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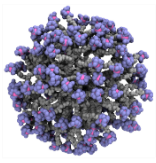

COST ACTION CA 17140
NANO2CLINIC
CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

2nd CA17140 STSM VIRTUAL CONFERENCE

BOOK OF ABSTRACTS

FEBRUARY 23, 2023

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In vivo tracking of Clathrin nanoparticles radiolabelled with Technetium-99m and Gallium-68

09:30 – 09:45 *Short oral*

A.Baião, F. Castro, C. Oliveira, R. Meel, and B. Sarmento

CD44v6-Targeted Chemotherapeutic Nanosystem Combined with Immunotherapy for Colorectal Cancer

09:45 – 10:00 *Short oral*

A. Apostolopoulou, P. Bouziotis and P. Koźmiński

Synthesis and characterization of Gold Nanoparticles and synthesis of a DOTA-derivative for the development of a novel cancer theranostic agent

10:00 – 10:15 *Short oral*

M.A. Karageorgou, Marija Mirković, S. Vranješ-Djurić, E. Stiliaris, P. Bouziotis and D. Stamopoulos

Technetium-99m labeled iron oxide nanoparticles coated with 2,3-dicarboxypropane-1,1-diphosphonic acid as a potential dual-modality SPECT/MR contrast agent

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M. Garofalo, M. Wieczorek, I. Anders, M. Staniszewska, S. Salmaso, P. Caliceti, B. Rinner, K. Pancer and L. Kuryk

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Novel oncolytic adenovirus Ad5/3-D24-ICOSL-CD40L co-administered with anti-PD-1 exhibits anti-cancer effect in H226 humanized mesothelioma model – Part II

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M. Garofalo, K. W. Pancer, M.a Wieczorek, M. Staniszewska, S. Salmaso, P. Caliceti1, L. Kuryk

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Fernando Torres Andon

Nanomedicines to target and reprogram tumor associated macrophages

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N. Fernández-Bertólez, A. Touzani, L. Martínez, A.T. Reis, S. Fraga, J.P. Teixeira, C. Costa, E. Pásaro, B. Laffon, V. Valdiglesias

Biocompatibility evaluation of CeO2 nanoparticles to be employed as nanodrugs in brain cancer nanomedicine

14:30 – 14:45 *Short oral*

A. P. Stavropoulou, E. K. Efthimiadou, P. Rocchi

Synthesis and biological evaluation of hybrid gold nanoparticles conjugated with an Anti-Sense Oligonucleotide

14:45 – 15:00 *Short oral*

K. Żelechowska-Matysiak, E. Salvanou, P. Bouziotis, A. Majkowska-Pilip

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DOX-PEG-198AuNPs-PEG-Tmab - multimodal radiobioconjugate for targeted radionuclide therapy of HER2+ cancers

❖ Physico-chemical characterization of nanodrugs Part II (15:00 – 16:30)

Session chair: Giovanna Lollo

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Bruno Sarmento

From nanoparticle mucodiffusion to receptor-mediated transcytosis, all aboard to fight colorectal cancer

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How CD147-specific nanomedicines end: from target engagement in 2D towards validation using a novel vascularized in vitro glioblastoma model

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F. Sousa, A. Cruz, F. J. Ferreira, J. Bessa, B. Sarmento, I. Mendes Pinto

Nanoencapsulated Bevacizumab Inhibits Glioblastoma Vascularization via Intratumoral VEGF Trapping

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16:15 – 16:30 *Short oral*

A. Tintaru, E. Laurini, A. Muselli and S. Pricl

Cancer Nanomedicine via Dendrimers Nanocarriers

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Session chair: Catarina Pacheco

16:30 – 16:45 *Short oral*

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Synthesis and characterization of nanofibers functionalized with nanoparticles with anticancer properties

17:15 – 17:30 *Short oral*

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T. Piotr, B. Klajnert-Maculewicz, A. Janaszewska, T. Strašák and M. Müllerová

Evaluation of cationic carbosilane dendrimers as nanovectors of therapeutic nucleic acids for cancer nanomedicine

17:30 – 17:45 *Short oral*

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18:00 – 18:15 *Short oral*

J. R. Ruiz, F. J. Mata, S. García-Gallego and G. Varchi

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❖ Physico-chemical characterization of nanodrugs Part III (18:15 – 19:30)

Session chair: Catarina Pacheco

18:15 – 18:30 *Short oral*

V.C. Ursachi, G. Dodi, S. Cordeiro, A.R. Fernandes, V. Balan

Functionalized chitosan based magnetic nanoparticles with potential in cancer therapy applications: physio-chemical characterization and biological studies

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L. Storozhuk, O. Sandre and N.T.K. Thanh

Iron Oxide Nanoflowers for Magnetic Hyperthermia Cancer Therapy

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E. Tomaszewska, D. Wróbel, K. Ranoszek-Soliwoda, K. Bednarczyk, G. Celichowski and J. Grobelny

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19:00 – 19:15 *Short oral*

N. Kalčec, A. Ljulj, L. Božičević, V. Vrček, D. Marson, S. Pricl, F. Separovic, R. Prassl and I. V. Vrček

Transformations of L-DOPA during the synthesis of gold-based nanodelivery systems for LAT- 1 targeting

19:15 – 19:30 *Short oral*

N. Peranić, M. Loparić, I. Vinković Vrček

Nanomechanical tool for characterization of cellular immune response

❖ Closure (19:30 – 19:45)

Presenter: Maria Francesca Ottaviani (Grant Awarding Coordinator)

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PLENARY LECTURES

Nanomedicines to target and reprogram tumor associated macrophages

Fernando Torres Andón[1,2], Clément Anfray[2], Aldo Ummarino[2], Tamara Dacoba[1], Carmen Fernández Varela[1], Iago Fernández Mariño[1], Alfonso Calvo[3], Alberto Mantovani[2], Paola Allavena[2], Eduardo Fernández Megía[7], Flavia Castro[8], María José Oliveira[8], José Crecente-Campo[1], María José Alonso[1]

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[2] Clinical and Research Hospital Humanitas, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), 20089 Rozzano, Milan, Italy.
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In the tumor microenvironment of solid tumors, tumor-associated macrophages (TAMs) play a key immunosuppressive role that limits the ability of the immune system to fight cancer and hinder the antitumoral efficacy of most treatments currently applied in the clinic, including immunotherapy[1]. As a continuation of our recent work on the combination of TLR agonists for the treatment of cancer through reprogramming TAMs into M1-like antitumor macrophages[2], we are working on the development of polymeric nanocapsules (NCs) loaded with these drugs to improve their stability, pharmacokinetic profile and systemic toxicity, thus allowing their application in the clinic.

We have developed a series of polymeric nanocapsules (NCs) loaded with poly(I:C) and/or R848. Among a series of lead 5 prototypes, protamine-NCs loaded with poly(I:C)+R848 were selected taking into account their size, charge and stability, and *in vitro* evaluation focused on the ability of the NCs to polarize macrophages towards M1-like antitumor effectors. The protamine-NCs loaded with poly(I:C)+R848 tested after intratumoral injection in lung cancer and fibrosarcoma murine models were able to prevent growth of primary tumor and inhibit metastasis. For the intravenous administration, we implemented an additional polymeric layer of hyaluronic acid functionalized with mannose to target the CD206 receptors overexpressed on the surface of TAMs. These NCs presented higher accumulation in a fibrosarcoma model.

In the frame of the COST action Nano2Clinic we have explored the antitumoral efficacy of selected TLR agonists in very aggressive and breast cancer models resistant to immunotherapy (i.e. anti-PD1). In particular, we tested *in vitro* models using immuno-spheroids with macrophages and human breast cancer cells and *in vivo* orthotopic murine models of triple-negative-breast cancer cells.
The polymeric nanocapsules loaded with the combination of poly(I:C)+R848 reprogram TAMs into M1-like antitumor macrophages in solid tumors: controlling the sustained activation of innate and adaptive immune responses, resulting in inhibition of tumor growth, prevention of metastasis and furnishment of antitumor immune memory. After intravenous administration of NCs functionalized with mannose present preferential accumulation in macrophages in a fibrosarcoma model. Preliminary results of these TLR-loaded-NCs various immunocompetent pre-clinical tumor models will be presented. Our work aims to provide information about benefits with respect to unmodified drugs,

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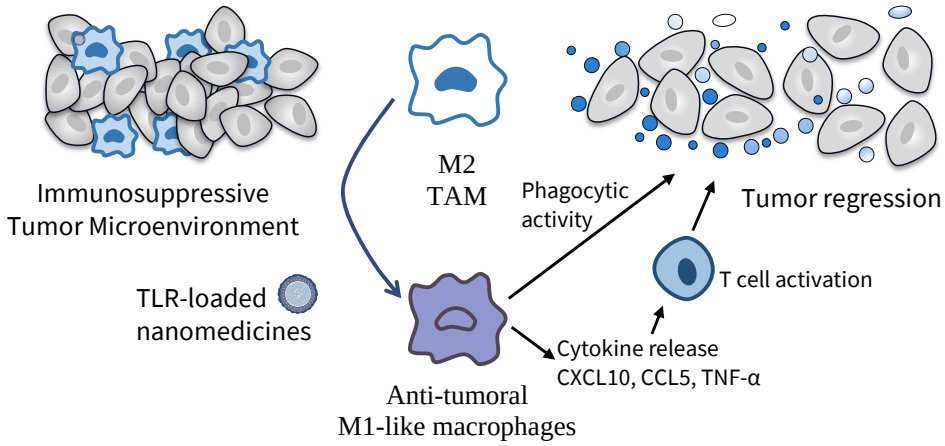
tumor targeting and reduced side effects, towards the final goal of clinical translation of nanomedicines to improve outcomes of patients with cancer.

- Acknowledgements

This work was supported by the 2^2-INTRATARGET project (A20/00028) funded by the ISCIII under the umbrella of the ERA NET EuroNanoMed GA N 723770 of the EU Horizon 2020 Research and Innovation Programme. FTA was supported by a grant by "Fundación de la Asociación Española Contra el Cáncer", an "Oportunious" program grant by Xunta de Galicia and the CA17140 COST Action.

FIGURE

Figure 1. Nanomedicines targeting and reprogramming tumor associated macrophages to fight against cancer.



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From nanoparticle mucodiffusion to receptor-mediated transcytosis, all aboard to fight colorectal cancer!

