avian influenza virus HA protein which could have vaccine potential.

This is a proof of principle of the potential of plant-produced influenza vaccines as a feasible pandemic response strategy for South Africa and other developing countries.

United States Patent **8,535,930**
Williamson , et al. **September 17, 2013**

Expression of proteins in plants

Abstract

The invention relates to a method of producing a HPV polypeptides and/or an ***influenza*** virus H5 polypeptide in a plant comprising the steps of cloning a HPV gene; and/or an ***influenza*** virus H5 gene or nucleic acid encoding their functional equivalents into a vector adapted to target components present in the plant, infiltrating at least a portion of the plant with the vector or transforming plant tissue with the vector so as to transiently express the HPV polypeptides and/or an ***influenza*** virus H5 polypeptide, and/or to create a transgenic plant; and recovering the HPV polypeptides and/or an ***influenza*** virus H5 polypeptide expressed by the plant. The invention further relates to vectors, transgenic plants or parts thereof and the progeny of such plants used in or which come about as a result of the method.

The invention relates to a method of producing a HPV polypeptides and/or an ***influenza*** virus H5 polypeptide in a plant

News releases

Medicago in the news

Useful links

ShareThis

Medicago successfully produces VLP vaccine candidate for H7N9 virus responsible for recent influenza outbreak in China

