Clinical Applications of Nanotechnologies in the Field of Cancer

January 28 & 29, 2010
Montpellier, France

CLARA
CANCÉROPÔLE LYON AUVERGNE RHÔNE-ALPES

Cancéropôle
Grand Sud-Ouest
Bordeaux • Limoges • Montpellier • Nîmes • Toulouse

ibec Institute for Bioengineering of Catalonia

SUMMARY

PROGRAMME

THURSDAY, JANUARY 28, 2010

9h15 — **WELCOME & OPENING**
Objectives of seminar and context of nanomedicine applied to clinical practice
Peter PAUWELS, Evelyne CREMER, Josep SAMITIER

9h30 - 10h00
Research on nanomedicine applied to cancer in Europe and European Technology Platform: Spanish and French technology platforms
Josep SAMITIER, François BERGER

10h00 - 11h00 — **SESSION 1: Presentation of nano research activities: IBEC/Biopol L´H-Barcelona, Cancéropôle Grand Sud-Ouest and Cancéropôle Lyon Auvergne Rhône-Alpes**

10h00 - 10h20
Institut de Bioenginyeria de Catalunya (IBEC) / Biopol L´H
Josep SAMITIER, IBEC, Barcelona

10h20 - 10h40
Cancéropôle Grand Sud-Ouest & Fondation InnaBioSanté
Christophe VIEU, LAAS CNRS, Toulouse

10h40 - 11h00
Cancéropôle Lyon Auvergne Rhône-Alpes
François BERGER, INSERM-UJF, Grenoble,
Marc JANIER, Hospices Civils de Lyon

11h00 - 11h30 — **BREAK & POSTERS**

11h30 - 15h45 — **SESSION 2: Transfer of nanotechnology to clinical applications related to cancer**
Chairpersons: Josep SAMITIER, IBEC, Barcelona
and François BERGER, INSERM - Université Joseph Fourier, Grenoble

11h30 - 12h00
Ultrasound triggered, image guided, local drug delivery
Chrit MOONEN, UMR5231 CNRS, University Victor SEGALEN, Bordeaux

12h00 - 12h30
In vitro diagnostics: nanobioanalytical platforms
Pilar MARCO, IBEC/IQAC-CSIC, Barcelona

12h30 - 12h50
Optical imaging and cancer: from mice to man
Jean-Luc COLL, INSERM U823 - Université Joseph Fourier, Grenoble

12h50 - 14h15 — **LUNCH & POSTERS**

SESSION 2: Transfer of nanotechnology to clinical applications related to cancer

14h15 - 14h45
A new platform of bio-inspired nanomedicines in oncology
Jean-Pierre BENOIT, INSERM U646, Université d'Angers

14h45 - 15h15
Abraxane and the nab-platform : nanotechnology from the bench to the clinic
Neil DESAI, Senior VP Research & Development, Abraxis Biosciences

15h15 - 15h45
Round Table I: What are the hot key-issues on the transfer of nanotechnology to clinical applications? How can nanomedicine contribute at the theragnostic level? Key-issues for oncospecialists. What basic research can bring to clinicians, the added value and technological limits.
Moderators: Josep SAMITIER, Barcelona
and François BERGER, Grenoble

15h45 - 16h00 — **BREAK & POSTERS**

16h00 - 18h20 — **SESSION 3: Ethics and regulatory issues related to nanotechnologies in the field of cancer**
Chairpersons: Simó SCHWARTZ Jr., CIBBIM-Hospital Vall d'Hebron, Barcelona
and Dominique MASSET, AFSSAPS, Paris

16h00 - 16h30
A philosophical perspective on nanotechnology
Bernadette BENSAUDE VINCENT, CNRS - Université Paris 10

16h30 - 17h00
Toxicology assays and protocols in nanomedicine
Simó SCHWARTZ Jr., CIBBIM-Hospital Vall d'Hebron, Barcelona

17h00 - 17h20
Impact of nanotechnologies on patients: questions for ethics
Marie CHARAVEL, Université Pierre Mendes France, Grenoble

17h20 - 17h50
Evaluation agencies
Bridging the gap between research and medical decision-making
Marta AYMERICH, Facultat de Medicina, Universitat de Girona
Perception and risk evaluation of health products using nanotechnologies
Dominique MASSET, AFSSAPS, Paris

17h50 - 18h20
Round Table II: Legal and regulatory framework: Spanish and French positions
Moderators: Oriol SOLA-MORALES, CATHAR, Barcelona, Dominique MASSET, Paris and Jacques DESCOTES, Lyon

18h20 - 19h30 — **APPETISERS & POSTERS**

19h30 - 22h30 — **DINNER**

FRIDAY, JANUARY 29, 2010

8h30 - 12h15	**SESSION 4: Proof of concept of clinical applications in oncology** *Chairpersons: Gabriel CAPELLA, ICO/IDIBELL, Barcelona and Gilles DIVITA, Centre de Recherche de Biochimie Macromoléculaire, Université Montpellier, CNRS, Montpellier*
8h30 - 9h00	The DIACPROL project: building a nanosensor ("electronic nose") for prostate cancer diagnosis *Jaume REVENTOS, Biomedical Research Unit, Vall d'Hebron Research Institute and Hospital, Barcelona*
9h00 - 9h30	Nano-fingerprinting as a new device for theragnostic medicine applied to glioblastoma *François BERGER, INSERM U836, Université Joseph Fourier, Grenoble*
9h30 - 10h00	Peptide-based nano-particles for targeted delivery of therapeutic siRNA *Gilles DIVITA, Centre de Recherche de Biochimie Macromoléculaire, Université Montpellier, CNRS, Montpellier*
10h00 - 10h30	**BREAK & POSTERS**
10h30 - 11h00	Multimodality imaging in oncology utilizing targeted drug delivery systems *Kalevi KAIREMO, University of Helsinki South Karelia Central Hospital, Finland*
11h00 -11h30	Pancreatic cancer gene therapy: non viral gene delivery *Eric PEROUZEL, Cayla Invivogen, Toulouse*
11h30 - 12h15	**Round Table III:** How to go further? Clinicians addressing their practice in oncology. Examples with proof of concept. Perspectives and Follow-up. *Moderators: Gabriel CAPELLA, Barcelona and Gilles DIVITA, Montpellier*
12h15 - 12h30	**CLOSING REMARKS** Institut de Bioenginyeria de Catalunya (IBEC) Cancéropôle Grand Sud-Ouest & Fondation InnaBioSanté Cancéropôle Lyon Auvergne Rhône-Alpes
12h30 - 14h30	**LUNCH**

PRESENTATIONS ABSTRACTS

CLARA
CANCÉROPÔLE LYON AUVERGNE RHÔNE-ALPES

Cancéropôle Lyon Auvergne Rhône-Alpes
SPEEDING UP PROGRESS

Axis I: Nanotechnology, Imaging and Cancer

Coordinators: François BERGER (INSERM - Université Joseph Fourier, Grenoble) and Marc JANIER (Hospices Civils de Lyon - UCBL, Lyon)

This priority axis aims to design new tools to detect and treat cancer, as well as to better understand the mechanisms of malignant tumors via imaging and nanotechnology.

As nanotechnology originates from a non-medical technological field, its use requires complementary expertise. Thus, topics such as nanoparticles, radiotracers, nanotracers, biomarkers, imaging and regulatory aspects of nanoparticles have mobilized the region's researchers. Coordinated by a steering committee that represents research, clinical and industrial partners from Clermont-Ferrand, Grenoble, Lyon and Saint-Etienne, the members of this network have been contributing to develop and boost the scientific activities of the axis.

With a portfolio of 11 regional projects funded by the National Cancer Institute (INCa), in addition to several ones receiving the support from the French National Research Agency (ANR) and European bodies, mainly breast, lung and brain cancers have been accessed by various innovative methods.
The pioneer funding program *Proof of Concept,* under its general call for projects or under the specific category Cancer Nano Transfer, has allowed the emergence of different approach strategies.

- Ganglio Tool / ProTool - pre-clinical validation of new nano-invasive methods of collecting molecular information in oncology for both tissues and biological fluids:
  - development of a tool for minimally invasive lymph node samples and test in the pre-clinical and clinical phase of lymphomas and other mediastinal metastatic adenopathies;
  - use of chemically-altered nanoballs to capture the low-concentration proteome, which can be activated in pathological tissues.

- *In vivo* intra-tumor activation of photosensitive nanoparticles was used to treat glioblastoma: preclinical findings indicate a reduction of tumor growth in grafted mice. Although models of xenografts are available, further studies need to better target tumor cells.
- Optical imaging: surgery instrumentation in fluorescence guided biopsies such as primary and metastatic sarcomas and prostate cancer.
- Hybrid radiosensitizer nanoparticles allowing treatment of radio-resistant tumors, such as melanoma and chondrosarcoma.
- Lipid nanoparticles for imaging and targeted drug delivery.

In addition to basic, pre-clinical and clinical research projects currently developed, core programs have been structuring research through two actions: one more transversal and another targeted.

- *Regulatory issues related to clinical transfer: assistance unit for innovative nano-medicines:* this unit proposes both a training program as well as assistance/coaching on the regulatory aspects of nanotracers development. Launched in partnership with Lyon Civil Hospitals (HCL), it is composed of a quality specialist with expertise in regulatory aspects, a specialist in pharmacotoxicology and an expert in charge of clinical evaluation.

- *IMPACT2 project: Innovative Medical Personalized Anti Cancer Theranostic Therapies:* this program involves implementing an integrated approach to nanomedicine for personalized prescription of antiangiogenics.This cross-disciplinary program brings together oncologists, biologists and technology specialists as well as economist in order to validate a process to predict response to targeted therapies.The ethics will be also investigated early.

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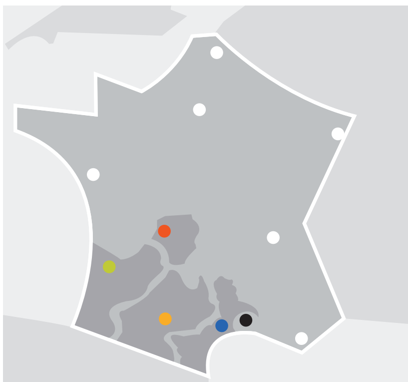
The Cancéropôle GSO, a link between researchers and clinicians in four regions

The Cancéropôle Grand Sud-Ouest (GSO) brings together scientific and medical research teams from Bordeaux, Limoges, Montpellier, Nimes and Toulouse to encourage collaborations, skill and tool sharing in research projects with a translational approach.

Its collective ambition is
- **To validate new therapeutic targets** with integrated biological and clinical studies relying on reinforced technological platforms.
- **To develop *in vitro* and *in vivo* preclinical study models** that correspond to human pathologies
- **To promote industrial partnerships** and the development of new structuring products

The Actors	And also
Over **300** teams and **1 500** researchers and clinicians	**3** clusters (pôles de compétitivité) : Prod'innov (Bordeaux), Eurobiomed (Montpellier), Cancer-Bio-Santé (Toulouse)
• **7** Universities, Inserm and CNRS • **5** University Hospitals and 3 Comprehensive Cancer Centers • Over **100** core facilities • **7** pharmaceutical companies : Amgen, Merck-Serono, Novartis, Pfizer, Pierre Fabre Medicaments, Roche and Sanofi-Aventis and also Astrazeneca, Celgene, GSK, Lilly in the MatwinClub • A network of small biotechnology companies across the 4 regions • **4** Regional Administrations: Aquitaine, Limousin, Languedoc-Roussillon and midi-Pyrénées.	
A whole territory is mobilizing to better meet the expectations of patients.	

