$^{155/161}$Tb-labeled radioconjugates for cancer therapy with Auger electrons

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Introduction & background: (state of the art and goal/motivation for the project)

Auger-emitting radionuclides clinically used for SPECT imaging (e.g. ^{99m}Tc, ^{123}I, ^{67}Ga or ^{111}In) started also to be envisaged for selective and targeted radiotherapy. This interest is related with the short penetration (< 0.1 µm) of Auger electrons in biological tissues, typically less than one cell diameter, which make Auger-emitting radionuclides best suited for the eradication of disseminated cancer metastases, with minimization of deleterious effect to non-target surrounding tissues.

To profit from the advantages inherent to Auger-emitting radionuclides, it is essential that their radiocompounds specifically recognize the target tumoral cells and are transported to their nucleus to elicit DNA damage. For this goal, the most obvious strategy relies on the design of multifunctional radioconjugates that contain a specific targeting vector (*e.g*. peptide or antibody) to warrant a selective uptake by the tumoral cells and a chemical entity (*e.g*. a nuclear localization signal (NLS) peptide or a DNA intercalator) to promote the internalization in the cell nucleus and/or in a close proximity to DNA. In our research group, we have explored the later approach for the Auger-emitter ^{99m}Tc, based on pyrazolyl-diamine ^{99m}Tc(I) tricarbonyl complexes containing bombesin (BBN) analogues and acridine orange (AO) DNA intercalators.[1,2] Our studies showed that ^{99m}Tc(I) complexes having a triglycine (GGG) linker between the BBN[7-14] sequence and the pyrazolyl-diamine chelator framework presented a high cellular internalization in prostate cancer cells (PC3 cell line), and a remarkably high nuclear uptake in the same cell line. The presence of the GGG linker had a great influence on the nuclear internalization of the compounds, which might be due its possible cleavage by lysosome enzymes, like cathepsin-B.[3] Upon cleavage, the released radiocomplex showed a better ability to cross the nuclear membrane and reach the DNA. This result might open new strategies to design best performing metallated radioconjugates for cancer therapy with Auger electrons, as proposed herein.

Project description: (detailed description of the project, translational, pre-clinical, imaging, treatment, new method)

The main goal of this project is to assess the potential interest of $^{155/161}$Tb-radioconjugates as new tools for anticancer Auger therapy. The $^{155/161}$Tb-radioconjugates to be designed and pre-clinically evaluated are stabilized by macrocyclic chelators of the DOTA type [4] carrying a bioactive vector (e.g. a BBN analogue or a PSMA inhibitor), a cleavable linker and a DNA-targeting moiety. The presence of the cleavable linker is expected to promote the intracellular release of a radiocomplex (carrying a DNA intercalator) with a better ability to reach the nucleus and interact with DNA. Related complexes will be obtained carrying a mitotropic moiety (e.g. a phosphonium derivative) for an augmented and selective accumulation of the radiocomplexes in the mitochondria. Mitochondria is considered a relevant target for the action of Auger electrons aiming at the erradication of tumor cells. However, so far, mitochondria targeting by Auger-emitting radioconjugates remains almost unexplored [5]. The new $^{155/161}$Tb-radioconjugates will be firstly evaluated in human prostate cancer cell lines to assess their cellular and nuclear uptake, as well as their radiotoxic effects. At a later stage, studies will be also conducted in appropriate animal models.

Materials and Methods : (planned experiments, where, licences for radioisotopes/animals, timeline)

To achieve the objectives of the project the following experiments will be carried on at the Radiopharmaceutical Sciences Group of C^{2}TN/IST:

-Optimization of the radiolabelling of the DOTA chelators with ^{155}Tb and ^{161}Tb ;

-Cellular studies of the ^{155}Tb and ^{161}Tb radioconjugates in human prostate cancer cell lines (cell uptake/ nuclear internalization)

-Biological effects of exposure to the $^{155/161}$Tb-radioconjugates radiolabeled will be studied by the clonogenic assay and screening of different cellular radiation biomarkers (cytogenetic biomarkers, such as micronuclei or chromosomal aberrations or gama-H2AX). To have a better insight on the role of Auger electrons in the observed radiobiological effects, similar studies will be also conducted for congener ^{177}Lu radioconjugates.

The laboratory facilities and team members are properly licensed by the National Authorities to work with the proposed radioisotopes and animals.

References and Funding: (literature, funding of project, other projects/grants linked)

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Isotope requests : (which isotope, activity, number of deliveries over period, purity grade)

^{155}Tb and ^{161}Tb, 50-100 MBq per batch, 4 - 6 deliveries/ 9 months, high radionuclidic purity.