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PROPOSAL
to the ISOLDE COMMITTEE

USE OF RADIOACTIVE ION BEAMS FOR BIO-MEDICAL RESEARCH

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SUMMARY

With this Proposal we wish to replace the two previous proposals P42 and P48 (corresponding to the ISOLDE Experiments ISC 330 and 331, respectively including the Addendum 1 dated 04.05.94.). Based on experimental results obtained during the last 4 year research in the frame work of the two proposals and considering modern trends in radiopharmaceutical developments we propose as a first main direction to study systematically relationships between physico-chemical parameters, the concentration and specific activity of tracer molecules and the corresponding biological response. This kind of studies requires highest achievable quality and a universality of radio-tracers, available at ISOLDE. Special attention in this concern is paid to bio-specific tracers (receptor-binding ligands, bio-conjugates etc.) aiming to search for new and more efficient radiopharmaceuticals for radionuclide therapy. The second direction is to support clinical radionuclide therapy by a quantitative follow up of the radionuclide bio-distribution in vivo using PET (Positron emission tomography). ISOLDE produced positron emitting radionuclides (^{83}Sr, ^{142}Sm for example) are potential candidates for this kind of clinical research. A third direction is to evaluate the application of exotic radionuclides (as the alpha-emitting ^{149}Tb) for radionuclide therapy.

1. Background:

1.1. Status of Nuclear Medicine

Nuclear Medicine is the application of radioactive tracers in vivo. The radioactive tracers used are named radiopharmaceuticals. Together with high technology imaging devices based on single photons or positron annihilation radiation we can obtain today detailed information on the function or malfunction of different metabolic processes in vivo with satisfactory high spatial resolution. Important aspects of research in radiopharmaceutical developments today are:

i. Increase specificity of functional imaging which requires radiotracers of new qualities, for instance tracers of better specific activity or tracers labelled with new radio isotopes showing dedicated decay characteristics.

ii. Quantitation of nuclear medical imaging techniques is still an important question.

iii. The third aspect for research activities is the whole field of radiopharmaceuticals for therapy.

The above three mentioned aspects are subjects which can be significantly supported by using the radioactive ion beams produced at ISOLDE.

1.2. Special features of ISOLDE produced radio-tracers

There are three main aspects which makes ISOLDE produced radio-isotopes so valuable for use in bio-medical research:

i. **Availability of exotic or uncommon radio-isotopes:** Spallation reaction makes available a complete range of isotopes having as complete a diversity of types and energies of radiation, of half-life, and of chemical properties as one would wish. Of special interest is the standardised availability of the full range of radio-lanthanides from just two standard ISOLDE target systems.

ii. **High purity:** The way of production guarantees that the radio-isotope preparations obtained at ISOLDE are really what we name carrier free preparations.

iii. **Ion beam quality:** The radioactive isotopes are obtained primarily as single charged ions of 60 keV energy. Thus, implantation may become an additional approach for new labelling technologies.

2. Bio-medical research based on radioactive ion beams at ISOLDE

2.1. Study of the relationships of bio-behaviour and specific activity

Radiopharmceuticals showing high functional specificity can not be used in unlimited concentration. The in vivo biodistribution and bio-kinetic behaviour very much depend on the concentration of binding sites in vivo. This is especially true for ligands binding to receptor. The concentration of receptors is limited. When the tracer concentration is too high all receptors are blocked. This may lead to wrong

diagnostic results, to misinterpretation of the result obtained, or can cause dangerous clinical syndromes. Therapeutic effects may be lost when carrier containing radiopharmceuticals are used. Especially in radionuclide therapy we are forced to use high specific activities to ensure that the radiotracer really is specifically adsorbed in the lesions selected for targeted therapy. Unfortunately, the radionuclides used in therapy often cannot be produced carrierfree using the classical production routes. Systematic studies are required to determine carefully the relationship between specific activity of the tracer and the biological response. For this aspect clearly carrierfree radionuclides are required.
We propose to use ISOLDE produced radioisotopes in this field:

