Investigation of production possibilities of Terbium radionuclides for cancer diagnosis and therapy

Closing report

The aim of the research program

It has recently become widely accepted that element of Terbium is a good candidate for theranostics. This consists of the use of a positron emitter of an element together with a therapeutic radionuclide of the same element. This way the diagnosis and therapy are combined and it becomes a personalized medicine. Both the ^{161}Tb(Therapy)/^{152}Tb(PET) and ^{149}Tb(Therapy)/^{152}Tb(PET) pairs can be used for this purpose. Additionally, ^{155}Tb is useful for SPECT studies. Unfortunately, the widespread application of Terbium radioisotopes-labeled macromolecules is limited because of the lack of well established and reasonable production routes. During the research program, therefore we decided to develop new production methods for the most promising Tb radionuclides using low and middle energy cyclotrons. Our basic aim was
(1) to determine the optimal conditions for the production of the SPECT nuclide ^{155}Tb, the PET nuclide ^{152}Tb and the therapeutically relevant ^{149}Tb and ^{161}Tb radioisotopes and,
(2) to elaborate highly efficient separation method of the terbium radioisotopes from the different (Gd, Tb) target matrixes.

During the research the following tasks were performed and results were achieved:

A) Preparation of thin Gd (enriched and natural isotopic composition) samples for production related nuclear data measurements.
B) Excitation function measurement for ^{nat}Gd+p, ^{nat}Gd+n, ^{152}Gd+p, ^{155}Gd+p and ^{159}Tb+p nuclear processes.
C) Elaboration of high efficiency separation methods for Tb/Gd and Tb/Dy systems
D) Additional results related to the research program

A) Preparation of thin Gd (enriched and natural isotopic composition) samples for production related nuclear data measurements.

We have developed a method to prepare 2-5 μm thick adhesive and mechanically stable Gd (both natural and enriched) layers deposited on Al backing via electro-deposition in a specially designed small volume electrolytic cell. Previous methods could produce much thinner targets only for nuclear physics experiments. High purity Al target-backing foils served as cathode while a Pt ring was used as anode. As an electroplating solution, $GdCl_3$ was dissolved in 0.5 ml 0.01 M HCl what was than completed to 20 ml with absolute ethyl alcohol resulting 3 mg/ml Gd^{3+} concentration. From this solution 2.5 ml was used for electro-deposition. The optimal quality Gd layer was prepared at 25 mA current. The electrolysis takes usually 50-55 minutes resulting smooth and adhesive Gd layer up to 3 mg/cm^2. Several irradiations were done to check the performance of the electrodeposited layers. The targets were activated with different intensity of 18 MeV proton beam of the ATOMKI cyclotron for 2 h. Based on this study it was concluded that the targets are capable of resisting beam currents up to 100 nA without material loss and structural damage. Our electro-deposition method seemed to be useful for preparing very expensive enriched Gd targets for production related nuclear data measurements.

B/1.) Investigation of the ^{nat}Gd+d and ^{nat}Gd+n nuclear processes for ^{161}Tb production

The excitation function of the ^{160}Gd(d,n)^{161}Tb (^{161}Tb: $T_{1/2}$= 6.88 d, $I(\beta^-)$ =100%) nuclear reaction was evaluated in collaboration with the Japanese colleagues. Three experiments were performed to get cross sections from the threshold energy up to 30 MeV. In Debrecen, two stacks, each containing 5 pieces of natural gadolinium foils, were irradiated with 10 MeV deuterons, while at the Japanese cyclotron one stack (9 pieces) was activated up to 30 MeV energy. The activity measurement of the irradiated samples was performed using gamma-ray spectroscopy without chemical separation. Based on the measured cross sections, thick target yields were calculated to evaluate the optimum irradiation circumstances. It was found that the available yield is 0.162 GBq/C using a thick target of 150 micron (between the threshold and 10 MeV). Highly enriched target material (>95%) can remarkably increase the yield (by about five times). However, the co-formed ^{160}Tb ($T_{1/2}$= 72.3 d) has much higher cross sections in the overlapping energy regions, therefore the time window for application of ^{161}Tb is limited. Due to the high ^{160}Tb contamination at EOB (above 10%), this production methods is limited to pre-clinical and animal studies. The production related nuclear data are in acceptable agreement with the only available literature data (published after the start of our program) but the theoretical predictions (THALYS code) significantly underestimate the measured values. We have also measured cross sections for ^{155}Tb and ^{156}Tb that represent the major radio-impurities in the final product if targets with lower ^{160}Gd enrichment level are used.

