

# Radionuclide Antibody-Conjugates, a Targeted Therapy Towards Cancer

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**Abstract:** Targeted alpha therapy (TAT) is an investigational procedure which utilises monoclonal antibodies (*mAbs*), peptide conjugates and/or other chemical compounds. These bio-vectors are able to transport a dose of alpha particles to destroy cancer cells. Radionuclide antibody-conjugates (RACs), labelled with beta emitters, have already been used in humans. More recently, TAT has been introduced to treat oncological diseases mainly leukaemia and lymphoma. Encouraging results have also been obtained in solid neoplasms with the administration of anti-tenascin. This chimeric antibody labelled with astatine-211 was delivered in patients with recurrent brain tumours into a surgically created cavity. Conversely, a clinical trial using a standard TAT approach to treat patients with metastatic melanoma, observed the shrinkage of the solid tumour mass. This response in melanoma may lead to an alternative mechanism for TAT, called tumour-anti-vascular-alpha-therapy (TAVAT), and forms the basis of a novel approach to the treatment of cancer disease states. In this paper, we will concentrate mainly on the application of TAT using antibodies. In particular, an investigation into the major general features connected with the use of alpha emitters in cancer therapy will be discussed. The prospective role of TAT with RACs will also be outlined briefly, especially focussing on the most important therapeutic strategies to date based on antibodies radiolabelled with beta emitters.

**Keywords:** Targeted alpha therapy (TAT), radionuclide antibody-conjugates (RACs), radionuclide generators, alpha particle, monoclonal antibodies, tumour-anti-vascular-alpha-therapy (TAVAT).

## 1. INTRODUCTION

The concept of targeted therapy towards the treatment of disease causing agents was first postulated by Paul Ehrlich over 100 years ago. He envisaged the creation of an ideal therapeutic agent termed the 'magic bullet' which went directly to specific cellular targets in order to attack disease. Ehrlich's vision is now being realized in the treatment of cancer with development of targeted therapies, mainly based on designer monoclonal antibodies. The production of rodent monoclonal antibodies using hybridoma technology developed by Milstein and Köhler in 1975, led to antibodies having a single specificity towards the cognate antigen [1].

This technology of tailoring the monoclonal antibodies has been exploited by numerous biopharmaceutical companies to develop delivery vehicles for radionuclides to image and treat a variety of cancers [2]. This led to the hypothesis that a cancer patient would first receive a radionuclide antibody capable of imaging the tumour volume. The images of the tumour are obtained by using one or more combinations of the following methods: planar imaging; single photon emission computed tomography (SPECT) and positron emission tomography (PET). These techniques can be extended to hybrid imaging-using systems incorporating PET (or SPECT) with computed tomography (CT). More recently, PET scanners have also been hybridized - with a magnetic resonance imaging (MRI) instruments - to obtain a PET-MRI machine, already available in the clinical setting [3, 4].

Moreover, if the cancer (tumour site) is shown to retain an appropriate level of the antibody - through the application of these imaging techniques - based on gamma or positron emitters: it would be reasonable for the patient to receive a therapeutic dose of the same antibody - labelled with a radionuclide emitting alpha or beta radiation - which is capable of killing the cancer cells.

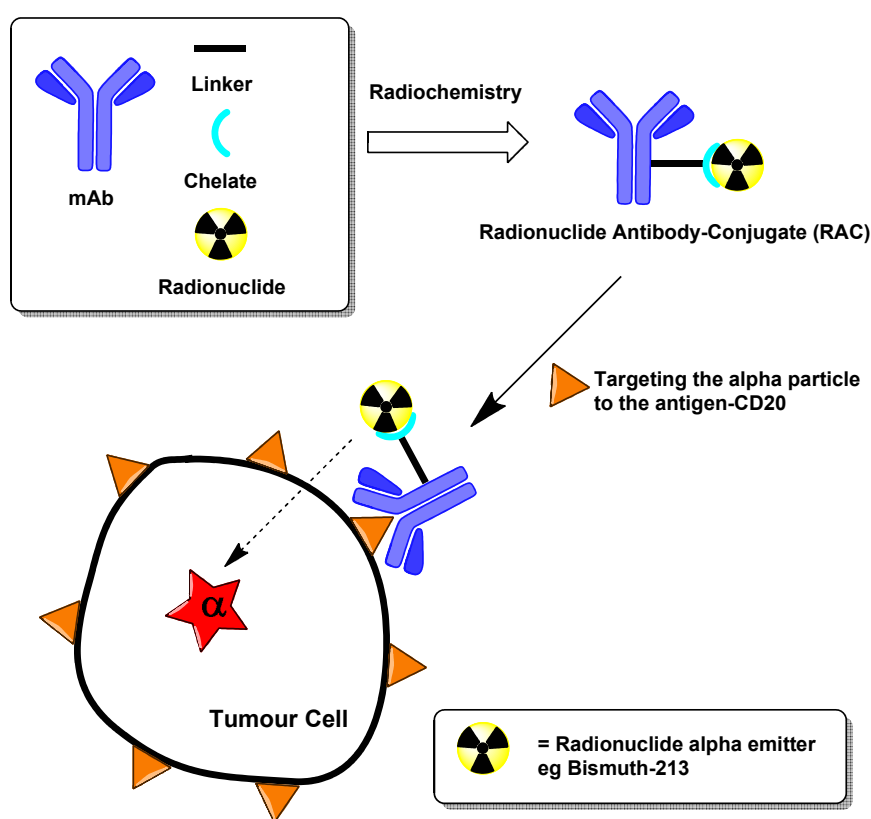
In early clinical developments this hypothesis using radionuclide monoclonal antibodies failed to make a marked impact on cancer therapy. The problem was mainly associated with the murine origin of monoclonal antibodies which produce the formation of immune-complexes and/or other non-specific radiochemical forms (due to *in vivo* metabolism). Consequently, this has the effect of significantly reducing the amount of specific uptake at the level of the tumour site. In addition, toxic immunogenic responses in humans were occasionally observed.

The limitation of utilising murine antibodies has been circumvented by the use of chimeric, humanized, or fully human monoclonal antibodies [5]. Furthermore, the other disadvantage was in the treatment of solid tumours where, in the large majority of cases, the delivered radiation dose was not able to induce a sufficient response on the tumour mass without producing significant toxic effects. Subsequently, the analysis of the radiation dose transported to tumour sites must be compared with the radiation dose delivered to surrounding normal tissues. Consideration must also be given to other normal and/or non-neoplastic sites capable of concentrating radioactivity and to the excretory organs i.e. emunctories (e.g. kidneys). This provides a need to acquire quantitative pharmacokinetic information - which can be used to calculate the percentage of injected dose per gram of tissue - of the therapeutic radionuclide to enable limiting damage to normal tissue [6-8].

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**Table 1. Potential Alpha Emitters for Therapeutic use.**

$\alpha$ -Emitters for Therapy			
Radionuclide	Decay Emissions	Mean $\alpha$ -Particle Energy [MeV]	Half-Life
$^{211}\text{At}$ to $^{207}\text{Pb}$ (stable)	2 !, 2 EC	5.9	7.2 h
$^{212}\text{Bi}$ to $^{208}\text{Pb}$ (stable)	2 !, 2 " #	6.05	60.6 min
$^{212}\text{Pb}$ to $^{208}\text{Pb}$ (stable)	2 !, 3 " #	-	10.6 h
$^{213}\text{Bi}$ to $^{209}\text{Bi}$ (stable)	2 !, 3 " #	5.9	45.6 min
$^{223}\text{Ra}$ to $^{207}\text{Pb}$ (stable)	4 !, 2 " #	5.7	11.4 days
$^{225}\text{Ac}$ to $^{209}\text{Bi}$ (stable)	5 !, 3 " #	5.8	10 days
$^{227}\text{Th}$ to $^{207}\text{Pb}$ (stable)	5 !, 2 " #	5.9	18.7 days

**Fig. (1).** Schematic diagram of TAT performed with a radionuclide antibody-conjugate (RAC) targeting a tumour cell.

The history of radio-immunotherapy (RIT) was made by antibodies labelled with beta emitters; but only recently, after years of discordant results, a clinical role for RIT therapy towards various cancers has now been accepted [9]. In very recent years, the use of alpha emitters has been proposed for radiolabelling of many molecules [10].