Bruno Sarmento

i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

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Our group is focused on the development of drug delivery systems, with special attention on nanotechnology, and their application to the pharmaceutical and biomedical fields. There is a particular interest in establishing bioengineering targeted nanomedicines for oral delivery of anti-tumor drugs for gastrointestinal tumours as gastric and colorectal cancer. The group also developed and validated novel in vitro cell-based intestinal model to evaluate the permeability and performance of drugs and drug delivery systems and proposed an innovative multicellular 3D colorectal cancer spheroid model used to screen efficacy of anticancer nanomedicines.
In this presentation, our most recent achievements of the establishment of nanomedicines, with passive and active targeting features for cancer cells will be described. Our approach involves innovative nanomedicines, with deep and comprehensive physical-chemical, in vitro and in vivo evaluation. Particular attention will be given to chitosan-based micelles for oral delivery of poor-soluble anticancer drugs and CD44v6-targeting nanomedicines containing chemotherapeutic and anti-angiogenic active molecules.

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SHORT ORAL PRESENTATIONS

Funded by the European Union

In vivo tracking of Clathrin nanoparticles radiolabelled with Technetium-99m and Gallium-68

O.L. Gobbo[1], S. Xanthopoulos[2] and P. Bouziotis[2]

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Introduction

The poor prognosis for brain tumours has been largely attributed to obstacles created by the blood–brain barrier (BBB). The BBB blocks accidental exposure to molecules and nanostructures such as viruses but, unfortunately, also blocks intentional traditional treatment of the tumours. We have designed an antibody-loaded Clathrin nanoparticle (CNP) which should be able to reach brain tumours by crossing the BBB or via intranasal (IN) administration. While a previous experiment has shown that these CNP could carry gadolinium across the BBB [1], we have investigated in vivo that our CNPs are able to reach the brain through the BBB or by IN administration using radiotracers such as Gallium-68 and Technetium-99m.

Results

Mice receive nasal administration either 68Ga-Clathrin or 99mTc-Clathrin and were culled after 90 minutes. The organs were harvested, weighed and quantification of radiotracer uptake was realized with a gamma counter (Figures 1 & 2).

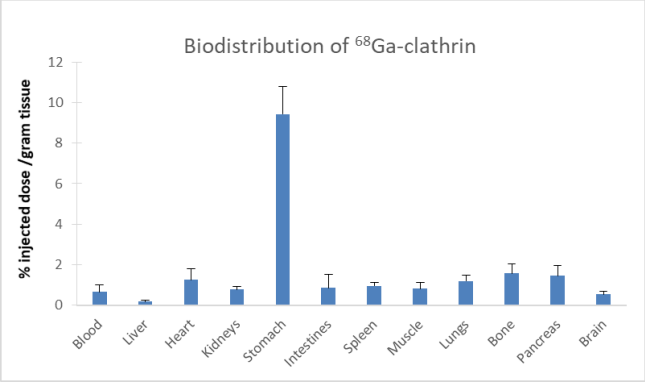

Figure 1. Biodistribution of 68Ga-clathrin in mice (n=3)

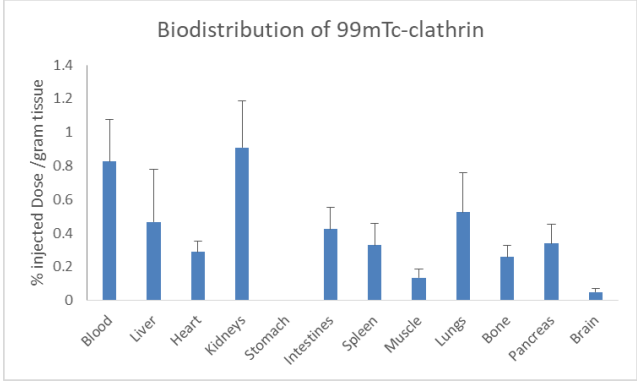

Figure 2: Biodistribution of 99mTc-clathrin in mice without stomach value (n=4)

Conclusion

With the small amount of clathrin we had for this study, we managed to get preliminary data showing that both Technetium-99m and Gallium-68 can be used for radiolabeling of clathrin.
Also, intranasal administration did not show an extraordinary concentration of clathrin in the brain. Preliminary data showed that IN administration of clathrin did not provide distinct advantage over iv delivery for a molecule passing through the BBB.

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CD44v6-Targeted Chemotherapeutic Nanosystem Combined with Immunotherapy for Colorectal Cancer

A. Baião[1,2,3], F. Castro[1,2], C. Oliveira[1,4], R. Meel[5], and B. Sarmento[1,2,6]

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Colorectal cancer (CRC) is one of the most frequently occurring and deadliest cancers worldwide, mainly due to its progression and metastatic behavior. Common chemotherapies for CRC are associated with severe side effects as they act non-selectively. However, monoclonal antibodies blocking the programmed cell death protein-1 (PD-1) and its ligand, PD-L1 axis, such as atezolizumab, have shown remarkable clinical efficacy in patients with various cancers, including microsatellite instability-high (MSI-H) CRC patients [1]. Thus, to improve CRC patients' outcomes, we propose an innovative combination therapy based on theco-administration of targeted polymeric nanoparticles loaded with irinotecan, and anti-PD-L1 atezolizumab, for a strong synergistic effect on anti-tumor efficacy. Nanoparticles will be targeted to the cluster of differentiation 44 containing exon 6 (CD44v6) since its overexpression in cancer cells has been associated with signaling pathways involved in CRC progression, being a potential therapeutic target. We have been studying CD44v6 targeting through functionalized polymeric nanoparticles decorated with a specific antibody fragment, which showed specific binding and internalization to cancer cells expressing CD44v6 [2]. Thenanosystem also had the potential to intracellularly deliver an anti-cancer drug into the cells. Future work includes the optimization of the nanosystem loaded with a chemotherapeutic, andthe biological impact assessment of the combination therapy using *in vitro* and *ex vivo* CRC models. The aim of the Short-Term Scientific Mission (STSM) Grant was to learn more aboutnanoimmunotherapeutics development and their evaluation using primary cells and animal models [3]. Consequently, we will apply the expertise obtained from the host institution on the nanosystems tailored to the immune system and primary cell-based assays to assess the anti-cancer potential of the combination therapy.

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Synthesis and characterization of Gold Nanoparticles and synthesis of a DOTA-derivative for the development of a novel cancer theranostic agent

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The purpose of this STSM was to synthesize 20 nm Gold Nanoparticles (AuNPs) at the Host Institute, using a modified Turkevich method. As a next step, the synthesized nanoparticles were characterized with techniques such as Dynamic Light Scattering (DLS) which greatly contributed to the improvement and quality of the study. Moreover, the AuNPs were supposed to be modified with a DOTA-derivative bearing a free thiol group which was synthesized at the Home Institute. With this modification, strong Au-S bonds are formed that stabilize gold nanoparticles [1]. At the Host Institute, we also had the chance to improve the synthesis of the DOTA-derivative and, as a result, to improve our final product, i.e. AuNPs functionalized with the DOTA-derivative. The synthesized DOTA-derivative was further purified by High Performance Liquid Chromatography (HPLC) and characterized by Mass Spectrometry (MS).

The general aim of this project is the development of new radiolabeled nanostructures with diagnostic and therapeutic properties for the diagnosis and treatment of metastatic prostate cancer. This can be achieved through the functionalization of Gold Nanoparticles with molecules that target the Prostate Specific Membrane Antigen (PSMA) which is overexpressed in metastatic prostate cancer and is a target for imaging and treatment. The modified nanoparticles can then be labeled with diagnostic [Gallium-68 (^{68}Ga)] and therapeutic radioisotopes [Lutetium-177 (^{177}Lu) and Actinium-225 (^{225}Ac)], via a suitable chelator (e.g. 1.4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid, DOTA). Thus, the labeled nanoparticles will target the PSMA that is overexpressed on prostate cancer cells, resulting in their accumulation in the tumor (targeted therapy). In the case of radioisotopes emitting positrons, such as ^{68}Ga, it will be possible to perform imaging with Positron Emission Tomography (PET). In the case of nanoparticles labeled with alpha- or beta-particle emitting isotopes, such as ^{177}Lu and ^{225}Ac, we can achieve selective targeting and killing of cancer cells without causing damage to healthy tissues [2].

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Technetium-99m labeled iron oxide nanoparticles coated with 2,3-dicarboxypropane-1,1-diphosphonic acid as a potential dual-modality SPECT/MR contrast agent

M.A. Karageorgou[1], Marija Mirković[2], S. Vranješ-Djurić[2], E. Stiliaris[1], P. Bouziotis[3] and D. Stamopoulos[1,4]

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Dual-modality contrast agents (DMCAs) constitute a powerful tool in diagnostic applications, since they are able to overcome the limitations associated with one imaging modality and assure enhanced interpretation of diseases *in vivo*, by providing synergistic information [1]. For this reason, radiolabeled iron oxide nanoparticles are widely employed in diagnosis over the last decade as single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) DMCAs [1]. In this respect, our study focuses on the synthesis and both *in vitro* and *in vivo* evaluation of ^{99m}Tc labeled iron oxide (namely, Fe_3O_4) nanoparticles coated with 2,3-dicarboxypropane-1,1-diphosphonic acid (noted as DPD), as a potential SPECT/MRI DMCA [2,3]. *In vitro* studies in phosphate buffer saline (PBS) pH 7.4 and human serum were performed to roughly evaluate the stability of the DMCA *in vivo*. Referring to the *in vivo* evaluation, biodistribution, MR imaging and gamma-camera imaging studies of the ^{99m}Tc-DPD-Fe_3O_4 DMCA were performed in healthy mice to study its imaging efficacy. The results showed that the DMCA exhibited high radiolabeling yield (95%) and proved to be stable *in vitro*. The biodistribution study showed noticeable liver accumulation up to 24 h post injection, followed by the kidneys and spleen at lower percentages. Both gamma camera and MRI imaging studies performed in healthy mice provided consistent results with the ones obtained from the biodistribution study, ultimately indicating the efficacy of ^{99m}Tc-DPD-Fe_3O_4 DMCA as potential SPECT/MRI DMCA in future diagnostic applications [2,3].