To overcome the high ^{160}Tb contamination problem of the Gd+p method, we have investigated the ^{nat}Gd(n,γ)^{161}Gd→^{161}Tb route. This way no ^{160}Tb radio-contaminant will be in the final product after the proper chemical separation. Based on the promising separation results (see below) of Tb from Gd and Dy, we have performed some neutron activation experiments for production of ^{161}Tb using mg amount of gadolinium–oxide and our high intensity neutron source (^{9}Be+p(18 MeV)). The activity measurement was performed using gamma-ray spectroscopy before and after chemical separation of Tb. (The OTKA foundation extended our research contract by 1 year to investigate this production route in detail at the circumstances of the Debrecen accelerator-based neutron source.) Our present irradiation scenario resulted in a rather low yield for ^{161}Tb (around 8000 Bq C^{-1} g^{-1}). It is expected, however, that activation of a highly enriched ^{160}Gd sample (0.1–0.5 g) for 3–4 days with a high beam current (above 100 μA) could produce around 3.7–11.1 MBq ^{161}Tb in radionuclide contamination-free form, enough for in-house labelling studies and preclinical experiments.

B/2.) Investigation of the ^{159}Tb(p,5n)^{155}Dy→^{155}Tb and ^{155}Gd(p,n)^{155}Tb nuclear processes for ^{155}Tb production

We have investigated the possible production routes of ^{155}Tb ($T_{1/2}$= 5.32 d, EC=100%) both for high and low energy accelerators (via the ^{159}Tb(p,5n)^{155}Dy($T_{1/2}$=10 h)→^{155}Tb and ^{155}Gd(p,n)^{155}Tb reactions, respectively). For the high energy activations, $^{nat}Tb_4O_7$ targets (thickness: 2.5 mg/cm^2), prepared by sedimentation method, were activated in South–Africa up to 66 MeV. Three target stacks were bombarded with beam current of 50 nA for 2 h in each case. The activity measurement of the irradiated samples was performed non-destructively via gamma-ray spectroscopy. Our measured excitation functions leading to the formation on ^{152,155,157,159}Tb were also compared with the theoretical predictions of ALICE/ASH and TALYS codes. Based on the measured cross sections, thick target yields were calculated to evaluate the optimum production circumstances for ^{155}Dy→^{155}Tb. It was found that supposing a bombardment time of 10 h, 0.5 h waiting time (between EOB and the start of the first chemical separation), 1 h processing time (ion-exchange procedure), 39 h waiting time (period required for the accumulation of ^{155}Tb from ^{155}Dy decay) and 0.5 h processing time (for the second chemical separation), the available ^{155}Tb yield can be

maximize. The exit energy of the energy window should be taken as 35 MeV and the entrance energy could be varied between 50 and 70 MeV. These circumstances provide yields from 39.8 GBq/μAh at an incident energy of 50 MeV to 116.6 GBq/μAh at an incident energy of 70 MeV. The radionuclidic purity is better than 99.9% at all incident energies below 60 MeV but decreases monotonically above 60 MeV to a value of about 88% at 70 MeV.
For the estimation of the 'low-energy' production possibility of ^{155}Tb, the excitation function of ^{155}Gd(p,n)^{155}Tb reaction was measured in Debrecen (E_p<18 MeV). Five activation were done with the same four target samples ($^{155}Gd_2O_3$; enrichment level: 99.82%), resulting 20 data points for this (p,n) reaction. The irradiations lasted to 2 h and the beam intensities were below 50 nA. The activity measurements, yield estimations and theoretical work were similar to the South-African circumstances. In a production energy window (from threshold up to 11 MeV), radionuclidically pure ^{155}Tb can be produced, with an estimated physical yield of 5.6 MBq/μAh. Above this energy the (p,2n) reaction, leading to the formation of ^{154}Tb (^{154m2}Tb: $T_{1/2}$= 22.7 h), will introduce significant radio-contamination to the product.