The Cancéropôle GSO benefits of recognised competency and expertise in clinical and cognitive research in particular for:
- **Malignant haematological disorders** (leukaemia and lymphomas)
- **Digestive tract cancers** (colon, pancreas, liver)
- **Breast cancer**
- And rare tumors: **sarcomas** and **gliomas**
- **Nanotechnological approaches**

Five partnership axis have been designed to accelerate dynamics between different teams and regions.
- Axis 1 "Cellular signalisation and therapeutic targets"
- Axis 2 "Genetic instability, cell cycle and epigenetics"
- Axis 3 "Predictive factors of the therapeutic response"
- Axis 4 "Biotherapy and therapeutic innovations"
- Axis 5 "Human and social sciences and epidemiology"

Task forces and different transverse networks reinforce the scientific activity such as several clubs working on: Biobank, Bioinformatics, In Vivo Imaging, Experimental Models and the pharmaceutical companies club.

Nanomedicine is present through different approaches in Bordeaux, Toulouse and Montpellier. In the diagnosis field, biological and medical imaging researches are conducted in those three regions. In Toulouse, high sensitivity detection using nanoscale devices (biochips, biosensors) are internationally recognised researches.

The three regions display research teams, which are working on new treatments (protein engineering, drug screening), cancer therapy (hyperthermia), drug delivery and targeting.

Toulouse is selected as one of the three French nanotechnologies integration centers promoted by the Nano Innov Plan (launched in May 2009).

Toulouse and Montpellier have national labelled centers engaged on Nano-technology research: LAAS (Laboratory of Analysis and Architecture of Systems) - Toulouse and CTMN (Micro and Nanoelectronics Technology Center of Montpellier University) - Montpellier.

In the French south west area, interactions between nanotechnologies experts and oncologists need to be enhanced to take advantage of the remarkable expertise in nanotechnologies.
To ensure public acceptance, clinical applications will have to be very carefully validated on different biological and ethical points of view.

FONDATION InNaBioSanté

The InNaBioSanté Foundation was created by Amgen, GlaxoSmithKline, Pierre Fabre, Siemens and Total. It is recognized as a public utility under the French research and innovation system by decree of May 5, 2006.
The Foundation's mission is to promote and finance health research and health-related economic development in, specifically, the fight against cancer.

It does so by creating new relationships among four generic technologies:
- **Information technologies,** which rely on information technology and multimedia, with applications including bioinformatics, molecular modeling and biomathematics;
- **Nanotechnologies,** which allow manipulation of form and size on a nanometric scale, with applications ranging from optics to biology and chemistry (for example, molecular engineering and the development of tailor-made drugs);
- Biotechnologies that perform procedures or act on living organisms and products derived from living matter (including, for example, monoclonal antibodies and the drug production);
- Radiotherapy and imaging techniques used in the diagnosis and locoregional treatment of cancers (including, for example, external radiotherapy, targeted radiotherapy, radium therapy and molecular imaging).

Excellence in research
The InNaBioSanté Foundation is committed to encouraging cross-disciplinary research of the highest quality. It is implementing a new strategy for identifying, funding and supporting innovative projects in the fight against cancer.
It offers research teams the material and financial backing they need to dedicate themselves fully to their research goals.
In the area of medical research, nanotechnologies will thus offer significant opportunities to improve healthcare, from prevention to patient care and access to more effective treatments.

The Foundation's action plan
The InNaBioSanté Foundation process is based on a comprehensive, cross-disciplinary approach to fund innovative, complementary projects at the crossroads of technology and the life sciences.
- Funding for public/private research partnerships upon call for statements of intent;
- Support for creating innovative companies that emerge from closer ties between research and industry;
- Funding for technological platforms;
- Support for new researchers while they study at centers of excellence in France and abroad;
- Funding for "chairs of excellence" to draw internationally renowned researchers;
- Organization of seminars and national and international conferences.

SESSION 2: TRANSFER OF NANOTECHNOLOGY TO CLINICAL APPLICATIONS

Ultrasound triggered, image guided, local drug delivery

Chrit MOONEN
Laboratory for Molecular and Functional Imaging: From Physiology to Therapy
UMR 5231 CNRS/Université « Victor Segalen » Bordeaux 2, Bordeaux

Local drug delivery has the potential of increasing the local therapeutic effect while limiting systemic toxic effects. Targeted or non-targeted drug-carrying nanoparticles may be used for local drug delivery. The objectives of image guidance are; 1) target identification and characterization; 2) temporo-spatial guidance of actions to release or activate the drugs and/or permeabilize membranes; 3) Evaluation of biocodistribution, PharmacoDynamics; 4) Physiological read-outs to evaluate the therapeutic efficacy.
Local release may be triggered by membrane fusion, phagocytosis, pinocytosis, but also by external physical means such as (focused) ultrasound. Nanoparticles may be designed specifically to enhance ultrasound induced bio-effects, notably cavitation. Most microbubbles consist of air- or perfluorocarbon-filled microsphere stabilized by an albumin or lipid shell with a size in the range of 1-10 µm.
Drugs can be attached to the membrane surrounding the microbubble, they can be imbedded within the membrane itself, they can be bound non-covalently to the surface of the microbubble and can be loaded to the interior of the microbubble, either in an oil or aqueous phase. These microbubbles can be targeted to specific (pathologic) sites using targeting ligands. Alternatively, spatial targeting can be achieved by focusing the ultrasound beam (with at best a width of half the wavelength, e.g. about 1 mm for 1 MHz ultrasound).

Thermosensitive liposomes have been suggested for local drug release in combination with local hyperthermia more than 25 years ago. Liposomes may carry both hydrophilic and hydrophobic drugs in their aqueous interior and lipid bilayer membrane, respectively. The circulation half-life may be increased by incorporating polyethylene glycol (PEG)-lipids in the bilayer.
Focused Ultrasound (FUS) is the only clinically viable technology that can be used to achieve a local temperature increase deep inside the human body in a non-invasive way. The recent developments of measuring and controlling temperature with MR guided Focused Ultrasound should lead to improved control of locally releasing drugs with temperature sensitive nanocarriers.
Image guided local drug delivery must be accompanied by evaluation of pharmacodistribution, in order to predict outcome. Imaging may provide a non-invasive assessment of such parameters. Similar to the encapsulation of drugs in nanocarriers, contrast agents can be included that report on the local release of drugs and subsequent tissue distribution.
Despite very promising early results, there is a clear need for better quantification of the results, development of suitable (mult-modality) contrast agents for pharmacodistribution, optimization of triggering procedures, and of drug/gene nanocarriers.

New developments and opportunities for cancer diagnostics using biosensors

Pilar MARCO
Applied Molecular Receptors Group (AMRg). Advanced Chemical Research Institute of Catalonia of the Spanish Council for Scientific Research. (IQAC-CSIC http://www.iiqab.csic.es/amrg). Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN. www.ciber-bbn.es), Barcelona, Spain

Cancer arise as result of disruption of normal cell signaling pathways that results in a decisive growth advantage in compare to other cells. Unfortunately, there not a single oncogene or tumor suppressor gene is universally altered, but different patterns of genetic changes have been observed even among tumors from the same location. During the last years a plethora of molecular biomarkers have been identified. However, in spite of this intense research and the rapid explosion of new technology platforms reported for cancer diagnostics, prognostics, therapeutics and monitoring disease recurrence, few of these technologies/biomarkers have transitioned to routine clinical diagnostics. In this presentation, information will be presented regarding new biosensor technologies with potential application for biomarker analysis. Merging these technologies with the actual cancer biomarker knowledge could provide diagnostic systems that assist clinicians in disease diagnosis, prognosis, treatment and recurrence. Since their conception biosensors have created great expectations due to their capabilities to generate electrical or optical signals as consequence of selective biomolecular recognition reactions occurring on the surfaces of transducer materials. In this context, the latest micro(nano)technological advances have given rise to a wide variety of potential diagnostic approaches. Materials and nanoparticles with defined physical properties may be used to develop functional hybrid biomaterials used to develop biosensors with improved features and capabilities for diagnostics[1-5]. At the moment, the unique properties of the materials at the nanolevel are pointing to the possibility to develop more sensitive and flexible biosensing systems. Regarding molecular recognition elements, although a variety of synthetic based recognition elements are being investigated (aptamers, peptide, polymers, etc.), antibodies continue to be the most used elements. Thus, antibodies have fascinating features regarding selectivity and affinity, added to the possibility to be produced against almost any kind of biomarker.
Although biosensor devices have already been employed in the clinic for the diagnosis/prognosis of certain diseases, their implantation on cancer diagnostics is slow due to the complexity of these diseases. Simultaneous monitoring of a large panel of biomarkers and integration into devices able to perform multis-step sample processing is required. Moreover, a key aspect is the acceptance of existing molecular biomarkers and discovery of new ones that respond with sufficient reliability to indicate disease.

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[2]Bratov, A.; Ramón Azcón, J.; Abramova, N.; Merlos, A.; Adrian, J.; Sánchez Baeza, F.; Marco, M. P.; Domínguez, C. Biosensors and Bioelectronics 2008, 24, 729 735.
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[4]Adrian, J.; Pasche, S.; Voirin, G.; Pinacho, D. G.; Font, H.; Sánchez Baeza, F.; Marco, M. P.; Diserens, J. M.; Granier, B. TrAC Trends in Analytical Chemistry 2009, 28, (6), 769 777.
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Optical imaging and cancer: from mice to man

Jean-Luc COLL
INSERM U823 - Université Joseph Fourier, Grenoble

Early and accurate detection of tumors, like the development of targeted treatments, is a major field of research in oncology. The generation of specific vectors, capable of transporting a drug or a contrast agent to the primary tumor site as well as to the remote (micro-) metastasis would be an asset for early diagnosis and cancer therapy. Our goal is to develop new treatments based on the use of tumor-targeted delivery of large biomolecules (DNA, siRNA, peptides), able to induce apoptosis while dodging the specific mechanisms developed by tumor cells to resist this programmed cell death. Nonetheless, the insufficient effectiveness of the vectorization systems is still a crucial issue. In this context, we generated new targeting vectors (as the RAFT-RGD) and nanoparticles for drug and biomolecules delivery. To undertake this work, we also developed several optical imaging systems allowing the follow-up and evaluation of our vectorisation systems. Based on our recent work I will present a general overview of how optical imaging can help for the development of targeted-therapeutics and image-guided surgery in oncology.