A clinical precedent is set on the practice of using alpha therapy towards bone metastases with radium-223 chloride marketed as Alpharadin®. To date, the most promising approach in cancer therapy is connected with the possibility of using radiolabelled antibody-conjugates (RACs) [11].

Currently, there are around 100 radionuclides that emit alpha radiation; the majority of them produced in nuclear

reactors (Table 1). Only a few are considered useful as therapeutics agents, including bismuth-213 (generator produced) [12], astatine-211 (cyclotron produced) [13], actinium-225 (generator produced) [14] and thorium-227 (generator produced) [15]. These radiolabelled therapeutic agents - used to seek tumours including monoclonal antibodies - can be utilized in the treatment of a variety of cancers such as lymphomas, leukaemia and melanomas [16]. This is exemplified by radium-223 (half-life = 11.4 days) towards self-targeting of bone metastases by virtue of its property to mimic calcium.

The main focus of this paper is related to the use of radiolabelled antibodies as agents for radio-immunotherapy

(RIT). Following labelling with alpha emitters, radionuclide antibody-conjugates (RACs) become the prototype and the most important criteria for targeted alpha therapy (TAT) can be performed using other targets and bullets, as in the case of peptides and somatostatin receptors.

This technique relies on the emission of alpha particles in which the radionuclide (e.g. actinium-225, bismuth-213, astatine-211) is held in a crown shaped chelate (e.g. derivatives of DTPA, DOTA) connected preferably to a low molecular weight drug (Fig. 1). Alternately it may be more frequently linked to a monoclonal antibody, antibody fragments or peptide *via* a linker-chelate [17,18]. Therefore, it is paramount to get the right combination of radionuclide, linker-chelate and/or peptide, drug substrate or antibody for a particular cancer [19-21]. Various studies have shown that the ideal radionuclide for targeted therapy must have all these basic characteristics [22-24]:

- The energy emitted from the radionuclide should be lower than 40 keV;
- The photon to electron emission ratio should not exceed 2 units;
- The ideal half-life of the radionuclide-for practical and treatment purposes-should be in the range of 30 minutes to 10 days;
- To generate 'medical' radionuclides the daughter radionuclide must be stable with a half-life greater than 60 days;
- Must be able to execute robust and simple radiochemical transformations, to incorporate the radioactive label into carrier substrates, as rapidly as possible for patient use.

In parallel with the characteristics of a suitable radionuclide, acting as a therapeutic agent, TAT must be effective towards the interaction of the RAC with an ideal target. This target must be a specific antigen (or a receptor) expressed on the membrane of all tumour cells. These may also include all neoplastic lesions, which are absent or significantly lower towards the expression on normal cells.

Furthermore, for the strongest therapeutic efficacy, the *in vivo* radioactivity should be ideally distributed only on the basis of the antigen-antibody interaction which occurs in neoplasm. Conversely, *in vivo* distribution is also dependent either on the interaction of RACs with normal and/or non-malignant reactive cells. This includes non-specific uptakes of the RAC and all the other *in vivo* produced new radiochemical forms, including catabolites, complexes and free radionuclides. Ideally, the therapeutic efficacy of the radiation dose must not produce a high accumulation radiation dose at the excretory sites.

Consequently, the best results of TAT may be obtained in neoplasm characterized by the presence of tiny lesions. In addition, multiple lesions present in various stages of leukaemia, lymphomas and melanoma can theoretically also be treated by the TAT approach. There is least effectiveness in the majority of solid tumours.

To achieve TAT efficacy requires that the neoplasm does not lose sight of its target. In fact, a targeted therapy may become ineffective when in follow-up appears, hence the so called escape phenomenon [25]. This means that a pheno-

typic change of neoplastic cells will result in the loss of the specific membranes antigen towards the radiolabelled antibody. This event is more frequent in tumours having a polyclonal origin and/or evolving to undifferentiated forms [26].

The emphasis of this paper should be centred on standard TAT which is traditionally based on the intravenous administration of the alpha emitting radiopharmaceutical and not on the use of alternative routes of administration such as the intra-tumour, intra-arterial and intra-peritoneal procedures [16,27-28].

Consequently, a brief discussion will follow on the most interesting aspects of targeted therapy already in clinical practice - using antibodies or peptides, radiolabelled with beta emitters - reaching out to the future of radiolabelling with these or similar compounds incorporating alpha emitters. Moreover we will include a paragraph on Alpharadin<sup>®</sup> which is not strictly in the family of targeted therapies directed at a tumour target. This alpha emitting radiocompound is suited towards a clinical setting in the treatment of bone metastases, arising from advanced prostate cancer.

## 2. PROPERTIES: ALPHA PARTICLES AND LINEAR ENERGY TRANSFER

In 1927 Regoud and Lacassagne used alpha emitting radionuclides to target tumours due to their high linear energy transfer (LET) properties to selectively kill cancer cells [29]. This LET property is a measure of the number of ionizations (ionization density) taking place when the charged alpha particle passes through 5 and 10 cell widths in living tissues [30]. These twofold charged alpha particles have an energy range between 5-9 MeV and travel in straight lines to deposit its energies between 80-100 keV/\$m in its short path.

This indicates that alpha-emitting radionuclides must be delivered at close proximity of the DNA in cancer cells for the radiation to be effective. The maximum rate of energy of the alpha particles increases to 300 keV/\$m at the end of each track compared to beta particles which have low LET value of 0.2 keV/\$m. Damage to cells varies between low and high LET radiation values. At higher LET levels, there is a tendency to break double-stranded DNA compared to low LET levels. It has been calculated that LET ranges are of the order of 100-200 keV/\$m, causing the maximum breakages in tumour DNA. This is because ionizations equate to a distance of 2 nm between the double-stranded DNA helix.

However, it can take one to three alpha-particle interactions with the cancer cell nucleus to kill it compared to 1000 to 5000 track events from beta particles to achieve a similar effect resulting in the death of the cancer cell. Therefore, the main advantage of using alpha emitters is that they do not destroy the surrounding healthy cells in the vicinity of cancer cells [31].

Consequently, cellular repair after alpha particle induced damage is much less efficient than other forms of radiation due to the high propensity for lethal double strand DNA breaks. Resistance to agents which cause double strand DNA breaks is minimal given the high lethality (toxicity) of this form of DNA damage. Radiation associated with an alpha-particle is potentially advantageous in terms of inducing cell death in the relatively quiescent clonogenic tumour stem-like cells that are capable of being destroyed by alpha-emissions.

The limitation of using beta emitting radionuclides in treating cancer is that most of the radiation leaves the targeted cells. This of course is detrimental to normal cells close to the neoplasm. Therefore, the therapeutic dose cannot be achieved without complications, sometimes severe, due to the damage determined in the surrounding healthy cells. Since the range of alpha particles are more frequently three to five times the diameter of a cancer cell, their effect is particularly suitable to treat neoplastic clusters. Also, the harmful effect remains almost entirely limited to the concentration of cells in the neoplasm, saving the surrounding normal tissue.

Subsequently, the radiation dose is almost totally restricted to the high density of the cancer cells. The drawback of using alpha radionuclides is that the total sum of radioactivity includes both the original injected radiocompound and all the new *in vivo* radiochemical metabolites that mostly concentrate in the tumour volume. Also, surrounding tissues and emunctories contain a very low amount of radioactivity.

Producing a high lethal effect on cells independently by their condition as neoplastic or normal cells it becomes mandatory for a cost/effective therapeutic agent to be used for the lowest uptake of alpha radionuclides in the normal tissues [32]. The variation in damage caused by the alpha particle with respect to the beta emitters on non-concentrating (either normal or neoplastic) cells surrounding radio-targeted cancer cells, can be also explained by the crossfire effect. The crossfire effect is the capability of a radiocompound with therapeutic action to kill neoplastic (or normal) cells non-concentrating the agent, when adjacent to the radioactive uptake site, in a range up to the maximum length of the radiation effect.

