Incorporating $^{131}$I into a PAMAM (G5.0) dendrimer-conjugate: design of a theranostic nanosensor for medullary thyroid carcinoma†

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We report the synthesis and purification of a targeting probe for Medullary Thyroid Carcinoma (MTC) by incorporating $^{131}$I into PAMAM (G5.0) dendrimers. Both the $^{131}$I labeled control dendrimer ($^{131}$I-PAMAM (G5.0) without attached targeting peptide) and the MTC-targeting dendrimer ($^{131}$I-PAMAM (G5.0) attached to VTP (vascular targeting peptide)) were labeled with the radioisotope $^{131}$I by applying the iodogen method. The resulting G5.0 dendrimers were purified by means of ultracentrifugation. The labeling efficiencies and radiochemical purities vs. time were determined by paper chromatography. The radiolabeling efficiencies of $^{131}$I-PAMAM (G5.0) and $^{131}$I-PAMAM (G5.0) were 93 ± 1% and 85 ± 2%, respectively. $^{131}$I-PAMAM (G5.0) did show small, but significant changes in radiochemical purity as a function of time after labeling. The highest observed highest purity was 82 ± 2%. $^{131}$I-PAMAM (G5.0)-VTP did display larger changes in radiochemical purity as a function of time after labeling, maximally 80 ± 2%. The stability of the two probes and their binding behavior to the human medullary thyroid cancer cell line (TT) were observed in vitro. Compared to the negative control group (consisting of Na$^{131}$I), the TT cell binding rate of $^{131}$I-PAMAM (G5.0)-VTP was significantly increased at 48 h and 72 h ($P < 0.01$). The TT cell binding rate of $^{131}$I-PAMAM (G5.0)-VTP at 48 h and 72 h was not significantly different when compared to the positive control group ($^{131}$I-PAMAM (G5.0) group) ($P > 0.05$). These findings have been confirmed by performing MTT assays. These results confirm earlier findings, which demonstrated fast uptake of PAMAM (G5.0) by various cell types.

Introduction

Medullary thyroid carcinoma (MTC) is not a real thyroid carcinoma, because it originates from parafollicular cells (C cells), which secrete calcitonin.2,3 Since C cells neither express the thyrotropin receptor NIS (sodium iodide symporter) nor take up iodine, $^{131}$I radiation therapy is ineffective.3 Furthermore, a majority of MTC patients are diagnosed with terminal cancer.3

Early MTC is known to invade the regional lymph nodes and then transfer to further organs (i.e. lungs) and bone. Thus, the complete surgical resection of all tumour tissue is difficult or impossible.4 With advances in molecular biology, numerous researchers focus on targeted therapies and biological therapies of MTC.2,5,6 Compared to the large antibody molecules, small peptides are advantageous, because they can avoid macromolecular protein immunogenicity, slow clearance, and other shortcomings.7 In addition, small peptides can effectively bind to targeted receptors and be labeled with radioactive elements, such as $^{131}$I, by means of straightforward chemical procedures.8

This study was designed as the first step in developing a nanosensor for early in vivo MTC diagnosis and subsequent treatment by using a $^{131}$I- and VTP-labeled G5.0 PAMAM (Polyamidoamine) dendrimer. The targeting efficacy of this radiolabeled nanosensor towards cultured TT cells will provide a first reference for the clinical treatment of MTC. We anticipate that this study will be followed by studying the targeting and treatment efficiencies of the radiation nanosensor in a mouse xenograft model for MTC.

Experimental

Materials and equipment

Na$^{131}$I (Chengdu Gaotong Isotope Ltd), fetal bovine serum (FBS), F12 medium and trypsin (Gibco company), ultrafiltration tube (Millipore Corporation), 12-well cell culture plate (Clone company), phosphate-buffered saline (PBS) (Shanghai Yuanye Biotech Ltd), iodogen beads (Sigma-Aldrich), Xinhua No. 1...
chromatography paper (Hangzhou Xinhua paper company), human medullary thyroid cancer cell line (TT) (GuangZhou Jennio Biotech Ltd), radionuclide activity meter (Beijing Huarui Science & Technology Development Ltd), γ counter (USTC Zonkia). Labelled PAMAM (G5.0) nanosensors and the VTP peptide sequence were synthesized at Kansas State University (see below). The MTT cell proliferation assay was performed using PBS buffer (phosphate-buffered saline: 8.0 g NaCl, 2.0 g KCl, 14.4 g Na2HPO4·7H2O, 2.4 g KH2PO4 and 0.80 L bidist. H2O, pH = 7.4) was obtained from Fisher Bioreagents.