Near-infrared fluorescence (NIR; 650-900 nm) can be imaged in 2D or 3D. The strong reflection of incident light and autofluorescence of the skin affect the sensitivity when working in reflectance. Switching to Fluorescence Molecular Tomography (FMT) mode greatly improves the quality of whole-body fluorescence imaging. It offers 3D volumetric imaging, true quantification very little affected by depth, optical tissue properties and heterogeneity, and autofluorescence. It is thus an emergent diagnostic tool for the localization and quantification of fluorescent probes, at a depth of a few cms, in some organs, like breast or prostate. In such situations, this technique may be considered as an alternative to the classical ionizing radiation imaging techniques, or a complement to morphological imaging as ultrasounds. It presents the advantage of providing a simple, inexpensive, non-invasive and accurate diagnosis. More recently a tomographic bench has been set up (CEA-LETI, Grenoble) in order to extend fluorescence molecular imaging to the diagnosis of human prostate cancer. The major challenge here is the screening of deep tissues.

In parallel with these technical developments, adapted, smart imaging agents based on the RAFT-containing polymers or nanoparticles are developed for multimodal imaging systems. Such agents will also be presented.

A new platform of bio-inspired nanomedicines in Oncology

Jean-Pierre BENOIT
INSERM U646, Université d'Angers, Angers

Nano-medicine, an emerging new field created by the fusion of nanotechnology and medicine, is one of the most promising pathways for the development of effective targeted therapies with oncology being the earlier and the most notable beneficiary to date. Indeed, drug-loaded nanosystems provide an ideal solution to overcome the low selectivity of the anticancer drugs towards the cancer cells in regards to normal cells and the induced severe side effects, thanks to their passive and/or active targeting to cancer tissues. Nanoparticle and liposome-based systems encapsulating drugs are already used in some cancer therapies (e.g. Abraxane®, Myocet®, Daunoxome®, Doxil®…). Nevertheless improvements are needed in drug targeting such as more stable formulations that do not leak, more versatile systems able to encapsulate any type of drugs, control of active and sub-cellular targeting…

In this context, we have developed new bio-inspired nano-cargos, the lipid nanocapsules, that mimic the lipoproteins, with sizes below the endothelium fenestration (Ø < 100 nm). Moreover these new nano-cargos have the ability to carry a large variety of drugs, lipophilic and hydrophilic small molecules, peptides and proteins, siRNA and DNA, radiopharmaceuticals, constituting a real platform of nanomedicine.

The lipid nanocapsules (LNC) have been prepared according to an original method based on a phase inversion temperature process recently developed and patented[1]. Their structure is a hybrid between polymeric nanocapsules and liposomes because of their oily core which is surrounded by a tensioactive rigid membrane. Their size can be adjusted below 100 nm with a narrow distribution. Importantly, these properties confer great stability to the structure (physical stability> 18 months). Blank or drug-loaded LNC can be prepared, with or without PEG (PolyEthyleneGlycol)ylation that is a key parameter that affects the vascular residence time of the nano-cargos[2]. Other hydrophilic tails can also be grafted such as dextran, chitosan, polylysin... Different anticancer drugs (paclitaxel, docetaxel, etoposide, hydroxytamoxifen, doxorubicin, siRNA,…) have been encapsulated. They all are released according to a sustained pattern. Preclinical studies on cell cultures and animal models of tumors (glioma and lung carcinoma) have been performed, showing promising results[3].

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SESSION 3: ETHICS AND REGULATORY ISSUES RELATED TO NANOTECHNOLOGIES IN THE FIELD OF CANCER

A philosophical perspective on nanotechnology

Bernadette BENSAUDE VINCENT
Université Paris OUEST/IUF, Paris

In many countries nanoresearch programs have been launched with a lot of hype: they promised clean and cheap energy, remediation of polluted sites, targeted cancer cures, enhanced human performance and longevity. This extensive use of promises raises the issue of credibility. How can we trust them?

In parallel various efforts have been made to develop more "responsible innovation" with anticipation of impacts of the applications of nanotechnology and engagement of the public upstream. I will first review some of these attempts - and then try to assess the results of such initiatives in Europe. How is "responsible innovation" displayed in the domains of concerning nanotoxicology, ethical and societal impacts, and public engagement?

Toxicology assays and protocols in nanomedicine

Simó SCHWARTZ Jr.
CIBBIM-Nanomedicine, Vall d'Hebron University Hospital, Barcelona

Obtention of new disease specific biomarkers are a must to achieve success in nanodiagnostics and there is an increasingly growing request of them to confront several diseases and clinical conditions (i.e. unknown infections or cancer). Also for developing targeting strategies needed to biofunctionalyze nanoparticles against them to deliver traceable particles (imaging) and therapeutic drugs (drug delivery). Pharmacological redesign of known drugs by linking them with nanocarriers from different nature (polymeric carriers, liposomes or magnetic nanoparticles among others) should be regarded as a new instrument to modify their pharmacological properties including therapeutic activity and sensitivity, pharmacokinetics and toxicology. In that sense, there is a need to provide the Industry and academia with optimal in vitro and in vivo validation tools for "proof of concept" demonstrations and preclinical studies of new nanotechnology based biomedical approaches. Only those nanotechnology-based designs able to reach adequate standards in terms of efficacy and safety will have the opportunity to reach the clinical setting. Here we will provide several examples of in vitro and in vivo validation strategies used to test new drug delivery systems against cancer models.

Impact of nanotechnologies on patients: questions for ethics

Marie CHARAVEL, Caroline SALAS, Marc GANDIT, Michel DUBOIS, François BERGER
Université Pierre Mendes France, INSERM, Floralis, CHU Grenoble, CLARA

For the past thirty years, medicine has evolved rapidly from clinical examination of the patient towards technical examination of its body. In oncology, technologies intervene at different levels of the take care of cancer:

- before the appearance of symptoms, with the screening of cancer and precancerous states;
- during the development of symptoms, with the extension outcome (bilan d'extension) of the cancer and one's consequences on the organism;
- during the treatment of cancer, with the assessment of clinical response to the treatments;
- during the surveillance following cancer, to attempt to predict the relapse of cancer before symptoms appear.

Tomorrow, but rather today, nanomedical research will allow to better improving diagnosis and the therapy of cancer by carrying therapeutic agents towards a specific target.

It is common to underline the power of technology in medicine and to question ethics on: researcher's behavior; prevention of risks; principle of precaution front uncertainties; values and goals of such technological progress front political intentions and commercial wagers, etc. In the meantime the outcome of its human application, in the frame of the take care of patients, is poorly explored. The application of new technologies in oncology reveals several questions: What does it mean for a patient to learn by screening that he is affected by a disease? What kind of perception has the patient of its body by being informed by a technology approach that he develops a disease? How does the patient perceive the disease without clinical manifestation of a symptom but on the basis of a technological examination? What perception has the patient of the treatment with its side effects in a context where the disease has not yet developed a clinical symptom? What kind of relationship does the patient maintain with its physician, and beyond the medicine approach that interferes on the basis of technology-mediated information and not per se on the basis of patient complaints?
These questions are interposed at the center of an ethical debate on the interaction of man with the development of new technologies in medicine. If we deplore that these questions have almost not been approached in a pragmatic way, an ethical debate on nanotechnology applied to oncology requires firstly evaluations based on accurate information which give a code of conduct for a socioethical integration. Thus, it is the right time to initiate a global ethical debate related to the progress made in the field of nanomedicine, on the basis of an anticipating and prospective approach.

Bridging the gap between research and medical decision-making

Marta AYMERICH
Facultat de Medicina, Universitat de Girona, Spain

Although not all the scientific community agrees about the meaning of translational medicine, there has been a growing body of scientific literature in the last years that have reach some consensus[1]: if we understand the biomedical research as a continuum, two translational blocks can be identified[2]: the first one (T1) allows the translation of lab based biomedical research into the clinical research, it aims at transferring new understandings of disease mechanisms gained in the lab into the development of methods for diagnosis, therapy, and prevention and their first testing in humans; and the second block (T2) bridges the know-do gap between clinical research and medical decision-making, it aims at ensuring that new treatments and research knowledge actually reach the patients or populations for whom they are intended and are implemented correctly. In this T2 field expertise in clinical epidemiology and evidence synthesis, communication theory, informatics, behavioral science, public policy, health economics, organizational theory and other implementation sciences are needed[3].

Despite the efforts made by the US Veterans Health Administration (with the QUERI initiative, www.queri.research.va.gov/), the US Agency for HealthCare Research and Quality (with the TRIP initiative, www.ahrq.gov/research/ trip2fac.htm#Initiative), and health technology assessment agencies in Europe[4-5], the science of implementation remains underdeveloped and much more work is needed.

Although there have been a number of reviews of implementation research that have consistently shown that the majority of interventions can achieve moderate improvements in healthcare[6-7], a limited number of studies provided a rationale for their choice of implementation strategy. Therefore, generalising from the findings of these studies to other healthcare settings is difficult due to the limited understanding of the characteristics of the professional behaviour, health care organisations, and health system that might influence the effectiveness of research implementation[8].

Moreover, a critical factor concerning effectiveness of research translation into decision-making is indeed even before that clinical research takes place, that is, in clinical research prioritization. Thus, methods which identify research gaps in health needs and which help to set priorities for research topics can be helpful in translating research knowledge into decision-making. Prioritization is usually based on the feasibility of carrying out the research, which in turn depends on the methodological strengths of the project and the abilities and experience of the research team. The relevance of the research topic is presumably always implicitly present in decision-making on funding for research, but it is rarely taken into account in a transparent and explicit manner. To ensure research implementation, the use of sound and explicit criteria to prioritize between research topics is paramount. In addition, criteria have to include the potential of translating knowledge into clinical practice as well as to reinforce the recommendation of taking into account the current state of evidence for designing new research[9], together with answering questions relevant to clinical and health policy decision makers[10].

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Perception and risk evaluation of health products using nanotechnology

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Nanoparticles are defined as particles at dimensions between approximately 1 and 100 nanometres. They have considerable general industrial applications and although only a few nanoparticle medicinal products are available at the present time, a spectacular development of these products can be expected over the years to come. In the short and medium term, the main use of nanoparticles medicinal products (NMP) is for vectorization of active principles, corresponding to several products already marketed general scientific and/or regulatory data related to the toxicological evaluation of NMPs are currently lacking.

"Conventional" toxicological approach proposed by current guidelines for medicinal products in general (ICH, FDA, EMEA) has been accepted up until now for approved NMPs or NMPs currently under evaluation by health authorities. However, some criticisms have been raised concerning the currently available methods of experimental evaluation that are considered to not adequately assess the properties of nanoparticle products. The most frequent criticisms essentially concern pharmacokinetic and toxicokinetic studies, which are considered to not realistically take into account the specificities related to the nanoparticle structure. The relevance of in vitro tests has also been questioned, as the sedimentation rate and diffusion capacity of nanoparticles must modify the conditions of exposure (dose-duration) of the models used (for example, genotoxicity tests). Finally, the lack of data on long-term effects is often emphasized.

Consequently, like certain consumer groups in the U.S.A., we may need to recommend the development of completely new regulations based on "adapted" safety assessment tests for nanomaterials, including NMPs. This maximalist proposal is totally idealistic and scientifically unjustified according to the very great majority of the scientific community. How many years for development and validation would be necessary to achieve such a result? This major revision also does not appear to be justified by the available scientific data. Some manufacturers consulted and most of the task force also consider that toxicological evaluation of NMPs should not be appreciably different from "conventional" evaluation, but with certain specific adaptations (inappropriate nature of repeated-dose studies for NMP used as a single dose in man, such as in medical imaging). The plan adopted for elaboration of these recommendations is based on this approach, i.e. adapt the safety assessment strategy, when necessary, without modifying the basic principles.

