Published in final edited form as:

Clin Cancer Res. 2013 February 1; 19(3): 530–537. doi:10.1158/1078-0432.CCR-12-0298.

Molecular Pathways: Targeted α -Particle Radiation Therapy

Kwamena E. Baidoo, Kwon Yong, and Martin W. Brechbiel

Radioimmune & Inorganic Chemistry Section, ROB, NCI, NIH, Bethesda, Maryland, USA

Abstract

An α -particle, a ^4He nucleus, is exquisitely cytotoxic, and indifferent to many limitations associated with conventional chemo- and radiotherapy. The exquisite cytotoxicity of α radiation, the result of its high mean energy deposition (high linear energy transfer, LET) and limited range in tissue, provides for a highly controlled therapeutic modality that can be targeted to selected malignant cells (targeted α -therapy (TAT)) with minimal normal tissue effects. There is a burgeoning interest in the development of TAT that is buoyed by the increasing number of ongoing clinical trials worldwide. The short path length renders α -emitters suitable for treatment and management of minimal disease such as micrometastases or residual tumor after surgical debulking, hematological cancers, infections, and compartmental cancers such as ovarian cancer or neoplastic meningitis. Yet, despite decades of study of high-LET radiation, the mechanistic pathways of the effects of this modality remain not well defined. The modality is effectively presumed to follow a simple therapeutic mechanism centered on catastrophic double strand (ds) DNA breaks without full examination of the actual molecular pathways and targets that are activated that directly impact cell survival or death. This Molecular Pathways article provides an overview of the mechanisms and pathways that are involved in the response to and repair of TAT induced DNA damage as currently understood. Finally, this article highlights the current state of clinical translation of TAT as well as other high-LET radionuclide radiation therapy using α -emitters such as ^{225}Ac , ^{211}At , ^{213}Bi , ^{212}Pb and ^{223}Ra .

Keywords

Targeted α -therapy; Clinical trials; Cancer therapy; DNA; DNA damage; Ovarian cancer; Hematologic cancer; α -Radiation; Radiation therapy; Metastasis; Monoclonal antibodies

BACKGROUND

An α -particle is a naked helium-4 nucleus, therefore, it is relatively heavier than other subatomic particles emitted from decaying radionuclides and nuclear reactions such as electrons, neutrons and protons. With a +2 charge, α -particles are more effective ionization agents, have a high linear energy transfer (LET), in the range of 100 KEV/ μm , and are highly efficient in depositing energy over a short range in tissue (50 – 100 μm). An α -particle deposits 500 times more energy per unit path length than an electron or β^- -particle. Unlike low LET radiation (conventional x-, γ -, and electron-like radiation), the cytotoxic efficacy of α -particle radiation is indifferent to dose fractionation, dose rate or hypoxia, and also overcomes the resistance to chemotherapeutics encountered in conventional chemo- and radiation therapy. The α -emitting radionuclides that are medically

*To whom correspondence should be addressed: Martin W. Brechbiel, Radioimmune & Inorganic Chemistry Section, Radiation Oncology Branch, NCI, NIH, 10 Center Drive, Building 10, Rm B3B69, Bethesda, Maryland 20892-1002, USA. Phone: 301-496-0591; Fax 301-402-1923; martinwb@mail.nih.gov.

Author Conflicts of Interest: the authors have no conflicts of interest to disclose.

relevant and available for potential clinical use at this time are ^{211}At , ^{212}Bi , ^{213}Bi , ^{225}Ac , ^{223}Ra , ^{212}Pb , ^{227}Th , and ^{149}Tb .

The use of α -particle radiation as a therapeutic modality was recognized almost concurrently with the discovery of particle radiation by Rutherford in 1898 from which evolved the use of radium radionuclide brachytherapy applications (1). While there are several isotopes of radium, ^{223}Ra (Alpharadin) has recently risen to the forefront for clinical translation to treat bone metastases (*vide infra*) (2), while ^{224}Ra has had application in the treatment of bone diseases such as ankylosing spondylitis (3). However, the targeting of radium radionuclide relies solely upon the physicochemical nature of this element which dictates the innate unaided biodistribution properties of the radium ion and as such does not qualify as a targeted α -therapy (TAT). For TAT, a molecular target is chosen and the α -emission delivered to that chosen location and site. In fact, at this time the necessary chemistry to perform TAT with radium is not yet available (4).

A highly desirable goal in cancer therapy that has eluded clinicians is the ability to target malignant cells while sparing normal cells. If significant differential targeting is achieved by the vector, then a toxic payload on the vector will deliver a lethal dose preferentially to those cells expressing higher concentrations of the target molecule thereby sparing nearby normal cells. TAT seeks to achieve this goal by utilizing highly cytotoxic α -particle radiation carried to specific sites of cancer by appropriate vectors. The short path length of the α -particle addresses the concern of sparing normal tissue by limiting energy delivery, upon which cell killing depends within the cell where it is delivered, and as indicated above, reverses resistance to chemotherapy or conventional radiotherapy. The short path length also renders α -emitters suitable for treatment and management of patients with minimal disease such as micrometastases or residual tumor after surgical debulking, hematological cancers, infections, and cancers such as ovarian cancer or neoplastic meningitis that present as single layers or sheets of cells on compartment surfaces.