2.1.1. ISOLDE produced ^{153}Sm in ^{153}Sm EDTMP therapy

^{153}Sm is commercially produced via ^{152}Sm (n,g) ^{153}Sm reaction in nuclear reactors. Consequently the specific activity is limited and depends on neutron flux density available, the enrichment of the target material and the duration of activation. The precise knowledge about the influence of the specific activity on the biokinetic and biodistribution will help to optimise the therapy. In previous studies we measured carefully the relationship between EDTMP-ligand concentration and biokinetic behaviour of carrier free radio-lanthanides [1]. From Fig.1 we learnt that the ligand concentration plays an extremely important role when low molecular chelators are used as ligands. We propose now to perform similar biokinetic studies using well defined specific activities of the radio-lanthanides with special emphasis to ^{153}Sm. A similar experimental design is proposed for ^{83}Sr as well as outlined below under Top.2.3.

2.1.2. Radio-lanthanides for labelling of bioconjugates

Bioconjugates are molecules which consist of a protein or peptide conjugated with a bifunctional ligand. The basic molecule contains a specific bioselective function which usually determines the biokinetic behaviour. The bifunctional ligand consists of a linker molecule and a chelator. The function is to connect a radioactive isotope stable to the basic molecule or body. The concentration of binding sites in vivo are usually very limited. Consequently we should bring only the same or even a lower number of labelled molecules as tracer into the body in order to achieve maximum effect. The whole field suffers form the limited availability of high quality absolutely carrierfree radionuclides which can be used. ISOLDE produced radio-lanthanides are ideal for this kind of studies. In our earlier experiments (IS 330) we could demonstrate that monoclonal antibodies as well as Octreotides can be successfully labelled with any of the radio-lanthanides available at ISOLDE [2,3] (Fig.2 and Fig.3)
This field is advancing well.

i. In the case of monoclonal antibodies a new technique "bifunctional monoclonal antibodies" is under development. The principle is to create new "receptor" binding sites in vivo which show higher binding affinity to small molecules which are then labelled with a radio-tracer. Due to the smaller molecule size the biokinetics becomes faster and highe tumor to tissue ratios are obtained. More

convenience for clinical application is expected. ISOLDE produced radio-lanthanides are ideal radio-tracers for this kind of systematic studies.

ii. It has been proposed to use fragments of monoclonal antibodies (fab) [6] only. The fragments carry the full bio-selective information of the mab but they are much smaller molecules. Because of the smaller size fab's show a faster clearance. All approaches to adapt radioactive metal ions to proteins (bifunctional ligands) can be used for fab's as well. We plan to use ISOLDE produced radio-lanthanides for this kind of systematic study.

iii. Low molecular weight peptides are intensely investigated concerning their potential for diagnosis and therapy (see for instance [4]). Thus, Octreotide, a somatostatine analogue consisting of 8 aminoacids, can be labelled with lanthanides via a bifunctional ligand. Since the receptor concentration in vivo is limited only Octreotides of high specific activity can show a better tumor accumulation. Presently, intense research activity is directed to modify the molecule with the aim to improve the organ distribution. ISOLDE produced carrier free radioactive metal ions will definitely support this developments of new and more efficient types of radionuclide therapy.

2.1.3. Relationship between biokinetic behaviour and chelate stability

As mentioned above and written in the previous proposals a suitable chelating group plays an important role in biocojugates. Generally we wish to use the monoclonal antibodies or receptor binding molecules as carrier or vehicle to transport selectively a radioisotope into tumor cells. Suitable isotopes of therapeutic interest are usually b^- emitter, difficult to obtain in the required high quality. In addition different chelating groups may be possible. Different linker molecules carrying polyamino-polycarboxylic acid groups have been proposed as chelators. The trend goes to chelating groups providing highest possible complex stability constants. DTPA is a chelator which provides satisfactory high complex formation stability constants for a wide range of metal ions, including the lanthanides. Complex formation is very fast and easy. Higher complex stability is obtained with DOTA, a cyclic chelator. However, the complex formation requires usually stronger conditions (increased temperature and time). If the chelate complex stability is unsatisfactorily low, then a dissociation in vivo would form free unchelated radiolabelled species which follow their own biodistribution. From our preliminary studies we learnt that the liver is the critical organ. This is the case for 225-Ac labelled benzylamino-DTPA monoclonal antibodies (Fig.2), while for yttrium or heavy lanthanides a partial dissociation of the complex compound would be less critically, because the liver accumulation of those ionic species is relatively low. However, in case of yttrium instead of the liver the bone uptake may now become critically. It is assumed that DTPA derivatised at the carbone backbone in the linker molecule provides satisfactory complex stability for most of the lanthanides and yttrium (Fig.3). We propose to perform systematic studies in order to establish clear relationship between the chelate complex stability and the biological behaviour. Radio-lanthanides, produced at ISOLDE are the most ideal instrument for this kind of study. The tracers are carrierfree and we can change the stability constant without any changes

