Neurotensin receptor imaging: ^{68}Ga-DOTA-NT-20.3 uptake on pancreatic ductal adenocarcinoma cell line

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the most common malignancy of the pancreas, accounting for approximately 96% of all pancreatic tumours. Due to the lack of specific signs and symptoms, as well as aggressive growth behavior, in majority of cases treatment is palliative achieving median overall survival of only 8.5 months. In patients with PDAC, ^{18}F-FDG PET/CT has an established role in preoperative staging, suspicion of recurrent disease as well as in the follow-up of therapeutic treatment. Unfortunately, ^{18}F-FDG PET/CT imaging did not significantly increase the accuracy of PDAC diagnosis.
Neurotensin receptors are overexpressed in several cancer types including pancreatic ductal adenocarcinoma: approximately 75% up to 88% of PDAC express neurotensin receptor 1 (NTR-1), a possible target for neurotensin (NT) fragment DOTA-NT-20.3 labeled with ^{68}Ga.
The aim of this study was to test the affinity, uptake kinetics and rate of interaction of ^{68}Ga-DOTA-NT-20.3 with human pancreatic adenocarcinoma cell line AsPC-1, so as to assess whether this new radioligand might be applied *in vivo*.

Materials and Methods: NT peptide fragment 6-13, has been appropriately modified and derivatized with the DOTA chelator. This compound was named DOTA-NT-20.3 (Thanks to the patent proprietor IASON GmbH, Graz, Austria, who generously provided the peptide compound).
^{68}Ge/^{68}Ga generator was eluted with 8 mL 0,1 M HCl. A cleaning elution was carried out to eliminate the ^{68}Zn present in the generator, which can affect the synthesis yield. For the preparation of ^{68}Ga-DOTA-NT-20.3, 150-800 MBq of ^{68}GaCl3 (app. 8.0 mL) and 50 µg of precursor, dissolved in 50 µL TraceSelect Water, were used in an automatic synthesis module. 2 mL of CH_3COONa/CH_3COOH 0.8 M buffer and 400 µL EtOH/H_2O at 50/50 % (v/v) was added to 50 µg NT-20.3 and to 0.8 mL $^{68}Ga^{3+}$ solution eluted from column. The reactor was heated to 80±2°C for 3 min and then to 110±2°C for 7 min. The product was diluted to 8 mL with 0.9 % saline solution. Final product pH ranged between 4.0 and 5.0.
Quality control: 20 µl of ^{68}Ga-DOTA-NT-20.3 were injected into HPLC equipped with a ZORBAX Eclipse Plus C18 column, radiometric NaI(Tl) crystal and UV 254 nm (diodes array) detectors. The mobile phase was constituted by solvent A (acetonitrile and 0.1% trifluoroacetic acid) and solvent B (water and 0.1% trifluoroacetic acid). HPLC ran at 25°C with a 0.6 ml/min flow and a gradient of 00:00-07:00 min: 100-30% B.
^{68}Ga-DOTA-NT-20.3 retention time was approx. 7 min.
Cell treatment: AsPC-1 cells were seeded at a density of $2x10^5$ cells per dish and growth adherent to the plastic surface.
^{68}Ga-DOTA-NT-20.3 experiments were performed 24h post seeding. AsPC-1 cells were incubated for 40 min with increasing concentrations of ^{68}Ga-DOTA-NT-20.3 in order to reach activities of about 50, 100, 300, 500 kBq. About 100 kBq of ^{68}Ga-DOTA-NT-20.3 were added in uptake vs. time experiments. Uptake was evaluated at different times: 40, 60, 80 min. Control samples underwent the same procedure as other samples, but were incubated with fresh medium only. All experiments were performed at 37°C.