To make TAT possible, the development of monoclonal antibodies and other targeting vectors were required concomitantly with the development of suitable conjugation chemistries that would securely sequester α -emitters such as ^{225}Ac , ^{211}At , ^{213}Bi , ^{212}Pb (5–7). The physical limitations regarding what antibodies, peptides, or other targeting vectors might be labeled with an α -emitting radionuclide is only limited by their tolerance to the conjugation conditions required for attaching chelating agents or other prosthetic groups (for sequestering the radionuclide) to the targeting vectors. It is necessary that the conjugation and labeling conditions lead to the retention of effective targeting properties of the conjugate. As such, very few antibodies have been reported as difficult or impossible to radiolabel. It is important to choose chelating agents or prosthetic groups that bind the radionuclide strongly to limit dissociation of the radionuclide from the vector *in vivo*. However, the real limitations of use reside in the actual applications of the radiolabeled product, e.g., the optimal targeting time profile vs. radionuclide physical half-life can dictate choice of an α -emitting radionuclide or render it non-feasible. For example, there is little point in treating a lesion that requires days to optimize antibody delivery with a radionuclide that has half-life measured in minutes. Matching half-lives remains a significant criterion. Significant efforts regarding the development of all of these have moved forward to clinical investigation along with, in most cases, limited investigations into the mechanisms of action.

Mechanisms of Cell Death

The primary molecular target of ionizing radiation, and specifically for high-LET α -particle radiation, has been accepted to be DNA (8). The seminal work of Soyland et al very clearly demonstrated this by their studies wherein the physical cellular path taken by an α -particle through cells defined cytotoxicity (9). Traversal through the cytoplasm failed to be cytotoxic

while traversal through the nucleus as well as the actual distance traveled through the nucleus was correlated to cytotoxicity. Additionally, the high-LET effects were not observed when cells were irradiated by β^- -emissions or Auger electrons localized at the cell membrane or in the cytoplasm (10). An entire host of DNA damage can be expected including double strand (ds) breaks, cross-linking, and complex chromosomal rearrangement (>3 breaks in >2 chromosomes) to which the high efficiency of cell death may be attributed. The overall impact however, exceeds what can be explained by ascribing the target simply to DNA. Delayed toxicity attributable to increases in intracellular reactive oxygen species (ROS) as well as mitochondrial involvement has been invoked to explain the extra effects. Bystander effects in which DNA damage occurs in cells adjacent to directly irradiated cells can also result from extracellular ROS (11,12). Thus, there are complex multiple molecular pathways that are involved in the therapeutic application of targeted α -particle radiation.

The therapeutic benefit of α -irradiation is cell death as a result of the high dose and damage to DNA that is incurred. Cell death is brought about through a number of mechanisms such as apoptosis, autophagy, necrosis, and mitotic catastrophe. To ensure the maintenance of the integrity of the genome, the cell is endowed with a myriad of redundant DNA repair mechanisms. Failure of these systems from catastrophic cellular injury results in cell death. A summary of many of the cellular responses and pathways that are involved in cell death and repair after DNA DSBs are depicted in Figure 1 which, outlines some of the participating genes and complexes. DNA damage is possibly sensed by the ATM/ATR system which activate down stream complexes such as *p53*, PARP DNA-PK and PI3K to control cellular responses that regulate cell proliferation, DNA replication, checkpoints, recombination, and the repair and regulation of DNA damage (10). Another group of kinases are involved in cell death which includes MAPK8. Cell cycle checkpoints are generally observed and are associated with arrests to permit the performance of repair through various mechanisms, e.g., homologous and non-homologous end joining and then progression, or to initiate the apoptotic process. The pathway taken is dependent upon the degree of damage; higher percentages of unrejoined ds breaks remain after high-LET radiation due to the more complex nature of these breaks being more difficult to repair. There is a recent authoritative review that provides an in depth assembly of information regarding DNA ds breaks due to ionizing radiation and coordination between cell cycle progression and the relevant repair mechanisms; however, it remains unclear what aspects of the pathways is applicable towards TAT at this time (13).