in the basic molecule. Reference nuclides as ^{111}In and ^{67}Ga as well as other metallic radionuclides as radio-yttrium and actinium will be included in this systematic study.

From our systematic biokinetic studies involving DTPA-, DOTA- and other chelating groups in peptides (such as Octreotide) in combination with ISOLDE produced radio-lanthanides, Ga, In and Cu we expect more and complete information on the influence of the complex stability and the biokinetic behaviour The answer to this question will help to make better and more efficient radiopharmaceuticals for therapy.

2.2. ^{149}Tb a suitable radionuclide for radionulide therapy

Cancer proceeds through a number of quite separate stages in the development of lethal disease. Early stages offer the potential for control if alpha emitting radioimmunotherapy (RIT) is applied. Later stages may be more appropriate for beta RIT. Prophylactic therapy of metastatic cancer requires the localisation of dose to the cancer cell and rules out radioactive beta emitting radionuclides. Alpha emitting nuclides, however, are much more appropriate toxins, as their efficacy depends on the energy and the range of the alpha particles emitted.

The lethal dose for subclinical lesions of 10^4 cells is 20 Gy. In order to limit the systemic dose to 2 Gy to the body, a 10:1 tumor to tissue dose ratio is required. For 100 cancer cells in circulation only 10 Gy and a 5:1 dose ratio is required. But for beta therapy, only 2.5 % of the beta energy is deposited in a cancer cell with ^{131}I, so only 1:40 dose ratio is achievable. Thus control of isolated cells is not efficient because there is a 5 x 50 = 200 fold short fall in dose ratio and beta radionuclides therapy cannot reasonably be expected to control cancer cells ore small cancer cell clusters. Alpha particles have higher linear energy transfer (LET) than betas, so fewer hits are required to kill a cell. The short range of alpha means much lower dose to surrounding normal cells if a tracer molecule with cancer specific properties (such as mab) is used. Cross fire is not an important aspect of dose delivery as it is with betas and microdosimetry calculations are required to determine median cell dose and cell survival probabilities.

Among the existing alpha emitters the ^{149}Tb turns out to be the most promising candidate for RIT because of the low energy of the alpha particles emitted. Presently the ISOLDE facility is the only facility where this isotope is available in the required quantity (0.5-1 GBq) and quality (carrier-free) [5]. The half life of ^{149}Tb is very much shorter than that of beta-emitting isotopes used in radionuclide therapy today. As monoclonal antibodies may take as long as 24 - 48 hours to reach peak uptake in solid tumors, such tumors are not the target for ^{149}Tb alpha radionuclide therapy. Uptake times required for cell in transit or pre-angeogenic lesions are expected to be very short, and such, the short half-life of ^{149}Tb may be of advantage. Thus, the alpha emitting ^{149}Tb offers a more enlightened approach to the control of early stages cancer or leukaemia.

The following experiments based on ^{149}Tb are planned:

* cell culture uptake studies
* cell culture surviving experiments
* animal survival studies
* phantom PET studies to learn about feasibility of quantitation using positron branching in the ^{149}Tb decay.

ISOLDE is so far the only facility in the world where we have access to ^{149}Tb in the required amount and quality.

