

FIRB - 2012

Line [01, 02, 03], Position	Luca Santi, Associate Professor				
Research Title	<u>Plant-derived bioengineered virus nanoparticles for brain cancer targeting and drug delivery.</u>				
Horizon 2020: field [cross]	SH-social sciences, humanities		PE-mathematics, physics, etc.		LS-life sciences X

Part I – General Principal Investigator/Responsible of the Unit 1

A.A. General Informations

Full name, position	Luca Santi, Prof. Associato non confermato				
Born (year)	1973	CUN Area	SSD		
		05	BIO15, Biologia Farmaceutica		
Department	Dipart. di Scienze e Tecnologie per l'Agricoltura, le Foreste , la Natura e l'Energia, (DAFNE)				

A.B. Research Project

Keywords

Short summary:

Brain blood barrier
Glyoma
Virus nanoparticles
Targetted drug delivery
Plant production

Five bioengineered chimeric viral nanoparticles (CVNPs) will be designed and built in order to achieve the individual exposure of five different peptides on their exterior surface. The purpose is to generate a gain of function aimed to a specific targeting and internalization of the CVNPs by the endothelium of cerebral capillary cells and by brain tumor cells.

This strategy opens the possibility to achieve targeted and contained drug delivery. Unmodified *Tomato bushy stunt virus* derived nanoparticles (UVNPs) and CVNPs will be produced and purified from *Nicotiana benthamiana* plants, a tobacco close relative, extensively used for biopharmaceutical production in plants.

In particular the research units will be engaged to test the five candidate chimeras to define the best performing in terms of *in vitro* activity: on a glioblastoma derived cell line, stability and biostability, yield, loading ability and strength of the ligand-receptor interaction.

Best performing CVNPs will be assayed *in vivo* using a well known brain tumor mouse model.

A.C. Summary of Scientific Achievements

A.C.1 Product type	Volume number	Data Base (ISI, Scopus, Pub Med etc)	Year	pages
Non-food/feed seeds as biofactories for the high-yield production of recombinant pharmaceuticals	9	PLANT BIOTECHNOLOGY JOURNAL	2011	p. 911-921
Plant-made pharmaceuticals for the prevention and treatment of autoimmune diseases: where are we?	9	EXPERT REVIEW OF VACCINES	2010	p. 957-969
Application of a multidimensional gas chromatography system with simultaneous mass spectrometric and flame ionization detection to the analysis of sandalwood oil.	7	JOURNAL OF CHROMATOGRAPHY A	2010	p. 721-29
Viral nanoparticles as macromolecular devices for new therapeutic and pharmaceutical approaches	2	INTERNATIONAL JOURNAL OF PHYSIOLOGY, PATHOPHYSIOLOGY AND PHARMACOLOGY	2010	p. 161 -178
Plant derived veterinary vaccines	33	VETERINARY RESEARCH COMMUNICATIONS	2009	p. S61-S66
Plant – derived recombinant F1, V, and F1-V fusion antigens of Yersinia Pestis activate human cells of the innate and adaptive immune system	22	INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY	2009	p. 133-143
Production and characterization of an orally immunogenic Plasmodium antigen in plants using a virus-based expression system	7	BIOTECHNOLOGY JOURNAL	2009	p. 846-855
An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles.	26	VACCINE	2008	p. 1846-1854
Viral vectors for production of recombinant proteins in plants.	216	JOURNAL OF CELLULAR PHYSIOLOGY	2008	p. 366-377
Protection conferred by recombinant Yersinia pestis antigens produced by a rapid and highly	103	PNAS	2006	p. 861-866

scalable plant expression system.				
Rapid, high-level production of hepatitis B core antigen in plant leaf and its immunogenicity in mice.	24	VACCINE	2006	p. 2506-2513
Virus-like particles production in green plants.	40	METHODS	2006	p. 66-76
Genetics of barley Hooded suppression.	167	GENETICS	2004	p. 439-448
The GA octodinucleotide repeat binding factor BBR participates in the transcriptional regulation of the homeobox gene Bkn3.	34	PLANT JOURNAL	2003	p.813-826
In vitro interactions between barley TALE homeodomain proteins suggest a role for protein-protein associations in the regulation of Knox gene function.	27	PLANT JOURNAL	2001	p. 13-23

A.C.2 Scientometric [optional]

