TARGETED ALPHA THERAPY: FROM ALPHA TO OMEGA

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Abstract

This review covers the broad spectrum of Targeted Alpha Therapy (TAT) research in Australia; from in vitro and in vivo studies to clinical trials. The principle of tumour anti-vascular alpha therapy (TAVAT) is discussed in terms of its validation by Monte Carlo calculations of vascular models and the potential role of biological dosimetry is examined.

Summmary of this review is as follows:

1. The essence of TAT
2. Therapeutic objectives
3. TAVAT and Monte Carlo microdosimetry
4. Biological dosimetry
5. Preclinical studies
6. Clinical trials
7. What next?
8. Obstacles

1. THE ESSENCE OF TARGETED ALPHA THERAPY

1.1. Immunotherapy

Since the development of the hybridoma [1], monoclonal antibodies (MAb) have been raised against many antigens over-expressed by cancer and other cells [2]. Most of these MAbs exhibit antibody-dependent cell-mediated cytotoxicity, caused by lysis of antibody-coated target cells by effector cells with cytolytic activity and Fc receptors. Cell-mediated cytotoxicity arises from cytolysis of a target cell by effector lymphocytes, such as cytotoxic T lymphocytes or natural killer cells and may be antibody-dependent or independent. These toxicities tend to have moderate efficacy. Some antibodies work by neutralizing or blocking receptors, and these tend to be the more effective.

1.2. Radioimmunotherapy

Benign or partial blocking MAbs that are cancer cell specific can still be effective if labelled with a toxin. Then the MAb becomes a targeting vector to take the toxin to the targeted cancer cells. In such cases, the half-life of the MAb in the body should preferably match the half-life of the toxin. Toxins can be chemicals or radioisotopes and are mostly chelated to the MAb to form relatively stable immunoconjugates (ICs). Chemical toxins can have long half-lives in the body, eg ricin, which increases their toxicity for normal tissue. Radioisotopes have a wide range of half-lives and radiation decay properties.

Nuclear imaging uses long-lived gamma emitters, which allow blood clearance as the tumour increases its uptake of the conjugate over time, so improving contrast. Iodine-131and ^{123}I, ^{201}Tl, ^{67}Ga and ^{111}In are some reactor and cyclotron produced radioisotopes for this purpose. Beta emitting radioisotopes, predominantly ^{131}I, are used for therapy. The radioisotopes are generally conjugated with a bi-functional chelator attached to the targeting antibody to form the radio-immunoconjugate (RIC). However, the early success of beta emitting radioimmunotherapy has been modest [2].

In recent times, high linear energy transfer (LET) radiation in the form of auger electrons and alphas particles have been studied. Auger and Koster-Kronig electron transitions cause the emission of multiple low energy and very short-range electrons, such that the LET can still be high. However, the short range requires access into the nucleus to cause single (SSB) and double (DSB) strand breaks in the DNA. Alphas particles have ranges from 20 to 80 μm and the radioisotope sources can be located on cell membranes or nearby cells. The LET is typically ~100 keV/μm with a high probability of causing DSBs (although the ratio SSB/DSB ~ 20, compared with ~ 60 for low LET radiation) [3].

Alpha radiation is ideal for killing isolated cancer cells in transit in the vascular and lymphatic systems and for inducing tumour regression by killing tumour capillary endothelial cells. Apoptosis [4] is the dominant form of cell death with high LET radiation. A programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding tissues. Apoptosis plays a crucial role in developing and maintaining health by eliminating old cells, unnecessary cells and unhealthy cells.

Over the past 20 years targeted Alpha Therapy (TAT) has progressed from in vitro studies to in-vivo experiments and on to clinical trials [5]. The radiobiology and microdosimetry is well understood but the key to its application is in the biological targeting. The dose to normal tissues always provides a limitation to the injected dose and that received by the tumour. However, TAT can achieve cancer regression within the maximum tolerance dose (MTD) for normal tissue.

TAT was originally thought to be an ideal therapy for "liquid" cancers, e.g., leukaemia and micrometastases, as the short half-lives of the radioisotopes were sufficient to target these blood borne cancer cells and the short range ensured that the targeted cancer cells received the highest radiation dose.

The difficulty of proving efficacy against micrometastic disease cannot be underestimated [6] but phase 1 trials of end stage AML cancer patients have established the tolerance dose and provided evidence of efficacy.

Tumour anti-vascular alpha therapy (TAVAT) [7] offers the potential of shutting down leaky tumour capillaries and so inhibiting tumour growth. This effect has been ascribed to tumour regressions observed in a phase 1 trial of TAT for metastatic melanoma [8].

Alpha radiation is a very effective in causing DNA damage and when targeted can be highly cytotoxic to cancer cells. With energies from 2-8 MeV and ranges of 20-80 μm, alpha emitting radioisotopes can deliver high linear energy transfer radiation with increased rates of double strand breaks that can induce apoptosis in cells within the alpha particle range. However, if mismatch DNA repairs of DSBs occur, then alpha radiation has a much higher probability (×20) of causing genetic damage and secondary cancer than electrons or photons.

2. THERAPEUTIC OBJECTIVES

The primary objective in immunotherapy is to increase efficacy by improving delivery and specificity. At the same time, toxicity must be reduced to allow increased doses to be delivered to control the target cancer. Cancer management begins with surgery to remove solid tumours, radiotherapy to control local tumour and reduce local recurrence (as in post-lumpectomy for breast cancer) and chemotherapy to control systemic disease or for palliation. Immunotherapy is a developing approach that could make a major contribution to the management of cancer.

There are three separate objectives that must be addressed:

1. Kill isolated cancer cells in transit in the lymphatic and vascular circulation,
2. Regress pre-vascular and lymphatic cancer cell clusters or spheroids,
3. Regress tumour vasculature and tumours.

Each objective requires a different approach.

2.1. Isolated cancer cells in transit

These cells have special problems relating to cell cycle and bioavailability as summarized below.

- Cells may be outside the cell cycle in the G0 phase. As such, these cells may be relatively insensitive to radiotherapy and chemotherapy.
- Systemic cancer targeting is required, but only a small fraction of the dose will reach its target so short range cytotoxic action is essential to reduce normal tissue damage, which will be the dose-limiting factor.
- High labelling efficiency is required to reduce saturation of targeted antigens by unlabelled MAb.
- Short half-life is preferred as cells in the vascular system can be reached quickly. The long half-life of chemotoxins in the body is not indicated for such targets. As such, alpha radiation is best suited for the toxic agent.
- Lymphatic administration may be required to eliminate cells in transit from primary lesions.
- The therapeutic response is difficult to determine [6]. However, magnetic cell separation of cancer cells in the peripheral blood with the magnetic microspheres coated with the targeting MAb could solve this problem [9].