2.3. Quantitation using positron emitters

This aspect is related to clinical tasks. Radionuclide therapy (RIT) aims to bring selectively radioactive isotopes into tumor cells and deposit locally a high rediation dose without damaging the surrounding healthy tissue. With two exception (^{131}I as iodide and ^{89}Sr as Sr^{2+}) one needs the help of biospecific carrier molecules which carry the radiotracer into the target cell. As outlined above suitable carriers are monoclonal antibodies, fragments, peptides or even low molecular weight chelating ligands. One of the problems today is the fact that we cannot measure the individual regional uptake of the therapeutic tracer in vivo, thus we can not determine the real dose deposited in a target region. Consequently one can not establish clear relationship between a deposited radiation dose and the corresonding response. In order to answer this question we need an individual quantitation of the tracer concentration in regions of interest in vivo. Positron emission tomography (PET) is the only technique today, which allows to determine absolute radioactivity concentration in vivo. Using positron emitting isotopes of the same element which is used in therapy we are really able to quantify the uptake and determine deposited doses. Suitable candidates to perform such in vivo dosimetry are for example ^{83}Sr, ^{86}Y, ^{142}Sm. The ^{83}Sr (T1/2=32.4 h) is a suitable positron emitter which can be used to monitor the ^{89}Sr uptake in bone metastasis in palliative pain therapy. The ^{86}Y (T1/2=14.7 h) has been proposed to do the same when ^{90}Y CITRATE or ^{90}Y EDTMP is used. In our previous Proposal we demonstrated that ISOLDE produced ^{142}Sm provides excellent PET images and consequently ^{142}Sm is a suitable positron emitter to monitor ^{153}Sm. We propose to support both, the ^{89}Sr and ^{153}Sm EDTMP radionuclide therapy with clinical PET studies based on ^{83}Sr and ^{142}Sm. Both positron emitters are available at ISOLDE in excellent quality and quantities.

3. Beam time request

The isotopes of interest along this proposal are produced using mainly three ISOLDE targets (in combination with the hot surface ionisation ion source):

Nb-foil target	($^{83, 82}$Sr; $^{86, 85}$Y)
Ta-foil target and	(radio-lanthanides)
(or combined Nb-foil / Ta-foil target)	
U-carbide	(neutron rich light radio-lanthanides, ^{225}Ra)

In contrast to usual proposals we are able to plan our experiments according to the beam time schedule relatively flexible. Usually we need for the experiments between 10^{13} and 10^{15} collected atoms depending on the half life of the nuclide. This can be usually achieved during 1/2 - 2 shifts collection time. The collection of A=225 from U-carbide requires minimum 4 shifts.

For our research program we request in total 50 shifts for a two year period, or 25 shifts per year divided into

Ta-Target	10 shifts
Nb-target	7 shifts
U-carbide	8 shifts.

A combined Ta-Nb foil target is welcome, increases flexibility and enables us to have more frequently access to ISOLDE beams.

