Development of a new prostate cancer theranostic radiopharmaceutical

Author: Laura Melendez-Alafort[1]
Co-author(s): Debora Carpanese[1]; Guillermina Ferro-Flores[2]; Blanca Ocampo-García[2]; Clara Leticia Santos-Cuevas[2]; Laura De Nardo[3]; Nicola Salvarese[4]; Giulio Fracaso[5]; Cristina Bolzati[4]; Antonio Rosato[3]

[1]*Veneto Institute of Oncology IOV-IRCCS*
[2]*Instituto Nacional de Investigaciones Nucleares*
[3]*University of Padova*
[4]*Institute of Condensed Matter Chemistry and Energy Technologies ICMATE-CNR*
[5]*University of Verona*

Corresponding author: lmalafort@yahoo.com

Prostate cancer (PCa) is the second leading cause of cancer deaths for adult men in the Western world. Although radical prostatectomy and local radiotherapy are largely successful for patients with localized cancer, available treatments for metastatic PCa have demonstrated weak curative efficacy. Consequently, new tools to improve the detection of recurrent PCa, and to identify and treat metastases, are imperatively needed. Antibody-based constructs represent a good strategy to develop theranostic agents. Currently, the murine mAb ^{111}In-capromab pendetide (ProstaScint®) is the only product that has been approved by the Food and Drug Administration (FDA) as a diagnostic radiopharmaceutical for PCa. ProstaScint® showed promising results in clinical diagnosis, but as a whole antibody exhibits low tumour targeting with a maximum uptake at 6-7 days post-injection and delayed clearance from non-target tissues. These issues limit its use as theranostic agent. Recently, preclinical studies of an anti-PSMA single-chain variable fragment of IgGD2B mAb (scFvD2B) labelled with ^{123}I, showed high tumour affinity, improved antigen-positive tumour uptake, with shorter circulatory half-life, and decreased uptake in non-target tissues. The aim of this work was to develop a new PCa theranostic radiopharmaceutical based on the scFvD2B radiolabel with ^{177}Lu.

The scFvD2B was conjugated to the chelating agent DOTA by using different stoichiometric molar ratios. The number of DOTA per scFvD2B and the affinity constant (Kd) for each construct was determined to choose the conjugated with higher specific targeting activity against PSMA receptors. The select DOTA-scFvD2B conjugate was labelled with $^{177}LuCl_3$. Stability of ^{177}Lu-DOTA-scFvD2B was studied using HPLC analysis after incubation at 37 °C with fresh hu- man serum, cysteine, glutathione or EDTA solutions (300-fold excess), at time points ranging from 0.5 to 192 h. In vitro cell studies were performed to determine the binding specificity and cellular internalization of ^{177}Lu-DOTA-scFvD2B. Biodistribution studies were performed in both healthy and PCa-bearing mice to evaluate ^{177}Lu-DOTA-scFvD2B pharmacokinetics and assess its tumour detection potential using SPECT imaging.

DOTA-scFvD2B Kd values showed that the construct characterized by 1:5 (scFvD2B:DOTA) molar ratio is the one with the greatest number of DOTA per scFvD2B which maintains the high specificity for the PSMA receptor. ^{177}Lu-DOTA-scFvD2B possessed high in vitro stability, the radiochemical purity of the radioconjugate accomplished at 192 hours after dilution was higher than 98%. Biodistribution studies performed in healthy mice after intravenous administration of the radioconjugate demonstrated that DOTA did not significantly change the scFvD2B pharmacokinetic properties. Indeed, ^{177}Lu-DOTA-scFvD2B showed a favourable biokinetic profile with a rapid blood clearance. Moreover, SPECT/CT imaging studies carried out in mice bearing PCa tumours in lungs proved good and specific tumour detection properties of ^{177}Lu-DOTA-scFvD2B from 6 to 192 hours post-injection.

In conclusion, ^{177}Lu-DOTA-scFvD2B high stability and specific affinity for the PSMA receptors in vitro and in vivo make this radioconjugate a promising PCa theranostic radiopharmaceutical. However, further dosimetric studies have to be performed to establish its therapeutic potential.