PID: 31886 – In vivo trafficking of fluorescent and radiolabelled cisplatin-cross-linked DNA nanoblocks for cancer therapy

Owner: [redacted]

Moderator:

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Proposal Status: Reviewer Selected

VID: 55781 – Technical Evaluation
In vivo optical imaging – In vivo multimodal preclinical imaging (canSERV)
CanSERV: Centre for Advanced Preclinical Imaging

canSERV | canSERV Second Open Call

VID: 55784 – Technical Evaluation
Preclinical PET-CT and SPECT-CT imaging – In vivo multimodal preclinical imaging (canSERV)
CanSERV: Centre for Advanced Preclinical Imaging

canSERV | canSERV Second Open Call

PART A. Registration: Administrative data of the applicant

Gender

Man

Career stage

Category C - Recognised researcher

Country, where the research team or its majority works

Czechia

PART B. General information on the Proposal

Application acronym

ITDN

Fields

Advanced Technologies for Personalised Oncology

Abstract

The drug delivery potential of DNA nanostructures in trafficking chemotherapeutic cargo for cancer treatment is currently gaining significant interest. They offer not only biocompatibility but also programmability and addressability of functionalization sites, allowing for the assembly of complex nanoarchitectures with multiple functionalities suitable for theranostics. We will assemble DNA nanoblocks cross-linked using cisplatin, which doubles as a stabilizing and chemotherapeutic agent, and decorate the nanostructures with fluorescent dyes or radioisotope chelators for in vivo imaging as well as folate molecules for tumour targeting. To complement our ongoing in vitro experiments, this project will explore the in vivo stability, biodistribution, and tumour targeting of the decorated nanostructures. Both fluorescence imaging and positron emission tomography will be used to track the nanostructures in mice xenografted with HeLa cells as a folate-receptor-alpha-positive model. By analysing the in vivo behaviour of these nanostructures, we will be able to optimize them for drug delivery applications and contribute to the current need for in vivo explorations to facilitate the clinical translation of these promising drug delivery systems.

PART C. Administrative data of your home institution

PIC

999511863

Legal name

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Name and address (street, town, postcode, country, email, webpage) of department in which the research supported by the service is to be carried out

Please note that a support letter from your home institution will be required before starting service provision.

PART D. Research proposal

Please read the Service Field Guideline as reference for the fields below

Yes

Please dedicate an individual paragraph on describing the work you would like to conduct with each requested service

The work will focus on observing the in vivo biodistribution through time of labelled cisplatin-cross-linked DNA nanoblocks in tumour-xenografted mice to investigate the distribution of the nanoblocks in major organs and tumour tissues and also extract blood samples at different time points to profile the bioavailability and pharmacokinetics.
The first phase concerns fluorescence imaging to gain a preliminary understanding of the biodistribution and tumour accumulation of fluorescently labelled pure DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks functionalized with folic acid. The mice will be injected with saline solutions of the respective nanoblocks and the animals will be imaged at different time points. For this requested service, we plan to utilize the In Vivo Xtreme (Biospin Bruker, Ettlingen, Germany) in vivo optical imager in CAPI (Center for Advanced Preclinical Imaging, First Faculty of Medicine of the Charles University in Prague) capable of multispectral VIS-NIR fluorescence imaging, and our dye of choice is the IRDye800 with an emission maximum situated at 812 nm, which is ideal to avoid the auto-fluorescent wavelength windows for the major organs. Extracted blood samples at different time points will be placed in well plates for spectrophotometry analysis (fluorescence spectrometry for fluorophore concentration and ICP-MS for platinum content) ex vivo.
The second phase will utilize the Albira PET-SPECT-CT (Biospin Bruker, Ettlingen, Germany) in CAPI to perform a PET scan on mice injected with radiolabelled DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks. Using PET, we should be able to accurately quantify the amount of radioisotope in each major body organ since there will be less signal attenuation as opposed to fluorescence imaging. The PET will also allow 3D reconstruction to spatially resolve organs and tissues where the radiolabelled nanostructures accumulate. Currently, we have DFO-chelator-functionalized DNA nanoblocks ideal for sequestrating positron emitters like 89Zr and we have the possibility of also changing the chelator to DOTA, suitable for plenty of nuclides used in PET like 64Cu and 68Ga. Extracted blood samples at different time points will be placed in well plates for spectrophotometry analysis (radiography for radioisotope concentration and ICP-MS for metal content) ex vivo.