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Oncolytic adenovirus Ad5/3-D24-ICOSL-CD40L as a novel strategy for mesothelioma therapy – Part I

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Leveraging the patient's immune system to fight cancer is not a novel strategy and has been investigated by many researchers worldwide [1]. Oncolytic viruses (OVs) induces anti-tumour responses through selective self-replication within cancer tissue and mediates systemic immunostimulation [2]. Talimogene laherparepvec (T-VEC), is the first FDA-approved oncolytic virus product for melanoma treatment given locally. However, the systemic administration of oncolytic vectors still faces unmet challenges, including the presence of neutralizing antibodies and inefficient targeting delivery efficacy. Currently, state-of-the-art progress has been made in the development of systemic delivery of oncolytic agents, which demonstrates a promising step toward broadening the scope of cancer immunotherapy and improving the clinical efficacy. Considerable attention has been given to extracellular vesicles (EVs). EVs have been proposed as drug delivery tools for anti-cancer therapies. The aim of the study was to design and produce, by using engineering tools, a novel oncolytic adenovirus AdV5/3-D24-ICOSL-CD40L expressing potent co-stimulatory molecules enhancing clinical efficacy through the modulation of anti-cancer immune responses in mesothelioma therapy. Furthermore, we wanted to investigate whether mesothelioma-originated EVs can be used as a vehicle for targeted delivery of AdV5/3-D24-ICOSL-CD40L to mesothelioma cells. The efficacy was confirmed *in vitro* in three mesothelioma cell lines - H226, Mero-82 and MSTO-211H, and subsequently antineoplastic properties in combination with anti-PD-1, was evaluated in xenograft H226 mesothelioma nude BALB/c mouse models. Anti-cancer efficacy was attributed to reduced tumour volume. The combinatorial regime was the most potent in immunogenic cell death induction compared to monotherapy. Our findings support the systemic administration of EVs formulations with OVs as a potential strategy aimed at treating primary and metastatic cancers. Collectively results support further assessment of the AdV5/3-D24-ICOSL-CD40L in combination with checkpoint inhibitors as novel therapeutic perspectives for the treatment of mesothelioma.

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Novel oncolytic adenovirus Ad5/3-D24-ICOSL-CD40L co-administered with anti-PD-1 exhibits anti-cancer effect in H226 humanized mesothelioma model – Part II

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Malignant mesothelioma (MM) is an aggressive and very rare type of carcinoma [1]. The worldwide incidence of this malignancy has increased over the last decade, and an elevation in the number of cases in the future is anticipated. Although new treatment options are currently available, they are not curative, and new treatments are highly needed to provide hope for patients. In this study we evaluated the anti-tumour efficacy of the combinatorial therapy: AdV5/3-D24-ICOSL-CD40L, with an anti-PD-1 antibody in humanized mesothelioma NSG model. Subsequently we have assessed the biodistribution profile of the vector complexed with extracellular vesicles (EVs) in syngeneic mesothelioma AB12 model. Anti-cancer efficacy was attributed to reduced tumour volume and enhanced infiltration of tumour infiltrating lymphocytes, including activated cytotoxic T cells (GrB+CD8+). We additionally observed a negative correlation between tumour volume and CD4+ tumour infiltrating lymphocytes. Gene set enrichment analysis suggests that the combinatorial therapy results in changes to the expression of genes belonging to the "adaptive immune response" GO category. The presence of the fluorescent signal (EVs-virus-ICG labelled) at the tumour site may suggest tumour targeted delivery. Collectively, combinatorial therapy involving oncolytic adenovirus with checkpoint inhibitors may improve anti-cancer effectiveness and survival by targeted cancer cell destruction and triggering of immunogenic cell death.

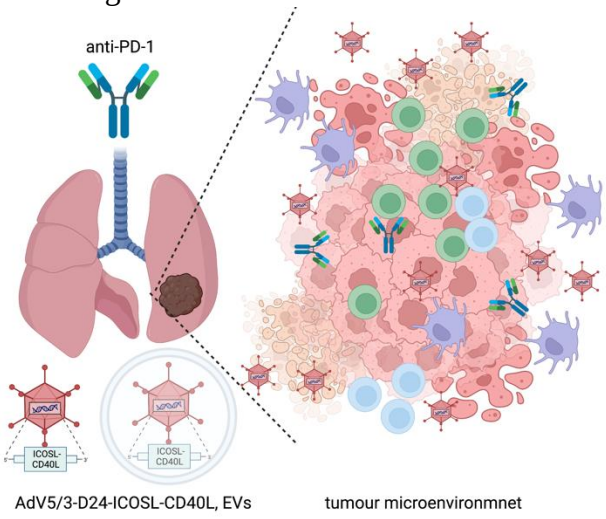

Figure 1. Schematic overview of the project.

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Synthesis of Magnetic Nanoparticles for Application in Anticancer Drug Delivery

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Magnetic nanoparticles (NPs) coated by piezoelectric perovskite (ABX_3) structure are promising for controlled and targeted drug delivery, as well as on-demand externally controlled drug release at target site. Under an external magnetic field, these NPs can generate a sufficient electric field around the plasma membrane, causing a change in cell membrane porosity, and providing open pores for NP entry and drug delivery. Moreover, they are capable of differentiating normal and cancer cells due to differences in their threshold field for electroporation, enabling their specificity towards cancer cells[*].

Our aim was to synthesize monodisperse magnetic NPs which have potential applications in cancer treatment as a drug carrier. For this purpose, superparamagnetic spherical cobalt ferrite (CF) NPs were synthesized by thermal decomposition method. Then, they were coated with polyvinylpyrrolidone (PVP) or meso-2,3-dimercaptosuccinic acid (DMSA) via ligand exchange reaction to make them water dispersable. Further functionalization of water- dispersable NPs with $BaTiO_3$ was carried out by sol gel method. The structural and magnetic properties of the NPs were characterized by X-ray diffraction (XRD), transmission electron microscope (TEM), Fourier-transform infrared spectroscopy (FTIR), zeta sizer, thermogravimetric analysis (TGA), vibrating sample magnetometer (VSM) option of SQUID and inductively coupled plasma mass spectrometry (ICP-MS). The results show that DMSA- coated NPs are colloidally stable, however, $BaTiO_3$ coating procedure needs optimization to get the nanoparticles without impurity. Magnetoelectric property of the NPs should be investigated for future studies.

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Magnetic nanosystems interactions with human induced pluripotent stem cells

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The field of molecular imaging in living systems, has expanded tremendously over the past century [1]. Magnetic iron oxide nanoparticles have attracted growing attention due to their biocompatibility, magnetic functionality, surface-to-volume ratio and potential applications in biomedicine, especially as magnetic resonance imaging (MRI) diagnostic vehicles [2]. In this study, we report a strategy divided in several steps, for the development and evaluation of potential MRI contrast agent candidates, namely: (1) synthesis and characterization by multiple methods of magnetic iron oxide nanoparticles conjugated with silica and glucose chains, (2) cell viability assays using MTT tests performed on FiPS mR5F-6 human induced pluripotent stem cells (hiPSCs) line and (3) *in vivo* MRI biodistribution of the obtained magnetic nanoparticle probe on healthy experimental animals using 1 T PET/MRI scanner. In summary, magnetic silica-glucose Fe_3O_4 nanoparticles were obtained by a two-step facile chemical route: iron oxide core obtained by co-precipitation method followed by multifunctional silica-glucose shell chain coverage. The physicochemical properties of the functionalized magnetic materials, in terms of size distribution, surface charge, structure and magnetic properties, were evaluated. Dynamic light scattering data indicated adequate size distribution and a positive zeta potential. The magnetic nanosystems contain magnetic iron oxide core, silica and glucose shell as confirmed by FT-IR spectroscopy, and have magnetic properties as showed by vibrating-sample magnetometer analysis. The MTT results revealed that the conjugates were non-cytotoxic for hiPSCs and showed at least 80 % cell viability, even at higher concentrations after 48 h incubation. The *in vivo* studies confirm that these new molecular imaging probes based on magnetic nanoparticles can be used as MRI contrast agents.

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INNOVATIVE OPHTHALMIC FORMULATION FOR THE PREVENTION AND TREATMENT OF PTERYGIUM AND OTHER RELATED DISEASE

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Pterygium is a progressive disease of the human eye arising from sub-conjunctival tis- sue and extending onto the cornea. Due to its invasive growth, pterygium can reach the pupil compromising visual function. Currently available medical treatments have limited success in suppressing efficiently the disease. Previous studies have demonstrated that curcumin, polyphenol isolated from the rhizome of Curcuma longa, induces apoptosis of human pterygium fibroblasts in a dose- and time-dependent manner showing promising activity in the treatment of this ophthalmic disease [1]. However, this molecule is not very soluble in water in either neutral or acidic pH and is only slightly more soluble in alkaline conditions, while its dissolving in organic solvents drastically reduces its potential use for biomedical applications. A nanoformulation of curcumin stabilized silver nanoparticles (Cur-AgNPs) seems an effective strategy to increase the bioavailability of curcumin without inducing toxic effects [2]. In fact, silver nitrates have been used safely for the treatment of many ophthalmic conditions and diseases for a long time and the concentration of AgNPs in this formulation is quite low. The synthesis of this new compound was achieved through a modified Bettini's method [3] adapted to improve the quality of the product intended for human use. Indeed, the pH of the reaction was changed to 9, the temperature of the reaction was increased from 90 ◦C to 100 ◦C and after the synthesis the Cur-AgNPs were dispersed in Borax buffer using a dialysis step to improve the biocompatibility of the formulation. This new compound will be able to deliver both components (curcumin and silver) at the same time to the affected tissue, representing an alternative and a more sophisticated strategy for the treatment of human pterygium. Further in vitro and in vivo assays will be required to validate this formulation.

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INNOVATIVE OPHTHALMIC FORMULATION FOR THE PREVENTION AND TREATMENT OF PTERYGIUM AND OTHER RELATED DISEASE

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The aims of my STMS has been to investigate the synthesis of curcumin-caged Silver nanoparticles for the treatment of pterygium, a cancer of the front of the eye arising from sub-conjunctival tissue that is able to invade the cornea, as defined by The New York Eye Cancer Centre. Due to its progressive growth, pterygium can reach the pupil reducing visual function, leading up to blindness. Several medical therapies, such as the administration of thiotepa, 5-fluoruracil, mitomycin C and VEGF inhibitors have not led to very satisfactory outcomes. It has been shown that curcumin, possesses potent antioxidant, anti-inflammatory and anti-carcinogenic properties [1]. In previous studies has been demonstrated that curcumin significantly inhibits human pterygium fibroblast proliferation and induces apoptosis of these cells in a dose- and time-dependent manner [2]. The chemical structure of curcumin, unfortunately, makes it very little soluble in water at acid and neutral pH but quite water soluble in alkaline conditions, soluble in dimethyl sulfoxide (DMSO), ethanol and acetone, drastically reducing its biomedical applications. Various studies have reported that the bioavailability of curcumin could be increased by conjugating it with metal nanoparticles [3]. The purpose of this study is to investigate as the bond with Silver nanoparticles aids curcuminoids in permeating into cell membrane, solving the pharmacokinetics problems of curcumin in order to improve its bioavailability. I investigated the effects of *in vitro* treatment with Curcumin-Silver-Nanoparticles (Cu-AgNPs) on human-derived pterygium keratinocytes culture, exploiting the synergic action of both curcumin and silver as nanocompound.