B/3.) Investigation of the ^{155}Gd(p,4n)^{152}Tb and ^{152}Gd(p,n)^{152}Tb nuclear reactions for ^{152}Tb production

Similarly to the formation of ^{155}Tb, also two routes were studied for ^{152}Tb ($T_{1/2}$= 17.5 h, β^+=17 %)) production. At high energy, only one stack (containing 10 (^{155}Gd, enrichment level: 99.8%) and 10 (^{nat}Gd) samples) was irradiated with 66 MeV protons at the accelerator of the iThemba LABS of South-Africa. The activity measurement of the irradiated samples was performed non-destructively via gamma-ray spectroscopy. Excitation function of ^{nat,155}Gd(p,4n)^{152}Tb nuclear reactions were in excellent agreement with our preliminary and the available literature results, and made possible to get evaluated databases for this reaction. THALYS and ALICE theoretical calculations also supported the experimental results. The expected yield for the ^{155}Gd(p,4n)^{152}Tb reaction reaches around 600 MBq/μAh already in the 30-40 MeV energy window. The yield is excellent, but the co-formed ^{153}Tb($T_{1/2}$= 2.34 d), ^{154}Tb($T_{1/2}$= 22.7 d) and ^{155}Tb($T_{1/2}$= 5.32 d) represents high radio-contamination level even if 100% ^{155}Gd is used.
Two experiments were performed to get cross sections from the threshold energy of the ^{nat,152}Gd(p,n)^{152}Tb reaction up to 18 MeV using the ATOMKI accelerator. Each stack, containing 5 (^{nat}Gd) and 3 (^{152}Gd, enrichment level: 30.8%) Gadolinium foils, were irradiated for 3 and 6 h with beam intensity of 50 nA. The activity measurements, yield estimations and theoretical work were similar to the South-African circumstances. Based on the excitation function curve of this (p,n) reaction, thick target yields were calculated to evaluate the optimum production circumstances for this route. It was found in the case of ^{152}Gd(p,n)^{152}Tb reaction, that the (p,2n) reaction, leading to the ground state of ^{151}Tb($T_{1/2}$= 17.609 h), will introduce a significant radio-contamination above about 12 MeV. Therefore, an energy window from threshold up to only 12 MeV can provide a radio-nuclidically pure ^{152}Tb product. Physical yield of about 40 MBq/μAh is expected at lower energy accelerators. Although the yield is acceptable for routine production purposes the 'drawback' of this method is the use of highly enriched (>99.5%) and extremely expensive target material. If it is used for production purposes, the elaboration of high efficiency recovery methods for ^{152}Gd is important.

B/4.) Investigation of the ^{152}Gd+p nuclear reactions for ^{149}Tb production

Excitation function of the ^{152}Tb(p,4n)^{149}Tb ($T_{1/2}$= 4.118 h, $I(\alpha)$ =16.7%) nuclear reaction was measured from its respective threshold energy up to 66 MeV. One target stack containing 6

enriched Gd targets was activated at the big accelerator of iThemba LABS. The targets were prepared by sedimentation method (from gadolinium-oxide) using enriched ^{152}Gd (30.8%). Although this enrichment level is not ideal, these measurements are nevertheless expected to provide useful information for this important reaction. The excitation function curve leading to the formation of ^{149}Tb was also compared with the theoretical predictions of ALICE/ASH and TALYS codes. Both codes described properly the shape of the excitation function curve (after using an about 0.3 scaling factor). Based on the measured cross sections, thick target yields were calculated to evaluate the optimum production circumstances for ^{149}Tb. With an energy window of 66 MeV down to threshold (about 30 MeV), a physical yield of 2.556 GBq/μAh can be expected for ^{149}Tb. This is a very high yield but, unfortunately, do not lead to radionuclidically pure products, because of the co-formation of ^{150,151,152}Tb radioisotopes (via ^{152}Gd(p,3n)^{150}Tb, ^{152}Gd(p,2n)^{151}Tb and ^{152}Gd(p,n)^{152}Tb reactions, respectively). However, shorter irradiation time (5-10 h) and an appropriate cooling period (10-15 h) could decrease the EOB contamination level at least to acceptable level of animal studies. Additionally, mass separation after the chemistry could significantly decrease further the radio-contamination of the product.