Investigation of TAT mechanisms in vitro

There are a limited number of reports of mechanistic investigations into TAT that provide specific detail of the involvement of the various repair proteins. Many of the investigations have been performed using *in vitro* cell culture systems. Petrich et al described a TAT study of an ^{211}At labeled anti-CD33 monoclonal antibody (mAb) (~1/1000 molecules labeled) that directly compared the same mAb conjugated with calicheamicin (~1:1 molecules labeled). At effectively the same protein concentration, an equivalent degree of DNA ds breaks resulted (14). Dilution of the toxin conjugate to the 1/1000 activity, use of unlabeled control mAb, or control “free” ^{211}At resulted in no DNA ds breaks in HL-60 cells. The degree of ds breaks from the ^{211}At labeled mAb was demonstrated to be dose dependent. Induction of radioactivity dependent apoptosis related to caspase activation was also observed. Taken as a whole, this study demonstrates the exquisite potency of antibody based TAT that also overcomes the resistance that has been seen with calicheamicin conjugates. One might speculate that this relative degree of effectiveness will carry through when compared to other toxin or drug conjugates.

Human lymphocytes irradiated by Na^{211}At have been studied to assess the relative expression of the radiation responsive genes by Turtoi and Schneeweiss (15). Genes that

were investigated for their response to the high-LET radiation included: *BBC3* (B-cell lymphoma 2 binding component 3), *CD69* (cluster of differentiation 69), *CDKN1A* (cyclin-dependent kinase inhibitor 1A), *DUSP8* (dual specificity phosphatase 8), *EGR1* (early growth response 1), *EGR4* (early growth response 4), *GADD45A* (growth arrest and DNA-damage-inducible, alpha), *GRAP* (growth factor receptor-bound protein 2-related adaptor protein), *LAP1B* (*TOR1AIP1*; torsin A interacting protein 1), *IFNG* (interferon gamma), *ISG20L1* (interferon-stimulated exonuclease gene 20 kDa – like 1), *c-JUN* (jun oncogene), *MDM2* (mouse double minute 2), *PCNA* (proliferating cell nuclear antigen), *PLK2* (polo-like kinase 2), *RND1* (rho family GTPase 1), *TNFSF9* (tumour necrosis factor superfamily member 9) and *TRAF4* (tumour necrosis factor receptor-associated factor 4). The objective of the study was to evaluate the potential of the genes as measures of α -particle biodosimetry. While not a TAT study per se, the list of studied genes provides an indicator of the response and repair, proliferation, and growth factors, as well as pro-apoptotic factors that could be involved in the response to α -irradiation. With the exception of *GRAP*, all were dose dependently up-regulated over various ranges of exposure; *GRAP*, linked to transmission of extracellular stimuli for induction of proliferation, differentiation, or apoptosis, was significantly down-regulated independent of dose.

Several studies regarding the molecular mechanisms of ^{213}Bi (^{212}Bi) TAT have been reported. Macklis et al reported on the observation of the classic patterns of apoptosis, membrane blebbing, chromosomal condensation, and characteristic DNA fragmentation displays from murine EL-4 lymphoma cells undergoing TAT with ^{212}Bi (16). Supiot et al reported on treating multiple myeloma cells (LP1, RMI 8226, and U266) with a ^{213}Bi labeled anti-CD138 antibody in combination treatment with paclitaxel or doxorubicin (17). Interestingly, while pre-treatment with either drug resulted in G₂-M arrest, only one cell line showed an increase in DNA fragmentation (comet assay). No increase in apoptosis was observed in all of the studied cell lines. While radiosensitization from combination therapy was noted, involvement of apoptosis was ruled out as a mechanism for cell death. Seidl et al have executed far more extensive studies targeting d9-E-cadherin with ^{213}Bi labeled d9mAb using human gastric cancer cells (HSC45-M2) (18). These studies demonstrated that cell killing was dose dependent. Visible effects of α -irradiation of HSC45-M2 cells were evident in the formation of micronuclei and severe chromosomal aberrations. However, cell death was not inhibited by z-VAD-fmk and thus was independent of caspase 3 activation, the mode of cell death was therefore concluded to be different from apoptosis. Seidl et al also performed gene expression profiling and a time course microarray for the whole genome (19). Out of the 682–1125 genes that showed up-regulation and 666–1278 genes that showed down-regulation at one time point each, there were 8 genes that appeared up-regulated and 12 genes that were down-regulated throughout the course of study. Of those that were up-regulated, *COL4A2*, *NEDD9* and *C3* had not been previously found to be linked to high LET radiation response; complementarily, this observation held for the down-regulated *WWP2*, *RFX3*, *HIST4H4* and *JADE1*. This discovery process also yielded genes that were not previously associated with any biological process or molecular function; *ITM2C*, *FLJ11000*, *MSMB* were consistently up-regulated while *HCG9*, *GAS2L3*, *FLJ21439* were complementarily down-regulated. Such findings bring to light additional new targets that might be involved in the selective eradication of malignant cells and provide further insight into mechanisms and pathways of response to α -emitter based therapies.