**Synthesis of peptides**

Three functional peptide sequences were synthesized via standard Solid Phase Peptide Synthesis (SPPS) method. Briefly, preloaded trityl-resin was swelled in DCM for 20 min. After washing with DMF, Fmoc-protected amino acids were added, with N,N,N’,N’-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) as coupling agent, diisopropylthylamine (DIEA) as base, and anhydrous DMF as solvent. Upon completion of the reaction, the excess amino acid, HBTU, and any other side products were removed by washing the resin with DMF. Next, the Fmoc protection group was removed in 20% piperidine/DMF solution, yielding free NH2 groups for further amide bond formation to elongate the peptide chain. After addition of the amino acids sequentially, the peptide sequences were cleaved from the trityl-resin using a 95/2.5/2.5 trifluoroacetic acid/triisopropylsilyl ether/water (TFA/TIPS/H2O) cocktail, and then precipitated in cold ether. The collected product was dissolved in DI water, and then was dried by lyophilization. The final product was characterized by HPLC and MALDI-TOF. The procedure is described in detail in our previous work. The following oligopeptides were synthesized: KYKYKYC for 11 binding and GPLPLRCK§ for enhanced uptake by C cells. Their MALDI-TOF spectra are shown in the ESI section.

**Synthesis of maleimide-glycine acid chloride (Mal-Gly-Cl)**

Glycine (5.0 g, 66.6 mmol) and maleic anhydride (6.6 g, 66.6 mmol) were suspended in 80 mL of acetic acid and allowed to react for 3 h at RT. The resulting white precipitate was collected by filtration, washed with cold water, and dried in high vacuum at 45 °C. The obtained white solid was used without further purification. 10.95 g, 63.3 mmol, 95%. Glycine maleic acid (10.95 g) and 2.1 equivalents of triethylamine (12.6 g) were refluxed for 2 h in 400 mL toluene at 140 °C (oil bath) using a Dean-Stark apparatus to remove the formed water. Upon completion of the reaction, the toluene solution was decanted while still hot and then concentrated to dryness. To the resulting solid, 150 mL ethyl acetate was added, and the mixture was acidified with 2 M HCl until all the solid was dissolved. The organic phase was washed with water (30 mL, three times), brine (30 mL, one time), and then dried with anhydrous MgSO4. Finally, the drying agent was filtered off, and the solvent was concentrated to yield 3.40 g Mal-Gly-OH (yield 36.5%) the product was characterized by proton NMR (400 MHz, CDCl3): δ = 10.73 (broad, 1H), 6.81 (s, 2H), 4.34 (s, 2H) (Scheme 1).

The obtained maleimide glycine was converted to the active maleimide glycine acid chloride by stirring 1.0 g of Mal-Gly-OH in 6.0 mL of dry oxalyl chloride under an Ar atmosphere for 2 h at RT. The excess oxalyl chloride was removed by means of a Schlenk technique using a liquid N2 trap. This reagent was prepared freshly prior to the coupling reaction with the G5 PAMAM dendrimer. A stock solution was prepared in anhydrous DMF with 50 mg mL⁻¹ concentration (stock solution A).

**Synthesis of PAMAM G5 dendrimer–maleimide conjugate**

5.0 mL 5% PAMAM G5 dendrimer methanol solution (by weight percentage) was transferred to a 25 mL round bottom flask, methanol was removed via standard Schlenk technique. 10 mL of dry DMF was added via a syringe, the flask was flushed with dry argon and sealed. After stirring for 24 hours, a clear homogenous PAMAM G5 dendrimer DMF solution formed with 100 mg mL⁻¹ concentration. (Stock solution B). The conjugation of PAMAM G5 dendrimer with maleimide-glycine acid chloride was achieved by the mixing of stock solution A and B with designated ratio, in the presence of 1 equivalent dry triethyl amine relative to Male-Gly-acid chloride. After stirring at RT for 24 hours, the reaction mixture was diluted with 10 mL of DI water, and the unreacted small molecules were removed by dialysis in a membrane bag with molecular weight cut-off of 10 000. The purified product was concentrated to dryness by lyophilization.