In order to clarify and encourage innovative development of NMPs, the French Health Safety Agency has release recommendations for the toxicological evaluation of NMPs. These recommendations have been formulated in the usual order of other guidelines. The Task Force emphasizes that, in view of the wide range of structures, physicochemical and biological properties, therapeutic uses, etc., case-by-case assessment of the most relevant study programme for a given NMP will always be essential.

SESSION 4: PROOF OF CONCEPT OF CLINICAL APPLICATIONS IN ONCOLOGY

The DIACPROL project: building a nanosensor ("electronic nose") for prostate cancer diagnosis

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The possibility to diagnose and monitor diseases, and in particular some cancers, by measuring the concentration of volatile markers in body fluids (or in the breath) has gained acceptance in recent years. Electronic noses for this purpose should present extremely low detection limits, high odour specificity and an unmatched chemical space, thus having the potential to reach the medical diagnostic application targets. Bioelectronic noses based on biological olfactory receptors satisfy these requirements thus constituting a unique emerging technology onto which to base these challenging diagnostics electronic noses.

Bladder and prostate cancers represent a serious sociosanitary problem due to their high mortality rates and to the difficulty in patient care and treatment. The existing diagnosis and monitoring tools for both bladder and prostate cancers are either strongly invasive or considerably non-specific. Therefore, the diagnosis and monitoring of bladder and prostate cancers need of a new family of diagnostic tools, highly specific, highly sensitive and non-invasive.

The objective of the DIACPROL project is to build an electronic nanoanalytical platform based on olfactory receptors for the non-invasive and specific diagnosis and monitoring of bladder and prostate cancers through their odorant signature within urine.

In order to realise the proposed nanobioplatform, advances in Molecular Biology, Biotechnology, Micro/Nanotechnologies, Electronic Instrumentation and Computing Science will be required. The high sensitivity and selectivity of the olfactory receptors, coupled to the integrated high-gain low-noise electronics and specific odorant identification algorithms will provide the maximum sensitivity, specificity and reliability to the proposed nanobioplatform, being able to detect even minute traces of the volatile markers and thus contributing to an early diagnosis of the diseases.

Nano-fingerprinting as a new device for theragnostic medicine applied to glioblastoma

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Availability of molecular biomarkers usable for early detection and indicative of prognosis and therapeutic response prediction is a clinico-biological priority. The availability of a non invasive strategy to catch the low concentrated/and or tissue proteomic compartments as well as to none invasively monitor tissue response to therapy is a crucial issue. In the medico-biological state of the art, this is by now performed by invasive biopsy, and the success for the validation of remote non invasive peripheral biomarkers has been low. In this context, the development of micro/nanotools devoted for intra-tissular characterisation is indispensable.
Nanotechnologies introduced several high sensitivity devices, which should provide the availability to detect very low concentrated biomarkers in the peripheral compartment including blood or CSF for example. Association of different materials at different steps of the blood biomarker procedure including paper, magnetic nanobeads or polymers open new opportunities to re-invent the old, expensive and non reproducible blood puncture procedure. Similarly surface chemistry at the nano-level opens the opportunities to catch the inaccessible low concentrated proteome that is crucial for biomarker studies. Several micro-nano devices will be presented providing from the blood interface to lab-on-chip analysis providing a more efficient and robust access to the deep proteome compartment.
The recent success of targeted therapy clearly demonstrated that direct tissue molecular investigation may be the best modality to annotate the relevant active pathways providing efficient therapeutical targets. We demonstrated that proteins could be captured from the microsurgery tools directly introduced in the human brain. We optimized the tool by the addition of specific chemical and micro/nanostructuration modifications. A small silicon chip was produced, and plugged on the metallic stylet. We first introduced chemical modification on the surface, mimicking in vivo the SELDI-TOF concept, devoted to the capture of a specific subproteome.The results obtained clearly demonstrated the adequate concentration of a specific subproteome provided the individualization of supplementary peptides. The chemical modifications also provided the conservation of the spatial location of the proteins inside the tissue. We also significantly increased protein capture by the addition of specific micro/nanostructuration modifications on the surface of the device. Extension to genomic as well as cell investigation was also validated as well alternative prototypes were developed to target other locations such as liver, lung, sarcoma, prostate and breast cancer.
In conclusion, we validate the concept of molecular biopsy using a specific silicon chip harboring surface modifications. This will be helpful, to help diagnosis, prognosis and therapeutic prediction during conventional micro-invasive approaches in oncology in connection with poly-omic characterization. This micro-harvesting procedure was translated at the nanolevel, using magnetic nanobeads with specific surface modifications in serum and pathological tissues.

Multimodality imaging in oncology utilizing targeted drug delivery systems

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Nano-engineered particles have been developed to reach specific molecular targets on diseased cells and have been used in various experimental and clinical conditions. The medical application involves diagnostic and therapeutic applications and a large deal of this research concerns malignant disease. Various approaches have been tried to effectively reach the cancer cell and PEGylated liposomes have demonstrated targeting and controlled release of antineoplastic drugs. For cancer diagnostics nanoparticles have been engineered to optimize magnetic resonance imaging, ultrasound imaging and nuclear medicine imaging. The summary of the characteristics of these particles in nanoparticle applications is presented in Table[1].

Table 1. Summary of the Differences Between Imaging Modalities and their Possibilities for Nanoparticle Applications. The Method Characteristics (Spatial Resolution, Depth Resolution, Temporal Resolution, Sensitivity, the Amount of Needed Molecular Probe are Modified from the Data in the Literature [77]

Modality	Spatial resolution	Depth	Temporal resolution	Sensitivity (mol/L)	Molecular probe	Nanoparticle design
PET	1-2 mm	No limit	10 s-min	10^{-11} - 10^{-12}	ng	Label outside, in the membrane, or inside (radionuclide)
SPECT	0.5-1 mm	No limit	min	10^{-10} - 10^{-11}	ng	Label outside, in the membrane, or inside(radionuclide)
Bio-luminescence	3-5 mm	1-2 mm	sec-min	10^{-15} - 10^{-17}	g-mg	Label inside (or outside), luminescent compound
Fluorescence	2-3 mm	<1 mm	sec-min	10^{-9}-10^{-12}	g-mg	Label outside or inside, fluorescent compound
MRI	25-100 µm	No limit	min-hrs	10^{-3}-10^{-5}	g-mg	Label outside, in the membrane, or inside, paramagnetic atom, particles
CT	50-200 µm	No limit	min	10^{-1}-10^{-4}	N/A	Label inside (or outside), contrast media
Ultrasound	50-500 µm	mm-cm	sec-min	10^{-1}-10^{-4}	g-mg	Label inside, gas filled particles

**Table from K. Kairemo, P. Erba, K. Bergström, E.K.J. Pauwels. Nanoparticles in Cancer. Curr Radiopharm 2008; 1; 30-36*

Peptidoliposomal drug derivatives were constructed for targeted drug delivery and tested in tumor bearing animals. The first targetor was a CTT peptide known to target matrix metalloproteinases-2/-9, essential components of tumor cell migration, neovascularization and tumor stromal destruction. The drug delivery system was tested in several tumor models, most often in nude mice bearing human ovarian cancer xenografts. The affinity of radiolabelled (111In) peptides was determined in a competitive assay binding human breast ca cells and was approximately 20 nM. The biodistribution was detected by tissue sampling in mouse models, and higher uptakes than 15 % ID/g tumor were observed with the naked peptide, 20 % ID/g tumor with the peptidomicellar construction (17 nm particle), and 35 % ID/g tumor with the peptidoliposome (100 nm particle). The bioactivity of this peptide/nanoparticle was always tested using peptideELISA. The drug concentrations in the nanoparticles were measured by HPLC, inclusive their metabolites.

In experimental therapy trials targeted drug delivery systems were compared with non-targeted drug and free drug in bioequivalent concentrations. The targeted liposomal doxorubicin increased survival these animals as compared

to non-targeted system and free drug. The targetor principally increased survival of animals approximately 35% as compared to treatment with pegylated liposomes without targetor. The concentrations in tumor at 0-96 hours demonstrated approximately 40 % increment in AUC (targeted drug vs. non-targeted drug). Similar results were obtained with other drugs. The peptidomicellar construction was further developed for GMP and designed for clinical trials.

Another bioactive peptide obtained by phage display is a specific ligand to the leukocyte ß2 integrins, αMß2 integrin(CD11/CD18). This LLG peptide is a nonapeptide which is dependent on two disulfide bridges that constrain the peptide structure. This peptide is known to inhibit the ß2 integrin-mediated leukocyte cell adhesion and binds to the cation-sensitive I-domain of the integrin a subunit. Biodistribution of the phage display peptide LLG (14 amino acids) and its conjugates in the targeting moiety were studied in various animal models. Additionally peptidoliposomal derivatives were constructed for targeted drug delivery.

The affinity of radiolabeled (111In) peptides was determined in a competitive assay was found to be of high nanomolar range. The biodistribution, detected both by external imaging and tissue sampling, did not reveal any specific accumulation sites and the peptide was rapidly excreted via kidneys. In several animal inflammatory/infectious disease models excellent targeting signals based on biodistribution analysis were observed. We used E.coli LPS and thioglycolate inflammation models in mice (several locations), S. aureus infections in rats (thigh muscle) and E. coli infections in rabbits (thigh muscle). Special attention was paid to different isomeric conformations (disulfide bridge orientation) and their effect to biodistribution.

The possibilitities to use LLG as a therapeutic agent had been studied, absolute tumor-to-blood ratio at 24 hrs using labeled peptide as leukemic tumor targeting agent was 4.7. Pegylation of LLG increased circulation time. The LLG can also function as a therapeutic agent on surface of liposome, resulting in a even longer circulation half-life. Using liposome we can modify drastically the pharmacokinetics and dynamics of the peptide. Our animal models (mice, rats, rabbits) demonstrated excellent in vivo targeting of infectious and inflammatory tissue.

Some constructs with multiple signals (radiolabels, MRI contrast, gas bubbles, autofluorescent compounds) were developed.

Peptide-based nano-particle for in vivo delivery of siRNA

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The development of short interfering RNA (siRNA) has provided great hope for therapeutic targeting of specific genes responsible of patholological disorders. However their clinical application remains limited by their poor cellular uptake, low bioavailability, and insufficient capability to reach targets in vivo. We have designed a novel approach, based on short amphipathic peptides "NANOPEP" that promotes efficient delivery of siRNA into wide variety of mammalian cell lines and in vivo upon systemic and topical administrations. This carrier consisting of a balance between hydrophobic and hydrophilic domains and forms stable discrete "nanoparticles" with siRNA, through non-covalent interactions. Cellular uptake mechanism of NANOPEP/siRNA nanoparticles is dependent on the size of the particle and involves membrane potential and dynamic, which enables a rapid release of the siRNA into the cytoplasm and promotes a robust down-regulation of target mRNA. NANOPEP promotes siRNA delivery into primary cell lines and in vivo upon systemic administration without triggering any nonspecific inflammatory response.