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Study on Rose Bengal/polymersome and luciferase/polymersome hybrid structures

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Photodynamic therapy is based on application of photosensitizers that after irradiation by light with a specified wavelength produce reactive oxygen species and singlet oxygen, which start the cascade of reactions leading to cell death [1,2]. Furthermore, the efficiency of photosensitizers depends on local accumulation and specific cellular uptake, motivating research concentrated on the development of delivery systems. One of the promising nanocarriers is a pH-sensitive polymersome. A trademark of our system is the capability of selective opening and closing of the polymersome under the influence of pH change. Such properties enable the encapsulation of rose bengal and then its release under strictly controlled conditions [3]. Addition of luciferase may increase phototoxicity of rose bengal in hole nanosystem [4].

In the first step polymersome A were used in order to encapsulate enzyme – luciferase, and polymersome B for caring rose bengal, luciferin, and mixture of rose bengal – luciferin . Applying established protocols both polymersomes were assembled and encapsulated with proper compounds. Moreover, cryogenic transmission electron microscopy imaging of evaluated compounds was performed to prove the proper assembling and size of nanocarriers. In the second step asymmetrical flow field-flow fractionation experiments were performed. Finally, calibrated AF4 was performed for RB Psome B. In the third part, luciferase assay was calibrated and performed using established protocol as a control of enzymatic reaction leading to luminescence. Then using encapsulated enzyme in polymersome A the same assay was performed to evaluate ability of activation of luciferin by encapsulated luciferase.

The first results performed during the STSM open new possibilities to use evaluated nanosystems in photodynamic therapy. However, complexity of this nanosystem requires further studies to fully evaluate its properties. Future collaboration allows to improve tested nanosystem, evaluate in in vitro as well as in vivo experiments.

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Extracellular vesicles as a next-generation drug delivery vehicles for precision cancer treatment

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Extracellular vesicles (EVs) are naturally occurring cargo-delivery vesicles with a role in physiological and pathological processes, including tumorigenesis. It has been reported that cancer-derived EVs have the capability to selectively target the tumor tissue originating the vesicles and to deliver a large variety of macromolecules (1). Therefore, EVs are an interesting drug delivery tool for anti-cancer therapies, although the limited knowledge about their *in vivo* tropism still hinders their therapeutic applications (2). Herein, EVs were generated from cancer cell lines and loaded with the fluorescent dye DiIC18(5); 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine, 4-chlorobenz-enesulfonate salt (DID), which is often used for *in vivo* applications for its high tissue penetrance and low interference with tissue autofluorescence. Then, *in vivo* and *ex vivo* imaging technologies were used, as detection system to characterize the whole-body biodistribution of the EV-formulations. Interestingly, the systemic administration of EV formulations *in vivo* demonstrated a selective accumulation of the fluorescence signal 24 hours after injection at the tumor site, thus suggesting a tumor-specific tropism. Further studies will provide a rational basis for developing novel biocompatible EV-based nanoparticles for the tumor-selective delivery of therapeutic agents.

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Light up a fire inside the tumor by combining oncolytic viruses with immune-checkpoint inhibitors

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Cancer cells have evolved diverse mechanisms to avoid immune surveillance generating a "cold" immunosuppressive tumour microenvironment to evade the immune attack, resulting in tumour growth and progression [1]. In this scenario oncolytic viruses (OVs), which are vectors able to specifically kill cancer cells spearing the healthy ones, represent a novel class of innate immunity activators able to allow the "cold- to-hot" conversion of the tumor microenvironment. Interestingly, the combination therapies of OVs with check-point inhibitors are an encouraging strategy for lighting up a fire inside the tumour thus generating a more immunogenic tumor microenvironment [2]. We have hypothesized that by testing the administration of a newly cloned oncolytic adenovirus Ad5/3-D24-ICOSL-CD40L expressing co-stimulatory molecules ICOSL and CD40L we could induce the production of tumour infiltrating lymphocytes. We firstly showed that the therapy with the virus in combination with anti-PD1 was the most effective regimen in melanoma and mesothelioma animal models. Moreover, the observed anti-cancer effect positively correlated with cytotoxic CD8+ tumour-infiltrating lymphocytes exerting a central role in the tumour volume control. Altogether our findings highlight the potentialities of the combination approach for the treatment of solid malignancies such as melanoma and mesothelioma.

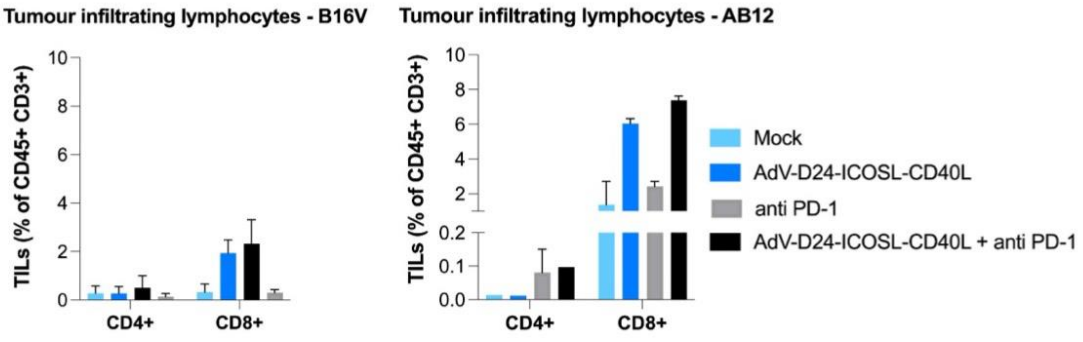

Figure 1. Anti-cancer and immunomodulatory properties of tested agents in immunocompetent mouse models

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Modification of dihydropyridine-based nanoparticles to enhance siRNA and drug delivery to glioblastoma cells

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Gene therapy, and specifically the use of small interfering RNAs (siRNAs), offers the possibility to treat diseases as glioblastoma, not effectively cured yet with the traditional pharmacology. Nevertheless, this approach presents some challenges, as the degradation of siRNAs in the extracellular medium by the action of RNases, and their difficulty to enter the cells by their own because of their negative charge, what makes them unlikely to interact with the cell membrane [1]. Several transfection vectors have been developed to give answer to this problem, from which non-viral vectors offer advantages as low immunogenicity, lower production costs, higher safety, and the possibility to perform a wide range of modifications on their surface, compared to the traditionally used viruses [2]. Among them, liposomes have been widely studied due to their biocompatibility, transfection efficiency, and the ability to carry both hydrophobic and hydrophilic drugs. Specifically, self-assembling lipid-like 1,4-dihydropiridines have been shown to be able to successfully transfect pDNA into different cell lines *in vitro* [3], showing inherent antimicrobial and antitumoral properties as well [4]. The aim of this work is to synthesize and characterize self-assembling lipid-like 1,4-dihydropiridine derivatives with the addition of arginine peptides and different dodecyloxycarbonyl substituents for siRNA transfection of glioblastoma cells, looking thus for an innovative non-viral gene therapy to treat this disease. Preliminary experiments have shown that part of the nanoparticles synthesized are able to complex the siRNA and show no toxicity in glioblastoma cells, despite are not able to protect it from RNase degradation. These results suggest the need of keeping doing modifications in these nanoparticles to achieve siRNA protection and, subsequently, an effective transfection.

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SCALE-UP MANUFACTURING OF DOCETAXEL-LOADED POLYMERIC NANOPARTICLES FOR TARGETED CANCER CHEMOTHERAPY

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Glioblastoma (GBM) is the most common and lethal type of primary brain tumor. The 5-year survival of GBM patients is still limited to a dismal 5%, highlighting the need to advance more effective GBM therapies. GBM tissue presents an abnormal expression of the L-type amino acid transporter 1 (LAT1), for which histidine (His) is an inexpensive and powerful targeting ligand [1]. Although His is expected to provide higher accumulation of drug nanoparticles (NPs) into GBM cells via LAT1 binding, consequently enhancing the anti-tumor response, it has been poorly explored in GBM-targeted therapies. Thus, this project proposes GBM-targeted, His-functionalized polymeric NPs loaded with docetaxel as a novel anti-cancer nanomedicine. The overall objective of this STSM was to profit from the technology transfer competences of the Host Institution to increase the efficiency of the production of His-functionalized polymeric NPs well-established at the Home Institution. The microreactor technologies of the Host Institution were expected to offer a highly reproducible up-scaling, cost- and environmental-friendly production of these docetaxel-loaded polymeric NPs, while still maintaining the quality of the existing lab-scale formulation.

His-functionalized polymeric NPs demonstrated scale-independent 250 nm size, 0.2 polydispersity index, 70% drug entrapment efficiency and a controlled drug release over 48 h. The GBM cell binding of the NPs was 2.5-times higher than non-His-functionalized NPs in all tested GBM cell lines (U-251 MG, U-373 MG, U-87 MG). The His-functionalized NPs were further validated in a GBM 3D spheroid construct, which was generated through high-throughput 3D modeling of U-251 MG tumor cells, tissue differentiated macrophages isolated from peripheral monocytes, and brain microvascular primary endothelial cells. Immunohistochemistry revealed the spatial distribution of tumor-associated vimentin, extracellular matrix fibronectin, CD68 macrophage marker and CD31 EC marker within the 3D spheroid construct. Docetaxel-loaded His-functionalized polymeric NPs drastically disturbed the morphology of the spheroid tumor core, suggesting a significantly higher level of cytotoxicity compared to the same dose of the free drug control.

This work allowed the exploitation of His functionalization to synthesize cost-effective GBM-targeted chemotherapeutic NPs with capacity to undergo a significantly higher accumulation within tumor cells and disrupt the tumor core of a tumor/macrophages/ECs crosstalk GBM 3D spheroid construct.