The alpha particle (helium nuclei) can only travel 40-100  $\mu\text{m}$  compared to beta particles that travel 0.2-12 mm. Therefore, it is evident that using a beta emitter produces a higher number of damaged non-concentrating cells, which are in close proximity to the site of uptake. Unfortunately, the low or absent crossfire effect may also create a disadvantage for alpha emitters. In fact, to produce a full therapeutic effect it is more important, with respect to the beta emitters, that the target site must be highly and homogeneously expressed in all neoplastic lesions.

These shortcomings may be partially resolved in the pathophysiological space to produce the so called bystander effect. This has the ability to kill non-irradiated cells due to the cause of negative detrimental signals received from nearby irradiated cells. Consequently, this phenomenon may reduce the problem of limited access and/or effectiveness in heterogeneous, bulky or poorly vascularized tumours.

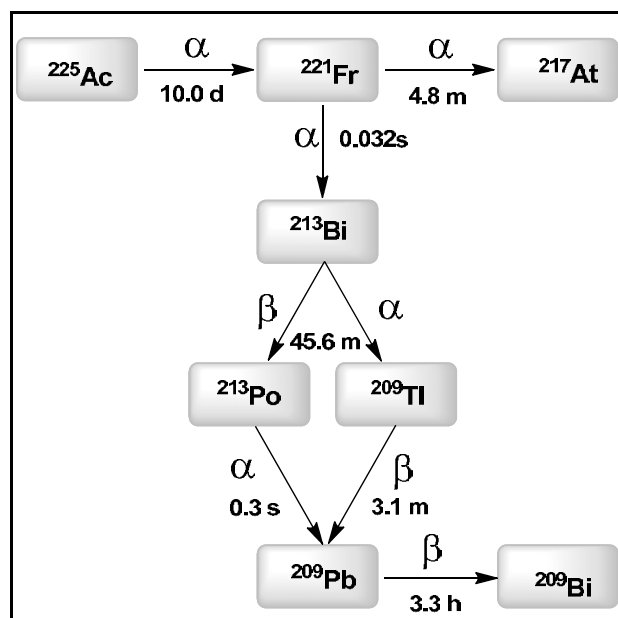
To conclude this paragraph, we emphasize that there are several advantages, especially of a radiobiological nature, to using alpha-emitting radionuclides in radiotherapeutic applications [16]. These include: (1) their relative biological effectiveness (RBE) being very high; resulting in a high probability of non-repairable DNA double-stranded breaks. This explains the distance between two strands of DNA as being almost the same as the distance between two ionizations of alpha particles; (2) the cytotoxic effectiveness of alpha-particles is much less dependent on dose rate, compared with beta-particles [33]; (3) the oxygen effect is absent for

densely ionizing radiation, such as alpha-particles, making it possible to treat both normoxic and hypoxic cell populations [34]; (4) the cytotoxic effect of high-LET radiation does not depend on the cell cycle status [35] and (5) the absence or low gamma component greatly reduces radioprotection issues, allowing treatment to be given on an outpatient basis [36].

### 3. RADIONUCLIDE GENERATORS

The process of developing targeted alpha therapy (TAT) into a viable treatment for cancer involves a need to supply the alpha emitter radionuclides at the site where the radioactive drugs are to be prepared and/or delivered. This is made possible by connecting either with radionuclides having a half-life long enough to allow their utilization far from the place of production, or with the availability of a generator system.

The most popular alpha emitter radionuclide generator in the preceding two decades utilises the actinium-225/bismuth-213 radiometal combination [37] (Fig. 2). Bismuth-213 was the first reported alpha-particle emitter used in a clinical trial as a potential cancer therapeutic. This radionuclide has a half-life of 45.6 min and mostly decays to become the alpha emitter polonium-213, *via* a branched scheme. This cascades to the emission of two alpha particles, with energies of 5.9 MeV and 8.4 MeV and yields of 2.01% and 97.8% respectively (Fig. 3).



**Fig. (2).** Decay chain of actinium-225/bismuth-213 radionuclide generator.

During the decay transitions of bismuth-213 three beta particles are also emitted with energies of 444, 659 and 198 keV; having yields of 98, 2 and 100 %, respectively. The latter of these beta particles is emitted by polonium-209, which has a half-life of 3.25 hours and decays to a stable bismuth-209. The radionuclide bismuth-213 also emits a gamma ray photon with the associated energy of 440 keV (26%). Due to this gamma ray emission, tumour imaging

becomes possible and the process may also be used to derive dosimetry data.

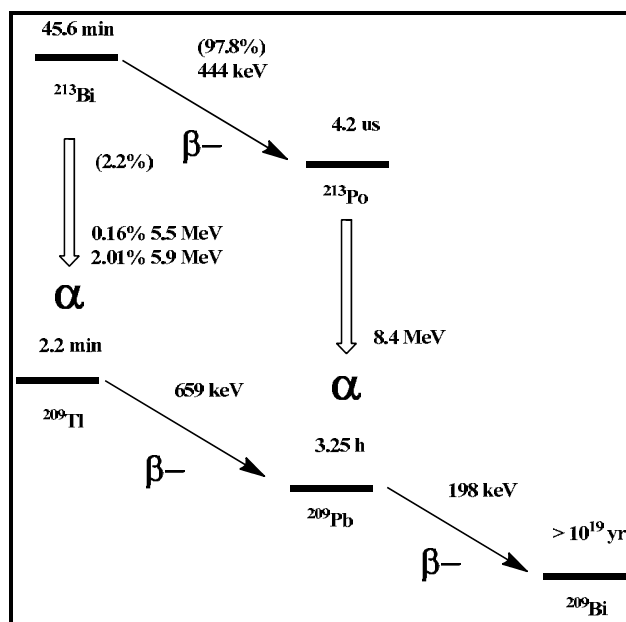


Fig. (3). Decay transitions of bismuth-213.

Accordingly, because of the high gamma energy, suitable collimators and crystals are needed for a reliable result. New techniques have also been developed to generate activities up to 100 mCi of bismuth-213 from the mother actinium-225, to be used in the synthesis of radionuclide antibody-conjugates (RACs) [38]. In its simplest form, the generator consists of a syringe filled with hydrochloric acid and an organic resin: this is connected at a junction containing another syringe filled with actinium-225 in a medium of hydrochloric acid. Under negative pressure, both solutions are mixed to generate the alpha emitter bismuth-213. This simple generator set-up is capable of formulating six doses of bismuth-213 every 24 hours cycle. In addition, up to 57 therapeutic doses may be available from the actinium-225/bismuth-213 generator for the treatment of patients [39,40].

Actinium-225 is a radiometal (half-life = 10 days) with six daughters produced in the decay chain up to a stable bismuth-209. For every decay of actinium-225, there are five successive alpha and three subsequent beta emissions, most of them of high energy having major radiations of 8.38 MeV (alpha) and 1.42 MeV (beta) [14,41].

The heaviest radio-halogen is astatine-211 which does not have an associated stable isotope. It decays at a half-life of 7.2 hours, via a branched pathway [42]. The first route of disintegration is by alpha emission to bismuth-207 (42%); followed by electron capture (EC) to stable lead-207. The second branch is by electron capture (EC) and leads to polonium-211 (58%); followed by alpha emission to stable lead-207. Astatine-211 is generally produced in a cyclotron by bombardment with alpha particles on a natural bismuth target [43].

The chloride salt of radium-223 marketed as Alpharadin<sup>®</sup>, was the first reported bone-targeting alpha emitter to be successfully used in clinical trials for palliative therapy of skeletal metastases [44]. This radiopharmaceutical shows

advantages with respect to alternative strategies, using beta emitters because of the higher linear energy transfer (LET) and the shorter range (<100  $\mu\text{m}$ ) of alpha particles. Alpha particles account for 95% of the 27.8 MeV emitted per decay which leaves less than 2% of the emissions from photons. This means that a high absorption dose will be delivered locally; although the number of photons for imaging will be low. Radium-223 has a physical half-life of 11.4 days with a complex radioactive decay chain that includes nuclides of radon, polonium, lead, bismuth, and thallium prior to reaching the stable isotope lead-207.