NANOPEP-carriers were applied to the delivery of siRNA targeting the cell cycle regulatory protein Cyclin B1 into cancer cells. We demonstrated that when associated with NANOPEP, sub-nanomolar concentrations of siRNA Cyclin B1 significantly knocked down Cyclin B1 protein levels resulting in cell cycle arrest in G2 arrest and blocked cancer cell proliferation. The surface of NANOPEP particles can be functionalized to significantly improve siRNA stability in vivo, thereby enhancing the efficiency of this technology for systemic administration following intravenous injection. We have validated the therapeutic potential of this strategy for cancer treatment by targeting cyclin B1 in various mouse tumour models and demonstrate that NANOPEP-mediated delivery of cyclin B1 siRNA prevents tumour growth in vivo following systemic intravenous injection. Moreover, we showed that functionalization of NANOPEP particles with other chemical groups or biological moieties can be applied to generate formulations to target specific cell types or tissues which can be of a major interest for future development. Given the biological response yielded through this approach, we propose that non-covalent, peptide-based delivery technologies hold a strong promise for therapeutic administration of siRNA.

Pancreatic cancer gene therapy: non viral gene delivery

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Pancreatic cancer is a formidable health problem with increasing incidence. Although this tumor represents only 2% of new cancer diagnoses it is the fifth most common cause of all cancer deaths. It is one of the most lethal malignancies with an overall 5-year survival rate below 4%. To this day no therapies with significant incidence on the disease progression have been established and most patients are treated with a palliative intention with gemcitabine (Gemzar).

Gemcitabine is a prodrug that is converted to its active triphosphate form in cells, however this process is largely inefficient in a pancreatic cancer setting. Our strategy is therefore to improve this reference treatment by increasing gemcitabine activation using a gene-directed enzyme prodrug therapy. Gene transfer is accomplished by intratumoral injection of a complex of plasmid DNA and a cationic polymer to mediate its cellular delivery. Preclincal data indicate that pancreatic tumors are render more sensitive to Gemzar following gene delivery.

The drug candidate is relatively easy to produce at the laboratory level but when moved to a GMP setting for regulatory approval significant hurdles can be encountered. This short talk will relate the experience of a small self-funded biotech in bringing a nanoparticule-based gene therapy protocol to the clinic. A phase I/II clinical trial has been initiated in collaboration with the Toulouse Hospital and is expected to start mid-2010.

POSTERS ABSTRACTS

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4- **Faure Anne-Charlotte:** Multifunctional nanoparticles for biomedical applications
5- **Garanger Elisabeth:** Block copolymer-based nanoparticles for therapy and imaging of different types of cancer
6- **Leclerc Lara:** Development of innovative pH sensor to evaluate phagocytosis of nanoparticles.
7- **Mornet Stéphane:** Towards nanosized manganite perovskites for hyperthermia
8- **Reventós Jaume:** Subtractive Proteomic Approach to the Endometrial Carcinoma Invasion Front
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22- **Sporer Christian:** Electrochemical Biosensors for Medical Diagnostics

SESSION 3: ETHICS AND REGULATORY ISSUES RELATED TO NANOTECHNOLOGIES IN THE FIELD OF CANCER

SESSION 4: PROOF OF CONCEPT OF CLINIC APPLICATION IN ONCOLOGY

SESSION 1: PRESENTATION OF ACTIVITIES

Poster 1

Creation of an animal facility module authorizing the use of radioelement

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Wishing to lead preclinical studies of validation of the therapeutic efficiency of new anticancer agents in brachytherapy and/or targeted radiotherapy, we were faced with the securing of the accommodation (hosting) of animals during the period when they are radioactive. Therefore, we developed an adapted structure for this type of test in Lyon. This structure must comply the regulation pressures concerning at the same time use, stocking and preparation of radioactive elements (including the approval of order of these products) and in those of the animal facility. In front of these specifications, the implementation of this structure dedicated to research, authorizing the use of radioisotopes, in a nuclear medicine department, has enabled us to have to take into account only the specific standards to an animal facility. The Radioelements module HEH is an appendix of the Common animal facility for Rodents of the site Rockefeller, and hosts treated animals under the responsibility of a dual-qualified staff, competent in radiation protection and allowed to experiment.

Poster 2

Peptide based nano-particle for the delivery of siRNA

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The development of short interfering RNA (siRNA), has provided great hope for therapeutic targeting of specific genes responsible of pathological disorders. However their clinical application remains limited by their poor cellular uptake, low bioavailability, and insufficient capability to reach targets in vivo. We recently proposed a new peptide-based "nanoparticle" system, based on short amphipathic peptides "CADY" that promotes efficient delivery of siRNA into wide variety of mammalian cell lines and in vivo upon systemic and topical administrations. CADY is a 20-residue secondary amphipatic peptide able to form stable discrete nanoparticles with siRNA, through non-covalent interactions. Cellular uptake mechanism of CADY/siRNA nanoparticles is dependent on the size of the particle and involves membrane potential and dynamic, which enables a rapid release of the siRNA into the cytoplasm. CADY strongly interact with phospholipids independently of their net charge and of their physical state, throughout both electrostatic and hydrophobic interactions. Binding to siRNA or phospholipids triggers conformational transition of CADY from unfolded state to an alpha-helical structure, which stabilizes CADY/siRNA nanoparticles and improves their interactions with cell membranes. CADY were applied for the delivery of siRNA targeting the cell cycle regulatory protein Cyclin B1 into cancer cells. We demonstrated that when associated with CADY, sub-nanomolar concentrations of siRNA significantly knocked down target at both mRNA and protein levels. We validated the therapeutic potential of this strategy for cancer treatment by targeting cyclin B1 in various mouse tumor models and demonstrate that CADY-mediated delivery of cyclin B1 siRNA prevents tumor growth in vivo following systemic intravenous injection without inducing any immune response. Thus we propose that non-covalent, peptide-based delivery technologies hold a strong promise for therapeutic administration of siRNA.

Poster 3

Urine proteomic approach for the early detection of prostate cancer

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Prostate specific antigen (PSA) serum measurement in combination with a digital rectal examination (DRE) and transrectal ultrasound guided biopsy (TURS) is currently the gold standard for prostate cancer (PCa) screening in Europe. Nevertheless, PSA and DRE lack significant specificity and biopsy lacks ideal sensitivity (12 30% false negatives). Therefore additional biomarkers are needed to supplement or potentially replace the currently used diagnostic techniques. We sought to determine a proteomic profile in urine able to distinguish between the presence and absence of PCa. We used a combination of proteomic technologies in aged matched urine supernatants obtained after DRE to identify differentially expressed proteins in patients with PCa. Firstly, we compared 9 histological confirmed PCa urine samples and 9 control samples (age-matched patients with the typical background of benign prostate hyperplasia (BPH), atrophy and chronic inflammation). Then, Two-dimensional gel-based proteomic approach (2D-DIGE) coupled with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and database mining experiment were performed to identify novel biomarkers of PCa in urine. We identified a proteomic profile of 26 potential biomarkers for the detection of prostate cancer in urine. The majority of this proteins belonged to secreted components of several well known functional of cancer and inflammation networks like NFkB, PDGFB and PSA. Finally, we are validating the candidate biomarkers using Selected Reaction Monitoring (SRM)-based assays in a bigger cohort of urine samples. These data demonstrate the ability of proteomic analyses to reveal novel biomarkers for PCa in urine, an important step forward in advancing accurate diagnosis which is currently the bottleneck for the ability to cure patients from PCa. These biomarkers could be used to develop a simple diagnostic test (ELISA) to be utilized in hospital and outpatient routines.

Poster 4

Multifunctional nanoparticles for biomedical applications

Faure AC.

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We recently developed core-shell nanoparticles combining medical imaging and therapy. They are composed of a paramagnetic gadolinium oxide core for magnetic resonance imaging, neutron capture therapy and radiotherapy and a polysiloxane shell which can be designed for fluorescence imaging and/or photodynamic therapy. If the composition and the size of the core and therefore the related properties are imposed by synthesis conditions, the functionalization of the polysiloxane shell can be carried out sequentially. As a result, fluorophores or photosensitizers are entrapped in the shell while hydrophilic functionalized poly(ethyleneglycol) (PEG) chains can be covalently immobilized on the surface of the polysiloxane shell. Because of the free circulation and of the specific biodistribution depending on the type of PEG molecules, the imaging and the therapeutic capacities of these nanoparticles can be advantageously exploited.

Poster 5

Block copolymer-based nanoparticles for therapy and imaging of different types of cancer

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Despite their relative efficacy on a wide range of cancers, current chemotherapeutics present the major drawback of non-selective toxicity, particularly cardio- and nephrotoxicity, leading to severe side effects. This prompted the development of biodegradable nano- or submicron-sized particles capable of targeting cancer cells and encapsulating drugs, hence protecting them from degradation and carrying them to the tumor while avoiding their spreading throughout sensitive organs. The identification and development of new nanoparticles for simultaneous delivery of therapeutics and diagnosis of breast and colorectal cancers and bone metastases is the major goal of the European project NANOTHER. This interdisciplinary program gathers 18 partners from 9 different countries. Our role within this project is to synthesize biodegradable, stimuli-responsive amphiphilic block copolymers capable of self-assembling into micelles or vesicles to be loaded with various cytotoxic molecules (drug, peptide, siRNA) as well as magnetic nanoparticles (MR detection and hyperthermia) and functionalized with targeting moities. For this purpose, PTMC-b-PGA, PEG-b-PBLG and Jeff-b-PBLG block copolymers were synthesized by ROP of γ-benzyl-L-glutamate N-carboxyanhydride initiated by amino functionalized PTMC, PEG and Jeffamine, respectively. Solvent precipitation techniques lead to well-defined and reproducible nanoparticles as evidenced by light scattering and electron microscopy.
Nanoparticle size and dispersity can be tuned by varying nanoprecipitation conditions. Among self-assembled morphologies, polymeric vesicles are the most promising since they can incorporate both hydrophilic and hydrophobic compounds thanks to their dual hydrophilic reservoir and thick hydrophobic lamellar membrane.

Poster 6

Development of innovative pH sensor to evaluate phagocytosis of nanoparticles

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Introduction: Inhaled nanoparticles (NP) exhibit variable toxicity levels which mainly depend on their physicochemical characteristics (size, morphology, cristallinity, chemical surface composition …). Biological effects using several classical tests (ROS, TNFá, LDH) are usually performed on alveolar macrophages collected from the respiratory system. In this frame, evaluation of NP uptake by macrophages appears as an important evaluation. This study presents the development of pH sensible NP in order to evaluate phagocytosis by macrophages and particularly the step of fusion between phagosomes and lysosomes.