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EXPLOITING THE USEFULNESS OF MICROFLUIDIC DEVICES TO MANUFACTURE NOVEL SYSTEMS IN THE FIELD OF CANCER NANOMEDICINE

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The 5-year survival of glioblastoma (GBM) patients is limited to a dismal 5%, highlighting the need to advance more effective therapies. GBM tissue abnormally overexpresses the L-type amino acid transporter 1 (LAT1), for which L-histidine (His) is an inexpensive and powerful targeting ligand. Thus, we propose the chemical modification of a conventional chemo-immunogenic drug, docetaxel, into a nanomedicine with surface-His (nano-DTX-His) to target GBM tissue via LAT1 adhesive binding and further augment localized cell death. Since nano-DTX-His cannot be used for intravenous therapies due its inability to cross the blood-brain barrier (BBB) per se, we further propose its modification with an acid-cleavable Angiopep-2 layer (nano-DTX-His-clv-Angiopep2) to favor BBB translocation. It is of important note that the choice of DTX is based on its IC50, which is around 20.000-times lower than the standard temozolomide [1]. Monodisperse nano-DTX-His was manufactured with c.a. 250 nm and a controlled docetaxel release over 48 h. The uptake of nano-DTX-His was 3.5-times higher than nano-DTX-ØHis in U-87 MG, U-251 MG and U-373 MG conventional GBM cell lines. In GBM invasive margin (GIN) patient cells, cell uptake was 8-times higher than the controls. 2D studies of cell viability in GIN cells demonstrated an anti-cancer potential of nano-DTX-His 50% superior compared to the controls. Nano-DTX-His-clv-Angiopep2 was able to provide higher BBB translocation of docetaxel compared to nano-DTX-His and nano-DTX-His-Øclv-Angiopep2 in hCMEC/D3 Transwell® systems. A biodistribution in vivo trial confirmed a 3-fold enhancement of NPs accumulation into the brain by using nano-DTX-His-clv-Angiopep2. Lastly, the in vivo anti-tumor efficacy was validated in GBM orthotopic models following either intratumoral or intravenous administration. Median survival and number of long-term survivors was increased by 50%. Histological, hematologic or blood biochemical systemic toxicity was not detected. Altogether, a preclinical proof of concept was provided to support nano-DTX-His-clv-Angiopep2 as an effective anti-GBM multistage chemotherapeutic strategy, with ability to respond to multiple fronts of the GBM microenvironment.

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Quantification of poly(aneu)ploid cells with confocal imaging

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Multinucleation is a frequently observed phenomenon, which occurs in normal tissues during *inter alia* early embryonic development or differentiation of bone resorbing osteoclasts. However, growing body of evidence links cancer resistance to chemotherapy and radiation with formation of giant, polynucleated cells. As long as physiologically relevant multinucleation is tightly controlled and comprises the pathways such as endoreduplication, acytokinetyc divisions or fusion of monocytes/macrophages, some cancer cells respond to damaging agents by entering a dormant phase after complete or partial multiplication of DNA. These poly(aneu)ploid cells have reported potency to repopulation and giving rise to entire tumors, thereby leading to cancer relapse. The number of these cells as well as their poly(aneu)ploidy may serve as predictive markers for chemo- or radiotherapy success, and indication for further anticancer approaches. Therefore, we decided to work out a method for quantification of poly(aneu)ploid cells in the culture based on the imaging of fluorescently stained DNA followed by automated software workflow. For this purpose we made use of p53-mutated breast cancer cell line – MDA-MB-231 and developed cisplatin- and doxorubicin-resistant subtypes, which are primed to formation of DNA-enriched cells. To induce multinucleation cells were exposed to one dose of doxorubicin and DNA was stained with DAPI. Non-resistant, untreated cells served as negative control. For nucleus area-based quantification of single, double and multinucleated cells the pictures of cells embedded in ProLong™ Gold Antifade Mountant with DAPI were acquired with Eclipse Ti2-E inverted microscope with C2 confocal system (Nikon, Austria) and the size of DAPI area was analysed with NIS_Elements / General Analysis (GA3) Software from Nikon. For nucleus volume-based quantification pictures of cell population (5-10, depending on the Z-score) were acquired using the confocal laser scanning microscope TCS SP8 (Leica Microsystems, Germany) and 3D images were performed in Leica Application Suite X (LAS X, Leica Microsystems, Germany). Counting and measuring fluorescently-labeled nuclei in an image field included adjustment of intensity, noise level, gamma, opacity, various projection modes, pre-threshold filtering, adjusting thresholds, performing binary image filtering, binary editing, and filtering object size. Subsequently, all data were exported as a spreadsheet. The grouped distribution series of both DNA area and volume data allowed to identify G1, S, G2 phases, which correspond to 1-2N of DNA, in the negative control. Even here, poly(aneu)ploid cells were detected and accounted for ~6.5%, whereas in cisplatin- and doxorubicin-resistant phenotypes accounted similarly for ~11%. Anthracycline caused a substantial increase in the fraction of multinucleated cells up to ~12% in the population of non-resistant MDA-MB-231 cells, and up to ~25-30% in drug-resistant subtypes. Inhibition of DNA repair pathways with FDA approved PARP inhibitors such as Olaparib and Niraparib, which are recommended for patients with deleterious or suspected deleterious germline *BRCA* mutations, led to considerable enrichment of poly(aneu)ploid cells up to ~60% in all studied phenotypes. Similar, but less striking effect was observed for ATP-binding cassette (ABC) inhibitors such as Tariquidar (P-glycoprotein) or MK-571 (ABCC), which limit drug efflux, thereby inducing higher accumulation of anticancer agents inside cancer cells. In summary, fluorescent staining of DNA associated with confocal imaging and picture processing allows to identify and quantify poly(aneu)ploid cells in 2D culture. Further efforts are needed to adapt the described method to search for giant cells inside 3D cell cultures and tumors.

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Evaluation and validation of the suitability of the standardised *in vitro* mammalian cell Micronucleus (MN) test (OECD TG No. 487) for testing genotoxicity of nanodrugs with potential use in cancer nanomedicine

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Standard toxicity tests may not be entirely adequate for evaluating nanomaterials, since their unique physicochemical properties are also responsible for unexpected interactions [1]. The *in vitro* cytokinesis-block micronucleus (CBMN) test is recommended for genotoxicity testing of pharmaceuticals intended for human use [2], but cytochalasin-B (Cyt-B) used in this test to block cytokinesis may interfere with nanoparticles (NP), leading to inaccurate results. Our aim was to determine whether Cyt-B could interfere with micronuclei (MN) induction by TiO_2NP in human SH-SY5Y cells, as evaluated by CBMN test. Cells were exposed for 6 or 24h, according to three treatment options: co-treatment with Cyt-B, post-treatment, and delayed co-treatment. Influence of Cyt-B on TiO_2NP cellular uptake, and MN induction as evaluated by flow cytometry (FCMN) were also assessed. TiO_2NP were significantly internalized by SH-SY5Y cells, both in the absence and in the presence of Cyt-B, indicating that this chemical does not interfere with NP uptake. Dose-dependent increases in MN rates were obtained in CBMN test after co-treatment and post-treatment, while FCMN assay only showed a positive response when Cyt-B was added concurrently with TiO_2NP, suggesting that Cyt-B might alter CBMN assay outcomes. Still, no differences were observed in the comparisons between the three treatment options evaluated. Post-treatment and delayed co-treatment with Cyt-B, proposed by OECD [3] for CBMN test when applied to nanomaterials, do not seem to be suitable alternatives to avoid Cyt-B interference under the specific conditions settled in this study. Consequently, further research is needed to define additional protocol alternatives of CBMN assay for accurately assessing genotoxicity of nanomaterials.

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Biocompatibility evaluation of CeO_2 nanoparticles to be employed as nanodrugs in brain cancer nanomedicine

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Cerium dioxide nanoparticles (CeO_2NP) have recently gained attention for their unique structure-dependent properties, antioxidant enzyme-like behaviour, ROS scavenging activity and great potential for biomedical applications. In addition to their antioxidant and anti-inflammatory activity, CeO_2NP are also known to exhibit anticancer potential, providing an attractive opportunity for use in cancer therapy, as a pharmacological agent and/or in drug/gene delivery systems [1]. Therefore, the main objective of this STSM was to evaluate the cytotoxic and genotoxic effects on human glioblastoma A172 cells exposed for 3, 24 and 48h to CeO_2NP (1-100µg/ml), to verify their safety to be used as possible nanomedicines for brain cancer treatment, specifically glioblastoma [2]. In addition, cell-specific differences in nanoceria effect were evaluated by comparing the results obtained with those observed in human neuronal SH-SY5Y cells exposed under the same experimental conditions. After carrying out the physicochemical characterization and analysing the cellular uptake of the CeO_2NP, potential alterations in cell viability (MTT assay) and induction of DNA double-strand breaks (γH2AX assay) caused by the exposure were determined. The possible NP interference with assay methodologies was previously addressed and eliminated when necessary. Results obtained showed that, although there was a significant dose- and time-dependent internalization of NP by both cell types, nanoceria induced scarce cytotoxicity or genotoxicity in both cell lines, being restricted to the highest doses and longer exposure time tested. In general, data obtained suggest a high biocompatibility of CeO_2NP under the tested conditions, except for glioblastoma cells exposed for 48h from 25 to 100µg/ml. These results provide a better understanding of the CeO_2NP interaction with nervous system cells and their possible adverse effects. However, further studies are necessary to delve into the differential behaviour of these NP depending on the nervous cell type tested.

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Synthesis and biological evaluation of hybrid gold nanoparticles conjugated with an Anti-Sense Oligonucleotide

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Abstract

Nanotechnology has emerged as a cutting-edge multidisciplinary technology with functional ability in diverse fields. Nanoparticles can improve the solubility, stability, biodistribution and pharmacokinetic properties of the drug, along with the capacity to target specific cells and tissues (1,2). Gold colloids have served chemistry, biology and medicine among other fields for over a century, and continue to develop their utility. They are used as "theranostic" agents for several pathologies, including cancer (3).

Glioblastoma multiform is a severely malignant type of glioma that is incurable with contemporary therapies due to the complexity it declares (4). The translationally controlled tumor protein (TCTP) is a protein that takes part in immune responses, cell proliferation and tumorigenicity and its high expression is found to be related with lower overall survival (5). Thus, targeting TCTP shall be a potent way of reducing glioma tumor growth.

In this Short-Term Scientific Mission, took place the conjugation of the anti-sense oligonucleotide which targets the TCTP to gold nanoparticles and its biological evaluation in vitro. According to the DLS results, the anti-sense oligonucleotide is conjugated to the gold nanoparticles and the nanosystem was biologically evaluated through MTT assay.