A.C.2 Scientometric [optional]		Additional evaluation parameters
Total Impact Factor (2010)	85,716	ISI
Total Citations	341	Scopus
Hirsch (H) index	10	Scopus
Normalized H index*	0,769	Scopus

*H index versus/divided by the academic seniority (time span from graduation)

A.D. Selected Publications - List of the publications (5, 10, 15 respectively for Line 1, 2 or 3). For each publication report: authors, title, reference data (journal, year, volume, pages)

B.1

B.1.A General Information, Unit 1 (UNITUS Research Unit)

Responsible of the Unit 1 is the General Principal Investigator

B.1.B. Research of the Unit

Keywords Summary [aim] [maximum 100 words]

Nanotechnology	UNITUS will design, build and produce in plants, the five candidate CVNPs. The material will be made available to all research units to conduct the tasks necessary for the fulfillment of the project. UNITUS will perform molecular characterization of the formulations and will test in mice the immunogenicity of the carrier and of the selected chimeras. Also the biological fate of the particles will be evaluated <i>in vivo</i> , testing different organs for biodistribution. Moreover UNITUS will collaborate with Nomad bioscience, Germany, for the production and purification of pharmaceutical grade CVNPs for the final <i>in vivo</i> studies.
Plant virology	
Bioengineering	
Protein cages	
Nanoparticles	

B.2

B.2.A General Information, Unit 2 (IBAF-CNR Research Unit)

Full name, position	Chiara Baldacchini, Researcher III Level		
Born (year)	1977	CUN Area	SSD
		02	FIS07
Department, University	Istituto di Biologia Agroambientale e Forestale, Consiglio Nazionale delle Ricerche		

B.2.B. Research of the Unit

Keywords Summary [aim] [maximum 100 words]

Nanoscopies	IBAF-CNR Research Member will be in charge of: determine the affinity with the brain cell membrane of the selected peptides, either isolated or located on the virus nanoparticle capsid; characterize the shape and the coat protein symmetry of either the unmodified and the chimeric viral nanoparticles, at the single-molecule level; investigate how the loading with doxorubicin modifies the topographic and mechanical properties of the chimeric viral nanoparticle with the highest affinity for brain cell membrane and their effects on brain cancer cells from line GL15.
Surface Plasmon Resonance	
Intermolecular interactions	
Single-molecule	
Cell mechanical properties	

B.2.C. Summary of Scientific Achievements

Product type	Volume number	Data Base (ISI, Scopus, Pub Med etc)	Year	pages
Chemically modified multiwalled carbon nanotubes electrodes with ferrocene derivatives through reactive landing	115	Journal of Physical Chemistry C	2011	4863-4871
Highly conductive redox protein-carbon nanotube complex for biosensing applications	21	Advanced Functional Materials	2011	153-157
Lying-down metallic single-walled carbon nanotubes as efficient linkers for metalloprotein-based	10	Journal of Nanoscience and Nanotechnology	2010	2753-2758

nanodevices				
Molecular charge distribution and dispersion of electronic states in the contact layer between pentacene and Cu(119) and beyond	77	Physical Review B	2008	205417
Symmetry lowering of pentacene molecular states interacting with a Cu surface	76	Physical Review B	2007	245430
Conductive atomic force microscopy investigation of transverse current across metallic and semiconducting single-walled carbon nanotubes	91	Applied Physics Letters	2007	122103
Self-organization of pentacene grown on Cu(119)	601	Surface Science	2007	4242-4245
Mixing of electronic states in pentacene adsorption on copper	99	Physical Review Letters	2007	046802
Molecule-metal interaction of pentacene on copper vicinal surfaces	601	Surface Science	2007	2603-2606
Yeast cytochrome c integrated with electronic elements: A nanoscopic and spectroscopic study down to the single-molecule level	19	Journal of Physics Condensed Matter	2007	225009
Molecular gap and energy level diagram for pentacene adsorbed on filled d-band metal surfaces	89	Applied Physics Letters	2006	152119
Core-shell photoabsorption and photoelectron spectra of gas-phase pentacene: Experiment and theory	122	Journal of Chemical Physics	2005	124305
Au(110) induced reconstruction by pi-conjugated molecules adsorption investigated by photoemission spectroscopy and low energy electron diffraction	566-568	Surface Science	2004	79-83
Cu(100) surface: High-resolution experimental and theoretical band mapping	68	Physical Review B	2003	195109
Electronic band states of long-range ordered aromatic thione	66	Physical Review B	2002	115407

molecules assembled on Cu(100)