The conjugation of peptide sequences was achieved by incubating of peptides with designated ratio with PAMAM G5 dendrimer–maleimide in PBS (1×) buffer (pH = 7.2) for 24 hours. The final products were purified by dialysis in a membrane bag with molecular weight cut-off of 10 000. The purified products were concentrated to dryness by lyophilization.

Specifically, 20 mg of G5-maleimide and 10 mg of peptide sequence KYKYKYC were dissolved in 2 mL 1× PBS buffer (pH

![Scheme 1](image1.png) Synthesis of maleimide-glycine acid chloride: (a) acetic acid; (b) trimethylamine in toluene, reflux, followed by ethylacetate/HCl; (c) oxalyl chloride.

![Scheme 2](image2.png) Attachment of maleimide-glycine acid chloride to PAMAM Starburst dendrimer, generation 5.0.
of both oligopeptides. According to Dendritech, the only source of sulphur is the cysteine at the C-terminal end G5.0 with KYKYKYC and GPLPLRC. This is possible, because Inductively coupled plasma (ICP) measurements to determine temperature. PAMAM (G5.0)–VTP was 1.41 ± 0.3% (by weight). Assuming that the labelling degree with KYKYKYC did not change during the co-labelling procedure, we estimate 9 ± 2 chemically attached GPLPLRC labels per PAMAM dendrimer.

**Determination of radioactive labelling efficacy**

The efficacy of PAMAM (G5.0) labelling with $^{131}$I was determined by comparing the radioactivity of purified $^{131}$I–PAMAM (G5.0) or $^{131}$I–PAMAM (G5.0)–VTP with the total radioactivity utilized in the labelling reaction.

**In vitro radiochemical purity analysis of $^{131}$I–PAMAM (G5.0) and $^{131}$I–PAMAM (G5.0)–VTP**

The radiochemical purities of two nanosensors were determined 0 h, 2 h, 4 h, 6 h, and 24 h after synthesis. At the same time intervals, the negative control group Na$^{131}$I was also measured. Xinhua No. 1 paper was used as stationary phase, and methanol/normal saline (4 : 1, 2 : 1 or 1 : 1) or acetone/normal saline (4 : 1, 2 : 1 or 1 : 1) were used as mobile phases. For each time point, 0.30 μCi of the control group (G5.0), MTC targeting group ($^{131}$I–PAMAM (G5.0)) and negative control group (Na$^{131}$I) were spotted on chromatography paper, separately. After performing paper chromatography and subsequent drying, the paper was cut into 10 even pieces and numbered as 1–10 from bottom to top (#1 is bottom part contains original spot). The radioactivity on each piece was measured by a $\gamma$-counter. The radiochemical purity was calculated according to the following formula:

$$\text{Radioactivity of the highest counting piece min}^{-1} \text{ blank)/ (total radioactivity of 10 pieces min}^{-1} \text{ blank} \times 10 \times 100\%.$$  

**In vitro TT cell binding experiments**

TT cells were cultured and then transferred into a 12-well plate with a concentration of $8 \times 10^5$ per well. A complete medium (FBS 10%) was used to culture cells for 48 h and then discarded. Then a maintenance medium (FBS 2%) was used for culturing four different groups, which were the PBS control, Na$^{131}$I negative control, $^{131}$I–PAMAM (G5.0) control and the MHC targeting $^{131}$I–PAMAM (G5.0)–VTP nanosensor. Except in the PBS control group, 30 kBq of Na$^{131}$I, $^{131}$I–PAMAM (G5.0) or $^{131}$I–PAMAM (G5.0)–VTP were added to each group in three replications. After 48 h and 72 h of culturing, the cells were digested with trypsin and then washed with 1.0 mL PBS per well and centrifuged at low-speed (2000 rpm) for 2 min. After washing 3 times and centrifuging, the supernatant was discarded, and cells were collected and their radioactivity was counted by using a $\gamma$-counter. The following equation was used to analyse the obtained results:
Cell binding efficiency = \frac{[\text{radioactivity of cells in one well min}^{-1}]}{[\text{radioactivity of cells in one well min}^{-1} + \text{radioactivity of the medium and combined 3 times washing for one well min}^{-1}]} \times 100%.