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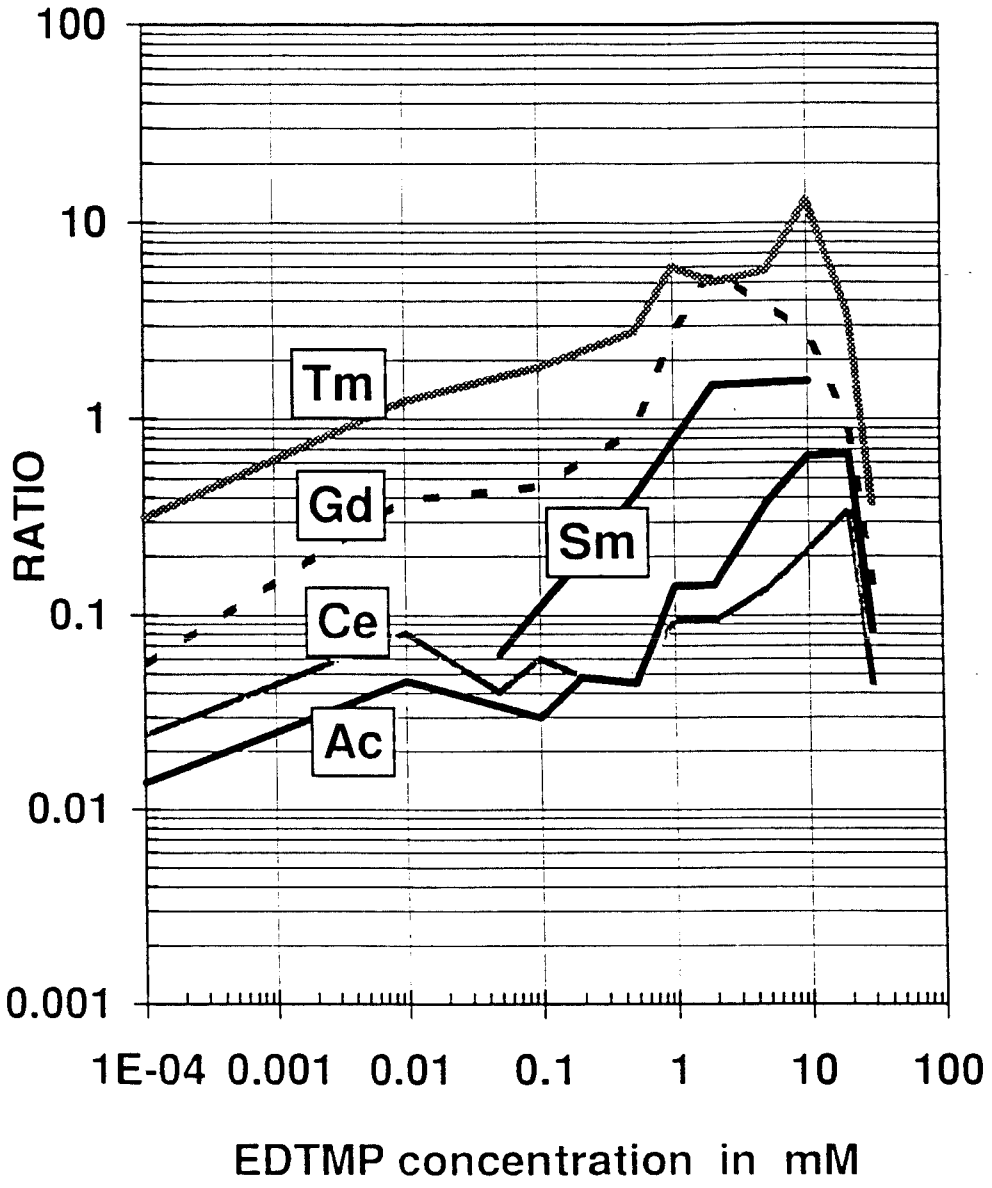

Fig.1

Tumor to liver ratio of radionuclide uptake for radio-lanthanides and ^{225}Ac in tumor bearing mice as function of the EDTMP ligand concentration.
We learn that with increased ligand concentration the tumor to liver ratio can change dramatically by a factor 50 due to a reduction of the liver uptake. The ratio obtained for the heavy lanthanides (^{167}Tm as a representative) is around 10 and decreases with growing ionic radius to about 2 for Sm, 0.7 for Ce to 0.3 for Ac. The radio-tracers used were carrier-free. For more details see [1].