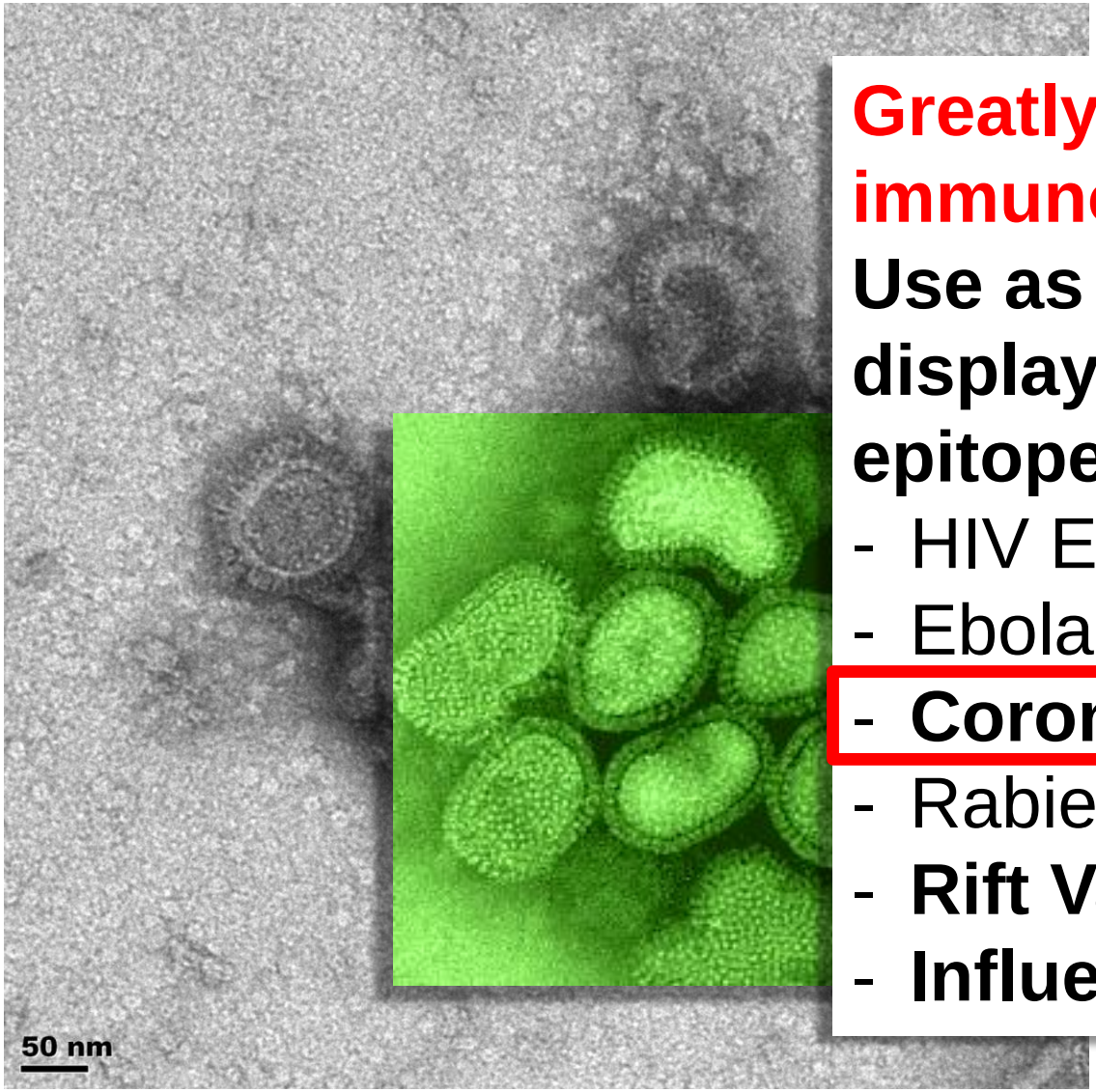
05/08/2013

-Medicago Ready to Collaborate with Authorities to Further Study the Potential of this Vaccine to Protect Against H7N9-

QUEBEC CITY, May 8, 2013 /CNW/ - Medicago Inc. (TSX: MDG; OTCQX: MDCGF), a biopharmaceutical company focused on developing highly effective and competitive vaccines based on proprietary manufacturing technologies and Virus-Like Particles (VLPs), today announced that it has successfully produced a new VLP vaccine candidate for the H7N9 virus that is responsible for the current influenza outbreak in China.

"To our knowledge, Medicago is the first to produce a VLP vaccine candidate against this potential pandemic strain demonstrating our ability to be a first responder in a pandemic scenario," said Andy Sheldon, President and CEO of Medicago. "Current influenza egg based vaccine manufacturers can take up to six months to produce a vaccine for any new strain, we have proven once again that we can cut this development time drastically.This combined with our vaccine production capacity at our pilot facility in Canada and our commercial facility in North Carolina and our best-in-class efficacy results for our H5N1 vaccine, strongly positions Medicago as a key player in addressing a potential pandemic."

H5 HA Budding as VLPs in Plants



Greatly increased immunogenicity

Use as platform for display of other GPs or epitopes:

- HIV Env
- Ebola GP
- **Coronavirus S**
- Rabies G
- **Rift Valley fever virus Gn**
- **Influenzavirus A M2e**

frontiers in Bioengineering and Biotechnology

Front Bioeng Biotechnol. 2015; 3: 197.
Published online 2015 Dec 8. doi: 10.3389/fbioe.2015.00197

PMCID: PMC4672040
PMID: 26697423

Production of H5N1 Influenza Virus Matrix Protein 2 Ectodomain Protein Bodies in Tobacco Plants and in Insect Cells as a Candidate Universal Influenza Vaccine

Sandiswa Mbewana,[1] Elizabeth Mortimer,[1] Francisco F. P. G. Pêra,[1] Inga Isabel Hitzeroth,[1,*] and Edward P. Rybicki[1,2]

US008460933B2

(12) **United States Patent**
Rybicki et al.

(10) **Patent No.: US 8,460,933 B2**
(45) **Date of Patent: Jun. 11, 2013**

(54) **EXPRESSION SYSTEM INCORPORATING A CAPSID PROMOTER SEQUENCE AS AN ENHANCER**

(75) Inventors: **Edward Peter Rybicki**, Cape Town (ZA); **Fiona Lesley Tanzer**, Cape Town (ZA)

(73) Assignees: **South African Medical Research Council**, Cape Town (ZA); **University of Cape Town**, Cape Town (ZA)