Investigation of TAT mechanisms in vivo

Even fewer investigations of the mechanisms of cell death implicated in TAT have been performed *in vivo*. The studies of the mechanisms that apply *in vivo* are extremely important because they are more relevant to the actual tumor environment. However, these studies are

exceedingly challenging and expensive to perform. Recent studies by Yong et al related to ^{212}Pb , an *in vivo* generator of ^{212}Bi , targeted to HER2 by conjugation to trastuzumab is, to the best of our knowledge, the first study to actually investigate the *in vivo* tumor response at the cellular level. (20). In this study, mice bearing human colon cancer LS-174T intraperitoneal xenografts were treated with trastuzumab radiolabeled with ^{212}Pb and compared to several controls. Significant apoptosis induction and DNA ds breaks were observed after 24 hours. Additionally, Rad51 protein expression was found to be down-regulated, indicating delayed DNA ds damage repair as compared to controls. Cell cycle was also impacted resulting in G2/M arrest, depression of the S phase fraction, and depressed DNA synthesis that persisted beyond 120 hours while DNA synthesis appeared to recover in the control tumors by 120 hours. The ^{212}Pb TAT also delayed open chromatin structure and expression of p21 until 72 hours suggesting a correlation between modification of chromatin structure and induction of p21. A second study from Yong et al examined the impact of TAT combination therapy wherein Gemzar (Gemcitabine), a standard of care therapeutic for pancreatic cancer and a well-defined radiosensitizer, was administered prior to the ^{212}Pb TAT in the same animal tumor model system (21). The ^{212}Pb TAT treatment again increased the rate of apoptosis in S-phase arrested tumors. In this instance, ^{212}Pb TAT administered after pre-treatment with Gemzar abrogated G2/M arrest which was associated with inhibition of Chk1 phosphorylation and increased apoptosis. This combination therapy also resulted in reduced DNA synthesis, enhanced DNA ds breaks, accumulation of unrepaired DNA, with down-regulation of Rad51, all correlating to a blockage in DNA damage repair. Again, modification in the chromatin structure of *p21* was indicated. Changes in the H3K4/H3K9 ratio indicated transcriptionally repressed chromatin states and delayed open DNA structure as a result of the failure of adequate *p21* induction. Thus, the impact of catastrophic ds DNA destruction as a result of high-LET α -particle traversal of the nucleus included significant interference with the homologous repair mechanism through the down-regulation of Rad51, inhibition of Chk1 phosphorylation, chromatin modification, apoptosis and perturbation of the cell cycle.

CLINICAL-TRANSLATIONAL ADVANCES

A strong case can be made for the use of TAT in the clinic. With exquisite and effective targeting of DNA, the principal molecular target, TAT could deliver better outcomes than the ongoing ravaging horde of “molecular targeted” drugs. In cancer therapy the simple facts are that 50% of therapies incorporate radiation as one of the more efficacious forms of therapy (22,23), and combination therapies outperform single modalities (24). The response rates that can be achieved with proper application of RIT are difficult to achieve otherwise and strongly suggest that TAT, when applied properly, could prove to be a significant therapeutic modality to incorporate in the clinic (25). Many of the overarching obstacles to clinical translation of TAT, however, include high costs of the radionuclide, unresolved chemistry, limited availability of the radionuclides, traditional opposition to and fear of radioisotopes, real or mere imagined perceptions as opposed to the use of more “traditional” drugs.

Appropriate use of TAT is defined by a combination of the radionuclidic properties including actual emissions and half-life, the choice of targeting vector, scale of disease, and accessibility of disease by the targeting vector such that the α -emitting radionuclide might be delivered within a realistic timeframe, and targeted volume or disease presentation. Thus, use of α -emitting radionuclides is envisioned as being exceptionally potent and appropriate for the treatment of small lesions and metastases, loco-regional or compartmentalized disease of similar presentation, and readily accessible disease such as leukemia and lymphoma. Furthermore, due to the limited range of the α -particle, normal tissue toxicity is expected to be quite low when using a TAT strategy. Lastly, while

generally accepted that there is no effective resistance to α -particle lethality and no oxygen or hypoxia limitations to efficacy making such therapies extremely potent in the therapeutic arenas, Haro et al provides a study on induced resistant clones of HL60 cells to high-LET radiation. While this study was not TAT per se with the α -emission originating from an ^{241}Am source, it demonstrated that it is possible to have a population of tumor cells that might be refractory to TAT (26). Regardless of these attributes, there have been a very limited number of clinical trials executed to date evaluating TAT (Table 1). However, there has been an increase in this activity recently, particularly spurred by the progress associated with Alpharadin (*vide infra*). The remainder of this discussion will focus on clinical achievements and progress associated with each α -emitting radionuclide.