Radium-223 localizes into bone as a consequence of being a calcium mimetic. This enables radium to be included in the bone mineral calcium hydroxyapatite, where it can substitute calcium during mineral formation. The utilization of radium-223 has several favourable features, which can be exploited in radionuclide therapy and benefits from being cheap to produce and readily available in large amounts [45].

The sources of actinium-227 (half-life = 21.7 years) could potentially be used as a long-term operating generator for radium-223. Actinium-227 can in turn be produced by neutron irradiation of the relatively commonly available radium-223. The long physical half-life of 11.4 days provides sufficient time for preparation and distribution, including long distance shipment. These advantages would justify its use for administration in a clinical setting. Radiation safety precautions associated with radium-223 therapy are minimal: following therapy with this radiopharmaceutical, the risk of radiation exposure to other than the patient is very small, with the main recommendation being maintenance of good hygiene practice [46].

In comparison with beta emitters, also used for palliative therapy of bone metastases, whilst samarium-153 (half-life = 46.7 hours) and strontium-89 (half-life = 50 days) are removed primarily by the kidneys; the major route of elimination of radium-223 is through the faeces, with only a small fraction by renal excretion thereby reducing chances of contamination.

#### 4. THE RADIONUCLIDE THERAPY SCENARIO: FROM RADIO-IODINE TO RADIONUCLIDE ANTIBODY-CONJUGATES (RACs) AND TAT

Radionuclide therapy has been proposed since the 1940s for the treatment of thyroid cancer using radioactive iodine-131, emitting both gamma and beta particle radiations. When iodine-131 concentrates in the thyroid follicular cells, it can result in destroying both the normal tissue or, after thyroidectomy, recurrences and metastases from differentiated thyroid carcinoma. Its therapeutic application is either in hyperthyroidism, with main reference to Graves-Basedow's disease (an autoimmune disorder of the endocrine system that stimulates and attacks the thyroid gland) and in malignancy. In patients with differentiated thyroid cancer, iodine-131 is used both for thyroid ablation of the remnant normal thyroid tissue after total or near total thyroidectomy and also for the treatment of recurrent disease and metastases [47].

The therapeutic efficacy in thyroid cancer especially in the case of differentiated thyroid carcinoma is possible because of two very favourable conditions: (1) the presence of

a very high percentage of neoplastic cells expressing the iodine symporter gene which allows the concentration of iodine-131, allowing very little uptake in normal tissue; (2) the diagnostic and therapeutic application of this approach is favoured by the usage of the simpler and more stable radiochemical form of radioiodine such as sodium salt [48].

In general, the radiation dose produced by radionuclide therapy is derived not only by the specific uptake of the administered radio-compound but also by the non-specific concentration - both of the original radiotracer and all the *in vivo* radioactive metabolites. Therefore, the ideal radiopharmaceutical for radionuclide therapy as previously reported for radio-iodine, must have the simplest and more stable radiochemical form, furthermore being advantaged by the lowest amount of non-specific uptake.

This goal may be quite easily achieved using small molecules having the capability to reach a high tumour/non-tumour ratio because of a favourable uptake mechanism, as happens in palliative therapy of bone metastases. Similarly, a favourable cost/effective result may be obtained using radioactive complexes concentrating on the basis of a mechanical and non-functional mechanism. This is the case for alternative routes of administration such as by using radiolabelled microspheres for intra-arterial therapy of liver neoplasms or radiocolloids for intra-articular synovectomy [49]. However, as a result of using radionuclide therapy radiotracers with a high molecular weight and/or by using biologically active molecules such as antibodies there exists more potential for complexity and unfavourable pharmacokinetics.

The result of a therapeutic effect depends on the specific interaction between the radioligand and target. Therefore, it is mandatory that the tracer, after radiolabelling, maintains its capability to link the binding site of the target without significantly losing its affinity.

Consequently, it is essential that, after administration, the radiopharmaceutical is distributed *in vivo* on the basis of its pharmacokinetics profile and is almost completely determined by the original radiotracer. Unfortunately, this scenario is infrequent since in a large number of cases the radiopharmaceutical may concentrate and follow a non-specific mechanistic pathway. Furthermore, the original radiocompound may be catabolized giving origin to new radiochemical forms, including the free radionuclide. In this outcome, a significant increase of non-specific uptake is determined, thereby reducing the theoretical effectiveness of the therapeutic agent [50].

Therefore, it is paramount to synthesize radiochemical compounds that possess functional activity to allow binding to the target and result in high *in vivo* stability. For this reason, although based on simple and reliable *in vitro* radiochemistry, the use of radio-iodinated ligands for radionuclide therapy tends to be less efficient because of the possible high *in vivo* de-iodination rate. This process causes the production of high amounts of free radionuclide and of other radiochemical forms.

Interestingly, the use of radio-iodinated molecules tends to remain restricted to few options, as in the case of the drug, iodine-131 *meta*-iodobenzylguanidine (MIBG) for neuroblastoma and in other neuroectodermal tumours. This is because of a high tumour/non-tumour ratio and the more fa-

vourable cost/effectiveness approach with respect to alternative therapeutic strategies. A favourable therapeutic result was obtained with Bexxar<sup>®</sup>, a monoclonal antibody radiolabelled with iodine-131 which will be discussed later on in this paper.

Consequently, we are continuously looking for therapeutic applications and robust radiolabelling procedures, as alternatives to radio-iodination. In particular, drug molecules have been designed to deliver high doses of radiation - *via* emitting radiometals - and to recognize certain specific receptors on the surface of tumours. This has been successfully demonstrated with the use of somatostatin analogues to treat neuroendocrine tumours (NET) [51].

This peptide receptor radionuclide therapy (PRRT) is a consolidated strategy using somatostatin (SST)-receptor ligands. These ligands are radiolabelled with a beta emitter, linked to the peptide *via* chelation, without altering its functional site. A patient with NET is encouraged to receive radionuclide therapy on the basis that a favourable tumour/non-tumour ratio can be achieved. This information is realized by a previous diagnostic scan performed using almost the same molecule, labelled with gamma or positron emitters. In this way, because of similar pharmacokinetics, the distribution subsequent to the *in vivo* administration of the radiolabelled therapeutic agent may be reliably provided.

Peptides have very favourable characteristics as diagnostic and therapeutic tools due to an excellent permeability, lack of antigenicity, minimal side-effects and rapid clearance from the body. In the presence of a high-affinity receptor binding, they are also easy to synthesize and chemically modify to link to chelators for insertion into the radiometal. Therefore they represent an optimal system for *in vivo* diagnosis and therapy using radionuclides [52,53].

The radiolabelling of antibody immunological agents for diagnostic and therapeutic purposes is more challenging due to potential negative effects on *in vivo* pharmacokinetics. The techniques of radio-immunoscintigraphy (RIS) and radio-immunotherapy (RIT) are based on a combination between the specific antigen which is highly expressed on neoplastic cells with respect to normal cells and the ability to recognise the radiolabelled antibody [54,55]. Even after resolving issues relating to immunogenicity contained within murine monoclonal antibodies, there are still drawbacks associated with antibodies being in human or humanized forms.

In fact, the whole radiolabelled antibody is affected by a low permeability allowing a slow and reduced extraction from the plasma. This give rise to a poor neoplastic concentration further complicated by non-specific uptake, producing unfavourable tumour/non-tumour ratios, which are incompatible with an effective radionuclide therapy [56].

These problems arise more frequently for solid tumours and are less evident for neoplasm as lymphoma and leukaemia. A possible improvement for RIS and RIT may be obtained using immunological molecules with a lower molecular weight than the whole antibody. However, these approaches produce different problems connected to a lower immunological affinity and/or reduced radiochemical stability which are observed.