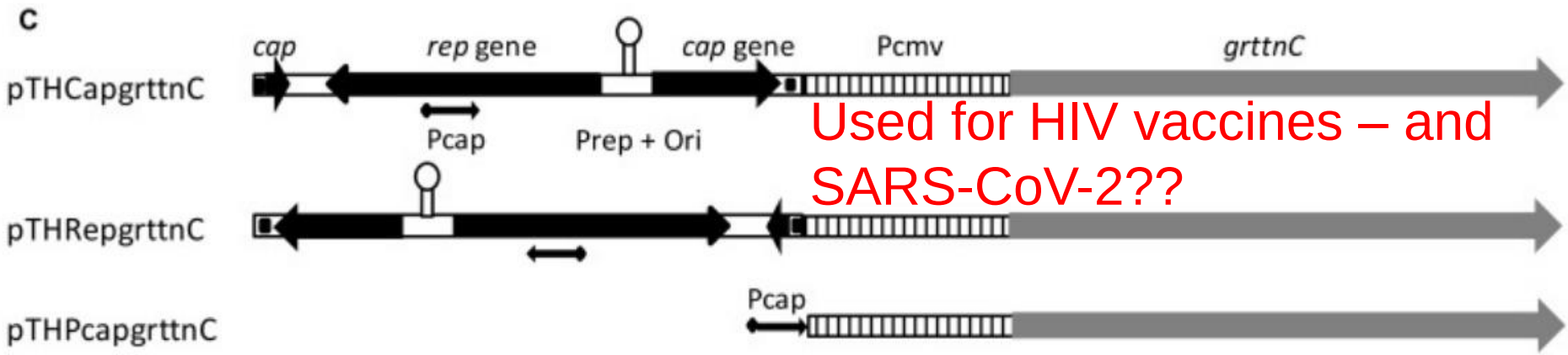
Gag by Using DNA Expression Vectors That Target Gag Antigen to the Secretory Pathway" J. Virology; 74(13):5997-6005.*

Yu et al."Lentiviral Vectors with Two Independent Internal Promoters Transfer High-Level Expression of Multiple Transgenes to Human Hematopoietic Stem-Progenitor Cells" Molecular Therapy, 7(6):827-838; 2003.*

Mankertz et al. "Analysis of transcription of Porcine circovirus type 1" J. Gen Virology; 83:2743-2751; 2002.*

Vandepapeliere P. "Therapeutic vaccination against chronic viral infections" The Lancet Infectious Diseases vol. 2:353-367; 2002.*

Hansen U. et al. "Sequences controlling in vitro transcription of SV40 promoters"The EMBO Journal vol. 2 No. 12 pp. 2293-2303,



Used for HIV vaccines – and SARS-CoV-2??

United States Patent Application **20150216961**
Kind Code **A1**
D'Aoust; Marc-Andre ; et al. **August 6, 2015**

ROTAVIRUS-LIKE PARTICLE PRODUCTION IN PLANTS

Abstract

A method of producing a virus-like particle (VLP) in a plant is provided. The method comprises introducing a first nucleic acid into the plant, or portion of the plant. The first nucleic acid comprising a first regulatory region active in the plant operatively linked to a nucleotide sequence encoding one or more ***rotavirus*** structural protein for example but not limited to ***rotavirus*** protein VP2. The nucleotide sequence may further comprise one or more than one amplification element and/or a compartment targeting sequence. A second nucleic acid might be introduced into the plant, or portion of the plant. The second nucleic acid comprises a second regulatory region active in the plant and operatively linked to a nucleotide sequence encoding one or more ***rotavirus*** structural protein, for example but not limited to ***rotavirus*** protein VP6. Optionally, a third nucleic acid and/or fourth nucleic acid might be introduced into the plant, or portion of the plant. The third nucleic acid comprises a third regulatory region active in the plant and operatively linked to a nucleotide sequence encoding one or more ***rotavirus*** structural protein, for example but not limited to ***rotavirus*** protein VP4. The fourth nucleic acid comprises a fourth regulatory region active in the plant and operatively linked to a nucleotide sequence encoding one or more ***rotavirus*** structural protein, for example but not limited to ***rotavirus*** protein VP7. The plant or portion of the plant is incubated under conditions that permit the expression of the nucleic acids, thereby producing the VLP.