The number of TAT clinical trials performed with ^{211}At has been quite limited. In part, this is a direct consequence of the limited number of production sites for this radionuclide (6). Nonetheless, Zalutsky et al investigated the feasibility and safety of this therapy in patients with recurrent malignant brain tumors using a chimeric antibody, ch81C6, that targets tenascin, a glycoprotein over-expressed in gliomas, as the vector for the first ^{211}At TAT trial (27,28). A total of 18 patients were treated with ^{211}At -TAT administered into a surgically created resection cavity (SCRC). This compartmentalized therapy strategy resulted in no reported cases of dose-limiting toxicity, no toxicity of grade 3 or higher with 96.7 % of the ^{211}At decays being retained within the SCRC. Results of the study were quite encouraging. Eight of 14 patients with recurrent glioblastoma multiforme survived for 12 months, 2 survived for 3 years and no patient required repeat surgery for radionecrosis. These results demonstrate that this application with ^{211}At -TAT was attainable, safe, and associated with therapeutic benefit for patients with recurrent central nerve system tumors. There have been no follow-up studies as yet.

A second ^{211}At -TAT clinical trial is ongoing at the University of Gothenburg, Sweden. Andersson and co-workers investigated the pharmacokinetics and dosimetry of ^{211}At -TAT in a phase I study in patients with recurrent ovarian carcinoma. In this trial the delivery vehicle was a F(ab')_2 fragment of antibody MX35 which targets the sodium-dependent phosphate transport protein 2b (NaPi2b) in human cancer cells (29). To date, nine patients have been infused with ^{211}At -TAT via a peritoneal catheter to assess the strategy of intracavitary administration. Results have demonstrated that ^{211}At -TAT by intraperitoneal administration is feasible and that therapeutic doses in microscopic tumor clusters can be achieved without significant toxicity to the patient.

To date, there has been only one clinical trial using ^{225}Ac . The targeting vector for this trial is a humanized antibody, lintuzumab, that targets CD33 on acute myeloid leukemia cells which had previously been investigated in clinical trials for RIT with β^- -emitters and ^{213}Bi TAT (30,31). This trial was based on results of pharmacokinetics, dosimetry and toxicity obtained in cynomolgus monkeys that indicated that ^{225}Ac TAT was feasible (30). The ongoing phase I clinical trial was initiated by the Scheinberg group at Memorial Sloan-Kettering Cancer Center with a primary goal to define both safety and the maximum tolerated dose of ^{225}Ac TAT in patients with advanced myeloid leukemia (AML) through a dose escalation series (31). The initial dose of $0.5 \mu\text{Ci/kg}$, which is several orders of magnitude less than doses routinely used in RIT with β^- -emitters, demonstrates the extreme potency that this radionuclide delivers as a therapeutic. Eighteen patients with relapsed or refractory AML were treated. The trial has been so successful in demonstrating that ^{225}Ac TAT targeted by lintuzumab had anti-leukemic activity across all dose levels that it is now being investigated in a multicenter phase I/II trial in combination with low-dose cytarabine for older AML patients at Memorial Sloan Kettering and the Fred Hutchinson Cancer Centers while additional centers are expected to open the study in the near future (32).

A somewhat larger number of TAT clinical trials have been initiated and executed with ^{213}Bi , in part, facilitated by the availability of this radionuclide from an on-site generator based on ^{225}Ac . Heeger and co-workers at the German Cancer Research Center initiated a phase I dose escalation trial to determine toxicity and feasibility as well as dosimetry and pharmacokinetics. Nine patients with B-cell malignancies were treated with a ^{213}Bi labeled anti-CD20 antibody (33). Toxicity was limited to mild leukopenia in two patients with two patients responding to the therapy. This trial has been continued at the University Hospital Düsseldorf, Germany.

The Allen group initiated a phase I dose escalation ^{213}Bi TAT study for metastatic melanoma using mAb 9.2.27 to target the core protein of chondroitin sulfate proteoglycan of cancer cells. Twenty-two patients with stage IV/in-transit metastasis were treated (34). Patients showed disease reduction at eight weeks based on the tumor marker melanoma inhibitory protein activity; 6% demonstrated complete response, 14% showed partial response, 50% stable disease and 30% progressive disease with no toxicity being registered during the study.

The Scheinberg group at Memorial Sloan-Kettering is credited with the first proof-of-concept ^{213}Bi TAT clinical trial again targeting CD33 with antibody HuM195 (lintuzumab) to treat 18 patients with advanced myeloid leukemia in a phase I trial. Fourteen patients had reductions in the percentage of bone marrow blasts, and had reductions in circulating blasts after therapy, all without detection of significant toxicity (35). Jurcic et al conducted a follow up study with ^{213}Bi TAT wherein 13 newly diagnosed patients and 18 patients with relapsed/refractory acute myeloid leukemia were first treated with continuous cytarabine infusion for 5 days (36). Myelosuppression was the primary toxicity and two of 21 patients treated with the maximum tolerated dose died. At all dose levels, marrow blasts reductions were observed and CD33 sites were found to be saturated by ^{213}Bi TAT lintuzumab.