2.2. Cell clusters

Cell clusters occur when the cell cycle is switched on in the appropriate seed/soil environment. However, endothelial cell growth factors expressed by cancer cells are still insufficient to stimulate the vascular extensions into the lesion.

- Clusters can only be reached by non-vascular transport.
- Long range cross fire, as for betas, is not indicated but useful short range cross fire can occur for alphas.
- Chemical toxins have no cross-fire effect and would be much less effective in cell clusters with limited penetration. The long half-life of chemotoxins in the body is not indicated for such targets.
- TAT was effective in in vitro studies of cancer cell spheroids [10].

2.3. Tumours

Tumour capillary permeability is an important parameter that determines in part the bioavailabilty of the cancer cells in a tumour. Leaky neogenic capillaries allow the extravasation of the IC into the perivascular space to saturate the targeted antigens expressed by contiguous and adjacent cancer cells.

- Long range cross fire effect gives beta radiation an important advantage and reduces the effect of heterogenous uptake of the beta-IC in the tumour.
- Intralesional injections with alpha ICs can overcome this problem [11].
- Chemotoxins will suffer from bioavailability and the lack of a cross-fire effect.
- The potential role of the bystander effect [12] could be of benefit here. This radiation-induced phenomenon causes unirradiated cells to exhibit effects as a result of signals received from nearby irradiated cells, causing a mutation in the nucleus of these cells. Cells that that are not directly hit by an alpha particle, but are in the vicinity of one that is hit, may also contribute to the genotoxic response of the cell population. In vitro studies show that when the medium containing irradiated cells is transferred to unirradiated cells, these cells show bystander responses when assayed for clonogenic survival and oncogenic transformation.
- Tumour capillary permeability causes a high density of ICs targeted to antigens around the capillaries, with a rapid drop off with distance from the capillaries [58].. Whereas this is a drawback for beta-emitting ICs, it enhances the toxicity of alpha-ICs to the capillary endothelial cells. The short range (~ 80 μm) of the emitted alpha radiation ensures a high radiation dose to the endothelial cell nucleus, inducing apoptosis. The capillary will close and if enough such capillaries close down, the tumour will regress. This process is called tumour antivascular alpha therapy (TAVAT) and explains the tumour regressions observed in the phase 1 clinical

trial of systemic TAT of metastatic melanoma [7]. This process could be assisted by tumour vascular disruption agents that increase tumour capillary permeability.

3. TAVAT AND MONTE CARLO MICRODOSIMETRY

3.1. Microdosimetry

TAT has the advantage of delivering therapeutic doses to individual cancer cells for low doses to normal tissues. The dosimetry for the labeled alpha particle is challenging because of the heterogeneous antigen expression among cancer cells and the nature of short-range, high-LET alpha radiation. It is therefore inappropriate to investigate the therapeutic efficacy of TAT by macrodosimetry. Microdosimetry of TAT is a function of the cell geometry, source-target configuration, cell sensitivity, and biological factors. A detailed knowledge of each of these parameters is required for accurate microdosimetric calculations.

The dosimetry of TAT is distinguished from that of beta immunotherapy or external beam radiotherapy in three different ways as detailed below [59].

(1) Short path length of alpha particles. The high energy of alpha particles is deposited in a short range. Some cell nuclei receive multiple alpha particle hits, while others receive no hits. The amounts of energy deposited vary greatly from target to target, leading to a wide frequency distribution.
(2) Small target volume. The alpha track length is comparable to cellular dimensions causing high LET within the small target volume. It is important to understand the differing biological effects on individual cells. Given the energy delivered along an alpha-particle track and its potential cytotoxicity, the dosimetry for estimating mean absorbed dose may not always yield physically or biologically meaningful information of radiation energy deposition in biological cells. Instead, stochastic or microdosimetric methodologies may be required.
(3) Non-uniform distribution of radioisotopes. The heterogeneous antigen expression and tumor uptake leads to variable spatial microdosimetric distributions of the alpha immunoconjugate (AIC). Spatial and temporal changes of the source activity in the target can also occur. When the distribution of radio-labeled antibody is non-uniform, techniques of dose averaging over volumes greater in size than the individual target volumes can become inadequate predictors of the biological effect. The specific energy is the most important quantity for microdosimetry as it can be used to calculate the cancer cell survival rate.

3.2. Specific energy

This is defined as (unit, SGy) the ratio of the energy deposited (Joule) to the mass of the target (kg) and has the same units as absorbed dose. The mean specific energy equals the absorbed dose. Although microdosimetry is concerned with the same concept of energy deposition per unit mass as dosimetry, the difference in the range of alpha particle and small size of the target volume introduces stochastic effects, which are negligible in conventional dosimetry. The stochastic quantity of specific energy can be used to investigate biological effects.

3.3. Monte Carlo models

Metastatic melanoma lesions experienced marked regression after systemic TAT in a phase 1 clinical trial [13]. This unexpected response was ascribed to Tumour Anti-vascular Alpha Therapy (TAVAT) [7], in which effective tumour regression is achieved by killing endothelial cells (ECs) in tumour capillaries and thus depriving cancer cells of nutrition and oxygen. Quantitatively analysis of the therapeutic efficacy and safety of TAVAT required building up Monte Carlo microdosimetric models [14].

The Monte Carlo code Geant4 was adapted to simulate the spatial non-uniform distribution of the alpha emitter ^{213}Bi. The Intraluminal model was designed to simulate the background dose to normal tissue capillary ECs from the non-targeted activity in the blood. The Perivascular model calculates the EC dose from the activity

bound to the perivascular cancer cells. The key parameters were the probability of an alpha particle traversing an EC nucleus, the energy deposition, the lineal energy transfer and the specific energy. These results were then applied to interpret the clinical trial. Cell survival rate and therapeutic gain were determined.

The specific energy for an alpha particle hitting an EC nucleus in the Intraluminal and Perivascular models is 0.35 and 0.37 Gy respectively. As the average probability of traversal in these models is 2.7% and 1.1%, the mean specific energy per decay drops to 1.0 cGy and 0.4 cGy, which demonstrate that the source distribution has a significant impact on the dose. Using the maximum activity of 25 mCi in the melanoma clinical trial [13], the specific energy to the tumour EC nucleus was found to be 3.2 Gy and to a normal capillary EC nucleus to be 1.8 cGy. These data give a maximum therapeutic gain of about 180 and validate the TAVAT concept. Thus TAVAT can deliver a cytotoxic dose to tumour capillaries without toxicity to normal tissue capillaries.