Nevertheless, after numerous and rigorous trials, various monoclonal antibodies have been approved for cancer therapy in humans (Table 2) [57]. Rituximab, a mouse-human chimeric monoclonal antibody directed to the antigen CD20, was approved by the US Food and Drug Administration (FDA) for the treatment of some forms of lymphoma [58]. These target cells that express surface CD20 are exclusively found between pre-B and mature B cells. Rituximab specifically lysis circulating B cells, while sparing stem cells and mature plasma cells.

Consequently, this unlabelled antibody has a cytotoxic effect *via* a variety of mechanisms, including those promoting apoptosis [59]. The therapeutic benefit, however, seems to be limited to the tumour cells directly affected by the antibody. Initially approved in the USA in 1997 for treatment of non-Hodgkin's lymphoma, its FDA approval has been expanded to encompass chronic lymphocytic leukaemia and in 2006 for rheumatoid arthritis. Most recently in 2011, it was also approved for Wegener's granulomatosis and microscopic polyangiitis, two rare types of vasculitis (inflammation of blood vessels). Rituximab in combination with cyclophosphamide / doxorubicin / vincristine / prednisone (R-CHOP) remains the standard frontline regimen for diffuse large B-cell lymphoma [60].

Today the second-generation of anti-CD20 monoclonal antibodies include ofatumumab, velatumumab and ocrelizumab. These are humanized antibodies to reduce immuno-

genicity and are capable of producing a more favourable pharmacokinetic profile. These non-radiolabelled antibodies can amplify the body's immune mechanism to destroy cells, expressing the target towards which the antibody is directed [61]. More recently, with respect to rituximab, two radionuclide-bearing monoclonal antibody therapies using Bexxar<sup>®</sup> and Zevalin<sup>®</sup> have been approved by the US FDA, and several more are in clinical trials (Table 3).

#### 4.1. Bexxar<sup>®</sup>

Bexxar<sup>®</sup>, developed by Kaminski and Wahl, contains the antibody tositumomab radiolabelled with iodine-131 [62]. This IgG2a anti-CD20 monoclonal antibody is derived from immortalized mouse cells. The patient first receives tositumomab followed by the infusion of tositumomab, radiolabelled with iodine-131: this is the same antibody covalently bound to the radionuclide iodine-131. The iodine-131 emits both beta and gamma radiation and decays with a half-life of 8 days (Fig. 4).

A successful clinical study involving 40 patients led to the approval in 2003 of Bexxar<sup>®</sup> for the treatment of rituximab-refractory, low-grade, follicular non-Hodgkin's lymphoma [63]. In 2005, Fisher *et al.* summarized the results obtained from 250 patients in 5 clinical trials which included a subset of patients [64].

In a clinical study involving 250 patients, 226 (90%) had stage III or IV disease and 46% had bone marrow involve-

**Table 2. FDA Approved Oncology Non-radionuclide Antibody-based Therapies Adapted from Burden *et al.* [57].**

Drug Name	FDA Approval	Type	Target	Therapy (Indications)
Trastuzumab Emtansine (Kadcyla <sup>®</sup> )	2013	Humanized IgG1	HER2	HER2 positive metastatic breast cancer
Brentuximab Vedotin (Adcetris <sup>®</sup> )	2011	Chimeric IgG1	CD30	Hodgkin's lymphoma, anaplastic large cell lymphoma
Ipilimumab (Yervoy <sup>®</sup> )	2011	Human IgG1	CTLA-4	Metastatic melanoma
Ofatumumab (Arzerra <sup>®</sup> )	2009	Human IgG1	CD20	Chronic lymphocytic leukaemia
Panitumumab (Vectibix <sup>®</sup> )	2004	Human IgG2	EGFR	Colorectal cancer
Cetuximab (Erbix <sup>®</sup> )	2004	Chimeric IgG1	EGFR	Colorectal cancer
Bevacizumab (Avastin <sup>®</sup> )	2004	Humanized IgG1	VEGF-A	Colorectal cancer
Alemtuzumab (Campath <sup>®</sup> )	2001	Humanized IgG1	CD52	Chronic lymphocytic leukaemia, acute myeloid leukaemia, Multiple sclerosis
Gemtuzumab (Mylotarg <sup>®</sup> )	2000	Calicheamicin conjugated humanized IgG4	Siglec3 (CD33)	Acute myeloid leukaemia
Trastuzumab (Herceptin <sup>®</sup> )	1998	Humanized IgG1	HER2	Breast cancer
Rituximab (Rituxan <sup>®</sup> )	1997	Chimeric IgG1	CD20	Non-Hodgkin's lymphoma, B-cell lymphoma, chronic lymphocytic leukaemia

**Table 3. Radionuclide Antibody-Conjugates (RACs) in Clinical Trials for Therapeutic Applications Adapted from Steiner and Neri [2].**

Radionuclide Antibody-Conjugates (RACs)	Clinical Phase	Description	Targeted Therapy
<sup>131</sup> I-Tositumomab (Bexxar <sup>®</sup> )	FDA Approved 2003	Treatment combination consisting of the unlabelled and iodine-131 radiolabelled murine CD20 targeting antibody tositumomab	B-cell lymphoma, Hodgkin's lymphoma, diffuse large B-cell lymphoma, non-Hodgkin's lymphoma, multiple myeloma
<sup>111</sup> In/ <sup>90</sup> Y-Ibritumomab tiuxetan (Zevalin <sup>®</sup> )	FDA Approved 2002	Murine CD20 targeting antibody ibritumomab linked by the chelator tiuxetan. Yttrium-90 used for cancer therapy, Indium-111 for SPECT imaging	B-Cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, non-Hodgkin's lymphoma
<sup>131</sup> I-Metuximab (Licartin)	Phase II	Iodine-131 radiolabelled murine antibody HAB18 F(ab') <sub>2</sub> fragment against the HCC-associated antigen HAB18G/CD147	Hepatic cancer
<sup>131</sup> I-3F8	Phase II	Iodine-131 radiolabelled anti-GD2 ganglioside murine IgG3 monoclonal antibody	Cancer, medulloblastoma, neuroblastoma
<sup>131</sup> I-L19 (Radretumab)	Phase II	Iodine-131 radiolabelled SIP composed of L19 that binds to the ED-B domain of human fibronectin	Non-small cell lung cancer, solid tumours, hematologic/blood
<sup>131</sup> I-F16 (Tena-Rad)	Phase II	Iodine-131 radiolabelled human monoclonal antibody against the A1 domain of tenascin-C	Haematological cancer, solid tumours
<sup>131</sup> I ch-TNT-1/B (Cotara)	Phase II	Iodine-131 radiolabelled chimeric monoclonal antibody chTNT-1/B for tumour necrosis therapy	Anaplastic astrocytoma, biliary cancer, colorectal cancer, glioblastoma multiforme, glioma, sarcoma
<sup>131</sup> I-BC8	Phase II	Iodine-131 radiolabelled murine anti-CD45 monoclonal IgG1 antibody	Acute myeloid leukaemia
<sup>111</sup> In-J591, <sup>177</sup> Lu-J591	Phase II	Indium-111/Lutium-177 labelled humanized monoclonal antibody to prostate specific membrane antigen/extracellular domain (PSMAext)	Prostate cancer
<sup>177</sup> Lu-DOTA-cG250	Phase II	Chimeric monoclonal antibody G250 conjugated to DOTA and radiolabelled with lutium-177	Kidney cancer (renal cell carcinoma)
<sup>177</sup> Lu-J591	Phase II	Lutium-177 radiolabelled humanized monoclonal antibody J591 targeting prostate-specific membrane antigen (PSMA)	Metastatic prostate cancer
<sup>90</sup> Y-hLL2 IgG; (Epratuzumab- <sup>90</sup> Y) LymphoCide <sup>90</sup> Y	Phase I/II	Yttrium-90/Lutium-177 radiolabelled human-mouse monoclonal IMMU-hLL2 targeting CD22	Follicular lymphoma, non-Hodgkin's lymphoma, acute lymphoblastic leukaemia
<sup>90</sup> Y-hPAM4 ( <sup>90</sup> Y Clivatuzumab tetraxetan)	Phase I/II	Human-mouse monoclonal hPAM4 IgG1 targeting human Mucin-1 conjugated to DOTA and radiolabelled with yttrium-90	Pancreas cancer

ment. The overall response rate (ORR) was 56% and the median duration of response was 12.9 months. A complete response (CR) was seen in 30% of patients. Many patients remained in remission beyond the 5 years that had elapsed at the time of the analysis. Kaminski *et al.* used Bexxar<sup>®</sup> as the initial therapy in 76 patients with stage III or IV follicular lymphoma. The ORR was 95%, and 75% of the patients had a CR [65].