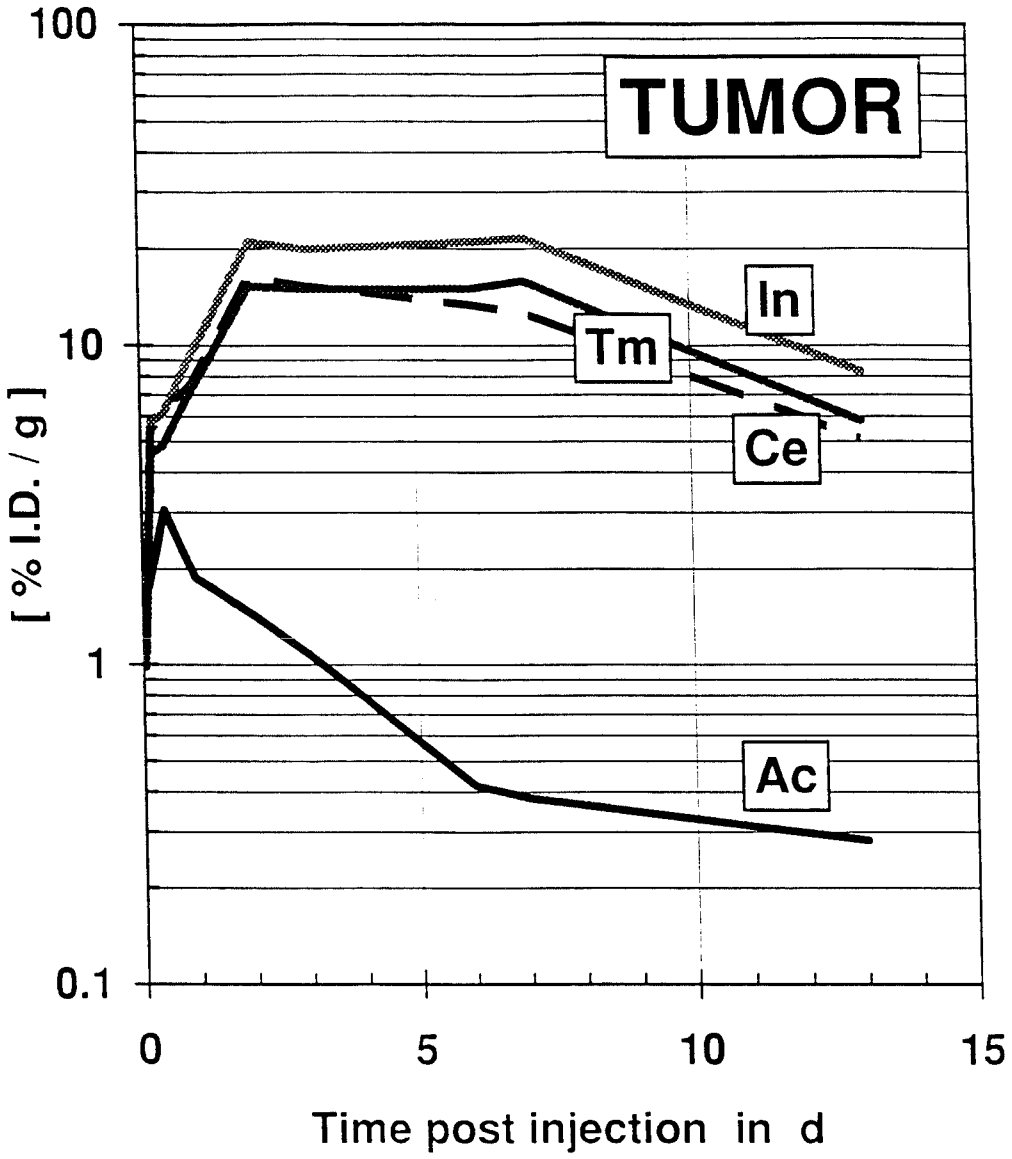

Fig.2

Tumor uptake of monoclonal antibodies labelled with ^{153}Gd, ^{169}Yb, ^{141}Ce and ^{225}Ac (carrier-free, produced at the ISOLDE facility at CERN) and ^{111}In (purchased from Amersham) in tumor bearing nude mice.

No differences can be seen between behaviour of the radio-lanthanides and ^{111}In labelled mab. However, the low and decreasing with time tumor uptake in case of ^{225}Ac is explained by the far insufficient stability of the Ac-DTPA complex binding. Chelators with significant higher stability constants are required for this element. For more details see [2].