The Merlo group performed a pilot ^{213}Bi TAT trial using substance P, a tachykinin peptide neurotransmitter which targets the neurokinin type-1 receptor (NK-1) which is consistently over-expressed in grade II – IV gliomas (37,38). In this pilot study, five patients were enrolled and treatments were administered via an implanted catheter system (intratumoural injection) Four patients received one therapeutic cycle while one patient received four therapeutic cycles. Again, the ^{213}Bi TAT agent was retained at the target site without local or systemic toxicity being observed. Pre-therapeutic functional scores (the Barthel Index) for the two glioblastoma multiforme patients was 75 and 80 which after TAT improved to 90 out of 100. Radiation-induced necrosis and demarcation of the tumors were detected by MRI (38).

The first phase I clinical trial employing ^{212}Pb TAT sponsored by Areva Med LLC opened at the University of Alabama, Birmingham in 2011. As one might expect, this trial is designed to determine dose-limiting toxicities and anti-tumor efficacy for treating intraperitoneal cancers, specifically, primarily ovarian cancer. Adverse events, immune response monitoring, as well as the assessment of efficacy through physical examination, radiographic imaging, and assay of tumor markers are being followed. Pharmacokinetics and excretion mechanism(s) from the peritoneal cavity are being determined by γ -camera imaging. While 3 patients have been treated completing the first cohort, no further information is available at this time (39).

As noted earlier, Alpharadin (^{223}Ra chloride) has been evaluated in 2 phase I trials and 3 double-blind phase II trials for castration-resistant prostate cancer and bone metastases and is moving onwards into phase III trials.² Of 292 patients treated with ^{223}Ra , less than 1% experienced grade 4 hematologic toxicity, 4% had grade 3 anemia, less than 3% presented

with grade 3 toxicity for platelets, neutrophils, or WBC, and mild reversible neutropenia was observed with repeated ^{223}Ra treatments (40). There was no indication of renal or hepatic toxicity. In one trial, median overall survival increased by 4.5 months as compared with the placebo group.

Conclusions

The limited clinical experience of targeted α -particle radiation therapy has demonstrated the potential of the modality for the treatment of smaller tumor burdens, micrometastatic disease, and disseminated disease where α -emitters may be efficiently delivered. The rational scientific matching of disease presentation with realistic accessibility and delivery based upon physical considerations is a key criterion to their success; however, development and growth of clinical TAT has been principally compromised by economics and limited supply issues.

The targeting of DNA inherent in this modality is highly efficacious, but events beyond the traversal of a cell by an α -particle require more study. The actual mechanisms by which cells die are not totally well-defined. There is a clear need for additional *in vivo* studies of the molecular mechanisms of response, repair, and cell death resulting from TAT treatment. There appears to be a host of additional genetic pathways that are activated *in vivo* that simply have not been recognized or studied until relatively recently (41). The studies need to be performed in relevant *in vivo* tumor models where assays of treated tumor tissue for pathways of response are investigated rather than the less relevant cell culture that predominate current experimentation. Combination therapy studies need to be performed under the same conditions. These data are of critical importance to grasp a real understanding of TAT that, like any other therapeutic modality, will no doubt enhance its utility and integration with other therapies. Full mechanistic understanding of the therapy will accelerate its development and clinical translation.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. We also thank Diane Milenic for assistance in assembling the manuscript.

References

1. Mazonera JJ, Gerbaulet A. The centenary of discovery of radium. *Radiotherapy Oncol.* 1998; 49:205–16.
2. Bruland OS, Jonasdottir TJ, Fisher DR, Larsen RH. Radium-223: From radiochemical development to clinical applications in targeted cancer therapy. *Curr Radiopharm.* 2008; 1:203–8.
3. Zhang Z, Siegert J, Maywaldt U, Kirch W. Cost-benefit analysis of [^{224}Ra] radium chloride therapy for ankylosing spondylitis (Bekhterev's disease). *Med Klin (Munich).* 2007; 102:540–9. [PubMed: 17634872]
4. Kim YS, Brechbiel MW. An overview of targeted alpha therapy. *Tumor Biol.* 2012; 33:573–90.
5. McDevitt MR, Sgouros G, Finn RD, Humm JL, Jurcic JG, Larson SM, et al. Radioimmunotherapy with alpha-emitting nuclides. *Eur J Nucl Med.* 1998; 25:1341–51. [PubMed: 9724387]
6. Zalutsky MR, Pruszyński M. Astatine-211: Production and availability. *Curr Radiopharm.* 2011; 4:177–85. [PubMed: 22201707]
7. Yong K, Brechbiel MW. Towards translation of ^{212}Pb as a clinical therapeutic: Getting the lead in! *Dalton Trans.* 2011; 40:6068–76. [PubMed: 21380408]
8. Franken NAP, Hovingh S, Ten Cate R, Krawczyk P, Stap J, Hoebe R, et al. Relative biological effectiveness of high linear energy transfer α -particles for the induction of DNA-double-strand breaks, chromosome aberrations and reproductive cell death in SW-1573 lung tumour cells. *Ocol Rep.* 2012; 27:769–74.