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DOX-PEG-^{198}AuNPs-PEG-Tmab - multimodal radiobioconjugate for targeted radionuclide therapy of HER2+ cancers

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Gold nanoparticles with unique properties, such as small size, high biocompatibility, low toxicity, and versatility due to the ease of surface functionalization are very promising candidates for potential clinical ways of drug delivery [1,2]. The aim of this study is the synthesis of novel multimodal radiobioconjugate containing simultaneously in one structure β- emitter - ^{198}Au (^{198}AuNPs), a chemotherapeutic – doxorubicin (DOX) and a guiding vector – trastuzumab (Tmab).

Based on Turkevich method, the synthesis of 30 nm gold nanoparticles using radioactive precursor (^{198}Au) was performed [3,4]. The DLS and TEM measurements confirmed the expected average size (~ 30 nm) and spherical shape. The zeta potential value showed high stability of ^{198}AuNPs without a tendency to agglomeration. The ^{198}AuNPs were successfully modified with bifunctional hydrophilic polymer PEG due to high chemical affinity of sulphur to gold. Subsequently, doxorubicin and trastuzumab were attached to activated carboxylic groups of PEG leading to form irreversible peptide bond.

^{198}AuNPs, ^{198}AuNPs-PEG-DOX, DOX-PEG-^{198}AuNPs-PEG-Tmab were tested in vitro on SKOV-3 and MDA-MB-231 cell lines showing high affinity and cytotoxicity. In vivo experiments, where the radioactive compound was administrated directly to the tumor of SKOV-3 (HER2(+)) mouse xenograft models showed high uptake of radiobioconjugate at the injection site. On the contrary, after intravenous injection, ^{198}AuNPs-PEG-DOX and DOX-PEG-^{198}AuNPs-PEG-Tmab were mainly located in the liver and spleen. Furthermore, DOX-PEG-^{198}AuNPs-PEG-Tmab radiobioconjugate resulted in the retardation of tumor growth after intratumoral injection in SKOV-3 tumor-bearing mice.

DOX-PEG-^{198}AuNPs-PEG-Tmab radiobioconjugate exhibits very good properties for the treatment of HER2-positive cancers by intratumoral or post-resection injection. Radiopharmaceutical is a promising candidate for targeted radionuclide nanobrachytherapy to enhance the therapeutic effect of patients suffering from the HER2 positive cancer.

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How CD147-specific nanomedicines end: from target engagement in 2D towards validation using a novel vascularized in vitro glioblastoma model

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Surface-decorated nanomedicines have been extensively exploited to carry and target anti-cancer therapies [1,2]. Therefore, relying on CD147 upregulation on glioblastoma (GBM) cells surface along with high affinity and specific binding between antagonist peptide 9 (AP-9) and CD147, we designed and prepared AP-9 decorated polymeric nanoparticles (NPs) [3]. Ideally, in comparison with non-decorated counterparts, AP-9 increases NPs tumour bioavailability and avoids off-target accumulation.
Here we aimed to 1) study the benefit of decorating polymeric NPs with different AP-9 densities and 2) develop and characterize a reliable GBM-on-the-perivascular niche model to test nanomedicines.
Using U-251MG cell line and normal human astrocytes (NHA) we demonstrated that a small increase in NPs surface decoration, from 2.5 to 5mol% of AP-9, is enough to increase NPs engagement and targetability towards tumour cells, in comparison with non-decorated NPs. As expected, and presumably due to lower expression of the target CD147, this tendency was not observed for NHA. Regarding the novel GBM model, U-87MG spheroids were developed using either ultra-low adhesive well plates or hanging drops. In both conditions spheroids grew at least for 12 days, despite being different in terms of size and shape. Using these spheroids, we set up the GBM-on-the-perivascular niche model, which also included primary human endothelial cells and fibroblasts with or without being previously embedded in a fibrin and collagen hydrogel. In the presence of a 3D matrix, stromal cells are preferentially located around the tumour spheroid and in close proximity with each other. Moreover, following 5 days they start developing elongated structures, which was not observed in the absence of the matrix.
Based on these early findings, future challenges include anti-GBM drugs encapsulation into AP-9 decorated NPs as well as the GBM-on-the-perivascular niche metabolic and mechanical characterization before its use to test decorated and loaded nanomedicines.

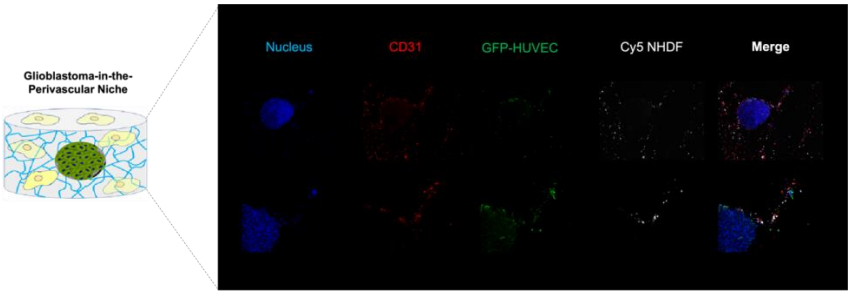

Figure 1. Glioblastoma-in-the-perivascular niche model. Schematic representation of the 3D model comprising a U-87MG spheroid and stromal cells distributed across a fibrin and collagen matrix. Representative fluorescent images of the sectioned model at day 5, after staining

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"Risk-benefit analysis of nano(bio)materials used in medical applications"

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The purpose of this short-term scientific mission (STSM) was to gain new knowledge and expertise in assessing the risk and benefits of nano(bio)materials used in the medical sector. The opportunity of being engaged in group discussions with experts in risk assessment of nano(bio)materials on specific topics such as how to perform hazard and exposure assessment to nano(bio)materials, how to estimate the associated risks and how to balance the identified risk with medical benefits. In order to deepen the methodological knowledge, during the STSM period, suitable case studies for performing risk-benefit analysis for nano(bio)materials have been identified. According to available information in the literature high aspect ratio nanoparticles (such as carbon nanotubes-CNT) have been deeply investigate and have been recognized as suitable case study materials[1,2]. In fact, CNTs have been successfully applied in pharmacy and medicine due to their high surface area that is capable of adsorbing or conjugating with a wide variety of therapeutic and diagnostic agents (drugs, genes, vaccines, antibodies, biosensors, etc.). Main applications of CNT in all areas of pharmacy and medicine from therapy to analysis and diagnostics have been identified and collected in a review document. The concepts of life cycle thinking, and occupational risks related to the synthesis, formulation, use, and end-of-life of nano-based medical technologies have also been deeply discussed. Collection of information and data specific for the case study of CNT, to support the human health risk assessment by using the software product SUNDS (www.sunds.gd) developed by the host institution team. Special attention was paid to practice to work with the SUNDS program during the STSM.

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Nanoencapsulated Bevacizumab Inhibits Glioblastoma Vascularization via Intratumoral VEGF Trapping

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Glioblastoma multiforme (GBM) is the most aggressive malignant brain tumor, being the median survival time of patients at around 15 months after disease diagnosis[1]. GBM has the constant need of vascularization, making this tumor one of the most vascularized and invasive solid tumors[2]. Bevacizumab, an anti-VEGF monoclonal antibody, was approved by FDA to be used as a single agent for patients with GBM[3]. Despite good results in the clinical trials, the low probability of bevacizumab in crossing the BBB limits its CNS accessibility and does not allow an improvement in the overall patient survival[4]. Therefore, an alternative to improve the efficacy of GBM treatment is highly needed and might be achieved by the combination of nanotechnology through controlled release nanosystems[5]. In this study, bevacizumab-loaded PLGA NP were successfully developed as an alternative to cross effectively BBB, accomplishing a better therapy[6]. No significant differences were also found by BrdU and ELISA assay for anti-proliferative and anti-VEGF properties between free and encapsulated bevacizumab, demonstrating the success of encapsulation. *In* vivo efficacy of bevacizumab-loaded PLGA NP was evaluated using a glioma zebrafish model to study the neoangiogenesis and tumor growth through the injection of GBM cancer cells. *In vivo* results showed a significant decrease in tumor area just for the bevacizumab-loaded PLGA NP group. Trying to understand the molecular mechanism behind the efficacy of nanoparticles, a cellular uptake in both cell lines was done to study the internalization of bevacizumab and its effect on VEGF secretion. A significant increase in the number of bevacizumab positive cells and a decrease in the number of VEGF producing cells was obtained for the bevacizumab-loaded PLGA NP group. These last results demonstrated that bevacizumab-loaded PLGA NP might cause a disorder in VEGF signaling pathway, being an efficient alternative to deliver intracellularly monoclonal antibodies.

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Cancer Nanomedicine *via* Dendrimers Nanocarriers

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Nowadays, the use of natural products in the treatment of a large spectrum of diseases represents one of the hottest research topics. The plants and their bioactive secondary metabolites constitute a potential source of crucially needed new effective drugs. Unfortunately, some of the currently available treatments suffer from some toxicity, administration difficulty and even resistance development. Our recent studies deal with new developed families of multivalent amphiphilic dendrimers (MUSE), which exhibit their great potential toward drug encapsulation and delivery to the targeted organs [1], [2]. In this context, the interaction between different families of MUSE dendrimers (Figure 1) and targeted bioactive products with physiological interest represents an important challenge tobe taken up [3].