Please estimate how many units (e.g., samples, models, hours or days of access) you will need. Please indicate the number of units for each service requested (e.g., Service xy – 5 Units)

We anticipate a minimum of three treatments or groups (cisplatin-cross-linked DNA nanoblocks, pure DNA nanoblocks, and controls with pure DNA-oligomer-fluorescent-dye conjugate or chelated radioisotope) with at least three replicates, so a minimum of nine mice are needed for each imaging modality, making a total of 18 mice. We also plan to image at six different time points after injection: immediately after injection, 1, 3, 6, 12, and 24 hours. With at least an hour devoted to imaging, we therefore need at least six hours for each imaging technique, so at least 12 hours are required and at least 4 days of access. For the xenograft model, we require HeLa cell cultures available at CAPI, and we will utilize the immunodeficient NU/NU nude mouse for imaging.
Service 1: In vivo optical imaging – 10 Units
Service 2: Preclinical PET-CT imaging – 10 Units

Expected results: Please list up to 3 expected results from the work with each service you plan to access

Service 1: In vivo optical imaging
a) In vivo fluorescence images at different time points after injection to profile the biodistribution and tumour accumulation of the nanoblocks.
b) Time-dependent fluorescence decay plot of blood samples from mice injected with fluorescently labelled DNA and cisplatin-cross-linked DNA nanoblocks, to profile blood kinetics and bioavailability.
c) Comparison of the bioavailability and stability of cisplatin-cross-linked DNA nanoblocks as opposed to pure DNA nanoblocks and pure DNA-oligomer-fluorescent-dye conjugate.
Service 2: Preclinical PET-CT imaging
a) Three-dimensional reconstruction of radioisotope signals in vivo at different time points after injection of radiolabelled DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks.
b) Quantification of time-dependent biodistribution, tumour accumulation, and blood kinetics of radioisotope labelled DNA nanoblocks from PET scans at different time points.
c) Comparison of the bioavailability and stability of cisplatin-cross-linked DNA nanoblocks as opposed to pure DNA nanoblocks and pure chelated radioisotope.

Please describe your expertise level in using the selected technologies/services:

I consider myself as a basic user having joined in vivo imaging experiments but always with the assistance of the assigned technical expert.

Scientific background of the Principal Investigator

I finished my PhD in chemistry from the University of Paris-Saclay in 2018 on low-energy electron-induced processes on condensed systems, with some applications to evaluating radiotherapeutic physico-chemical mechanisms. Since 2019, I have been employed as a post-doctoral researcher here at the J. Heyrovsky Institute in Prague, specializing in DNA nanotechnology for radiation damage research [Sala et al., Nanoscale 13, 2021; Sala et al., J. Phys. Chem. Lett. 13, 2022] and more recently on in vitro [Sala et al., ACS Appl. Nano Mater. 22, 2022] and in vivo applications of the technology. My research works aim to provide elucidation of mechanisms involved in radiotherapy, especially concerning DNA damage, and to take advantage of these mechanisms to propose possible radio-chemotherapeutic solutions. I am thus well versed in techniques used for the characterization and preparation of DNA nanostructures, such as atomic force and electron microscopies, optical microscopy and spectroscopy, and electrophoresis. Due to our recent in vivo and in vitro explorations with collaborators from the Institute of Biophysics in Brno and CAPI, I am also familiar with biological assays to test cytotoxicity, radiation response, and in vitro and in vivo imaging. In the previous year, I also had a couple of short-term missions to the radiochemistry department of the CIC biomaGUNE in San Sebastian, Spain to explore the radiolabelling of DNA nanostructures and their interaction with radionuclides. One of my main goals is to tie together our fundamental research on DNA-radiation interactions with real solutions for cancer therapy and diagnosis.

Please provide a short CV highlighting your expertise in the field of your access application

[redacted] *Open File*

Description of the research project for which you are requesting supporting service(s)

The research project explores the drug delivery applications of cisplatin-cross-linked DNA nanoblocks. We have shown the stability and controllable cytotoxicity of cisplatin-cross-linked DNA nanostructures, and we are planning to continue this on DNA nanoblock architectures whose size and compact structure are often associated with enhanced uptake [Sala et al., ACS Appl. Nano Mater. 22, 2022] and are now collaborating with the Institute of Biophysics in Brno for in vitro cytotoxicity experiments with model tumour cell lines. We have already optimized the structures for fluorescent imaging, and we plan to also perform an in vivo study of the drug delivery and tumour targeting potential of such structures to take our research to the next level.