C) Elaboration of high efficiency separation methods for Tb/Gd and Tb/Dy systems

It is well known that the rare earth elements have very similar chemical behavior therefore they cannot be separated from each other by conventional ion-exchange chromatography. The only possibility is to use the slight differences in the complex formation ability with several appropriate chelating agents. Two methods were tried: the direct Column Chromatography and the Reverse Phase separation in order to select the best method for separation of Tb and Dy radioisotopes from around 300 mg Gd and Tb matrix.

In the first method Silica gel was mixed with HDEHP (Bis(2-ethylhexyl)phosphate) and was loaded into a glass chromatographic column (50 cm long and 11 mm inner diameter). The irradiated Gd target was repeatedly dissolved in cc. HCl and evaporated into dryness 3 times. After that it was picked up in 1 ml 0.4 M HCl and was loaded into the separation column. The elution was carried out at 0.8 ml/minute flow rate by 1.7 M HCl, during that the column was kept on 50 ^{o}C, using water bath. 0.5 ml portions were collected and the radioactivity of the samples was measured by γ-spectrometer. The Gd distribution was calculated by measuring the radioactivity of the ^{159}Gd (364 keV, $T_{1/2}$= 18.5 h) radioisotope, formed by ^{160}Gd(p,pn)^{159}Tb nuclear reaction, while the Tb distribution was followed by the ^{154m}Tb (123 keV, $T_{1/2}$= 9 h) and ^{156}Tb (199 keV and 534 keV, $T_{1/2}$= 5.4 d) radioisotopes, formed by ^{154}Gd(p,n)^{154m}Tb and ^{156}Gd(p,n)^{156}Tb nuclear reactions, respectively. In the case of Gd it was possible to control the distribution also by measuring the weight of the 0.5 ml samples after evaporation to dryness. The distribution along the samples was the same as determined by radioactivity measurements, and the sum of the weights in the individual ampoules gave back the 300 mg Gd quantitatively. It is an important fact from the point of view of using expensive enriched Gd target.

The Gd peak was eluted between 27.0 and 34.5 ml, while the Tb distributes from 32.0 to 42.0 ml. Calculating the yield of Tb radioisotopes from the overlapped peaks collected from 33.5 ml to the end of the Tb elution peak we got 90 % with a content of 12 mg Gd contamination. If we collect the Tb radioisotopes from 34.0 or 34.5 ml, we got 78 and 64 % yields with Gd contaminations of 3 and 0.2 mg, respectively. In this latter case the separation is good, but more than 1/3-rd part of the radioactive Tb is lost. The same results were observed when we separated the radioactive Dy from Tb matrix.

To obtain better separation possibilities, the reverse separation was also tried where the metals are fixed on a conventional resin and are eluted with the complexing agents. For the

experiments, where we wanted to compare the efficiency of an analytical HPLC and a semi-preparative column method, we prepared targets using gadolinium of natural isotopic composition in pressed tablets form of gadolinium-oxide. These targets contained either 175 mg or 350 mg gadolinium. In the Dy experiment we made pressed tablets using equal amount (175 mg each) of gadolinium-oxide and terbium-oxide in order to get a mixture of Tb, Gd and Dy radioisotopes after target activation.