3.4. Alpha vs beta radiation

Our Monte Carlo calculations (unpublished) show that alpha emitters can deposit two to three orders of magnitude more energy than beta emitters into the target call nucleus for the same number of decays. This means that to deposit the same specific energy to cancer cells, a much higher activity is required for the beta-emitter which could cause serious normal tissue radiation damage.

Alpha emitting radioisotopes with multiple alpha decays give much higher specific energies than those for single decays; for example ^{223}Ra or ^{225}Ac emit four alphas, giving a much higher specific energy that ^{211}At or ^{213}Bi. However, the recoil of daughters can free RI from the chelator, increasing normal tissue toxicity, especially in the kidneys.

Because of their short range, alpha emitters deliver ~ 3000 times the specific energy of beta emitters for the nuclei of ECs in the Perivascular Model. Of the beta emitters, the high-energy betas from ^{90}Y and ^{32}P give the lowest specific energies. Even if all the antigenic sites are saturated out to 25 cell layers from the capillary, beta radiation spares some 50% of ECs (for D0 = 1.4 Gy), whereas all ECs are killed by alphas. The solid Tumor Control Probability (TCP)=1 for all alpha emitters, compared with TCP = 0 for beta emitters. These results are consistent with experimental data, which show superior efficacy with reduced complications for ^{213}Bi compared with ^{177}Lu in a mouse model [56].

4. BIOLOGICAL DOSIMETRY

4.1. Micronuclei dosimeter

Real time dosimetry of alpha therapy is difficult. Characteristic gamma rays could give an estimate of the macroscopic alpha dose but this is not the dose of interest. Rather, the microscopic alpha dose is required and direct measurement of alpha emission by MOSFET detectors requires implanted detectors in the blood or key organs An alternative approach is to use radiation induced micronuclei (MN) in lymphocytes as a direct measure of the biologically effective dose in the blood [15]. This is not real time dosimetry but would be of value in fractionated therapy to establish the biological dose after the first fraction.

Lymphocytes in the peripheral blood are exposed to background alpha and beta radiation on systemic administration of the AIC. The AIC is designed to target the cancer cells, not the lymphocytes and as such background dose levels are expected to be low. However, there will be random hits of lymphocytes by untargeted alpha decays of the radioisotope in the blood.

Post-irradiation peripheral blood is taken and lymphoyctes induced to divide at different times. Cells that have suffered DSB's of their chromosomes will exhibit both large and small sections, which may recombine over time. The micronuclei result from DSBs in the DNA, which form discrete entities of bilayer phospholipid enclosed DNA fragments within the lymphocyte prior to division [15]. These fragments can be observed by

staining and counting by microscope and/or automated shape recognition programs. Results for different post-irradiation times were compared with benchmark values before exposure. Repair processes were observed to reduce the MN count after a week or so.

The biological dose measured by the MN is not the whole body dose. It is the dose to the blood volume and to the vascular walls. The AIC, being a large molecule, is not expected to diffuse through the vascular walls and so the activity remains limited to the vasculature, which, however, includes the bone marrow. As such, the biological dose to the dose limiting bone marrow can be determined.

This biological dosimeter provides a direct measure of the stochastic radiation damage without the need to implant a detector to measure absorbed dose and determine RBE factors.

4.2. Equivalent dose

This is defined to take into account the ICRP radiation weighting factor of 20 because alpha particles are 20 time more likely to cause cancer and other stochastic effects for the same absorbed dose of electrons or photonsThe effective dose is a weighted average of individual organ equivalent doses. Both equivalent and effective dose values are associated with the unit Sievert (Sv).

The relevant end-points for treatment evaluation (i.e., toxicity and efficacy) are deterministic for which the end-points for alpha particle radiation have 3−7 times more toxicity or efficacy per unit absorbed dose than electrons or photons. The factor used to weight the absorbed dose for deterministic end-points is the relative biological efficacy (RBE), which is defined for specific end points. As such, the unit for biological dose is RBE.Gy, not the Sv. It is recommended that the absorbed dose (in Gy) should be listed separately for (electron and photon) emissions and for alpha-particle emissions [16].

4.3. Mutagenesis

The mutagenic potential of 213Bi conjugated to a human melanoma antigen-specific antibody (9.2.27) was examined [17]. The lacZ transgenic mouse model was used, which contains multiple copies of a lacZ target gene in every cell, allowing the quantification and comparison of mutagenesis in different organs. Mice received an ip injection of 16.65 MBq of 213Bi-cDTPA-9.2.27, and were sacrificed at 24 h, 1 week and 4 weeks post-injection. Pharmacokinetic studies gave the absorbed and effective doses for each organ.

The mutant frequency and mutant spectra were analysed for the brain, spleen and kidneys. The brain and spleen did not show significant increases in induced mutation frequencies compared to spontaneous background levels or changes in mutant spectra, these results being independent of p53 status. However, elevated mutation frequencies and persistent size change mutations were observed in the kidneys, but were not significant at the $P = 0.05$ level. The effect of p53 status was also evident, as p53 heterozygotes displayed higher mutation frequencies than their wild-type counterparts, suggesting a reduction in the p53 gene may lead to an increased susceptibility to mutagenesis. These effects were time dependent and levels returned to those of the controls at 4 weeks post-irradiation, albeit with a predominant residue of size mutations.

These effects were observed at activities very much higher than those expected for the therapy of human patients. As such, the induction of secondary cancer with the ^{213}Bi-cDTPA-9.2.27 AIC is not expected to be a significant problem in the clinic.

5. PRECLINICAL STUDIES

5.1. Ac:Bi generator

The Australian program [18] was based on ^{213}Bi, which is eluted from the ^{225}Ac generator [19]. The short half-life of ^{213}Bi, being 46 min, precludes consideration of long biological life times. Stable alpha-conjugates were synthesised in our laboratory by labeling chelated MAbs with ^{213}Bi to form the AIC. These were tested in vitro and in vivo for melanoma [20,21], leukaemia [22], colorectal [23], prostate [24,25,26], ovarian [27] and pancreatic cancers [28,29].

The short range of alpha particles, and the short half-life of useful alpha emitting radioisotopes argue against TAT with ^{213}Bi being at all effective in regressing tumours [30]. Consequently, our studies related to the killing of isolated cancer cells and cell clusters and the inhibition of tumour growth. To this end we developed the 2 day model, where treatment followed 2 days post-inoculation of cancer cells. Mice were then followed until tumours reached ~1 cm3. In all cases, complete inhibition of tumour development was achieved with 75−100 μCi local s.c. injection (i.e., in the same location as the cell inoculation). Higher activities were required for systemic (tail vein or intraperitoneal) injection of the AIC. However, efficacy decreases for longer growth times and larger tumours, but can be partially offset by multiple dosing.