Material and methods: Two types of fluorescent NP with variable and well-characterized sizes and surface coatings have been synthesized. One type with FITC fluorescence and one other with double fluorochrome (FITC core and pHrodo in a porous polysiloxane shell). Red fluorescence of pHrodo probe increase as pH acidify permitting available the distinction of engulfed NP after incubation with macrophages (cell line RAW 264.7). Observations were realized by confocal microscopy performing fluorescence spectral analysis. Confocal acquisitions were realized with the two types of NP including fluorescent labeling of cell nuclei with hoechst as well as actin labeling with phalloidin for cellular limits detection.

Results: This study allows the validation of pHrodo NP model and the confocal observation of NP in contact with macrophages. Thus, this approach leads to distinguish entirely engulfed NP (yellow labeling due to colocalisation of NP in acidic vesicles) from those which are just adherent to the cell membrane (FITC green labeling).

Perspectives: We already have developed a cytometric quantification of micrometric particles. This technique will be conducted at the NP level in order to develop a complete quantification phagocytosis protocol. Key words: Nanoparticles, macrophages, uptake, phagocytosis, confocal microscopy.

Poster 7

Towards nanosized manganite perovskites for hyperthermia

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Hyperthermia consists of heating a cancerous tissue either to destroy directly tumor cells by necrosis or to improve drug or radiation efficiency (S. Mornet et al. J. Mater. Chem., 2004, 14, 2161; E. Duguet et al. Nanomed., 2006, 1, 157). One promising route uses alternative magnetic fields allowing to magnetic materials to absorb electromagnetic energy and convert it into heat. Magnetic fluid hyperthermia takes advantage of magnetic nanoparticles dispersion which could be administered by intravenous injection. Among the numerous requirements, two are of great importance: (i) the temperature must be controlled in vivo in order to avoid overheating of safe tissue (42-45°C) and (ii) the nanoparticles must be surface-derivatized not only for ensuring their stealthiness in the blood compartment towards macrophages (e.g. pegylation) but also for being labelled with appropriate targeting ligands. Concerning the first requirement, our strategy is to take advantage of the temperature-dependence of the magnetic properties of manganese perovskites La1-xSrxMnO3, which Curie temperature (Tc) may be tuned from -130 to 100°C by varying Mn3+/Mn4+ ratio. So, as soon as medium temperature reaches Tc, particles are expected to loose their magnetic properties and therefore their heating ability. Such particles would be both heaters and fusers (S. Vasseur, et al. Magn. Magn. Mater., 2006, 302, 315). We are now able to performed the synthesis of nanoparticles (size < 50nm) in the solid solution La1-xSrxMnO3 on a large composition domain ($0 \leq x \leq 0.5$) with tuneable Tc and, consequently the hyperthermia heating temperature of these nanoobjects. Moreover, our synthesis process was widely optimized resulting on crystalline materials which exhibit a high chemical homogeneity and a nanometric size. The problem of aggregation has also been solved. First attempts of silica encapsulation (necessary to prevent cytotoxicity) will also be presented.

Poster 8

Subtractive Proteomic Approach to the Endometrial Carcinoma Invasion Front

Reventós J.

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Tumor invasion defines the transition between tissue-restricted carcinomas, related to good outcome as optimal surgery becomes possible, and metastatic tumors associated with poor prognosis and a dramatic decrease in survival. In endometrial cancer, myometrial infiltration represents a determinant parameter highly valuable in prognosis. To date, the identification of proteins involved in endometrial carcinoma invasion has been essentially conducted by immunohistochemical methods, without a global perception on the invasive front. Laser microdissection presents nowadays limitations to the profound spatiotemporal regulation from both the tumor and the surrounding stroma occurring at the invasive front. In this work, we attempted an alternative proteomic approach to characterize specific components of the tumor invasive front or its reactive stroma, by comparing the invasive area of an endometrial carcinoma with the noninvasive superficial tumor area and normal tissue from the same patients. This strategy led us to identify proteins involved in cellular morphology, assembly and movement, differentially expressed at the invasive front, as well as pathways like cell-to-cell signaling and interaction and a modulated response to oxidative stress as events related to endometrial carcinoma invasion. In conclusion, we could identify new players of myometrial infiltration by applying a subtractive proteomic approach to the endometrial carcinoma invasion front.

SESSION 1: PRESENTATION OF ACTIVITIES

Poster 9

ETV5 transcription factor is up regulated in ovarian cancer and has a role in tumor progression

Ruiz A.

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Epithelial ovarian cancer is the most lethal gynecological malignancy and the fifth leading cause of cancer deaths in women in the Western world 1. Largely asymptomatic, over 70% of the patients are already at an advanced stage at initial diagnosis. Five year survival rate for women with advanced stage disease is less than 20%. In contrast, the cure rate is almost 90% when women are diagnosed at an early stage 2. Ets transcription factors have been implicated in the regulation of gene expression during a variety of biological processes including cell growth and differentiation. In particular, Ets transcription factors are able to activate the transcription of proteases, MMPs and TIMPs. The activation of proteolytic enzymes is central to both tumor invasion and angiogenesis. In the present study we have investigated the role of the Ets transcription factor ETV5 in epithelial ovarian cancer. We have analysed ETV5 expression in ovarian tumor samples by quantitative RT-PCR and immunohystochemestry and found ETV5 up regulated in tumor samples compared to control tissue. We have also examined the biological effects of inhibiting ETV5 expression in ovarian cancer cells. Our findings suggest that the overexpression of ETV5 detected in ovarian cancer cells may contribute to ovarian tumor progression through the ability of ETV5 to enhance ovarian cancer cell proliferation. ETV5 upregulation also protects ovarian cancer cells from apoptosis and enhances cell attachment to the peritoneal wall during ovarian cancer cell dissemination.

Poster 10

Validation of an orthotopic murine model for endometrial cancer

Ruiz A.

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In our Institution we have been working to understand the infiltration and dissemination process of endometrial cancer and we have studied different molecules that participate in this process which could be used as targets in novel therapies. But these new treatments should be tested pre-clinically, and with this aim we have developed the present study. We propose the validation of a murine orthotopic model in endometrial cancer developed using endometrial carcinoma Hec-1A cells. Hec-1A cells were transduced with a construct to stably express the luciferase gene. To detect tumor growth we use a novel imaging technology (IVIS, In Vivo Imaging System), which detects the bio-luminescence generated by the luciferase gene. It is possible to follow the metastatic process in the xenografts, as well as define the response to different treatments. We performed a pilot study with three Swiss-Nude mice and observed that the,orthotopically implanted Hec-1A cells generate local tumor, infiltrate the miometrium, vascular and lymphatic vessels, and develop distant metastasis in the same way that clinical advanced endometrial malignancies do. After intrauterine injection of 1 million Hec-1A-luciferase cells, two of three mice developed tumor locally and presented metastasis in para-aortic, inguinal and mediastinic lymph nodes, lungs, diaphragm, liver surface, pancreas and pelvic fat. One mouse did not develop local tumor and expectedly no metastases were found. At present we are confirming these findings with a higher number of mice.

Poster 11

Alternating gradient field magnetometers as a tool for the investigation on the mechanical and magnetic behavior of magnetic nanoparticles in biological samples

Serrano JJ.

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Alternating gradient field magnetometers (AGFM) are basic instruments for characterizing magnetic materials due to two reasons: their extremely high sensibility, preserving a very low noise floor, and their ability to measure magnetic material dispersed in fluids. These two features make the AGFM a very useful equipment to characterize the magnetic and mechanical properties of MNPs in biofuids and, specifically, in biological samples. The Princeton Measurements Corporation MicroMag Model 2900 AGM System® installed in our laboratories as part of the MNPs Characterization Platform, a facility that belongs to the Spanish Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), has demonstrated its capacity to detect low-concentrated nanoparticles (Kisker's PMAV-250) inside 1321N1 human glioblastoma cell lines. Actually, depending on their aggregation state, their site (inside or outside the cells) or type of linkage to cells structures like membranes, the observed MNPs magnetic behavior changes. Therefore, from the AGFM's measurements it should be possible to obtain a lot of information about the interaction between the MNPs and the cells, including the possibility to identify the type of MNPs present in a given biological sample. As a conclusion, the AGFM can serve as very useful tool for both the research on MNPs in biomedical applications, and for the detection and, hopefully, the identification of engineered MNPs as contaminant in ex-vivo samples. We present some results and show the status of the research line.

Poster 12

Silicon Carbide Quantum Dots as an alternative antineoplastic drug

Barberis A.

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Emerging nanotechnology brings new hope for significant breakthroughs to be achieved in the near future in cancer therapy. This would imply, nevertheless, a deep knowledge about the interaction of nanoscale materials with complex biological systems. We already presented the preferential cytotoxic effect of Silicon Carbide Quantum Dots (3C SiC QDs) on cancer cells. In this study we confirmed the influence on viability and proliferation on oral squamous carcinoma (AT-84 and HSC) and immortalized cell lines (S-G) and investigated cellular localisation of 3C SiC QDs. Cytotoxicity tests were performed without removing 3C SiC QDs or washing them out after 18 hours. In both tests, 3C SiC QDs display no cytotoxicity after 24 hrs while after 48 and even more after 72hrs are cytotoxic with a dose dependent response, selectively higher for tumoral than for immortalised cells. To evidence a possible synergism, those same cell lines were also grown in presence of Cisplatin, a drug currently used in cancer chemotherapy. Concentration of Cisplatin used has been selected so that the citotoxicity could be compared with the one caused by 3C Sic QDs. Results shows that Cisplatinum do not improve significantly the efficacy of 3C Sic QDs, while 3C Sic QDs drastically enhance the power of the Cisplatinum. Comparison between the fluorescence micrographs and the microscope photos obtained under white light demonstrates that the fluorescing 3C SiC QDs are strongly localized inside the cells. The mechanism(s) of action of 3C SiC QDs in inhibiting cell proliferation remains to be investigated. The data presented in these report demonstrate that human squamous carcinoma cells are markedly more susceptible to 3C SiC QDs mediated toxicity than their immortalised counterparts. These findings may be of important clinical interest as one of the greatest limitations in chemotherapy is the inability of anticancer drugs to distinguish between tumoral and normal cells.

Poster 13

New lipid nanoparticles formulation for imaging and drug delivery purposes

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In the field of nanomedecine, lipid nanoparticles (LNP) present numerous advantages over other formulations for the protection and the targeting of lipophilic active pharmaceutical ingredients (API), as well as contrast agents. These nanoparticulate carriers are biocompatible, biodegradable, allow controlled release and can easily be produced by solvent-free processes. However, to fully develop an efficient drug carrier, drug loading capabilities of LNP had to be optimized, while controlling their physical properties (size and physical stability). Here, we report on the optimisation of LNP formulation for API and/or contrast agent encapsulation and delivery. LNP are prepared by emulsion templating through ultrasonication. They are composed of a lipid core, mixture of solid and liquid lipids, which is stabilised by a shell of surfactants, mixture of an hydrophilic and a lipophilic surfactant. An experimental design was used to model the physicochemical behaviour of the LNP system. Final formulations were chosen on the basis of[1]: a size favourable for passive tumor targeting by the EPR effect (50nm<dp).

Poster 14

Preparation, characterization and cellular studies of photosensitizer-loaded lipid nanoparticles for photodynamic therapy

Boisseau P.