9. Soyland C, Hassfjell SP. Survival of human lung epithelial cells following in vitro α -particle irradiation with absolute determination of the number of α -particle traversals of individual cells. *Int J Radiat Biol.* 2000; 76:1315–22. [PubMed: 11057739]
10. Pouget JP, Mather SJ. General aspects of the cellular response to low- and high-LET radiation. *Eur J Nucl Med.* 2001; 28:541–61. [PubMed: 11357507]
11. Friesen C, Glatting G, Koop B, Schwarz K, Morgenstern A, Apostolidis C, et al. Breaking chemoresistance and radioresistance with [^{213}Bi]anti-CD45 antibodies in leukaemia cells. *Cancer Res.* 2007; 67:1950–8. [PubMed: 17332322]
12. Janssens S, Tschopp J. Signals from within: the DNA damage-induced NF-kappaB response. *Cell Death Differ.* 2006; 13:773–84. [PubMed: 16410802]
13. Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: The molecular choreography. *Mutation Res.* 2012; 751:158–246. [PubMed: 22743550]
14. Petrich T, Korkmaz Z, Krull D, Fromke C, Meyer GJ, Knapp WH. In vitro experimental ^{211}At anti-CD33 antibody therapy of leukaemia cells overcomes cellular resistance seen in vivo against gemtuzumab ozogamicin. *Eur J Nucl Med Mol Imaging.* 2010; 37:851–61. [PubMed: 20107790]
15. Turtoi A, Schneeweiss FHA. Effect of ^{211}At α -particle irradiation on expression of selected radiation responsive genes in human lymphocytes. *Int J Radiat Biol.* 2009; 85:403–12. [PubMed: 19382019]
16. Macklis RM, Lin JY, Beresford B, Atcher RW, Hines JJ, Humm JL. Cellular kinetics, dosimetry, and radiobiology of α -particle radioimmunotherapy: Induction of apoptosis. *Radiat Res.* 1992; 130:220–6. [PubMed: 1574578]
17. Supiot S, Gouard S, Charrier J, Apostolidis C, Chatal JF, Barbet J, et al. Mechanisms of cell sensitization to α radioimmunotherapy by doxorubicin or paclitaxel in multiple myeloma cell lines. *Clin Cancer Res.* 2005; 11:7047s–52s. [PubMed: 16203801]
18. Seidl C, Schröck H, Seidenschwang S, Beck R, Schmid E, Abend M, et al. Cell death triggered by alpha-emitting ^{213}Bi -immunoconjugates in HSC45-M2 gastric cancer cells is different from apoptotic cell death. *Eur J Nucl Med Mol Imaging.* 2005; 32:274–85. [PubMed: 15791436]
19. Seidl C, Port M, Apostolidis C, Bruchertseifer F, Schwaiger M, Senekowitsch-Schmidtke R, et al. Differential gene expression triggered by highly cytotoxic α -Emitter-immunoconjugates in gastric cancer cells. *Invest New Drugs.* 2010; 28:49–60. [PubMed: 19139817]
20. Yong KJ, Milenic DE, Baidoo KE, Brechbiel MW. ^{212}Pb -Radioimmunotherapy induces G2 cell cycle arrest and delays DNA damage repair in tumor xenografts in a model for disseminated intraperitoneal disease. *Mol Cancer Therap.* 2012; 11:640–9.
21. Yong KJ, Milenic DE, Baidoo KE, Brechbiel MW. Sensitization of Tumor to ^{212}Pb -radioimmunotherapy by gemcitabine involves initial abrogation of G2 arrest and blocked DNA damage repair by Interference with Rad51. *Int J Radiat Oncol Biol Phys.* 2012 (in press).
22. Teshima T, Owen JB, Hanks GE, Sato S, Tsunemoto H, Inoue T. A Comparison of the structure of radiation oncology in the United States and Japan. *Int J Radiat Oncol Biol Phys.* 1996; 34:243–50. [PubMed: 12118558]
23. Physician Characteristics and Distribution in the US, 2008 Edition. (<https://www.astro.org/News-and-Media/Media-Resources/FAQs/Fast-Facts-About-Radiation-Therapy/Index.aspx>)
24. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2006; 354:567–78. [PubMed: 16467544]
25. Hagenbeek A VIII. Radioimmunotherapy in malignant lymphoma: an underused tool? *Ann Oncol.* 2011; 22(Supplement 4):iv51–3.
26. Haro KJ, Scott AC, Scheinberg DA. Mechanisms of resistance to high and low linear energy transfer radiation in myeloid leukemia cells. *Blood.* 2012; 120:2087–97. [PubMed: 22829630]
27. Zalutsky MR, McLendon RE, Garg PK, Archer GE, Schuster JM, Bigner DD. Radioimmunotherapy of neoplastic meningitis in rats using an α -particle emitting immunoconjugate. *Cancer Res.* 1994; 54:4719–25. [PubMed: 8062270]
28. Zalutsky MR, Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, et al. Clinical experience with α -particle emitting ^{211}At : treatment of recurrent brain tumor patients with ^{211}At -