Intralesional alpha therapy was performed on human melanoma xenografts in nude mice [31] and showed complete tumour regression over 4-8 weeks. Intralesional TAT of melanoma with 300 μCi gave complete regression of melanoma xenografts in nude mice, but was far less successful in breast and prostate tumours. These results paved the way for the intralesional phase 1 clinical trial [11], wherein the mouse host for the human melanoma was simply exchanged for a human host.

Acute activity tolerances are in the region of 24-36 mCi/kg for systemic (ip) injections. However, long term toxicity (~ 6 months in mice) in the form of delayed radiation nephrosis, reduces the MTD to ~9 mCi/kg in mice and between 3 and 9 mCi/kg in rabbits.

While kinetics and bio-distributions will depend on the type of vector used, the melanoma trial provided the basic data to determine specific organ doses for comparison with threshold dose levels and the probability of induced secondary cancer. These data are of considerable value in ensuring patient safety in further systemic phase 1 clinical trials.

5.2. PAI2–uPAR alpha therapy

The PAI-2-uPAR targeting system has several important advantages. First, PAI2 is a human protein, rather than a murine antibody, so overcoming problems of immune response. Second, it is a much smaller targeting molecule so can penetrate tissue more efficiently leading to faster targeting, which is important considering the short half-life of the AIC. Finally, pre-clinical studies of over-expression show that uPA is highly expressed in around 75% of pancreatic adenocarcinomas, using immunohistochemical staining, while expression of uPA mRNA in normal pancreas is only 6% of that for pancreatic adenocarcinoma [27,29,31]. Thus, although there is frequently a high production of uPAR, which predicts poor survival, when there this is countered by a high production of its inhibitor PAI-2 improved survival results. Therefore the provision of exogenous PAI-2 would not be expected to adversely effect survival.

The human recombinant PAI-2 protein was successfully tested in breast [32], ovarian [26,33,34], prostate [Li2002] and pancreatic [28] cancers. These conjugates are highly selective and cytotoxic to targeted cancer cells. In vitro cytotoxicity of alpha-conjugates is very much greater than beta conjugates, non-specific alpha-conjugates and free alpha isotope. The lethal pathway for alpha therapy is predominantly apoptosis [36].

Preclinical results shown in Figure 1 demonstrate complete inhibition of tumour growth at 4 mCi/kg dose at 2 days post-inoculation for local s.c. administration and 9 mCi/kg dose for systemic administration [33]. All treated groups showed responses varying from almost complete inhibition to delayed tumour growth compared with controls. However, the low MW of PAI2 means that renal filtration will lead to delayed radiation nephrosis [37].

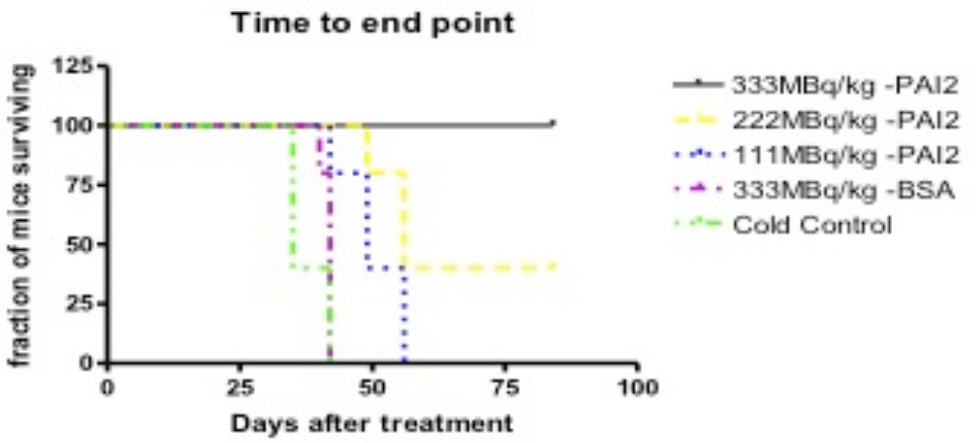

FIG. 1. Therapeutic Efficacy: The effect of a single intra-peritoneal injection of ^{213}Bi-PAI2 on subcutaneous CFPAC-1 human pancreatic cancer xenografts in nude mice. Single i.p. injections of 111, 222, 333 MBq/kg of ^{213}Bi-PAI2; 333 MBq/kg $^{of\ 213}$Bi-BSA (non-targeted hot control) and PAI-2 with no alpha conjugate (cold control) were given two days post-inoculation [33].

A clinical trial for stage IV pancreatic cancer patients who have either completed or declined standard systemic therapies would soon show efficacy because of the poor prognosis. Any delay in progression of the disease would be of clear benefit to the patient. Systemic TAT has the potential to regress pancreatic cancers and to eliminate micrometastases. It is expected that TAT would be indicated for the control of pancreatic cancer after resection of the primary tumour, when complete cell kill of micrometastases could be achieved, leading to elimination of clinical disease.

5.3. C595 anti-MUC1 alpha therapy

C595 is an IgG3, murine MAb raised against the protein core of human urinary epithelial mucin (MUC1). MUC1 is found to be frequently upregulated and abnormally glycosylated in a number of common malignancies, including breast, bladder, colon, ovarian, prostate and gastric cancer. Cancer-associated MUC1 is structurally different to normal MUC1 in that the former has shorter and less dense O-glycan chains, which exposes novel regions of the protein core.

The expression of tumour-associated antigen mucin-1 (MUC-1) on breast, prostate, ovarian and pancreatic cancer cell lines, in cell clusters and animal xenografts was detected by indirect immmunostaining. MAbs C595 (test) and A2 (non-specific control) were labelled with 213Bi using the chelator CHX-A" to form the AIC.
Preclinical results show inhibition of tumour growth and regression of cell clusters. Over 90% of primary prostate, pancreatic and ovarian tumours expressed MUC1 while 95% of normal tissues did not [33,36,37,38,54,55]. Further, MUC1 expression was found on the surface of cancer cell lines. The lethal pathway in all in vitro studies after TAT was found to be predominantly by apoptosis.