Statistical analysis

All data was analysed by employing the SPSS 17.0 software according to the ANOVA method. The results are shown as $X \pm SD$ (mean value $\pm$ standard error). $t$-Tests were conducted. $P < 0.05$ was considered as significant.

Results

Radioactive labelling efficacy

$^{131}$I–PAMAM (G5.0) and $^{131}$I–PAMAM (G5.0)–VTP have radioactive labelling efficacies of 92.2% and 77.6%, respectively.

Fig. 1 Radio-chemical purity determination of negative control Na$^{131}$I immediately after synthesis (0 h). The volume in each collection vial was 2.0 mL. (a) Acetone : normal saline 1 : 1; (b) acetone : normal saline 2 : 1; (c) acetone : normal saline 4 : 1; (d) methanol : normal saline 1 : 1; (e) methanol : normal saline 2 : 1; (f) methanol : normal saline 4 : 1.

Fig. 2 Radio-chemical purity determination of $^{131}$I–PAMAM (G5.0) immediately after synthesis (0 h). The volume in each collection vial was 2.0 mL. (a) Acetone : normal saline 1 : 1; (b) acetone : normal saline 2 : 1; (c) acetone : normal saline 4 : 1; (d) methanol : normal saline 1 : 1; (e) methanol : normal saline 2 : 1; (f) methanol : normal saline 4 : 1.

Fig. 3 Radio-chemical purity determination of $^{131}$I–PAMAM (G5.0)–VTP immediately after synthesis (0 h). The volume in each collection tube was 2.0 mL. (a) Acetone : normal saline 1 : 1; (b) acetone : normal saline 2 : 1; (c) acetone : normal saline 4 : 1; (d) methanol : normal saline 1 : 1; (e) methanol : normal saline 2 : 1; (f) methanol : normal saline 4 : 1.

Fig. 4 Radio-chemical purities of $^{131}$I–PAMAM (G5.0) at different time points. (a) Acetone : normal saline 1 : 1; (b) acetone : normal saline 2 : 1; (c) acetone : normal saline 4 : 1; (d) methanol : normal saline 1 : 1; (e) methanol : normal saline 2 : 1; (f) methanol : normal saline 4 : 1.

In vitro radiochemical purity analysis

The highest radioactivity of the negative control Na$^{131}$I was located in tube #9 or #10, depending on the employed mobile phase (Fig. 1) immediately after synthesis. $^{131}$I–PAMAM (G5.0) showed the highest radioactivity in tube #1 immediately after synthesis, disregarding of the mobile phase used (Fig. 2). However, $^{131}$I–PAMAM (G5.0)–VTP displayed high radioactivity immediately after synthesis in tubes #1, and #8 to #10, depending on the separation conditions (Fig. 3).

Radiochemical purity analyses of the same probe at different times after synthesis and different developing solvents

$^{131}$I–PAMAM (G5.0) did exhibit small, but significant changes in radiochemical purity as a function of time after labelling (Fig. 4). It had the highest purity of $82 \pm 2\%$ when using either acetone : normal saline (4 : 1) or methanol : normal saline (4 : 1) as mobile phase. Furthermore, there is a slower decrease of radiochemical purity for $^{131}$I–PAMAM (G5.0) when developed with
acetone : normal saline (4 : 1) compared to all other mobile phases. $^{131}$I–PAMAM (G5.0)–VTP did display a noteworthy change in radiochemical purity as a function of time after labelling. $^{80/C6}2\%$ was observed when using methanol : normal saline (2 : 1) was used as mobile phase. It then decreased slowly over time under most conditions, as anticipated when using $^{131}$I with a half-life time of 8.0197 days.

The major outcome of this series of experiments was that for $^{131}$I–PAMAM (G5.0) and $^{131}$I–PAMAM (G5.0)–VTP the highest radiochemical purities are obtained under slightly different conditions, thus reflecting the different isoelectric points (pI's) of the attached oligopeptides (see Discussion section). However, for both PAMAM-derived dendrimers, saline is required for breaking some, but not all hydrogen bonds between the primary –NH$_2$ groups of the PAMAM dendrimer and Xinhua No. 1 chromatography paper, thus enabling $R_f$-values for all dendrimer-derivatives between 0.35 and 0.40 (Fig. 5).