Due to the increasing interest in the use of DNA nanostructures for biomedical applications, plenty of in vitro investigations are being done regarding cellular uptake and cytotoxicity yet there are only a handful of in vivo investigations, which still do not have a consensus on the effect of various properties of DNA nanostructures on the biodistribution and pharmacokinetics of such nanomaterials when introduced in vivo [Jiang et al., Adv. Mater. 2023; Weiden and Bastings, COCIS 52, 2021; Jiang et al., Adv. Mater. 31, 2019]. Despite the promise offered by in vitro experiments, there is still a huge leap to translate the use of DNA nanostructures to the clinical level due to the lack of in vivo studies. Hence, we plan to contribute through this project by profiling the in vitro and in vivo capabilities of a type of DNA nanostructure (pure DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks) as a viable vehicle to traffic chemotherapeutic cargo into tumour cells and identify opportunities for optimizing stable DNA-based theranostic systems.

Our system utilizes cisplatin as both a stabilizing cross-linker for the nanostructure and at the same time, a therapeutic load. Cellular cytotoxicity and radiation-response studies with our collaborators at the Institute of Biophysics in Brno are now performed in vitro in two types of model cell lines on pure DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks. Currently, we are decorating the nanoblocks with fluorescent dyes for in vitro fluorescence imaging by confocal microscopy in collaboration with the Institute of Physics in Prague to ascertain cellular uptake. The next step is to incorporate active targeting strategies such as the introduction of folate/folic acid functional groups onto the nanostructures to enhance their uptake. This further motivated us to pursue in vivo explorations, in which we plan to involve the requested supporting services, to determine the biodistribution, bioavailability, kinetics, and tumor targeting of labelled and targeted DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks using both fluorescence imaging and positron emission tomography. Both techniques will allow the direct visualization of the nanostructures in vivo.

With the eventual combination of both in vitro and in vivo results, we hope to garner insights not only regarding the drug carrying and targeting potential of DNA nanoblocks but also understanding the interaction of DNA nanoblocks and similar nanostructures with living systems, which is crucial in the improvement and development of novel therapeutic tools against cancer.

Time frames of your research project

The first quarter (Month 1-3) of the project will be devoted to the procurement of necessary materials and reagents and optimizing the DNA nanoblocks for in vivo imaging. This involves optimizing the incorporation of fluorescent dyes and radioisotope chelators into the nanostructures. We have already optimized protocols for fluorescent modification and the tagging of radioisotope chelators like DFO and DOTA; however, we still need to optimize the labelling process with the radionuclides. In close collaboration with CAPI, we will perform the optimization of radiolabelling at the Institute of Macromolecular Chemistry in the Supramolecular Polymer Systems group.

The second quarter of the project (Month 4-6) will focus on fluorescence imaging. This also involves growing and monitoring cell cultures, the eventual xenografts, and preparing the mice for injection. This also includes ex vivo analyses of blood samples extracted at different time points.

The third quarter of the project (Month 7-9) will tackle PET imaging and will apply optimized procedures and protocols from the second quarter of the project, especially in preparing cell cultures and treatment groups. This also includes ex vivo analyses of blood samples extracted at different time points.

The last quarter (Month 10-12) will be devoted to additional data processing and interpretation, project evaluation, and the drafting of a manuscript for publication following possible publishable results from the project in conjunction with the in vitro explorations to be simultaneously undertaken with other collaborators during the course of the project.

A Gantt chart summarizing the work program and expected time frames are in the attached supporting file.

Please describe, how does the selected service(s) contribute to your research project?

The selected services will contribute immensely to our research project, not only in evaluating the in vivo potential of our drug delivery system but also to the current need to understand the fate and interactions of DNA nanostructures introduced in biological systems. In vivo studies on DNA nanostructures are scarce, despite numerous in vitro investigations showing the capabilities of DNA nanostructures of various configurations to target and deliver chemotherapeutic cargo to various types of model cells. We will also be able to optimize the labelling and imaging protocols for DNA nanostructures for both fluorescence and PET imaging through this project. Fluorescence imaging will initially profile the biodistribution, kinetics, and tumour accumulation of DNA nanoblocks and cisplatin-cross-linked DNA nanoblocks, while PET will complement these results with the accurate quantification of the biodistribution and a three-dimensional image reconstruction to accurately visualize and track the labelled nanostructures in vivo.