5.4. Orthotopic administration of ^{213}BI-Bevacizumab inhibits progression of PC3 xenografts in the prostate

Tumour growth requires development of new blood vessels – a process known as angiogenesis. The anti-angiogenic MAb Bevacizumab (BZ), which targets vascular endothelial growth factor (VEGF), is now part of the standard treatment for advanced colorectal cancer. Castrate resistant prostate cancers also express VEGF but are only marginally responsive to BZ alone or in combination with chemotherapy. . The efficacy of orthotopic administration of TAT with 213Bi-BZ was studied in the nude mouse, prostate cancer xenograft model [40]. PC3 human prostate cancer cells (106) were injected into the lower capsule of the mouse prostate gland one week prior to alpha-therapy. Mice were euthanized and assessed for tumour growth at 2 weeks post-therapy (2 mice), 4 weeks (2 mice) and 6 weeks (3 mice) post-therapy. The no-therapy control mice received a saline injection in

equal volume to each BZ administration. 213Bi- BZ was significantly more efficacious in inhibiting xenograft progression in the prostate gland compared with BZ antibody alone ($p = 0.009$) and when compared to the 'no therapy' protocol ($p < 0.0001$). Orthotopic administration of ^{213}Bi- BZ greatly improved the early control of organ confined prostate cancer compared to BZ alone ($P < 0.01$).

6. CLINICAL TRIALS

6.1. Overview

A great deal of preclinical work paved the way for the advance to clinical trials in recent years [41]. The Sloan Kettering Memorial Cancer Center has led the way, first with the application ^{213}Bi immunotherapy [42] and later with ^{225}Ac [43]. Other laboratories have concentrated on ^{212}Bi and ^{211}At. The advantages of the Bi radioisotopes are that they can be generated from long-lived parents, ^{225}Ac with 10 d and ^{228}Th with 1.91 y half-lives, which can be imported from overseas. The Ac-Bi generator has an additional advantage in that it decays in house and does not need long-term waste disposal. ^{211}At, with a 7 h half-life, needs to be used at or near the production site. While the half-lives of ^{213}Bi (47 minutes) and ^{212}Bi (61 minutes) are rather short, there is sufficient time for synthesis of the AIC, and for vascular distribution throughout the body. However, there is inadequate time for infusion into tumours, which can take 24−48 hours. This is one reason for the development of the ^{225}Ac alpha-conjugate, as the 10 d half-life allows plenty of time for infusion through the target tumours. On the other hand, the short range of the alpha products requires a high degree of homogeneity if all tumour cells are to be neutralised.

Targeting vectors must be specific for the cancers to be treated. As such, a number of vectors are being used or are to be introduced into the clinic. The following MAbs are in use: humanised HuM195 targets acute myelogenous leukaemia; the murine 9.2.27 targets the MCSP antigen on melanoma cells and GBM cells; the anti-CD20 for lymphoma; MX35 F(ab')2 for ovarian cancer; and the human-mouse chimeric anti-tenascin 81C6 for GBM. In the case of bone cancer, RaCl2 has a natural affinity for bone. Other proposed vectors are PAI2 against uPA, that is widely expressed by many cancers at their most malignant stage, and C595 a murine MAB against MUC-1, also of generic nature. The polysaccharide capsule binding MAb 18 B7 is proposed for fungal infection.

Seven clinical trials were reported the Berlin TAT symposium in 2011. ^{213}Bi was used for studies in acute myelogenous leukaemia (AML), melanoma and lymphoma; ^{225}Ac for AML; ^{223}Ra for bone cancer and ^{211}At for GBM and ovarian cancer. The Phase 1 ^{213}Bi trial for AML has been completed and the current trial is phase 2 with chemotherapy pre-treatment. The intralesional melanoma trial with ^{213}Bi has also been completed, being followed by a systemic trial with the same alpha conjugate. The following sections review the results of past and current clinical trials, and the objectives of proposed trials.

- Current and completed clinical trials are as follows:
- Completed Phase I study for acute myeloid leukaemia (AML) [42]
- Ongoing phase II study for post-chemotherapy of AML [52]
- Ongoing phase I study with ^{225}Ac [52]
- Completed phase 1 trial for intralesional melanoma [11]
- Phase 1 trial of systemic melanoma [13,44,45]
- Completed phase 1 trial of Glioblastoma [46]
- Competed pilot trial of GBM [47]
- Completed trial of ^{223}Ra for bone metastases [50,51]
- Phase 1 for lymphoma [48]
- Phase 1 trial in GEP-NET [53]
- Phase 1 trial of intraperitoneal TAT for ovarian cancer [57].

While solid tumours have never been envisaged as suitable targets for TAT, in contrast with liquid cancers and micrometastases [30], stage 4 advanced cancer patients are used in phase 1 trials for toxicity studies.

6.2. Intralesional metastatic melanoma

The intralesional melanoma trial [11] and the systemic melanoma trial [13] used the 9.2.29 MAb to target the melanoma-associated chondroitin sulfate proteoglycan (MCSP) receptor expressed by lesions of more than 90% of melanoma patients. This antigen is the same as the HMWMAA and thought to be identical with the NG2 murine antigen. The antibody is covalently coupled to the cDTPA chelator, and labelled with the 213Bi alpha-emitting radioisotope. The objective of these phase 1 trials with stage 4 melanoma patients was to determine the safety of the AIC and so far complications of any type or level have not been observed up to 25 mCi. However, unexpected tumour regressions were observed at quite low doses, such that a new concept was introduced to explain the clinical responses observed after systemic alpha therapy, called TAVAT [7]. Leaky neogenic capillaries allow extra-vascular diffusion of the AIC to target antigens on contiguous pericytes and cancer cells. Alpha emission kills the capillary endothelial cells, shutting down the capillaries with subsequent starvation of the tumour.

Intralesional Targeted Alpha Therapy (ITAT) for metastatic melanoma, being the first part of a program to establish a new systemic therapy. The benign targeting vector 9.2.27 was labelled with ^{213}Bi to form the AIC ^{213}Bi-cDTPA-9.2.27 (AIC), which is highly cytotoxic to targeted melanoma cells [38].

The safety and efficacy of intralesional AIC in patients with metastatic skin melanoma was investigated in 16 melanoma patients, all with melanomas that were positive to the MAb 9.2.27 [11]. AIC doses from 50 to 450 μCi were injected into lesions of different sizes, causing massive tumour cell death as observed by the presence of tumour debris. The AIC was very effective in delivering a high dose to the tumour while sparing other tissues. There were no significant changes in blood proteins and electrolytes. There was no evidence of a human-antimouse-antibody reaction. Evidence of significant decline in serum marker melanoma-inhibitory-activity protein (MIA) at 2 weeks post-TAT was observed.

Intralesional TAT for melanoma in human patients was found to be safe and efficacious to 1350 μCi. Tumours were resected at 8 weeks post-ITAT, to show massive cell debris in the injected volume, but no effect in untreated tumour or in the antibody only treated tumour in the same patient. Tumour to kidney activity ratios were ~ 3000. MIA, apoptosis and ki67 proliferation marker tests all indicated that TAT is a promising therapy for the control of inoperable secondary melanoma or primary ocular melanoma.

As such, intralesional TAT is indicated for uveal melanoma and brain metastases.