Inventors: **D'Aoust; Marc-Andre**; *(Quebec, CA)* **; Landry; Nathalie**; *(Quebec, CA)* **; Lavoie; Pierre-Olivier**; *(Quebec, CA)* **; Arai; Masaaki**; *(Osaka, JP)* **; Asahara; Naomi**; *(Osaka, JP)* **; Mutepfa; David Levi Rutendo**; *(Notthingham, GB)* **; Hitzeroth; Inga Isabel**; *(Cape Town, ZA)* **; *Rybicki*; Edward Peter**; *(Cape Town, ZA)*

PRESS RELEASE

June 20, 2013, 7:00 a.m. EDT

Medicago successfully produces plant-based Rotavirus VLP vaccine candidate

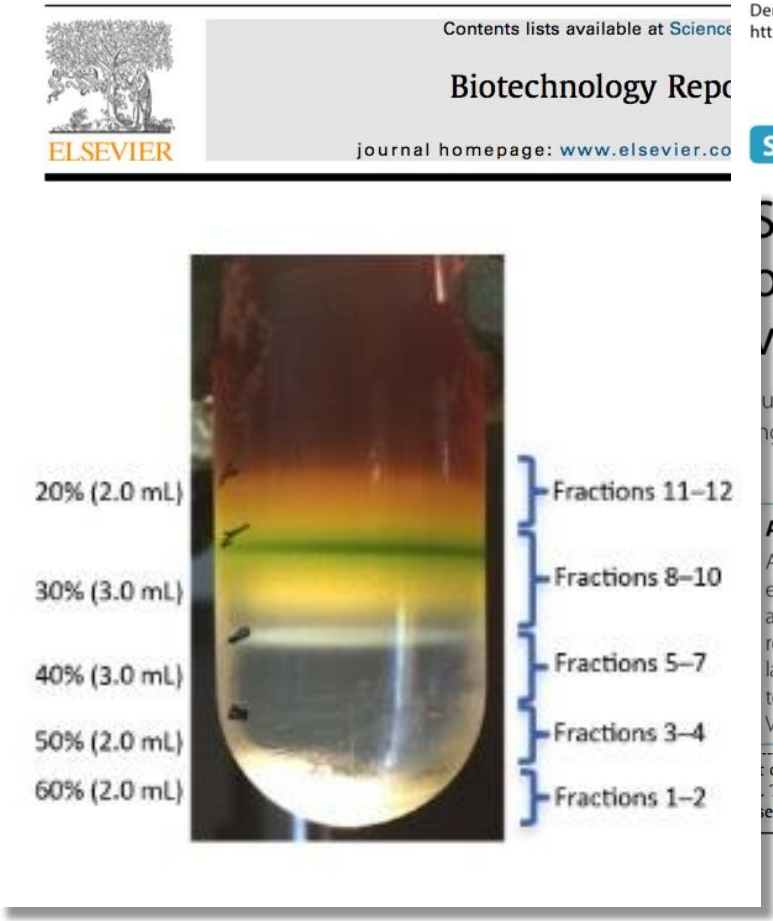
QUEBEC CITY, June 20, 2013 /PRNewswire via COMTEX/ -- First successful plant-based Rotavirus VLP containing all 4 Major proteins- -International Patent Application filed-