Targets were irradiated with our MGC-20 cyclotron for 3 hours (E_p=15 MeV, I_p=50 nA). These parameters were selected based on our cross section measurements. The ^{159}Dy [^{159}Tb(p,n)^{159}Dy] and ^{156}Tb [^{156}Gd(p.n)^{156}Tb] isotopes were used for indication of dysprosium and terbium in our separated samples, and the ^{159}Gd [^{160}Gd(p,pn)^{159}Gd] radioisotope was used for tracing the gadolinium.

The irradiated targets were dissolved in cc. HCl and evaporated to dryness. The targets were re-dissolved in 0.5 ml (for experiment on analytical column) and in 2 ml (for semi-preparative experiment) of 0.05 M NH_4Cl solution. For separation AG50W-X8 cation exchange resin was used with α-HIBA (α-hydroxyisobutyric acid) eluent. The α-HIBA elutes the elements with different speed depending on the concentrations. 0.14 M for was applied for dysprosium, 0.2 M for terbium and 0.5 M for gadolinium elution. The pH values of the eluents were set to 4.5 with NH_4CO_3. 200x5 mm chargeable column was used for the experiments on an analytical column, and 110x15 mm column was used in the case of semi-preparative experiments. The columns were filled with the pretreated cation exchange resin and connected to a HPLC pump. The dissolved targets were loaded onto the columns with 0.5 ml/min. The columns were washed with one column volume equivalent water after the loading. The separations were carried out by isocratic elution. The elution was followed up with the HPLC's gamma counter. 2 ml fractions were collected during the elution. After the separation processes, the fractions were measured with HPGe gamma spectrometer.

Separation of radioterbium from 175 and 350 mg irradiated Gd target was studied on the analytical column with both 1.0 and 0.6 ml/min elution speed. In all the four separation processes very similar results were received. The elution was started with the 0.2 M α-HIBA and it was continued until the activity dropped to 7 % of the maximum on the HPLC's gamma counter, then it was changed to 0.5 M α-HIBA. The obtained results were similar for all cases giving the gadolinium free part of the radioactive Tb 85 ± 2 %. These results were obtained with a relatively big target weight on an analytical column.

The separation was also performed with a semi-preparative column for comparison. The separation was carried out from 350 mg target at 0.6 ml/min elution speed. The bed volume of the semi-preparative column was almost 5 times bigger than the analytical. The same eluent concentrations were used. The shape of the curve, obtained on semi-preparative column, is different from the curve got on analytical one. 93 % of the terbium free from gadolinium could be collected on semi-preparative column. The 8 % separation deficit on analytical column is not significant, and can be easily compensated by more beam current or longer irradiation during a real production run.

In the case of neutron activation of Gd (for ^{161}Tb production), inactive dysprosium is also co-formed. After long activation, the amount of the produced Dy, having very similar chemical characteristics as Tb, could considerably decrease the chemical purity of the labelled radiopharmaceutical causing problems in the imaging procedure. Therefore, the terbium product has to be separated from the Dy before using for labeling. The separation of terbium from dysprosium and the gadolinium target material was carried out in one procedure on a semi-preparative column with 0.6 ml/min elution speed. The composition of the target was 175 mg Gd_2O_3 and 175 mg Tb_2O_3. The reason is that the natural monoisotopic ^{159}Tb produces the ^{159}Dy via the ^{159}Tb(p,n)^{159}Dy nuclear reaction but does not produce any radioactive Tb isotope with (p,pxn) reaction. Therefore we had to use some Gd in the target to get radioactive

Tb isotopes to monitor the separation of Dy from Tb. Three different eluent concentrations were used: 0.14 M α-HIBA was applied first followed by 0.2 M and finally with 0.5 M α-HIBA. The calculated Dy and Gd free part for the radioterbium is 99 %, therefore if dysprosium is produced during the irradiation, we can simple purify the terbium from both of the contaminants at the same time.