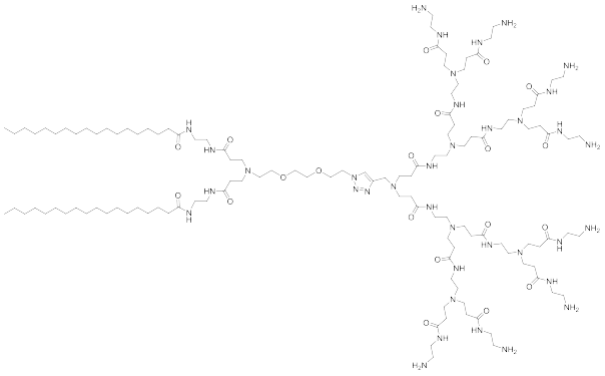

Figure 1. Structure of MUSE nanocarrier

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Stability study of mRNA-hybrid lipid nanoparticles in biological medium using fluorescence correlation spectroscopy

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The success of mRNA-based COVID-19 vaccines has impressively demonstrated the potential of nucleic acid-based therapeutics [1]. Safe and efficacious systemic delivery of mRNA have been enabled using lipid nanoparticles, however a selective delivery to specific organs and cells *in vivo* remains the major challenge in the development of mRNA-based therapeutics [1,2]. In the present work, we focused on the design, physico-chemical characterization and stability of lipid-hybrid nanosystems intended for the intravenous (i.v.) administration of mRNA [2,3]. In particular in the context of the STSM collaboration we studied the stability of the developed systems in complex biological medium as serum using fluorescence correlation spectroscopy (FCS). This stability study is of pivotal importance to understand the *in vivo* fate of nananosystems following i.v. injection. FCS is a microscopy-based technique that monitors the fluorescence intensity fluctuations of molecules diffusing in and out of the focal volume of a confocal microscope. By analysing the fluctuations of fluorescence intensity over time, FCS allowed the determination of the concentration of intact mRNA [4,5]. Cy5-labelled mRNA was used to monitor the fluorescence intensity of the intact nucleic acid, together with fluoresceinamine-labelled polymer for the coating hybrid lipid systems (HLRC). HLRC, prepared at different charge ratio (defined as ratio between positive amine groups (N) to negatively charged nucleic acid (P): N/P 1, 3, 3.5 and 5) were analyzed to quantify the mRNA and the fluorescent polymer complexed with the lipids after formulation and after 1 h of incubation in undiluted human serum (HS) at 37 ˚C. A high association of mRNA (above 90%) has been obtained for charge ratio between 3 and 5. In the case of charge ratio of 1, only 45.8% of the nucleic acid held on, probably due to the excess of mRNA present in the complex, which, as reported in the dynamic light scattering measurements, leads to a progressive instability. We also proven that the fluorescent polymer once associated to the system is highly stable. In fact, at charge ratio between 3 and 5 more that 70% remained associated. Overall, all the systems except N/P 1 can form stable complexes with both the genetic material and the shell polymer, not only in the optimized formulation conditions, but also in the presence of human serum [2].

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Synthesis of Cisplatin loaded Polyester-based dendritic structures for the targeted treatment of Osteosarcoma

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Osteosarcoma (OS) is the most prevalent form of bone cancer, affecting mostly children up to 19 years old. Though progresses in its treatment have been achieved in the past decades, the survival rate remains below 60%, dropping to 20-30% when metastasis or recurrence occur. One of the main drugs used for the chemotherapeutic treatment of OS is cisplatin, though its use is associated with severe side effects, tumor recurrence and often, a degree of permanent disability in patients [1,2]

In this STSM, we aimed to develop polyester-based dendritic structures with functionalized peripheral groups, to form a bone-targeting system and a control system. Afterwards, each system was to be loaded with cisplatin, for the targeted treatment of OS.

Both precursor systems were synthetized and characterized by nuclear magnetic resonance (NMR), mass spectrometry (MALDI-TOF), scanning electron microscopy coupled to Energy Dispersive X-Ray Analysis (SEM/EDX), transmission electron microscopy (TEM), infrared spectroscopy (FT-IR), size exclusion chromatography (SEC) and dynamic light scattering (DLS). Their cytotoxicity was evaluated in an OS cell line.

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Synthesis and characterization of nanofibers functionalized with nanoparticles with anticancer properties

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Electrospun polymer nanofibers are becoming more popular due to the possibility of their use in tissue engineering, regenerative medicine, transport of medicinal substances and as dressing materials. These materials are preferred caused by their high surface area-to-volume ratio, high porosity, good mechanical strength and ease of functionalization [1, 2]. Moreover, the ability to control the parameters of the electrospinning process allows to obtain nanofibers with the desired morphology and properties, while ensuring the reproducibility of the process. Nanofibers containing metallic nanoparticles may posses increased anticancer activity, which can be the result of the synergistic effect of the polymer and nanoparticles [1, 3].

The aim of the study was to synthesize nanofibers functionalized with various types of nanoparticles - nanofibers functionalized with gold, cadmium selenide or tantalum nanoparticles. The nanoparticles were synthesized in situ polyvinylpyrrolidone (PVP), to control size and stability of nanoparticles. Hybrid nanofibers were obtained by electrospinning process, immobilizing nanoparticles in a biocompatible and biodegradable PVP and poly(L-lactide-co-ε-caprolactone) (PL-b-CL) copolymer. Then, the fabricated nanofibers were subjected to physicochemical characterization, which included the assessment of their morphology, chemical composition and thermal stability using Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FTIR), Thermogravimetric (TGA) and Differential Scanning Calorimetry (DSC) analysis.

On the basis of preliminary studies, the anticancer potential of the produced nanofibers was also determined. The increase in mortality of A549 cancer cells *(human lung cancer epithelial cells)* treated with nanofibers is an introduction to the further development of research in which the fabricated nanofibers can be promising candidates in cancer therapies, especially those targeted at local cancerous changes. Therefore, the obtained nanofibers will be subjected to further analysis in order to determine their therapeutic effect.

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Evaluation of cationic carbosilane dendrimers as nanovectors of therapeutic nucleic acids for cancer nanomedicine

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Carbosilane dendrimers (CS-DDMs) with cationic groups at the periphery can interact with biomacromolecules and cellular structures. Due to their biocompatibility and efficiency, CS-DDMs have been intensively investigated for biomedical applications, especially for drug and gene delivery[1,2].

We have previously described biocompatibility and beneficial properties of CS-DDMs carrying onium salts[3] and carbohydrates (glyco-DDMs)[4,5] in the periphery. DDMs with phosphonium units in the periphery are less toxic compared to their more widely used ammonium analogues[3]. In addition, we have shown that these compounds are efficient transfection vectors for siRNA transfer into cells[6]. CS glyco-DDMs have been shown to be efficient nanocarriers of model drug doxorubicin with pH-dependent drug release and preferential internalization in tumor cells[4]. However, to our best knowledge, charged glycoDDMs suitable for complexation of genetic material have not yet been described.

Here we present a synthetic procedure for novel charged dendritic DDSs (cationic CS glyco-DDMs, hybrid CS-PAMAMs). Together with phosphonium CS-DDMs and commercial PAMAMs, the compounds were used in the first interstructural comparative assessment of cationic dendritic nanocarriers comprising cytotoxicity evaluation, complexation properties, and dendriplex stability investigation. The tested compounds displayed significant differences in cytotoxicity based on their structure and generation (number of peripheral units). Beside this, all tested scaffolds showed favourable complexation properties with siRNA, forming stable siRNA/DDM complexes. Such dendriplexes tend to release siRNA in an acidic environment, which holds promise for tumour-targeted therapy.

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Production of aptamer-guided liposomes for head and neck cancer treatment

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Conventional anticancer therapies present low specificity, leading to several side effects. To possibly solve these drawbacks, aptamers able to fold into G-quadruplex (G4) have been proposed to favour drug accumulation in cancer cells (1). AS1411 is a G4 aptamer able to recognize nucleolin, a protein overexpressed on cancer cell surface. This aptamer was tested in phase II clinical trials but showed low response rates and suboptimal pharmacokinetics (2). Nevertheless, AS1411 is being used as a targeting agent. Moreover, AS1411 derivatives were developed, with improved activity and higher affinity to nucleolin (1).

On account of what above stated, in the current work the use of AT11 (a AS1411 derivative), as targeting moiety was considered to functionalize liposomes with the aim to improve the selectivity of a potential anticancer drug, the acridine orange derivative (C_8) towards oral cancer cells.

In this respect, both empty and C_8-associated liposomes were produced by ethanol injection method; after preparation the liposome were further engineered by binding a AT11-TEG-cholesteryl moiety. The different liposomal formulations were dimensionally characterized by DLS, UV/vis spectroscopy and SDS-PAGE. Notably, the dimensions of the produced liposomes were in the range of 120-150 nm.

The effect of empty and C_8- liposomes on SCC154 cell line (squamous cell carcinoma of the tongue and Het1A cells (human oesophageal autopsy tissue by transfection with plasmid pRSV-T) was determined by MTT viability assay whereas the internalization was determined by confocal microscopy. When cells were treated with empty liposomes, cell viability was almost unaffected up to the concentration of 53.6 µg/mL. On the contrary, when cells are treated with the C_8-liposomes, a dose-response effect was observed on both cell lines. Moreover, when C_8-associated liposomes are conjugated with AT11 a clear selectivity of the liposomes towards the SCC154 cell line was observed. Interestingly, we demonstrated, by confocal microscopy analysis, that the AT11 conjugated liposomes are efficiently internalized and can reach the cytoplasm of the treated cells.

Overall, these findings suggest that the designed liposomal formulation represent a promising drug carrier for the therapy of oral cancers.

Acknowledgments

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pH responsive iron oxide nanoflower (IONFs) incorporated chitosan nanogels for cancer treatment

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The drug delivery systems are of great importance in cancer treatment and have become the interest of many scientists worldwide. Regarding to the recent trends for drug delivery systems design are mainly based on development of smart and nano size drug carriers which are targeted to cancer cells [1]. Targeted drug delivery to local treatment of cancer cells is possible without affecting healthy cells. Nanogels are promising materials to be used in targeted drug delivery systems due to their biocompatible character and injectable properties whereby they can be used to load drugs [2]. In additionally, pH sensitive drug delivery systems gain importance for an effective targeted delivery. This study aims to create pH responsive iron oxide nanoflower (IONPs) incorporated chitosan nanogels which have potential applications in cancer treatment as a drug carrier system. In this study, the IONPs were used according to the Storozhuk et al., 2021 protocol [3], and pH responsive IONPs incorporated chitosan nanogels were synthesized with different crosslinker agents. Chemical structure of nanogels was characterized by Fourier transform infrared (FTIR) and X-ray diffraction (XRD). Additionally, the particle size and zeta potential were estimated by dynamic light scattering (DLS) for pH responsive study and stability. Morphology of the nanogels (with and without iron magnetic nanoparticles) was analysed by transmission electron microscopy (TEM). Further studies need to be performed to assess the suitability of the carrier system for the purpose of cancer treatment.

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Carbosilane dendritic hydrogels for sustained drug release

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Cancer is one of the most frequent pathologies and one of the main causes of death worldwide. One of the solutions proposed to solve the drawbacks of current anti-tumor therapies is the use of nanotechnology. My work focuses on one type of nanoparticles, dendrimers, and their use for the synthesis of hydrogels as sustained and controlled release systems for antitumor drugs [1].

This STSM aimed to explore the potential of these dendritic hydrogels to selectively load and release antitumor drugs under certain stimuli, in which the host group has extensive experience.