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PhotoDynamic Therapy (PDT) has been established as a potent and less invasive treatment for different kinds of cancer[1]. Among various attempts to enhance the therapeutics efficacy of PDT, the specific delivery of the PhotoSensitizer (PS) in the tumor is expected to increase its clinical applications, since unwanted accumulation, especially in the skin, impairs the patients' quality of life (prolonged cutaneous photosensitivity). The aim of this study was to engineer Lipid Nanoparticles (LNP) with different sizes and various PS contents, using simple, solvent-free and easily scale up manufacturing processes. Meso-tetra (hydro-xyphenyl) chlorin (mTHPC) is one of the most potent photodynamically active substances in clinical use[2] and it has been successfully applied in the treatment of various indications, such as the head and neck[3], prostate[4], pancreatic cancers[5]. Here, a derivative of mTHPC was efficiently incorporated into the lipid core of LNP, leading to a large range of stable and reproducible mTHPC-loaded LNP with narrow size distribution. The photophysical and photochemical properties of mTHPC-loaded LNP were studied by measuring absorbance and fluorescence spectra, colloidal stability, particle size and zeta potential, as well as singlet oxygen luminescence. The photocytotoxicity of three selected mTHPC-loaded LNP (25 nm, 45 nm and 95 nm of diameter respectively) was evaluated on MCF-7 cells in comparison to free mTHPC under irradiation at 652 nm with adjusted times to reach a range of light fluence (from 1 to 5 J/cm²). All the physical-chemical, photophysical and biological measurements performed allow us to conclude that LNP is a promising nano-drug delivery system for PDT.

Poster 15

Nanoparticles for fluorescent imaging, Magnetic Resonance Imaging and hyperthermia treatment

Inard C.

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To induce a temperature increase of cancer tumors has been known for long time to gift benefits in cancer therapy. However means used to realize this treatment remain very invasive increasing notably the patient mortality. A very promising strategy is localised hyperthermia, combined or not with chemotherapy. The localised heating of magnetic objects addressed on tumors is obtained under alternative magnetic field. Monodisperse iron nanoparticles, of which we control the synthesis, are completely adapted to a therapy of this type. These unique objects show a magnetic moment intensity which has no equivalent and which should ensure a very high thermal efficiency. Their stability and biocompatibility will be obtained by the growth of a protective shell (silica for example) which will have to be functionalised by cancer antibody or tumor selective peptides. This project associates competences of chemists, physicists, and cancerologists. Challenges related to the in vivo action and which will be taken up are the stability, the biocompatibility, the addressing and the absence of toxicity. Moreover, we will profit from the collaboration of the company Nanomeps, which is specialised in nanomaterials production and could develop these products.

Poster 16

Synthesis of biocompatible gold nanoconjugates for diagnostics applications

Fernández F.

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Methods for diagnostics and treatment of human cancers involving nanosized objects have undergone significant evolution in recent years. Nanotechnology offers novel materials and techniques to improve detection and to enable the optimization of clinical and biomolecular diagnosis of cancer diseases. The use of gold nanoparticles (GNP) is increasing due to their optical properties as contrast agents in imaging technology and their conjugation potential to synthesize gold probes. The GNP can be conjugated to specific antibodies against different cancer biomarkers. In this work, a covalent functionalization of GNP (20 nm) to antibodies by using heterobifunctional reagents is described. Two thiolated alkanes containing polyethileneglycol (PEG) units supplied by Polypure were employed, an acid-PEG-thiol and methyl-PEG-thiol in 2.5:7.5 molar proportion to bind to GNP. These reagents are excellent mediators of conjugation supplying to the gold probe stability and biocompatibility. After the chemical acid activation with succinimide and carbodiimide the particles were attached to antibodies via active ester method. The resultant goldprobes showed to be immunochemically active by surface plasmon resonance (SPR) enhancing biosensor performance. The bioconjugation method offers the possibility to conjugate different antibodies getting a biocompatible gold probe with high potential in imaging diagnostics field.

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Poster 17

3D fluorescence imaging of drug delivery for lung cancer therapy

Josserand V.

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Defining appropriated models of lung tumors in mice that are similar in morphology, histopathology and molecular characteristics to human adenocarcinomas, and scaling down imaging methods to feet the small size of rodents are of the outmost importance for the pre-clinical validation of new treatments, identification of markers as well as to understand the sequences of molecular events leading to lung tumors formation. High-resolution microtomography (micro-CT) as well as MRI are the more commonly used methods to imaging lung tumor growth in animal models. Nonetheless, these methods require expensive machines, and are providing anatomical information mainly. Near infrared fluorescence (NIRF) is commonly used in a Reflectance mode (2D), but the strong reflection of incident light and autofluorescence of the skin affects the sensitivity, especially in deep and absorbing tissues like lung, spleen or liver. We developed a fluorescence diffuse optical tomograph (fDOT) which allows fluorescence imaging even in highly attenuating and heterogeneous regions like lungs, by correcting the light propagation model from optical heterogeneities by using the transmitted excitation light measurements. It allows non-contact measurements and does not require animal immersion in an optical adaptation liquid. We used a tumor-targeted NIR probe which has been demonstrated to recognize integrin avb3, a receptor over-expressed in neo-angiogenesis. After intravenous injection of the probe, the system records the outgoing transmitted and fluorescence light, then reconstructs the 3D fluorescence distribution resulting from the accumulation of the probe into cancerous nodules. Whereas no significant evolution were noticed using the 2D fluorescence reflectance imaging confirming that it is not adequate for such analyzes, by using the 3D imaging system, we were able to do the longitudinal quantitative follow up of lung tumor development in live animals.

Poster 18

Development of 3D-protein chip for cancer diagnosis

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Immunoassays are classical methods for in vitro diagnosis and protein microarrays allowing high-throughput and parallel screening of various biological interactions with tiny amount of biological solutions. So miniaturized immunoassays performed on microarrays have the potential to be a powerful tool for the diagnosis of cancer. One of the crucial parameters in the elaboration of protein microarrays is the design of a well defined surface chemistry, which enables to retain biological activity of immobilized proteins on solid supports.We have developed innovative and customized method for 3D-protein chip manufacturing. Our versatile and flexible 3D-chips elaboration gives opportunity to test various surface chemistries in parallel (one chemistry/microreactor) for optimizing each probe/target system and biological interaction conditions (buffer solutions, pH, protein concentrations). For that purpose, we have functionalized the surface of 3D-chip with various chemistries for the covalent immobilization of tumor antigens: NHS ester surface, Maleic Anhydride surface and Carboxymethyl dextran surface. Biological activity of immobilized tumor antigen was evaluated in immunoassay with its antibody labelled with biotin and detected with streptavidine-Cy3. Results indicate that this biological recognition and the reproducibility of the immunoassay are highly dependent on the surface chemistry. Thus, the technology we have developed offers the opportunity to screen large set of probes and various immobilisation/biological recognition conditions in one assay.

Poster 19

Drug development for early diagnosis and cancer therapy

Sancey L.

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Introduction- Early and accurate detection of tumors is a major field of research in oncology. The generation of specific vectors, capable of transporting a drug or a contrast agent to the tumor and metastasis would be an asset for early diagnosis and cancer therapy. Our goal is to develop new treatments based on the use of tumor-targeted delivery of large biomolecules able to induce apoptosis. Nonetheless, the insufficient effectiveness of the vectorization systems is still a crucial issue. In this context, we generated new targeting vectors (as the RAFT-RGD) and nanoparticles for drug and biomolecules delivery. We evaluated these vectorization systems in live mice with optical imaging systems.

Methods-Results- First, the affinity of the RAFT-RGD toward soluble integrins was determined using FCS. This tetrameric RGD compound presents a tenfold higher affinity for its specific receptor compare to the one of the monomeric cRGD (i.e. KD = 3.87±1.42 nM vs. 41.70±17.13). Its internalization, observed using confocal microscopy and ELISA, occurred after binding to integrin $\alpha v\beta 3$ via small clathrin-coat pits. RAFT-RGD is a good vector for delivering small peptides or siRNA but we want to use it also for the targeted delivery of nanoparticules. We show that specific targeting critically depends on the quality of the postfunctionalization of the particles and more specially on the presence of poly(ethylene glycol) derivatives, with adapted chain length and electrostatic charges.

Conclusions- Specific targeting and efficient internalization are important issues for drug delivery. From this study, the multimeric presentation of cRGD motifs appears to be a prerequisite for the development of efficient integrin targeting and cell internalizing vectors for drug delivery to tumors. Furthermore, specific postfunctionalization of nanoparticles with RGD motives should improve tumor targeting and therefore let us consider targeted therapy after local activation of the nanoparticles.

Poster 20

Plasticizer free ion selective membranes for application in biomedical sensors

Mir Llorente M.

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The fields of biotechnology and biomedicine are becoming increasingly important in research and industry. Within this ever growing research field, the ability to control the adsorption of biomolecules to solid supports plays a key role in achieving reliable and competitive devices. For applications in sensing, affinity chromatography, biocatalysis and microfluidic handling it is important to build both a non-fouling surface, but also to have suitable recognition sites in order to attach specific ligands with desired orientation and coverage to ensure a reproducible and reliable response.Thus, the design of a sensor platform that exhibits a reproducible and stable surface with resistance to non-specific binding and well controlled ligand immobilization is of great relevance. The current methodology for ISE construction consists on the mixture of a plasticizer membrane based on polyvinyl chloride membranes and an ionophore receptor entrapped into this matrix[1]. However, this kind of membranes has problems of irreproducibility and stability on the surface[2]. With the purpose of overcome these problems, novel ion selective membranes based on polythiophene polymers were developed and compared with the polyvinyl chloride membranes. The thiol group in the polythiophene structure wills self-interact through a strong dative binding on the gold surface increasing the stability of the system.

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Poster 21

Characterization of dextran coated nanoparticles uptake by glioblastoma cells

Milagre R.

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Dextran coated iron oxide nanoparticles have been used as an experimental magnetic label for magnetic resonance imaging. Dextran-coated iron oxide nanoparticles provide enhancement of intracranial tumors by magnetic resonance imaging (MRI) and can be visualized histologically by iron staining. When linked to tumor targeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target tumor antigens with high specificity. The aim of this study is to determine the magnitude of dextran coated nanoparticles uptake by glioblastoma cells (in vitro), in order to use them as contrast agents in RMI and in hyperthermia treatments, with the purpose to be applied in tumoral diagnosis and treatment in vivo. We report the development and validation of an efficient protocol to label human glioblastoma cells with two different size dextran coated magnetic nanoparticles. We optimized both the concentration and the incubation time, achieving effective labeling of the glioblastoma cells in vitro, without impairment of their functional properties, proliferation or cell survival. The data have allowed us to define labeling protocols based on optimized doses and incubation times that did not affect short-term cells survival and proliferation. Uptake into tumor cells has been corroborated with results of immunocytochemistry and confocal microscopy. To test the detectability of these nanoparticles by MRI, increasing concentrations of nanoparticles were injected intracerebrally by stereotactic injection into the striatum. The results show that MRI can efficiently detect low nanoparticles concentration, and that the intensity of the signal is proportional to the number of injected nanoparticles. These studies demonstrate the efficacy of iron-based MRI contrast agents in the brain and provide imaging parameters and time course data for future studies in brain tumors and neurological lesions. Concerning magnetic hyperthermia assays, initial results show that the temperature we have reached is not high enough to destroy glioblastoma cells, so we need to optimize some parameters in order to overcome present limitations. Future advances in our group will be based on the development of new magnetic nanoparticles with enhanced RF absorption properties and new protocols for the supply of the RF power.