D.) Additional results related to the research program

1.) During the formation of the required Terbium radioisotopes, different amount of stable (^{159}Tb) or rather long–lived (^{157}Tb ($T_{1/2}$= 71 y), ^{158}Tb: 180 y) Tb nuclides could be also produced in the targets. Of course, their amount in any final product strongly depends on the enrichment level of the target and the production energy window. To get any impression on the importance of these processes, (ie. how they decrease the specific activity of the final products), we have performed model calculations on the well measured ^{64}Ni+p and ^{64}Ni+d systems forming ^{64}Cu. (^{64}Cu is also a promising theranostics radioisotope). In both cases two non-radioactive copper (^{63}Cu and ^{65}Cu) means the 'cold'-contamination. The preliminary results draw attention to investigate in detail those processes that form stable or long-lived Terbiums.

2.) To determine the proton energy inside the target stacks at the high energy experiments, we have used the ^{22}Na/^{24}Na monitor cross-section ratios. Both isotopes are formed in the target backing (Al) and the monitor foils (Al) via the ^{27}Al(p,X)^{24}Na processes. Our studies revealed that due to the influence of the secondary neutrons coming from the experimental set-ups, the ^{27}Al(n,α)^{24}Na reaction interferes with the ^{27}Al(p,X)^{24}Na monitor and therefore it can not be used for monitoring purposes. We were the first to propose the ^{22}Na/^{7}Be activity ratios to replace the ^{22}Na/^{24}Na ratio, especially at higher energies. Similarly, we have pointed at the disadvantages of the ^{3}He-monitor reaction (^{nat}Ti(^{3}He,X)^{48}V). We are going to use this monitor reaction in our later Tb production experiments.

3.) During the activity measurement of the partly positron emitter ^{152}Tb (PET), it was observed that the source strengths, obtained by analyzing the 511 keV photo-peak, differed from those values that were obtained from characteristic gamma-lines. Our studies revealed that in the case of 'non-pure' positron emitters better overall agreement is obtained when in-flight annihilation loss corrections are explicitly performed. A set of non-pure radioisotopes (important for nuclear medicine) from a wide mass region (^{22}Na, ^{52}Fe, ^{52m}Mn, ^{61}Cu, ^{64}Cu, ^{65}Zn, ^{66}Ga, ^{68}Ga, ^{82}Rb, ^{88}Y, ^{89}Zr and ^{132}Cs) were involved into this study. The only radionuclide which could not be successfully assessed in this way is ^{88}Y, as a result of contamination of the 511 keV peak by positrons from pair production events that annihilated in materials close to the detector.

4.) For the cold contamination studies and the in-flight investigations, we had to produce some ^{64}Cu (teranostic radioisotope), ^{65}Zn and ^{66}Ga radioisotopes. Since we already had some highly (>90 %) enriched ^{67,68}Zn target foils therefore we could investigate a 'local' production possibility of these radionuclides parallel with the Terbium study. The production reactions are ^{67}Zn(p,α)^{64}Cu, ^{68}Zn(p,px)^{65}Zn and ^{67}Zn(p,2n)^{66}Ga, respectively. During this activations it was found that above 50 MeV, a considerable amount of cold ^{65}Cu could be formed via the decay of ^{65}Zn in the case of ^{67}Cu production via the ^{68}Zn+p route. Additionally, routine production of ^{64}Cu with low energy cyclotron was also elaborated. In the case of the ^{64}Cu production study, two activations of highly enriched ^{67}Zn (91.5%) targets (electrodeposited by us) were performed in Chiba, Japan. Five targets in each experiment were activated with 30.6

MeV protons, using beam current of 100 nA. Both irradiations lasted for 2 h. Based on the calculated yields, it was concluded that an 18 MeV proton accelerator could provide enough EOB activity (88.80 MBq/μA) even for transportation of the ^{64}Cu product to other local medical institutions.

5.) During the high energy experiments, several ^{nat}Zr foils were also used as energy degrader in the different stacks. We had possibility to measure the radioactivity of the different Nb, Zr, Y and Sr radioisotopes formed in the Zr matrix. Our present data showed good agreement with the available experimental results, and helped to strengthen the database of those nuclear processes that were involved in the formation (directly or via decay or both) of the investigated radioisotopes.

6.) We have prepared a 'propagative' article on the preliminary results of the present research project in Hungarian, and published it in 'Természet Világa' to highlight our study for a 'broader audience'.