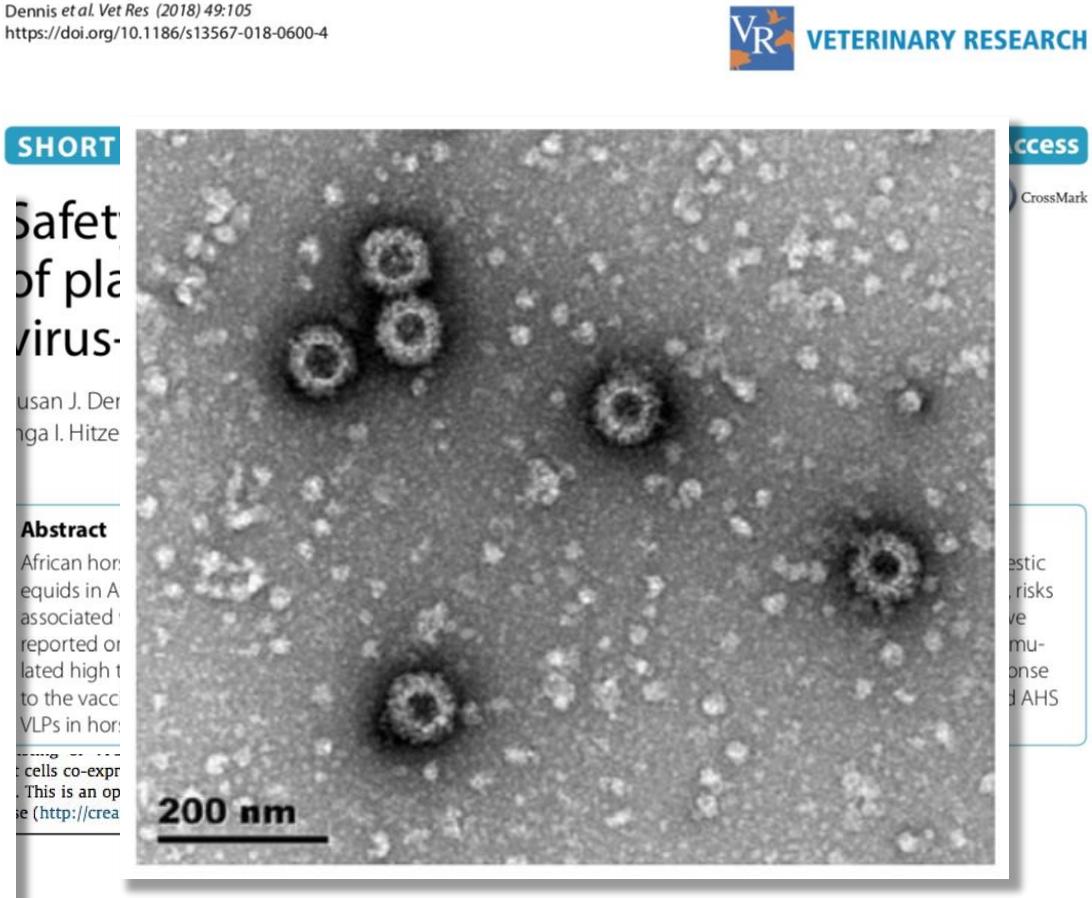
Medicago Inc. CA:MDG +4.00% (otcqx:MDCGF), a biopharmaceutical company focused on developing highly effective and competitive vaccines based on proprietary manufacturing technologies and Virus-Like Particles (VLPs), today announced the successful production of a Rotavirus VLP vaccine candidate comprising all four structural antigens of rotavirus (VP2, VP4, VP6 and VP7) using Medicago's plant-based manufacturing platform.

Plant-made Orbivirus VNPs as Vaccines

Expression and purification of BTV and AHSV VNPs

Sue Dennis, AHSV 4&5





Where should we go??

Rapid-response vaccines:

Speed of production, low cost and rapid scalability allow very rapid cGMP production of vaccines to:

- **Emerging disease agents** such as influenza Ebola / Marburg, CCHF viruses
- **SARS-CoV-2….**

"Orphan" vaccines:

Viruses like **Lassa fever**

Therapeutic vaccines for HPV-related cancers, HIV

Later: Biosimilars or generics:

HPV, HBV, HAV, brucellosis, *E coli*, cholera, TB….

With special thanks to the HPV green team, present and past:

Marc-André D'Aoust & Medicago Inc.

Rainer Fischer, Thomas Rademacher - RWTH Aachen University / Fraunhofer Inst

Neil Christensen – Univ Pennsylvania