**TT cell morphologies at different time points after exposure**

After 30 kBq of $^{131}$I–PAMAM (G5.0) or $^{131}$I–PAMAM (G5.0)–VTP were administered to TT cells at $t = 0$, the population of cells decreased. Some cells showed growth inhibition and the number of cells in suspension increased. However, no significant changes in cell morphology were observed (Fig. 6).

**TT cell binding efficacies at various time points after treatment**

Compared to the negative control group (Na$^{131}$I), $^{131}$I–PAMAM (G5.0) and $^{131}$I–PAMAM (G5.0)–VTP show significantly increased TT cell binding efficacies after 48 h and 72 h ($P < 0.01$). However, there is no significant statistical difference between the cell uptake of $^{131}$I–PAMAM (G5.0) and $^{131}$I–PAMAM (G5.0)–VTP ($P > 0.05$) (Table 1, Fig. 7).

**Discussion**

Thyroid cancer is rapidly increasing in China. A double-digit annual rise in the number of thyroid cancer cases in China was observed over the past decade, especially among young and middle-aged women. Currently, surgery is the main method to treat MTC. However, even early stage MTC can invade regional lymph nodes and spread to lungs, bones, and other distant organs. Surgery often cannot completely remove the tumour tissue and, consequently, the prognosis of MTC is very poor. To solve this problem, development of accurately
targeting novel drug formulations will become the key to successfully treating MTC. In this paper, a preliminary study comprising the synthesis, purification, activity evaluation and cell toxicity of a starburst dendrimer-based delivery platforms is described (Fig. 8).

Polyamidoamine dendrimers (PAMAM) are synthetic, stable and water soluble nanostructures. They have good biocompatibility and are suitable for carrying drug molecules. PAMAM dendrimers have gained much interest because their surface can be controlled through precisely defined chemical modifications whereas their interior can be used to bind drugs, metal cations, and nanoparticles. If targeting groups with specific binding ability to tumours, such as polypeptides, carbohydrates, folic acid, etc. are tethered to PAMAM dendrimers, targeted tumour diagnosis and treatment can be achieved.

To date, polypeptides, such as Gly-Pro-Leu-Pro-Leu-Arg (GPLPLR or VTP) were conjugated to liposomal formulations and used to target tumour tissue that is prone to neovascularization. Other researchers conducted in vitro experiments of liposomes conjugated with GPLPLR. This peptide was found to display high affinity towards umbilical vein endothelial cells by targeting membrane type-1 matrix metalloproteinase (MT1–MMP, MMP14), which is expressed in both, angiogenic endothelium and tumour cells. Consequently, GPLPLR caused the inhibition of tumour growth in Colon 26 NL-17 carcinoma cells. Furthermore, coupling the peptide sequences GPLPLR and AIPRG, which targets the neovasculature as well, can suppress growing colorectal cancer, melanoma and sarcoma cells in mice. With respect to selecting the best PAMAM dendrimer for this study, previous studies have shown that the fifth full generation (G 5.0) of PAMAM dendrimer featuring 128 primary surface amine groups possesses distinctly less toxicity towards mammalian cells than earlier lysine-based dendrimers.

In this study, a modified PAMAM (G 5.0) was reacted with Na$^{131}$I to form a nanosensor containing chemically bound $^{131}$I for the purpose of targeting human medullary thyroid cancer cells. The positive control group $^{131}$I–PAMAM (G 5.0) has a radio labelling degree of 93 ± 1% of the originally added $^{131}$I activity, while the targeting $^{131}$I–PAMAM (G 5.0)–VTP nanosensor has a labelling degree of 85 ± 2%. It is noteworthy that 18 ± 2 of the 128 primary amine groups of PAMAM G 5.0 are linked to KYKYKYC. Therefore, multiple attachments of $^{131}$I to KYKYKYCY could be possible. However, our calculation of the average labelling degree of both dendrimers with $^{131}$I has shown that each mole of G 5.0 was chemically linked to 0.81 moles of $^{131}$I, whereas each mole of G 5.0–VTP was coupled to 0.84 moles of $^{131}$I. Principally, higher (radio) labelling degrees can easily be achieved if the labelling reaction can be performed in higher concentration. However, increasing the $^{131}$I activity beyond 3 mCi will require enhanced protection measures against radiation damage. It is noteworthy that although the radio labelling degree of G 5.0–VTP is lower than of G 5.0, both dendrimers have a very similar labelling degree with $^{131}$I (0.81 vs. 0.84), due to the higher molecular mass of G 5.0–VTP.