B.2.D. Bibliometric parameters

Additional evaluation parameters

Total Impact Factor (2010)	63.923	ISI
Total Citations	313	Scopus
Hirsch (H) index	11	Scopus
Normalized H index*	1.1	Scopus

*H index versus/divided by the academic seniority (time span from graduation)

B.3

B.3.A General Information, Unit 3 (UNIPG Research Unit)

Full name, position	Paolo Blasi, Researcher		
Born (year)	1976	CUN Area	SSD
		03	CHIM/09
Department, University	Dipartimento di Chimica e Tecnologia del Farmaco, University of Perugia		

B.3.B. Research of the Unit

Keywords	Summary [aim] [maximum 100 words]
Physico-chemical characterization/stability	UNIPG Research Unit will be on charge of the physico-chemical characterization of the wild type and modified viruses. This will include: particle size and distribution, surface charge, and stability in physiological media. In addition, viruses will be loaded with fluorescent dyes and anticancer drugs and drug release, cell interaction as well as internalization will be studied by fluorescence microscopy and electrophysiological techniques. Anticancer efficacy will be, preliminary, evaluated on glioblastoma implanted on chick embryo chorioallatoic membrane while the most promising system/s will be characterized in vivo on rat implanted with brain tumors. Toxicity and biodistribution will be also investigated.
Drug loading and release	
Cell interaction/internalization	
<i>In vitro</i> and <i>in vivo</i> toxicity	
<i>In vivo</i> biodistribution and efficacy	

B.3.C. Summary of Scientific Achievements

Product type	Volume number	Data Base (ISI, Scopus, Pub Med etc)	Year	pages
Lipid nanoparticles as carrier for octyl-methoxycinnamate: In vitro percutaneous absorption and photostability studies	101	Journal of Pharmaceutical Sciences	2012	301-311
Lipid nanoparticles for brain targeting I. Formulation optimization	419	International Journal of Pharmaceutics	2011	287-295
The functional performance of microencapsulated human pancreatic islet-derived precursor cells	32	Biomaterials	2011	9254-9262
Simple and scalable method for peptide inhalable powder	39	European Journal of Pharmaceutical Sciences	2010	53-58

production				
Bioactive Long-Term Release from Biodegradable Microspheres Preserves Implanted A LG-PLO-A LG Microcapsules from In Vivo Response to Purified Alginate	27	Pharmaceutical Research	2010	285-295
Development of a scalable procedure for fine calcium alginate particle preparation	160	Chemical Engineering Journal	2010	363-369
Lipid nanoparticles for prolonged topical delivery: An in vitro and in vivo investigation	357	International Journal of Pharmaceutics	2008	295-304
Physicochemical characterization and release mechanism of a novel prednisone biodegradable microsphere formulation	97	Journal of Pharmaceutical Sciences	2008	303-317
Solid lipid nanoparticles for targeted brain drug delivery	59	Advanced Drug Delivery Reviews	2007	454-477
Preparation of large porous biodegradable microspheres by using a simple double emulsion method for capreomycin sulfate pulmonary delivery	333	International Journal of Pharmaceutics	2007	103-111
Preparation and in vitro and in vivo characterization of composite microcapsules for cell encapsulation	324	International Journal of Pharmaceutics	2006	27-36
Evaluation of Alternative Strategies to Optimize Ketorolac Transdermal Delivery	7	AAPS PharmSciTech	2006	Article 64
Long-term delivery of superoxide dismutase and catalase entrapped in poly(lactide-co-glycolide) microspheres: in vitro effects on isolated neonatal porcine pancreatic cell clusters. J. Control. Release 107 (2005) 65-77.	107	Journal of Controlled Release	2004	65-77
Ketoprofen controlled release from composite microcapsules for cell encapsulation: effect on post-transplant acute inflammation	107	Journal of Controlled Release	2005	395-407
Leucinostatin-A loaded	275	International Journal of Pharmaceutics	2004	61-72

nanospheres: characterization and in vivo toxicity and efficacy evaluation. Int. J. Pharm. 275 (2004) 61-72.
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B.3.D. Bibliometric parameters

Total Impact Factor (2010)	125,005
Total Citations	492
Hirsch (H) index	13
Normalized H index*	1.44

Additional evaluation parameters

JCR
Scopus
Scopus
Scopus

*H index versus/divided by the academic seniority (time span from graduation)