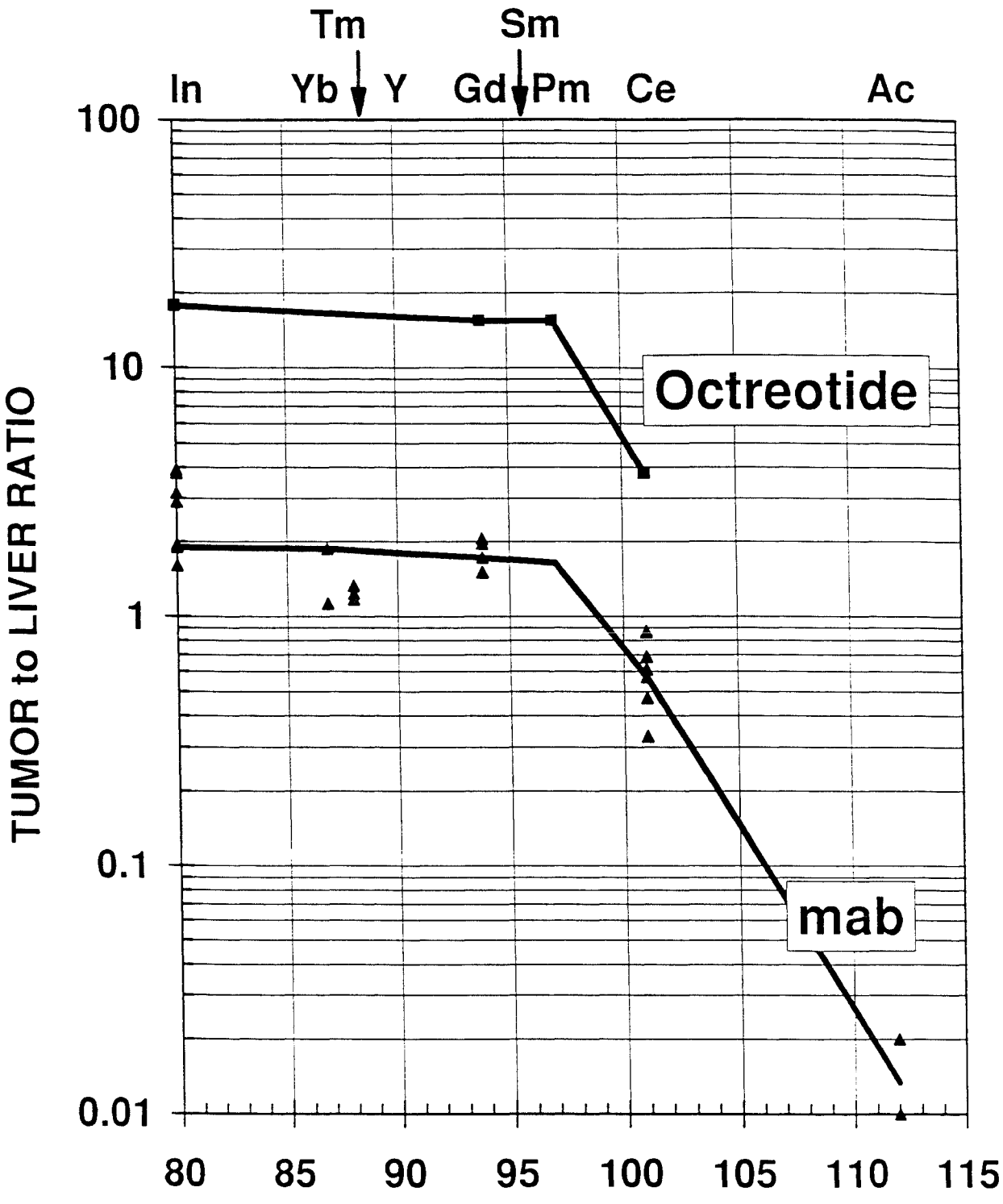

Fig.3

Comparison of the biokinetic behaviour of succinylaminobenzyl-DTPA-Octreotide and aminobenzyl-DTPA monoclonal antibodies labelled with ^{111}In, radio-lanthanides and ^{225}Ac.

The tumor liver ratio of tracer uptake is plotted versus the ionic radius of the metallic radionuclide. With the Octreotide generally significant higher tumor to liver ratios are obtained. For ions smaller than Pm no differences in the biokinetic behaviour can be detected. For the lanthanides below Pm and Ac the ratios value fall down because of a decreased in vivo stability indicating insufficient chelate complex stability. For more details see [2 and 3].