We explain the slightly different chromatography performance by G 5.0 and G 5.0–VTP with their labelling degree with iodine-binding and targeting oligopeptides. Whereas approx. 18 of the 128 primary amine groups of both PAMAM dendrimers (G 5.0 and G 5.0–VTP) are occupied with the attached labelling peptide (KYKYKYC), only G 5.0–VTP bears 9 targeting sequences (GPLPLR). Whereas KYKYKYC features an isoelectric point of pI = 9.43, GPLPLRC possesses a significant lower pI of 8.25. Therefore, the surfaces of G 5.0 and G 5.0–VTP feature slightly, but significantly different ionization states and octanol/water partitioning coefficient (log P$^{w}$), which are reflected in their different performance in paper chromatography.

As already discussed in the introduction, parafollicular cells (C cells) do not express the thyrotopin receptor NIS and therefore, they cannot uptake iodine directly. Consequently, $^{131}$I therapy is not possible without providing a pathway for iodine to be taken up. This experiment uses two synthetic iodide-carriers to transport $^{131}$I to TT cells in vitro. Since MMP14 is expressed in numerous solid tumours, it was of great interest whether the presence of the MMP14 targeting VTP peptide in $^{131}$I–PAMAM (G 5.0)–VTP can enhance the uptake of $^{131}$I by TT cells compared to the non-VTP labelled $^{131}$I–PAMAM (G 5.0). The results indicated that both $^{131}$I–PAMAM (G 5.0) and $^{131}$I–PAMAM (G 5.0)–VTP were taken up by TT cells at significantly higher rates than the negative control group Na$^{131}$I. However, there was no statistical difference in the uptake of $^{131}$I–PAMAM (G 5.0) and $^{131}$I–PAMAM (G 5.0)–VTP, as measured by $γ$ counting. The reason for this behaviour is the excellent uptake of PAMAM dendrimers by mammalian cells.

Similarly, but even more pronounced trend can be discerned from the results of the MTT assays. 48 h after the beginning of $^{131}$I exposure, the control group and the group of TT cells, which was given Na$^{131}$I, displayed undistinguishable cell viabilities. However, the viabilities of the TT cells treated with G 5.0 and G 5.0–VTP were significantly lower (43 ± 3% (G 5.0) and 52 ± 3% (G 5.0–VTP)). This trend was even more pronounced after 72 h. At that time interval, all three treatment groups showed significantly reduced cell viabilities ($p < 0.05$, compared with the control group of TT cells. In agreement with the data shown in Table 1, the decrease in viability was stronger for the group of cells treated with G 5.0 than with G 5.0–VTP.

Under in vitro conditions, there is no advantage of having an additional targeting group on the PAMAM dendrimer. However, targeting the microvasculature of MTC remains a viable strategy.
for treating inoperable cases and as adjuvant therapy after surgery. Therefore, our next step will consist in testing the MTC targeting efficacy of $^{131}$I-PAMAM (G5.0) and $^{131}$I-PAMAM (G5.0)-VTP vs. Na$^{131}$I in a xenograft mouse model of MTC.

**Conclusions**

The iodogen method was used to chemically bind $^{131}$I to a generation 5 (G5.0) PAMAM dendrimers. Two PAMAM dendrimers derivatives were synthesized, $^{131}$I-PAMAM (G5.0) and $^{131}$I-PAMAM (G5.0)-VTP. The vascular targeting peptide (VTP, sequence: GPLPLR) binds to the membrane-bound matrix metalloproteinase MMP14, which is expressed in the neovasculature and in solid tumours. The radiolabelling procedure was carried out with excellent yields and radiochemical purities for both PAMAM (G5.0)-based $^{131}$I-carriers. Both carriers are successfully taken up for treating inoperable cases and as adjuvant therapy after surgery. Therefore, our next step will consist in testing the MTC targeting efficacy of $^{131}$I-PAMAM (G5.0) and $^{131}$I-PAMAM (G5.0)-VTP vs. Na$^{131}$I in a xenograft mouse model of MTC.

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