6.3. Systemic therapy for metastatic melanoma

The aim of this study was to assess toxicity and response of systemic alpha therapy for metastatic melanoma using the AIC ^{213}Bi-cDTPA-9.2.27 [13]. Tools used to investigate the responses were physical examination, imaging of tumours, pathology comparisons over 12 weeks, GFR; CT, comparisons and changes in tumour marker over 8 weeks. Responses were based on RECIST criteria.

40 patients with stage IV melanoma/ in-transit metastasis were treated with activities of 55−947 MBq. Using RECIST criteria 50% of subjects experienced stable disease and 12% showed partial response. One patient showed near complete response after a 5 mCi iv injection of the AIC (20/21 lesions completely disappeared, see Figure 2) and was retreated at 12 months because of an excellent response to the initial treatment. Another patient showed response in a mandible tumour and reduction in lung lesions.

The tumour marker melanoma inhibitory activity protein (MIA) reduced over 8 weeks in most patients. However, there was a disparity of dose with responders. Toxicity at any level was not observed over the range of administered activities.

The observation of responses without any toxicity indicates that TAT has the potential to be a safe and effective therapeutic approach for metastatic melanoma.

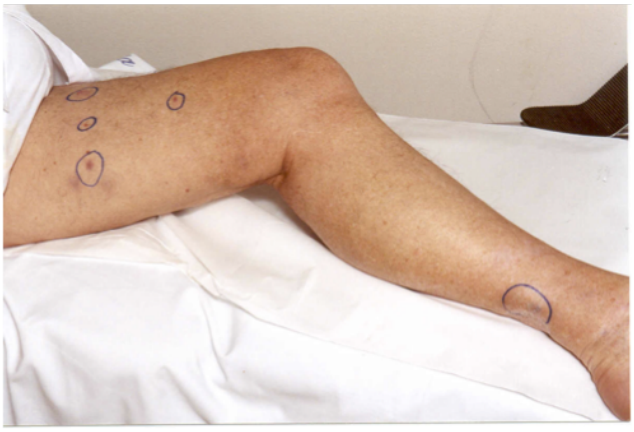

FIG. 2. 20 of the 21 melanomas completely regressed after systemic TAT. The initial sizes of the larger tumours are shown as blue rings. Post-TAT tumour beds revealed a complete absence of viable melanoma cells.

The observation of efficacy at quite low doses showed that this trial, while adequate as a phase 1, was inadequate to investigate the underlying factors that were contributing to the unexpected efficacy. As such, the trial was terminated in June 2007 without reaching the MTD and a new trial was designed to provide more detailed information but was not funded.

6.3.1. Improvements to the trial

The earlier trial used the cDTPA chelator to link the antibody and radioisotope, as this was the only commercially available chelator at that time. As delayed radiation nephrosis is the main concern, CHX-A", being more stable, is expected to reduce the renal uptake of free ^{213}Bi and so increase the maximum tolerance dose for the kidneys. Further, commercial production of CHX-A" is now available (Macrocyclics, USA).

The MAb 9.2.27 targets the MCSP antigen and if expression is low, antigens can be more readily saturated and blocked by unlabelled antibody, thus limiting tumour regression. One way around this problem is to increase the specific activity (SA) of the AIC. The AIC is usually prepared by minimizing the free radioisotope in the labelling process. However, our objective is to minimize the unlabelled antibody fraction. This then leads to a higher SA, less blocking of target antigens, and more effective therapy. Further, the higher specific activity will reduce the amount of antibody injected, even at higher activities, therefore reducing the HAMA effect.

Dose limiting toxicity is defined in terms of renal function; GFR Grade1 (normalized for age) or Grade 2 serum creatinine. GFR will be measured at 0, 26, 52 and 78 weeks. If there is > 25% decline GFR will be repeated in one month for verification. Serum creatinine will be measured at each visit.

A single administration of AIC was given in the first trial, (if needed 2−3 injections on the same day to achieve the required injected activity). With the higher activities, a fractionated dose regime will be more practical, and may give improved efficacy as tumours capillaries may be damaged, resulting in increased permeability for the AIC. Daily fractionation over 4−5 days would not be of concern for immune response (HAMA) as the time period is too short for the generation of an immune response (7−14 Days).

7. WHAT NEXT?

The future prospects of TAT have been previously described [53]. The dosimetry problems could be resolved with biological dosimetry and on-line MOSFET dosimeters. Therapeutic response could be monitored by magnetic cell separation of cancer cells in the blood with magnetic microspheres coated with the targeting MAb.

The limited supplies of Ac-225 available at present from separation from Th-229 are adequate for clinical trials. However, should TAT become a clinical procedure, then new supplies must be found. Accelerator production centres could ensure adequate supply of Ac-225 for international distribution and At-211 for local distribution.

Alpha therapy needs first to establish maximum tolerance doses for practical acceptance. This has been determined with ^{213}Bi-AIC for acute myelogenous leukaemia at ~ 1 mCi/kg [42]. The maximum tolerance dose has not yet been established for metastatic melanoma, but the efficacious dose for some melanomas is certainly less than 0.3 mCi/kg [45] and for intra-cavity therapy of GBM it is ~ 0.14 mCi/kg for ^{211}At-IC [46].

The next stage is to determine efficacy in phase II trials at the MTD or the effective dose. A current study of combined modalities for acute myelogenous leukaemia is ongoing at Memorial Sloan Kettering Cancer Center using chemotherapy to reduce the cancer load followed by ^{213}Bi-AIC to further reduce the cancer.

The melanoma trial showed significant efficacy in the phase 1 trial achieving 10% near complete or partial response, 40% stable disease and 13% long term survival of 2-5 y, without any evidence of adverse events [45]. The Duke GBM study achieved a median survival of 52 weeks for GBM [46].

It is now appropriate to consider that TAT can be applied to high risk subjects in remission, for whom recurrence is likely to arise from subclinical disease, as well as end stage patients for palliative therapy. Perhaps the most appropriate application could be for post-hormonal therapy in prostate cancer, when the PSA is at its nadir. TAT could target the hormone insensitive cells that survive and lead to fatal outcomes.

While these results are relatively impressive, the potential of TAT to reduce solid tumours by TAVAT remains just that. The demonstration of the synergy between tumour vascular disruption agents and TAVAT could bring about a sea change in cancer therapy.

Alpha therapy is still a work in progress, but great gains are being made in translating preclinical studies to clinical trials. Ideally suited to leukaemia, alpha therapy is demonstrating efficacy, but mostly at the MTD. However, this is not the case for GBM and metastatic melanoma.

The promise of TAT is greatly extended by the development of TAVAT for solid tumours. Metastatic melanoma results show surprising tumour regressions at doses very much below the MTD, and if further research is successful, TAVAT could change the prognosis for many end-stage cancers.