Please upload any additional files supporting your application (examples: figures with preliminary data, GANTT chart workplan etc.) Please provide a reference within the main text for all supplementary files

SupportingFile.pdf *Open File*

PART E. Ethics table

Please answer the questions below. If "yes" is selected, please specify in the text box. Details on required information can be found in the "How to complete your ethics Self-Assessment" guide (https://ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/common/guidance/how-to-complete-your-ethics-self-assessment_en.pdf)

1. HUMAN EMBRYOS/FOETUSES

Does your research involve Human Embryonic Stem Cells (hESCs)?

No

Does your research involve the use of human embryos?

No

Does your research involve the use of human foetal tissues / cells?

No

2. HUMANS

Does your research involve human participants?

No

Does your research involve physical interventions on the study participants?

No

Does your research involve conducting a clinical study as defined by the Clinical Trial Regulation 536/2014 (using pharmaceuticals, biologicals, radiopharmaceuticals, or advanced therapy medicinal products)?

No

3. HUMAN CELLS/TISSUES

Does your research involve the use of human cells or tissues (other than from Human Embryos/Foetuses)?

No

4. PERSONAL DATA

Does your research involve personal data collection and/or processing?

No

Does your research involve further processing of previously collected personal data (secondary use)?

No

Is it planned to export personal data (data transfer) from the EU to non-EU countries?

No

Is it planned to import personal data (data transfer) from non-EU countries into the EU or from a non-EU country to another non-EU country?

No

Does your research involve the processing of personal data related to criminal convictions and offences?

No

5. ANIMALS

Does your research involve animals?

Yes: Animals: NU/NU mice

The authorized technical staff of the service facility (CAPI) will be in charge of the handling and care of the mice involved in the experiments.

6. THIRD COUNTRIES

Will some of the activities be carried out in non-EU countries?

No

Does your research involve the use of substances or processes that may cause harm to the environment, to animals or plants?

No

In case non-EU countries are involved, do the activities undertaken in these countries raise potential ethics issues?

No

Is it planned to use local resources?

No

Is it planned to import any material (other than data) from non-EU countries into the EU or from a non-EU country to another non-EU country?

No

Is is planned to export any material (other than data) from the EU to non-EU countries?

No

Does your research involve low and/or lower-middle income countries?

No

Could the situation in the country put the individuals taking part in the research at risk?

No

7. ENVIRONMENT & HEALTH and SAFETY

Does your research deal with endangered fauna and/or flora and/or protected areas?

No

Does your research involve the use of elements that may cause harm to humans, including research staff?

Yes: Radioactive materials: Radionuclides for PET

Toxic chemicals: Cisplatin

The service facility is equipped with trained staff and up-to-date facilities for handling and disposal of toxic chemicals and small quantities of radioactive material for in vivo imaging.

8. ARTIFICIAL INTELLIGENCE

Does your research involve the development, deployment and/or use of Artificial Intelligence (AI)-based systems?

No

Could the AI based system/technique potentially stigmatise or discriminate against people?

No

Does the AI system/technique interact, replace or influence human decision-making processes?

No

Does the AI system/technique have the potential to lead to negative social and/or environmental impacts either through intended applications or plausible alternative uses?

No

Does the AI to be developed/used in the project raise any other ethical issues not covered by the questions above?

No

9. OTHER ETHICS ISSUES

Are there any other ethics issues that should be taken into consideration? Please specify

No

This open call is prepared in the context of a Horizon Europe project which have to comply with local, national and international ethical standards and regulations. For this ethics self-assessment, use the official guide "How to complete your ethics Self-Assessment" (https://ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/common/guidance/how-to-complete-your-ethics-self-assessment_en.pdf). If an ethics approval is relevant for your application, please note that we may reach out to you to ask you to provide it within the evaluation period with a short deadline (5-10 days). Please note that an English translation of the Ethics approval will be needed.

If you already have an approval, please upload it here

No file uploaded

I confirm that I have taken into account all ethics issues described above

Yes

PART F. Declarations

I declare to have the written consent of all participants on their participation and on the content of this proposal, as well as of any researcher mentioned in the proposal as participating in the project (either as other researcher, team member or collaborator)

Yes

I declare that the information contained in this proposal is correct and complete

Yes

I declare that all parts of this proposal comply with ethical principles (including the highest standards of research integrity as set out, e.g., in the European Code of Conduct for Research Integrity and including, avoiding fabrication, falsification, plagiarism or other research misconduct)

Yes

Proposal Team

Principal Investigator

[redacted]

Research Team