Specifically, the aim of the planned assays was to determine the ability of the hydrogels to capture and release anticancer drugs with poor water solubility. To this end, two different procedures were carried out: in the first, the drug was retained in the pores of the gel through non-covalent interactions; in the second, an attempt was made to covalently attach the drug to the network through the free OH groups present in the hydrogel. If successful, these approaches would allow access to different release profiles and kinetics, e.g. free diffusion of the drug from the hydrogels or a more controlled release due to covalent bond breaking.

As an anticancer drug, we selected camptothecin (CPT), a poorly water-soluble antitumor drug with a hydroxyl group available for covalent binding to the hydrogel.

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Functionalized chitosan based magnetic nanoparticles with potential in cancer therapy applications: physio-chemical characterization and biological studies

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Cancer is the second cause of death globally according to World Health Organization data. The search for new forms of cytotoxic agents with minimal side effects and maximal cytotoxicity, as well as for novel ways to reduce the administered doses of the cytotoxic agent, remains an urgent task, despite the large number of approaches to the treatment of cancer [1]. A noticeable interest in nanomedicine field is represented by magnetic nanocomposites based on biofunctionalized polymers due to the possibility to manipulate their characteristics such as size and shape, as well as the ability to improve cancer cell targeting [2,3].

The present study describes the physicochemical characterization and the biological properties of functionalized chitosan nanoparticles loaded with doxorubicin, a chemotherapeutic agent and a cell membrane calcium channel blocker, verapamil. Magnetic nanoparticles obtained by partial oxidation reaction followed by coverage with sodium oleate were embedded into a polymeric matrix and drugs solution by self-assembly method followed by ionic gelation with sodium tripolyphosphate in order to achieve the drug-loaded nanoparticles.

The physicochemical properties of different nanoparticles were evaluated using dynamic light scattering (DLS), cell viability assay, and elemental concentration using ICP-AES. The size, polydispersity index, and surface charge of the nanoparticles varied depending on the homogenization settings used. The average hydrodynamic diameter of the nanoparticles decreased when subjected to ultrasonic dispersion. Cell viability assay carried out on MCF-7 and MDA-MB-231 cell lines using MTS. The MDA-MB-231 and MCF-7 cell lines were used to test the cytotoxicity of various nanoparticle types as well as the IC50 values for doxorubicin and verapamil. At doses between 0.05 and 1 μg/ml, MTS studies demonstrated that they have no toxic effects after 48h. Therefore, the obtained nanoparticles will be subjected for further analysis in order to determine their therapeutic effect.

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Iron Oxide Nanoflowers for Magnetic Hyperthermia Cancer Therapy

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Cancer has a major impact on several sectors and human activities within society. This is reflected by the large number of newly diagnosed cases (375,400 annually for the years 2015-2018 in the UK) [1].

Magnetic hyperthermia (MH) using magnetic nanoparticles to generate heat in alternating magnetic fields offers a huge potential as an emerging modality for cancer treatment. IONPs have already been approved by FDA, constituting the most promising type of nanoparticles for MH. However, there is a bottleneck for the wide acceptance of MH therapy due to the quality limitations of commercial iron oxide nanoparticles (IONPs), which exhibit sub-optimal heating efficiency and are associated with high costs. We recently overcame these limitations with our novel synthetic procedure for iron oxide nanoflowers (IONFs) exhibiting heating rates that are 3 times higher than those of any commercially available nanoparticle alternative (Fig. 1.) [2].

An *in vitro* preliminary study was implemented using an optical imaging platform (Vivoptic) typically employed for the *in vivo* evaluation of diagnostic and therapeutic strategies at the University of Bordeaux. The platform was equipped with a bioluminescent cancer cell line expressing the gene of the luciferase firefly enzyme (LucF) PC3-CMV-LucF. The toxicity was evaluated by bioluminescence imaging in 24 hours after IONFs addition. Finally, the cell viability was calculated by the MTT assay. All samples show no toxicity at a rank of concentrations 0.02 to 0.2 mg/ml.

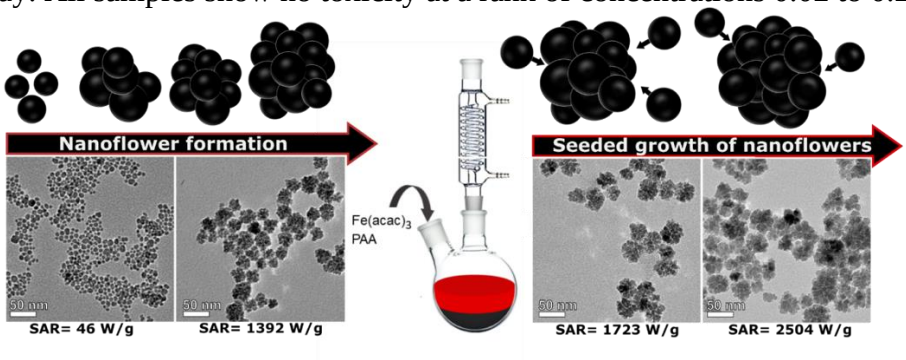

Figure 1. Schematic representation of the synthesis of IONFs with exceptional magnetic heating efficiency

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Preparation and characterization of functional metallic nanoparticles as potential drug delivery systems

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Metallic nanoparticles, especially gold (AuNPs) and silver (AgNPs), can be used as drug delivery systems because of its unique properties e.g. nanometre size, low toxicity, high reactivity and large surface area. However, the safe use of nanomaterials, especially in the case of biomedical application, requires its precise characterisation, in the case of both: i) nanomaterial size and shape and ii) identification of chemical structure of compounds present on its surface. Although the need of nanoparticles size and shape characterisation seem to be understood, and several techniques as DLS, SEM/STEM, TEM, XRD are routinely used for its characterization, the chemical description of nanoparticles chemistry still remains a challenge, especially in the case of the amount of ligands present on single nanoparticle.

The aim of this work was to determine the surface coverage of AuNPs and AgNPs with the size equal 5 nm, 13 nm, 20 nm, 30 nm and 40 with thiol ligand (sodium 2-mercaptoethanesulfonate (MES), 11-mercaptoundecanoic acid sodium salt (MUS)) using ICT technique. Nanoparticles were synthesized via chemical reduction method in water and precisely characterized with UV-vis, DLS and STEM techniques to determine its stability, shape, size and size distribution. The obtained results showed that the amount of ligands adsorbed on the metal surface depends on the size of the nanoparticles and the type of modifier.

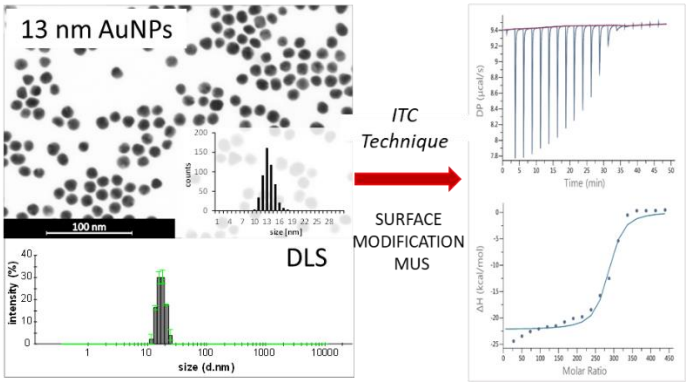

Figure 1. STEM image with corresponding size distribution histogram, DLS size distribution histogram, and ITC results obtained for 13 nm AuNPs modified with MUS ligand.

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Transformations of L-DOPA during the synthesis of gold-based nanodelivery systems for LAT-1 targeting

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LAT-1, the large neutral amino acid transporter-1, plays a crucial role in cancer growth and proliferation, making it a promising biomarker for imaging and treating human malignancies such as brain cancers. Thus, growing attention is being paid to the development of LAT-targeted drug delivery systems with blood-brain penetration ability [1,2]. For this purpose, gold nanoparticles (AuNPs) functionalized with L-DOPA have been developed [1]. The great potential of such systems to be used in brain cancer therapy prompts the need for evaluation of the processes occurring at the nano-bio interface [3]. In the presence of gold salts, L-DOPA can undergo various transformations such as oxidation, cyclization, and polymerization [4]. To avoid binding structurally altered molecules to the nanosurface and prevent any negative impact on human health, it is important to consider these transformation patterns. This study aimed to investigate the behavior of L-DOPA upon interaction with a gold nanosurface by using a combination of nuclear magnetic resonance (NMR) spectroscopy and computational methods. Experimental results showed that the final form of L-DOPA on the AuNPs surface is dependent on the molar ratio of reactants used during synthesis. When there was an excess of L-DOPA compared to Au, no alterations in the structure of L-DOPA were observed. However, an excess of Au was found to promote the oxidation of L-DOPA, which could result in various oxidation products being bound to the gold nanosurface. NMR spectra revealed that L-DOPA underwent intramolecular cyclization but could not differentiate oxidation products. On the other hand, computational simulations using density functional theory (DFT) and molecular dynamics (MD) techniques identified the most probable oxidation products for binding to the AuNP surface. The highest affinity to gold nanosurface was obtained for dopachrome compared to its tautomer and leukodopachrome. The obtained results represent valuable mechanistic data about the binding events at the surface of AuNPs to encourage their application as a drug-delivery systems in cancer therapy.

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Nanomechanical tool for characterization of cellular immune response

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A possible connection between cancer and inflammation was proposed in 19th century but until recently the exact role of inflammation in oncogenesis has largely been unknown. It has been shown now that cancer development is strongly influenced by both chronic and acute inflammation processes. Tumour microenvironment contains innate immune cells like macrophages and neutrophils, adaptive immune cells, cancer cells and their surrounding stroma.[1,2] Macrophages have the key role as one of the basic components of innate immunity and the inflammatory process is tightly regulated with macrophages playing a critical role in the initiation, maintenance and resolution of inflammation. During inflammation, mechanical properties of the cells may change and can be monitored with atomic force microscopy (AFM).[3] While it has been used extensively in material science for imaging and atomic force measurements, only recently has the AFM been employed for understanding the nano-bio interactions in physiological fluids.[5,6] That technique is based on the interaction between a sharp tip and the sample surface and presents a valuable tool in cancer research and diagnosis.

The aim of this work is to develop and optimize nanomechanical method for testing effects of inflammation on nanomechanical properties of THP-1 cell line. THP-1 cells are used as model of human monocytes and when differentiated can be a model for macrophage cells which were mentioned before. This research will provide information on how mechanical properties of cells can change during inflammation and will help understanding acute inflammation. Both contributions could be a helpful tool in early cancer detection. In addition, development and optimization of nanomechanical method will enable a new nanotechnological platform for further testing of other potential nanodrugs.

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