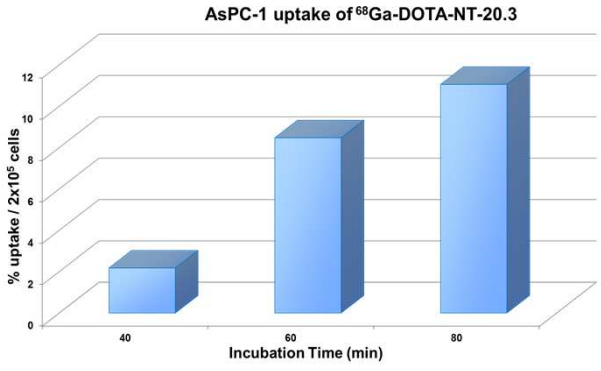
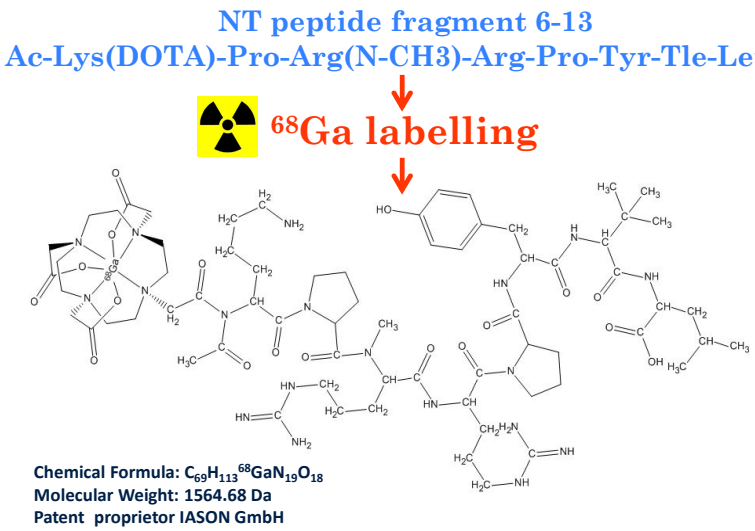


Figure 1: ^{68}Ga-NT-DOTA-20.3 uptake (%) in AsPC-1 cells *vs.* incubation time (min).



Chemical Formula: $C_{69}H_{113}{}^{68}GaN_{19}O_{18}$
Molecular Weight: 1564.68 Da
Patent proprietor IASON GmbH

Results: ^{68}Ga-DOTA-NT-20.3 uptake plateaued 80 min after incubation (fig.1). Bound peptide radioactivity was measured by gamma counter (RaytestGita). Kd (7.335 pmol) and Bmax (90.52 kBq) value were determined by nonlinear regression one-binding site model, using GraphPad Prism 5.01 software. Bmax value was used to calculate AsPC-1 binding sites, resulting in 1.09 x 106 sites per cell. It was performed at 37 °C, instead of 4 °C, thus in the presence of a significant NTR internalization percentage. Isotherm analysis for the determination of binding saturation showed a Hill slope (h) of 0.87 (fig.2).

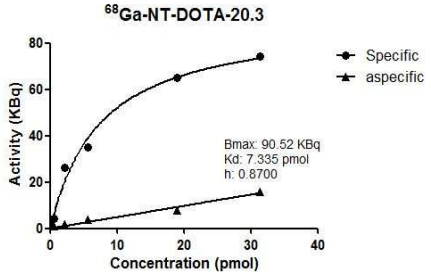


Figure 2: ^{68}Ga-NT-DOTA-20.3 activity (kBq) vs. concentration (pmol) in AsPC-1 cells

Discussion: The use of ^{68}Ga-DOTA-NT-20.3 in patients with PDAC is, in our opinion, the most promising clinical application for ^{68}Ga-NT analogue. Our decision to test the new NT analogue ^{68}Ga-DOTA-NT-20.3 on AsPC-1 was dictated by the need for an *in vitro* model which can be used for future *in vivo* application. Moreover *in vitro* studies on AsPC-1 cells are not present in the literature. We chose DOTA as a chelator despite the fact that it can potentially induce an alpha-helix-structure of others peptide altering its bioavailability. As regards NT, no detrimental effect on the affinity and distribution of DOTA chelator was observed either *in vitro* or *in vivo*.

Conclusion: Our preliminary *in vitro* results on ^{68}Ga labelled DOTA-NT-20.3 in a pancreatic adenocarcinoma AsPC-1 cell line indicate that ^{68}Ga-DOTA-NT-20.3 might prove to be a potential radiopharmaceutical for pancreatic cancer *in vivo* imaging.
Further *in vitro* experiments require a ^{68}Ga-DOTA-NT-20.3 uptake evaluation on non-cancer cells in the tumour microenvironment, as well as in acute and chronic pancreatitis.