#### 4.2. Zevalin<sup>®</sup> 'Better Together – Imaging + Therapy'

Zevalin<sup>®</sup> is the trade name of the ibritumomab tiuxetan radiolabelled with yttrium-90. Ibritumomab is a monoclonal mouse IgG1 antibody [57-66], while tiuxetan is a linker-

chelator, corresponding to a modified version of DTPA, whose carbon backbone contains an isothiocyanatobenzyl and a methyl group (Fig. 5). The drug is first labelled with the radiometal indium-111, a gamma-emitter and imaged using single photon emission computed tomography (SPECT) to verify that the antibody properly distributed within the body [67]. In 1% of patient groups, the distribution of Zevalin<sup>®</sup> is altered due to the excessive uptake in the bone marrow or kidneys [68].

Alternatively if the drug bio-distribution is satisfactory, therapy treatment is continued using a version of the Zevalin<sup>®</sup> antibody labelled with the strong beta-emitter yttrium-90 [69]. The radioactive yttrium-90 can supply a lethal

dose of radiation directly to the bound B-cells and indirectly to neighboring B-cells [70].

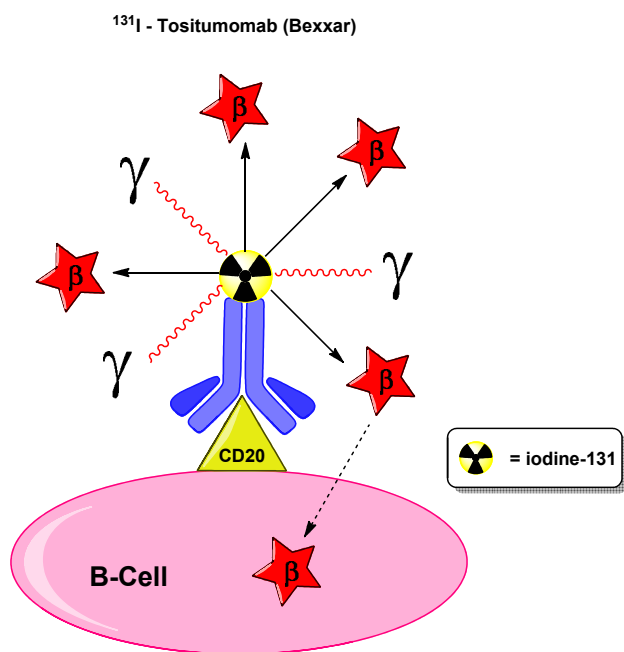


Fig. (4). Schematic representation of tositumomab radiolabelled with iodine-131 (Bexxar®) targeting a lymphoma B-cell.

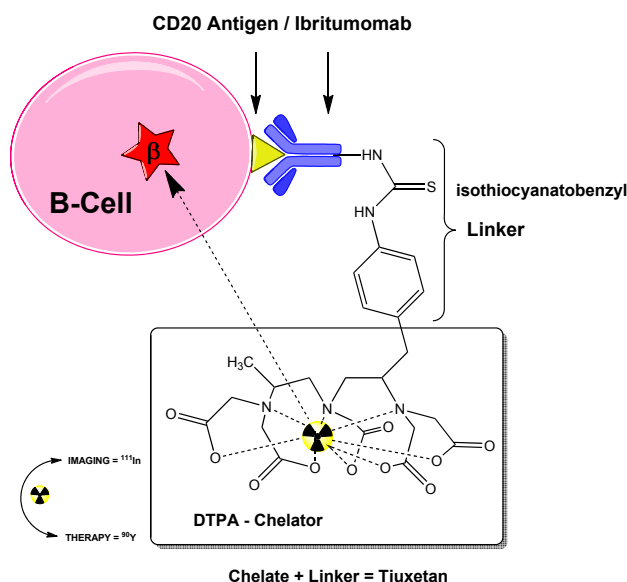


Fig. (5). Schematic representation of Zevalin® targeting a lymphoma B-cell with beta-particles.

Yttrium-90 is generated from its mother strontium-90 via emission of pure beta particles that decay to produce non-radioactive stable zirconium-90, with a half-life of 64 hours. The maximum energy of the beta emission is 2.29 MeV giving rise to an effective path length of up to 5.3 mm. The median biological half-life of Zevalin® in blood is 48 hours with clearance primarily through the urinary system. The primary adverse effects of Zevalin® therapy are similar to those determined by Bexxar®. These adverse reactions include: hematological toxicity with anemia, thrombocytopenia and neutropenia. The other side effects may be infection, chills,

fever, abdominal pain and allergic reactions. Patients should have a complete blood and platelet count each week for at least 6 weeks [71].

The major advantage of RIT with respect to the unlabelled anti-CD20 antibody therapy is derived from the potential to amplify the cytotoxic ability via a radiation cross-fire effect. This is caused by ionizing radiation producing an intense detrimental effect on neighbouring cells surrounding the deposited radionuclide. The range of the radiation in the tissue is about 250 cell diameters around the Zevalin-binding neoplastic cells. This radiation has the ability to penetrate into the surrounding tumour tissue delivering a lethal effect on the non-concentrating cells up to 5 mm from the uptake site [72].

Zevalin® therapy has useful indications for relapsed or refractory, low grade or follicular B-cell non-Hodgkin’s lymphoma. During a Phase III clinical trial with 143 patients with non-Hodgkin’s lymphoma, there was an overall response rate of 80% with yttrium-90 radiolabelled ibritumomab tiuxetan, compared with 56% with rituximab.

A second trial, found an overall response rate of 74% in rituximab refractory patients. These and other studies led to FDA approval in 2002 for the treatment of patients with relapsed or refractory low-grade follicular or transformed B-cell non-Hodgkin’s lymphoma; including patients with rituximab refractory follicular non-Hodgkin’s lymphoma. In 2008, Zevalin® was approved as the first-line consideration for follicular lymphoma in the European Union [73].

There is currently no clear clinical consensus as to which agent would be superior in a specific clinical setting, and there are currently no direct, large, randomized clinical trials comparing the efficacy of Zevalin® and Bexxar®. Jacene *et al.* in a paper evaluating the outcomes of Bexxar® and Zevalin® therapies in clinical practice concluded that both were well tolerated [74], but Bexxar® caused significantly less severe declines in platelet counts than did Zevalin®. Iagaru *et al.* published similar data, suggesting higher observed response rates for Zevalin® (77.8%) against Bexxar® (70.9%), but with more frequent adverse effects [75]. Due to the small number of patients in the study, it was not possible to determine whether differences in the outcomes or toxicities from the two agents were statistically significant.

## 5. TARGETED ALPHA THERAPY- CLINICAL STUDIES

### 5.1. Bone Metastases

If we extend the meaning of targeted therapy as a strategy, having the ability to target neoplastic cell membranes with radionuclides, then the application of radium-223 chloride (Alpharadin®) should also be included in this definition. In fact, being a mimic of calcium, radium-223 concentrates in the reactive and hyperactive normal bone surrounding skeletal neoplastic lesions and not directly on tumour cells [44].

Therefore, the uptake mechanism determined by the presence and the intensity of an osteoblastic activity means that a high radiation dose is given to osteoblastic bone metastases. This particular dose level is reduced at the bone marrow, which is surrounded by normal bone tissue, showing no signs of activity.