8. OBSTACLES

TAT has been tested extensively in preclinical studies and in phase 1 and 2 clinical trials. Yet its acceptance into the clinic has been limited. There are a number of possible reasons for this lack of progress, which are discussed as follows. First, there is the apparent failure of Radiation Oncologists & Medical Physicists to identify the most important therapeutic need, which is efficacious systemic therapy. This could be explained in part by the existence of the external beam lobby of Industry, Radiation Oncologists and Medical Physicists and the chemotherapy lobby of Industry and Oncologists. Further, the Nuclear Medicine profession thrives on imaging and relegates therapy to a minor activity.

There is a lack of commitment by oncologists for new approaches that are outside their training and occupation. As such, TAT is in no-mans land! However, Alpharadin (Ra-223) has succeeded with international clinical trials as a palliative therapy for prostate metastases to the bone because private funding was raised, which has opened the door for clinical trials.

REFERENCES

[1] KÖHLER, G., MILSTEIN, C., Continuous cultures of fused cells secreting antibody of predefined specificity. Nature **256** (1975) 495−497.

[2] GOLDENBERG, D.M., Targeted therapy of cancer with radiolabeled antibodies. J. Nucl. Med. **43** (2002) 93−713

[3] BRONS, S., Heavy ion production of single- and double-strand breaks in plasmid DNA in aqueous solution. Physica Medica 17 (Supplement 1) (2001) 217−218.

[4] KERR, J.F., A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. J. Path. Bact. **90** (1965) 419−435.

[5] ELGQVIST, J., (Editor), Targeted alpha therapy. Current Radiopharmaceuticals **4** (2011) (Issues 3 and 4).

[6] SGOUROS, G., Radioimmunotherapy of micrometastases: sidestepping the solid-tumor hurdle. J. Nucl. Med. **36** (1995) 1910−1912.

[7] ALLEN, B.J., et al., Tumour anti-vascular alpha therapy: a mechanism for the regression of solid tumours in metastatic cancer. Phys. Med. Biol. **52** (2007) L15−L19.

[8] RAJA, C., et al., Interim analysis of toxicity and response in Phase 1 trial of systemic targeted alpha therapy for metastatic melanoma. Canc. Biol. Therapy. **6** (2007) 846−852.

[9] MARTIN, V.M., et al., Immunomagnetic enrichment of disseminated epithelial tumour cells from peripheral blood by MACs. Exp. Haem. **26** (1998) 252−264.

[10] WANG, J., et al., Control of prostate cancer spheroid growth using ^{213}Bi-labeled multiple targeted alpha immuno-conjugates. Prostate **66** (2006) 1753−1767.

[11] ALLEN, B.J., et al., Intralesional targeted alpha therapy for metastatic melanoma. Cancer Biol Therapy, **4** (2005) 1318−1324.

[12] AZZAM, E.I., LITTLE, J.B., The radiation-induced bystander effect: evidence and significance. Hum. Exp. Toxicol. **23** (2004) 61–65.

[13] RAJA, C., et al., Interim analysis of toxicity and response in Phase 1 trial of systemic targeted alpha therapy for metastatic melanoma. Canc. Biol. Therapy **6** (2007) 846−852.

[14] HUANG, C.Y., et al., Monte Carlo calculation of the maximum therapeutic gain of tumor anti-vascular alpha therapy. Medical Physics **39** (2012) 1282−1288.

[15] SONG, E.Y., et al., The cytokinesis–block assay as a biological dosimeter for targeted alpha therapy. Phys. Med. Biol. **53** (2008) 319−328.

[16] SGOUROS, G., et al., MIRD Pamphlet No. 22: Radiobiology and dosimetry of alpha-particle emitters for targeted radionuclide therapy. J. Nucl. Med. **51** (2010) 311–328.

[17] ALLEN, B.J., et al., Mutagenesis induced by targeted alpha therapy using ^{213}Bi-cDTPA-9.2.27 in lac Z transgenic mice. Cancer Biol Therapy **8** (2009) 777-781.

[18] ALLEN, B.J., Systemic targeted alpha radiotherapy for cancer. Proc. Royal Society New South Wales **145** (2012) 443, 444, 19−33.

[19] FINN, R.D., et al., Refinements and improvements for Bi-213 production and use as a targeted therapeutic radiopharmaceutical. J. Labelled Compds Radiopharm. **40** (1997) 293.

[20] RIZVI, S.M.A., et al., Radioimmunoconjugates for targeted alpha therapy of malignant melanoma. Melanoma Res. **10** (2000) 281−289.

[21] ALLEN, B.J., RIZVI, S.M., TIAN, Z., Preclinical targeted alpha therapy for subcutaneous melanoma. Melanoma Res, **11** (2001) 175−82.

[22] RIZVI, S.M.A., et al., In vitro testing of the leukaemia monoclonal antibody WM-53 labeled with alpha and beta emitting radioisotopes. Leukemia Res. 26 (2002) 37−43.

[23] RIZVI, S.M.A., et al., In vitro and preclinical studies of targeted alpha therapy for colorectal cancer. Colorectal Disease, 3 (2001) 345−353.