Poster 22

Electrochemical Biosensors for Medical Diagnostics

Sporer C.

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The detection, identification and quantification of specific proteins or pathogens in human body liquids at very low concentration are of tremendous importance for early diagnostics of diseases and general health care. Biosensors based on electrochemical detection principles are especially interesting for medical diagnostics since they allow the development of highly sensitive multi-analytical platforms that combine the advantages of fast measurements with very low detection limits compared to optical detection methods like ELISA tests. We are developing biosensors based on electrochemical impedance spectroscopy (EIS) to detect the Immunoreactions between antigens and specific antibodies, anchored onto sensor electrodes. This versatile methodology enables us to quantify disease marker proteins in a broad concentration range from pathogenic levels down to pico-mole concentrations in the detection limits. With this technique we could also demonstrate the detection of pathogenic E. coli bacteria in samples at very low concentrations down to 10cfu/mL without the need for time consuming sample enrichment steps on culture plates, demonstrating the high potential of the methodology for medical diagnostics. Here we'll present recent results of our research on high performance nanobased multianalyte biosensors and discuss the possibilities of integrating the biosensor platforms into medical point-of-care devices.

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Poster 23

Regulatory issues related to clinical transfer: assistance unit for innovative nanomedicines

Huot L.

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The transfer of research findings to clinical applications is governed by strict rules implemented by regulatory bodies. There is currently a lack of available or adapted information on the regulatory evaluation on nanomedicines so that they may fall under two regulatory categories: human pharmaceuticals or medical devices. Thus, Cancéropôle CLARA in association with Hospices Civils de Lyon and Université de Lyon has established a support team: the Regulatory Unit. This group of experts on regulatory issues (Quality, Safety, and Efficacy/Clinical evaluation) is advising researchers from the academy and the industrial (biotech start ups) environments, for regulatory-targeted actions concerning nanomedicines. Actions to facilitate the transfer from "bench to bed" in the mid to long term include counseling of multidisciplinary research teams as well as training sessions on the development of new molecules or devices at the nanoscale.

Poster 24

Socio-ethical impact of new technologies on patients with brain tumors

Salas Toquero C.

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We present a protocol to assess the impact of the use of nanotechnology in the care of patients with brain tumors. This study takes place in the context of clinical diagnosis and considers the use of four types of biopsy, conventional biopsy to nanoshell biopsy. The impact will be evaluated according to two dimensions: on the one hand an abstract dimension explored in former patients interviewed on their opinion about four different kinds of biopsy, on the other hand a concrete dimension explored in patients in real situation asked to choose between two kinds of biopsy (conventional biopsy versus silicon biopsy).

Poster 25

A novel methylation panel for the early detection of colorectal tumors in stool DNA

Capella G.

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Introduction: The aim of the present study was to establish applicability of methylation status of a new panel of gene promoters in order to improve sensitivity and specificity of our previous results as non-invasive tools for the early diagnosis of colorectal cancer.

Materials and Methods: A quantitative measure of methylation levels of DNA representing 807 genes was performed in 45 paired tumor-normal samples from 45 patients using the Illumina GoldenGate methylation Cancer Panel I. The 5 genes showing the highest difference in methylation levels between normal and tumor tissue were selected. A Methylation Specific-Melting Curve Asssay was developed for the 5 genes (SLIT2, TGFB2, TWIST1, TMEFF2 and NPY). Methylation status was initially analyzed in stool samples of 10 patients with colorectal carcinoma, 10 patients with adenomas and 10 healthy subjects.

Results: 80% of carcinomas (8 of 10) and 70% of adenomas (7 of 10) were positive for at least one methylation marker in stool DNA. The relative contribution of every marker to the detection of adenoma and/or carcinoma was: 50% (10 of 20) for NPY, 50% (8 of 16) for TWIST1, 46% (6 of 13) for TMEFF2, 21% (4 of 19) for SLIT2 and 23% (3 of 13) for TGFB2. Only one marker (NPY) scored methylated in one healthy subject (Specificity of the technique 90%).

Conclusions: The new methylation panel of biomarkers showed in this study may identify colonic premalignant and malignant neoplasic alterations with good sensitivity and specificity. Validation is larger series is warranted.

Poster 26

An efficient electrochemical multianalyte immunosensor for in-situ cancer therapeutic monitoring

Valera E.

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Cancer is one of the main causes of mortality in the entire world. To reduce its mortality, early detection is crucial but also is extremely important the monitoring of the tumor evolution and the progress of the applied therapies. Therefore there is a need to develop systems to monitor the illness evolution and therapeutic monitoring at the doctors place. In this way, biosensors appear as a good tool. The amperometric immunosensors have demonstrated to be a simple and a sensitive solution for the detection of wide variety of compounds. However, the multi-analyte detection based on that transduction remains being a challenge, mainly due to the need for specific labels. In this work, a novel amperometric method is presented. In this approach, semiconductors, such as ZnS, CdS and PbS, etc, nanoparticles will be used as specific non-interfering labels. Likewise, specific antibodies, produced against the different markers, will be labelled with these nanoparticles and used and read simultaneously. The assay format is the classical immunochemical competitive between the cancer markers or chemotherapeutic drugs present in the sample and a specific antigen coated on the surface of magnetic particles (MPs) for a limited amount of the specific antibody. The use of magnetic ì-particles let us to reduce the matrix effect and combined with a magnetic working electrodes offers also a simplified way to perform the electrochemical assay. Briefly, the sample is mixed with the appropriate set of specific functionalized magnetic particles and the corresponding IgGs cocktail is added to perform the immunochemical assay. Then, the MPs are captured by a magneto-graphite composite electrode (m-GEC). By this method, we are sure that the nanoparticles are always very close the electrode. Finally, by means of the well-known anodic stripping techniques, all the different nanoparticles are simultaneously read, and the corresponding amounts of metal ions are expressed as a current signal.

Poster 27

Interest of the RAFT-RGD for measuring angiogenesis and for the surgery of cancers

Keramidas M.

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Neo-angiogenesis plays an important role in the development and maintenance of all solid tumors. Integrins are proteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions. The first integrin found associated with tumor vasculature is áVâ3 and it has been established that this integrin is highly expressed on activated endothelial cells and tumor cells but is not present in resting endothelial cells and most normal organ systems. These characteristics make it a suitable target for anti-cancer therapy. The RAFT-(cRGD)4 is a vector binding to the integrin ávâ3, that can be used to image tumor and metastasis. The RAFT platform is used as a suitable scaffold to independently present the RGD motif and reporter groups. The upper face is linked to 4 copies of the c(RGDfK) peptide for ávâ3 targeting, and the bottom face is linked to a fluorescent agent for imaging. Our goal is to use this molecule labeled with a Near-Infrared (NIR) dye for: i) Measuring angiogenesis in vivo non-invasively. A 3D-fluorescent optical imaging technology and a model of angiogenesis based on the implantation of a cellulose sponge in Nude mouse were developped. We demonstrate that the accumulation of RAFT-RGD on the endothelial cells invading the sponge is correlated with the amount of neo-formed blood vessels and can be measured non invasively using our 3D in vivo fluorescence imaging method. We believe that this method will allow a direct evaluation of the efficiency of anti- or pro-angiogenic treatments in real time, ii) Improving tumor surgery using optics. NIR image-guided surgery using RAFT-(cRGD)4 and a portable clinical imaging device (« Fluobeam ») greatly improved the quality of the surgery of very small metastasis by doubling the number of detected nodules and significantly reducing the duration of surgery. In conclusion, RAFT-RGD and fluorescent imaging are efficient tools to evaluate the formation of angiogenesis, and can be applied to improve the quality of surgery of cancers.

Poster 28

New modality of curietherapy with holmium oxyde submicronic particles

Taleb J.

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Aim: Demonstrate that sub-micronic holmium oxide (OxHo) particles (NanoH™) can be activated without alteration of the particle structure, directly injected in a tumor with high intra-tumor permanence, without overall adverse effect, and with efficient anti-tumor effect.

Materials and Methods: Tumor model is based on the double xenograft of murine breast cells (1372 MAT B III cell lineage) in Fischer rats. 300nm sized particles containing about 90% of holmium oxide were synthetized (NanoH™) and activated (166Ho) through neutron irradiation. The activated particles were suspended in a solution of PVA and 10% ethanol to obtain a solution with a specific activity of 56.5 MBq/100µl and mass particle concentration of 7.7%. Each 8-10 mm diameter tumor were injected, at day 7 after tumor grafting, with 15 to 200MBq, both with a hypodermic syringe and with a high-kinetic injection system (CERMA™) in 7 rats (14 tumors). The volume of the tumors was daily measured/calculated in all rats. Animals were euthanized at day 14 after tumor grafting. Results: In the control rat population, the average volume of tumors at day 7, 11, 14 and 16 were respectively: 0.012cm^3(SD=0.038), 1.5cm^3(SD=0.98), 4.84cm^3(SD=2.97)and 8.8cm^3(SD=4), with an average volume increase between D7 and D11 of 46%. One rat died at D10 due to anaesthesia problems. In treated rats the average tumor volume at day 7, 11, 14 and 16 days was respectively: 0.95 cm^3(SD=0.43), 0.98 cm^3(SD=0.51) and 1.3 cm^3(SD=0.37) with an average volume increase between D7 and D11=9.9%. In 9/12 tumors, growth of tumor after Ho particle injection at D11 was inhibited (average volume increase=-9%, -43% to+27%). In 3/12 tumors the growth was observed (average volume increase=71%, 41% to +113%), maybe related to a bad injection (outside the tumor).

Conclusion: Submicronic neutron-activated particles are promising new agent for curietherapy by direct tumor injection, with perspectives of additional treatment in glioma and in peritoneal carcinosis.

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NOTES

7 CANCEROPÔLES in France

for accelerating translational cancer research

Since 2003, the Cancéropôles have become part of the cancer research landscape in France enabling a better coordination of resources and means and breaking down barriers. They give rise to large-scale research networks and infrastructures fostering interdisciplinarity.

The 7 regional or interregional hubs named Cancéropôles bring together research units of scientific institutions (INSERM, CNRS, universities, CEA, ...), university hospitals, comprehensive cancer centers, pharmaceutical companies and biotech players.

Their goals are mainly: (1) structure and coordinate research between institutions, at regional and inter-regional levels, (2) define specific strategic orientations based on the fields of excellence of each Cancéropôle, (3) create synergies with innovation and economic development stakeholders and (4) ensure adequacy between regional and national R&D policies in oncology, as promoted by the french National Cancer Institute (INCa).

High-level quality research projects supported by the INCa through the Cancéropôles networks should allow to better understand the molecular mechanisms involved in oncogenesis which is crucial to define new therapeutic targets and to develop innovative treatments for the benefit of all patients.

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