Alternatively, an intense radiation dose may be given to the bone marrow when adjacent to hyperconcentrating benign lesions, such as active fractures and/or inflammation [45].

A major advantage for Alpharadin<sup>®</sup> is the homogeneous distribution of the radiocompound uptake occurring in a normal cell population, leading to the absence of the escape phenomenon. Moreover, any Alpharadin<sup>®</sup> that is not taken up by the bone metastases is rapidly cleared to the gut and excreted. The clinical efficacy of Alpharadin<sup>®</sup> has already been demonstrated. It is theoretically possible to allow its usage - in all indications defined for palliative therapy of bone metastases - using beta emitters. The main indication includes patients with prostate cancer showing otherwise untreatable secondary bone lesions [76].

Bone-targeting radiopharmaceuticals have extensively been researched and for many years used in a clinical setting for the palliative treatment of cancer that has metastasized to the skeleton. The radiocompounds are based on beta-particle emitters and include strontium-89 (Metastron<sup>®</sup>) and samarium-153 EDTMP (Quadramet<sup>®</sup>). These radiopharmaceuticals have been made commercially available for many years, whereas several others are under further clinical investigation.

Due to the relatively long radiation range, significant bone marrow exposure is associated with the use of beta-emitters [77]. This condition limits bone treatment with beta-emitters exclusively to pain palliation in patients with advanced prostate cancer, in the presence of otherwise untreatable skeletal metastases and more restrictively, in patients with breast cancer [78].

Radium-223 chloride is the first bone-targeting alpha emitter studied in clinical trials for skeletal metastases. These clinical trials have demonstrated the safety and efficacy of palliation of painful bone metastases in patients with castration-resistant prostate cancer. Alpharadin<sup>®</sup> targets bone metastases with a higher linear energy transfer (LET) and a shorter range (<100  $\mu\text{m}$ ) of radiation, allowing a lower radiation dose to the normal bone marrow [11]. A multinational, Phase III, double-blind, randomized study (ALSYMPCA) [79] compared Alpharadin<sup>®</sup> for the best standard of care with a placebo plus best supportive care in 922 patients with bony metastatic castration-resistant prostate cancer (mCRPC).

The overall survival rate in the control group was 11.2 months and 14.0 months in the group treated with TAT. Toxicity was minimal with 4% grade 3/4 thrombocytopenia and 2% grade 3/4 neutropenia [80]. The improvement in overall survival was observed for radium-223 when compared not only with the placebo, but also with beta emitters, is due to a decrease in myelosuppression [81].

This property allows consecutive doses to be administered safely and may be the basis for its more substantial anti-tumour effects and overall survival, compared with alternative radionuclide therapies [82]. Further studies of radium-223 should be considered for the potential escalation of dose. A combination therapy of using Alpharadin<sup>®</sup> (radium-223 chloride) with Taxotere<sup>®</sup> (docetaxel), a standard first-line chemotherapy drug for hormone refractory prostate cancer must be considered for treatment of skeletal metastases

from castration-resistant prostate cancer (CRPC) and other primary cancers [83].

## 5.2. Other Neoplasms

A Phase I trial for acute myeloid leukaemia (AML) was first carried out at the Memorial Sloan Kettering Cancer Center (MSKCC). The preclinical efficacy of bismuth-213 and actinium-225 in TAT has also been reported in lymphoma, melanoma, breast, prostate, ovarian and pancreatic cancers [83].

Various human clinical trials have taken place at the MSKCC using bismuth-213 conjugated to the murine monoclonal antibody M195 and the humanised HuM195 analogue (anti-CD33). These RACs containing the specific monoclonal antibody are directed towards a prostate antigen [84].

The murine M195 is directed against the CD33 antigen and is expressed by most of the myeloid leukaemic cells including the clonogenic progenitors of the leukaemia. HuM195 is able to modulate the response of human blood mononuclear cells, resulting in the death of the leukaemic cells. Since 2002, the safety profile, feasibility and anti-leukaemic effects have been demonstrated by Jurcic *et al* [85]. A conventional medical internal radiation dose (MIRD) approach estimated that the absorbed dose ratios between areas of leukaemic involvement and the whole body were 1000-fold greater than those seen with the corresponding beta emitters. This favourable behaviour is dependent both on the much reduced whole-body doses and the greater target organ doses for bismuth-213 in comparison with iodine-131 and yttrium-90 [86].

These pioneering studies with confirmation of clinical results from several Nuclear Medicine Departments in the treatment of 130 patients using TAT with bismuth-213 or actinium-225: it has been demonstrated that actinium-225, in a variety of *in vitro* tumour cell lines has the capability to kill cancer cells at doses of 1000 times lower than those associated with the corresponding bismuth-213 radiopharmaceuticals [87]. More recently, the potential application of TAT has also been proposed in treating lymphoma and leukaemia, in the selective T-cell and hematopoietic tissue ablation as a means of conditioning prior to bone marrow transplantation [87].

Conversely, on the application of a completely different pathophysiological profile, here we will only cite the research of Zalutsky *et al* [88]. This group administered the chimeric anti-tenascin monoclonal antibody 81C6 (ch81C6), labelled with astatine-211 (<sup>211</sup>At-ch81C6), into the surgically created resection cavity of patients with recurrent malignant brain tumours. Zalutsky *et al.* provided proof of concept that the regional administration of <sup>211</sup>At-ch81C6 is feasible, safe and associated with a promising anti-tumor effect in patients with malignant central nervous system tumours.

## 5.3. Targeting Melanoma

Melanoma is a skin cancer with a high mortality rate with approximately 8700 deaths per year in the United States. While it is easily treatable when caught in the early stages, difficulties arise in the treatment of patients with metastatic melanoma. In this case, a median survival of 8.5 months,

with an estimated 5-year survival of 6% remains substantially unchanged in the past 25 years [89]. Therefore new treatments involving antibodies targeted directly onto tumour cells and the utilization of active immune therapy *via* vaccination with tumour cells, are generating strong interest.

In this scenario, an alternative approach based on the use of target alpha therapy (TAT) with the monoclonal antibody 9.2.27 has been recently proposed [90]. This monoclonal antibody is tagged onto chelator cyclic anhydride of diethylenetriaminepentaacetic acid (cDTPA) incorporating the alpha emitter bismuth-213. Preclinical studies have shown that the radionuclide antibody-conjugate (RAC),  $^{213}\text{Bi}$ -cDTPA-9.2.27, produces a labelling efficiency of 96% and is highly effective in inhibiting DNA synthesis in melanoma cells.

It has to be remembered that in melanoma patients a very wide range of survival rates are observed with respect to the staging. In fact, the survival rates range from 99.9%, at stage 0 - melanoma *in situ*, to 7-19% at stage IV, in the presence of distant metastases. During the advanced stages of the tumour development, cancer cells are diffused from the primary tumour site and infiltrate firstly the lymphatic circulation system. Free flowing cancer cells can then group together and diffuse into vital organs determining their failure and subsequent death.

A clinical study examined 22 patients with stage IV melanoma metastatic cancer, treated with a range of activity from ~1.5 mCi to ~25.6 mCi of the radionuclide antibody-conjugate  $^{213}\text{Bi}$ -cDTPA-9.2.27. Using the RECIST criteria (Response Evaluation Criteria in Solid Tumors), the outcome of the therapy showed 14% of the patients with a partial response, 50% with stable disease and 30% in progression. In particular, one patient showed near complete response and was retreated because of his excellent reaction to the initial treatment. Another patient showed response in his mandibular tumour and a reduction in lung lesions. The tumour marker melanoma inhibitory activity protein (MIA) showed reductions over eight weeks in most of the patients.

This group of patients had not experienced toxic side effects over the range of administered activities demonstrating that TAT could be a safe and effective treatment for metastatic melanoma. Survival rates have been also analyzed from a previously reported Phase I study, using systemic targeted alpha therapy in patients with stage IV metastatic melanoma or in the presence of in-transit metastases. Following intravenous administration of 1.2-25 mCi of  $^{213}\text{Bi}$ -cDTPA-9.2.27, 38 patients were studied to observe response and toxicity.