[24] LI, Y., et al., ^{213}Bi-PAI2 conjugate selectively induces apoptosis in PC3 metastatic prostate cancer cell line and shows anti-cancer activity in a xenograft animal model. Brit J Cancer, 86 (2002) 1197−203.
[25] LI, Y., et al., Antigenic expression of human prostate cancer cell lines and primary prostate cancer sections for in vitro multiple targeted alpha therapy with Bi-213 conjugates. Int J Radiat Oncol Biol Phys: **60** (2004) 896−908.
[26] LI, Y., et al., Cytotoxicity of human prostate cancer cell lines in vitro and induction of apoptosis using 213Bi-Herceptin alpha-conjugate. Cancer Letters, **205** (2004) 161−171.
[27] SONG, Y.J., et al., Cytotoxicity of PAI2, C595 and herceptin vectors labeled with the alpha-emitting radioisotope bismuth-213 for ovarian cancer cell monolayers and clusters. Cancer Letters 234 (2006) 176−183.
[28] QU, C.F., et al., Preclinical study of ^{213}Bi labeled PAI2 for the control of micrometastatic pancreatic cancer. Clin. Exp. Metastasis **22** (2005) 575−586.
[29] QU, C.F., et al., Pre-clinical study of ^{213}Bi labeled PAI2 for the control of micrometastatic pancreatic cancer. Clin. Exp. Metastasis **22** (2005) 575−586.
[30] ALLEN, B.J., Can alpha-immunotherapy succeed where other systemic modalities have failed? Nucl. Med. Communications, **20** (1999) 205−207.
[31] XUE, A., et al., Prognostic significance of growth factors and the urokinase-type plasminogen activator system in pancreatic ductal adenocarcinoma. Pancreas **36** (2008) 160−167.
[32] ALLEN, B.J., et al., Preclinical studies of targeted alpha therapy for breast cancer using ^{213}Bi-labelled-plasminogen activator inhibitor type 2. Brit J Cancer, 88 (2003) 944−950.
[33] SONG, Y.J., et al., Cytotoxicity of PAI2, C595 and Herceptin Vectors labeled with the Alpha-Emitting Radioisotope Bismuth-213 for Ovarian Cancer Cell Monolayers and Clusters. Cancer Letters **234** (2006) 176−183.
[34] SONG, E.Y., et al., Pharmacokinetics and toxicity of ^{213}Bi-labelled PAI2 in preclinical targeted alpha therapy for cancer. Cancer Biol. Therapy **6** (2007) 898−904.
[35] LI, Y., et al., ^{213}Bi-PAI2 conjugate selectively induces apoptosis in PC3 metastatic prostate cancer cell line and shows anti-cancer activity in a xenograft animal model. Brit. J. Cancer **86** (2002) 1197−203.
[36] LI, Y., et al., Antigenic expression of human prostate cancer cell lines and primary prostate cancer sections for in vitro multiple targeted alpha therapy with Bi-213 conjugates. Int. J. Radiat. Oncol. Biol. Phys. **60** (2004) 896−908.
[37] ALLEN, B.J., et al., Targeted alpha therapy approach to the management of pancreatic cancer. Cancers: Special Issue "Pancreatic Cancer" **3** (2011) 1821−1843
[38] SONG, E.Y., et al., Bismuth-213 radioimmunotherapy with C595 anti–MUC1 monoclonal antibody in an ovarian cancer ascites model. Cancer Biol Therapy **7** (2008) 76−80.
[39] SONG, E.Y., et al., The cytokinesis–block assay as a biological dosimeter for targeted alpha therapy. Phys. Med. Biol. **53** (2008) 319-328.
[40] RIZVI, S.M.A., et al., Orthotopic administration of ^{213}Bi-Bevacizumab inhibits progression of PC3 xenografts in the prostate. Immunotherapy **4** (2012) 549−554.
[41] ALLEN, B.J., et al., Clinical trials of targeted alpha therapy for cancer. Reviews Recent Clinical Trials 3 (2008) 185−191.
[42] JURCIC, G.J., et al., Targeted alpha particle immunotherapy for myeloid leukaemia. . Blood **100** (2002) 1233−1239.
[43] McDEVITT, M.R., et al., Tumor therapy with targeted atomic nanogenerators. Science **16** (2001) 294, 5546, 1537−1540.
[44] ALLEN, B.J., et al., New directions for clinical trials of targeted alpha therapy for metastatic melanoma. Current Radiopharm. **1** (2008) 240−250.
[45] ALLEN, B.J., et al., Analysis of patient survival in a phase 1 trial of systemic targeted alpha therapy for metastatic melanoma. Immmunotherapy **3** (2011) 1041−1050.
[46] ZALUTSKY, M.R., et al., Clinical experience with the alpha-partilce emitting ^{211}At: treatment of recurrent brain tumours patients with ^{211}At-labeled chimeric anti-tenascin monoclonal antibody 81C6. J. Nucl. Med. **49** (2008) 30−38.

[47] CORDIER, D., et al, Targeted alpha-radionuclide therapy of functionally critically located gliomas with ^{213}Bi-DOTA-[Thi^{8},Met(O_2)^{11}]-substance P: a pilot trial. Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 1335–1344.

[48] SCHMIDT, D., et al., Phase 1 clinical study on alpha-therapy for non-Hodgkin lymphoma. In 4th Alpha-Immunotherapy Symposium Morgenstern A, Ed. ITU: Dusseldorf, Germany; pp 12, 2004.

[49] ANDERSSON, H, et al., Intraperitoneal alpha-particle radioimmunotheapy of ovarian cancer patients: pharmacokinetics and dosimetry of ^{211}AT-MX35F(ab')2 – a phase 1 study. J. Nucl. Med. 50 (2009) 1153−1160.

[50] NILSSON, S., et al., First clinical experience with alpha-emitting Radium-223 in the treatment of skeletal metastases. Clin. Cancer Res. **11** (2005) 4451−4459.

[51] NILSSON, S., et al., Bone-targeted radium-223 in symptomatic, hormone-refractory prostate cancer: a randomised, multicentre, placebo-controlled phase II study. The Lancet Oncology **8** (2007) 587−594.

[52] JURCIC, J., et al., Alpha-particle immunotherapy for acute myeloid leukaemia with Bismuth-213 and actinium-225. 7th Symposium on Targeted Alpha Therapy, Berlin July 17−19, 2011.

[53] KRATOCHWIL, C., et al, Dose escalation study of peptide receptor alpha-therapy with arterially administered ^{213}Bi-DOTATOC in GEP-NET patients refractory to beta-emitters. Ann. Congress European Assoc. Nuclear Medicine, Birmingham, Oct 15−19, 2011.

[54] ALLEN, B.J, Future prospects for targeted alpha therapy. Current Radiopharm. 4 (2011) 336−342.

[55] OU, C.F., et al., MUC1 expression in primary and metastatic pancreatic cancer cells for in vitro treatment by ^{213}Bi-C595 radioimmunoconjugate. Brit. J. Cancer 91 (2004) 2086−2093.

[56] OU, C.F., et al., In vivo and in vitro inhibition of pancreatic cancer growth by targeted alpha therapy using ^{213}Bi-CHX.A"-C595. Cancer Biology Therapy **4** (2005) e31-36.

[57] SEIDL, C., ^{177}Lu-immunotherapy of experimental peritoneal carcinomatosis shows comparable effectiveness to ^{213}Bi-immunotherapy, but causes toxicity not observed with ^{213}Bi. Eur. J. Nucl. Med. Mol. Imaging **38** (2010) 312−322.

[58] ANDERSSON, H., et al., Intraperitoneal alpha-particle radioimmunotherapy of ovarian cancer patients: pharmacokinetics and dosimetry of ^{211}At-MX35 F(ab')$^{2-}$-a phase I study. J. Nucl. Med. **50** (2009) 1153−1160.

[59] HUANG, C.Y., et al., (2012). Optimizing radioimmunoconjugate delivery in the treatment of solid tumor. Cancer Treatment Reviews **38** (2012) 854−860.

[60] HUANG, C.Y., et al., Microdosimetry for targeted alpha therapy of cancer." Computational and Mathematical Methods in Medicine (2012) Article ID 153212.