These responses were measured by physical examination, computed tomography at 8 weeks and blood sampling. Toxicity was monitored by blood pathology, urine analysis, glomerular filtration rate and human anti-mouse antibody response. The maximum tolerance dose was not achieved as there were no adverse effects of any type or level. However, an objective partial response rate of 10% was observed, with 40% of patients with stable disease at 8 weeks and a median survival of 8.9 months.

These preliminary results were unexpected because of the short half-life of the bismuth-213 and also the short range of the alpha-radiation. The analysis of clinical results demonstrated that melanoma-inhibitory activity, disease stages,

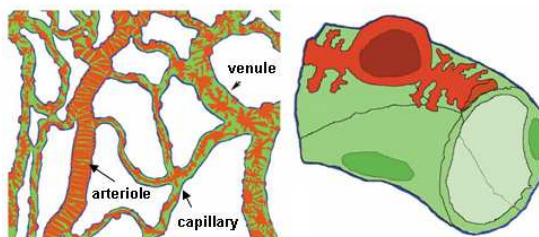
lactate dehydrogenase and treatment effects were significant prognostic indicators for survival [91].

## 6. TUMOUR ANTI-VASCULAR ALPHA THERAPY (TAVAT)

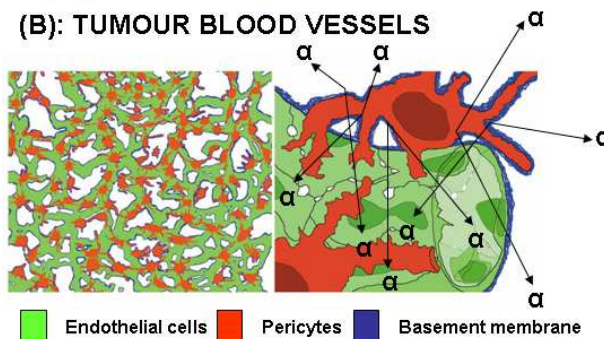
When a patient is injected with a cancer-specific antibody labelled with an alpha-emitting radionuclide (RAC), this antibody conjugate binds onto specific receptors expressed by cancer cells. This enables the accumulation of the RAC in the diseased tissue. The hypothesis was that targeted alpha therapy was only suitable for destroying isolated cancer cells and small pre-angiogenic cell clusters.

This uses the premise that short range alpha particle radiation is not able to penetrate at great distance into solid tumours. This hypothesis was in question when Allen *et al.* observed the regression of solid tumour mass in a Phase I clinical trial of metastatic melanoma when cancer patients were undergoing targeted alpha therapy (TAT). To explain this observation, a new therapeutic mechanism called tumour anti-vascular alpha therapy (TAVAT) was postulated by Allen *et al.* [92].

### (A): NORMAL BLOOD VESSELS



### (B): TUMOUR BLOOD VESSELS



**Fig. (6).** Schematic diagram adapted from Baluk *et al.* [93] showing (A) normal blood vessels compared to (B) the tiny spaces between the endothelial cells of tumour blood vessels. This allows the alpha-emitting RAC to target the melanoma-associated chondroitin sulfate proteoglycan (MCSP) antigen expressed by capillary pericytes and surrounding melanoma cells.

Tumour anti-vascular alpha therapy (TAVAT) is based on the abnormal vasculature structure and function of the tumour mass (Fig. 6). The endothelial cell walls of the tumour containing capillaries are leaky and this allows the RAC to pass through. The capillaries are enclosed by cells known as pericytes, which express the same antigen as melanoma cells. The RAC can escape from the bloodstream and accumulate at the pericytes and melanoma cells. The emitted alpha particles have the path length (80  $\mu\text{m}$ ) and rate

of energy loss to effectively kill endothelial cells resulting in the capillary closing and nutritional support to the tumour closing down. If enough capillaries are shut down, the tumour will regress and completely disappear [93].

TAVAT can lead to the treatment of other types of cancer and this can be achieved by designing conjugates with antibodies targeted to both cancer cells and pericytes. Melanoma is the only case in which both types of cell express the same antigen. Preclinical studies of the effect of TAVAT have only been observed in human subjects but not in mice [94].

## 7. A STRATEGY TOWARDS CARBON-14 LABELLING OF RACS

The number of biotherapeutics at different clinical stages (Table 3) targeting cancer is increasing [2]. Therefore, evaluation of the safety profile is paramount especially when using radiometals emitting alpha and beta particles [95]. Consequently, it is important that the binding of the radiometal to the chelate is not lost to the systemic circulation as this would reduce the efficacy of the RAC and produce unwanted side effects.

Despite advancement in analytical technologies, carbon-14 labelling of active pharmaceutical ingredients (APIs) remains the gold standard to facilitate absorption, distribution, metabolism, and excretion (ADME) studies [96]. A possible strategy towards the carbon-14 labelling of radionuclide antibody-conjugates (RACs) can be executed on the chemical linker part incorporating the chelate holding the radiometal payload. In these cases the carbon-14 study can be carried out on non-radioactive metal. This would enable ADME studies to be carried out in a safer manner.

The manufacture of a carbon-14 labelled RAC begins with the radiochemical synthesis of the linker to incorporate the label in the required position [97]. Ideally, the label should be placed in the most metabolically stable position in the linker to be able to survive in the systemic circulation of the RAC until it reaches the cancer cell. The carbon-14 labelled linker is then attached to a chelate. The other end of the carbon-14 labelled linker must be able to facilitate a bio-conjugation to the monoclonal antibody [98]. Finally, completion of the labelled synthesis of the non-radioactive metal payload is chelated [99] to give the carbon-14 labelled non-radioactive antibody conjugate which is an exact copy of the RAC to be used in ADME studies [100].

## CONCLUSION

Radionuclide antibody-conjugates (RACs) containing alpha emitters can deliver an effective controlled radiation dose to a neoplastic mass to kill tumour cells. In certain cases, the cancer becomes a disseminated disease and therefore it is important to deliver cytotoxic radiation not only to the primary tumour but also to its metastases bearing in mind limiting the radiation dose to healthy tissue cells. At the present, over 130 patients have received this experimental cancer treatment, called targeted alpha therapy (TAT) and the information gathered from these first clinical trials will contribute to future safety profiles for the administration of alpha emitters in patients.

Theoretically, an ideal application for targeted alpha therapy is in treating conditions such as lymphoma and leukaemia, in which neoplastic cells are in the systemic circulation. However, important results may be also obtained from clinical conditions such as tumour meningitis or pleural and peritoneal neoplastic effusions where the cancer cells are present, both as free-floating cells and spread along compartment walls.

The experimental technique TAT will require further studies to establish the maximum tolerance doses in the clinical arena. An increase of these targeted therapies in the Nuclear Medicine community will require further investigation into more efficient and practical routes to generate the radiometal and its subsequent incorporation into radiopharmaceutical drugs. Moreover, it will be necessary to facilitate further research and development into linker-chelation chemistry, transporting the radiometal (alpha emitters) to the tumour site, design of drug substrates and the need for further clinical trials for different types of cancers leading to its application towards personalised medicine.

Once clinically established, TAT would gravitate towards a major role in the treatment of small volume, minimal residual or micro-metastatic disease after partial cyto-reduction with chemotherapy. Further improvements could include combined therapies, multiple dose administration schedules and the use of combination therapies containing radiopharmaceuticals characterized by different physical characteristics. In parallel, important advances could be obtained with a loco-regional administration of the radiopharmaceutical, for example neoplastic pleural or peritoneal effusions or into a surgically created resection cavity after surgical de-bulking.

Furthermore, in order to progress and disseminate this exciting therapeutic strategy requires a wider radionuclide availability; mainly supporting the production of alpha emitters which show the best physical characteristics to promote efficacy and eliminate undesirable side effects. At the same time, it is absolutely essential for oncological understanding and culture to give increasing consideration to new radionuclide therapy frontiers. The recognition, accessibility and application of targeted alpha therapy (TAT) will be the future in the treatment of cancer.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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