- labeled chimeric antitenascin monoclonal antibody 81C6. *J Nucl Med*. 2008; 49:30–8. [PubMed: 18077533]
29. Andersson H, Cederkrantz E, Back T, Divgi C, Elgqvist J, Himmelman J, et al. Intraperitoneal α -particle radioimmunotherapy of ovarian cancer patients: pharmacokinetics and dosimetry of ^{211}At -MX35 F(ab')₂ a phase I study. *J Nucl Med*. 2009; 50:1153–60. [PubMed: 19525452]
 30. Miederer M, McDevitt MR, Sgouros G, Kramer K, Cheung NKV, Scheinberg DA. Pharmacokinetics, dosimetry, and toxicity of the targetable atomic generator, ^{225}Ac -HuM195, in nonhuman primates. *J Nucl Med*. 2004; 45:129–37. [PubMed: 14734685]
 31. ClinicalTrials.gov. Targeted Atomic Nano-Generators (Actinium-225-Labeled Humanized Anti-CD33 Monoclonal Antibody HuM195) in Patients with Advanced Myeloid Malignancies. Jun 9. 2011 <http://clinicaltrials.gov/show/NCT00672165>
 32. Jurcic, J. Memorial Sloan-Kettering (personal communication).
 33. Heeger S, Moldenhauer G, Egerer G, Wesch H, Martin S, Nikula T, et al. Alpha-radioimmunotherapy of B-lineage non-Hodgkin's lymphoma using ^{213}Bi -labeled anti-CD19 and anti-CD20-CHX-A''-DTPA conjugates. *ACS Meeting Abstracts*. 2009; 225:U261.
 34. Raja C, Graham P, Rizvi SMA, Song E, Goldsmith H, Thompson J, et al. Interim analysis of toxicity and response in phase I trial of systemic targeted alpha therapy for metastatic melanoma. *Cancer Biol Ther*. 2007; 6:846–52. [PubMed: 17495524]
 35. Jurcic JG, Larson SM, Sgouros G, McDevitt MR, Finn RD, Divgi CR, et al. Targeted α particle immunotherapy for myeloid leukemia. *Blood*. 2002; 100:1233–9. [PubMed: 12149203]
 36. Rosenblat TL, McDevitt MR, Mulford DA, Pandit-Taskar N, Divgi CR, Panageas KS, et al. Sequential cytarabine and α -particle immunotherapy with bismuth-213-lintuzumab (HuM195) for acute myeloid leukemia. *Clin Cancer Res*. 2010; 16:5303–11. [PubMed: 20858843]
 37. Kneifel S, Cordier D, Good S, Ionescu MCS, Ghaffari A, Hofer S, et al. Local targeting of malignant gliomas by the diffusible peptidic vector 1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid-substance P. *Clin Cancer Res*. 2006; 12:3843–50. [PubMed: 16778112]
 38. Cordier D, Forrer F, Bruchertseifer F, Morgenstern A, Apostolidis C, Good S, et al. Targeted alpha-radionuclide therapy of functionally critically located gliomas with ^{213}Bi -DOTA-[Thi⁸, Met(O₂)¹¹]-substance P: a pilot trial. *Eur J Nucl Med Mol Imaging*. 2010; 37:1335–44. [PubMed: 20157707]
 39. Bourdet, P. AREVA Med, (personal communication).
 40. Parker C, Aksnes A, Haugen I, Bolstad B, Nilsson S. Radium-223 chloride, a novel, highly targeted alpha-pharmaceutical for treatment of bone metastases from castration-resistant prostate cancer (CRPC): hematologic and safety profile with repeated dosing. *Ann Oncol*. 2010; 21(Suppl 8):277–8.
 41. Yong, K. NCI, (personal communication).

Figure 1.

Mechanisms of cell death by α -irradiation. Irradiation of cancer cells by α -radiation produces double strand breaks that evoke a myriad of cellular responses and pathways that include apoptosis, mitotic catastrophe, autophagy, necrosis, cell cycle arrest and DNA repair. Many genes and proteins are involved in these pathways some of which are depicted here. When cell death occurs by autophagy Beclin, LC3, ATG1, ATG5, and ATG7 are involved. CDK1 and Cyclin B are involved in mitotic catastrophe while RIPK1, TRAF2, PARP, and Calpains are involved in necrosis. Associated with the ATM/ATR and Ku/DNA-Pkcs complexes are a host of downstream systems that result in cell cycle arrest, apoptosis or DNA repair by non-homologous end joining or homologous repair.

Table 1

Clinical trials using α -particle emitters

Trial	Cancer Type	Radioimmunoconjugate	Outcome	Reference
Zalutsky et al.	Glioblastoma	^{211}At -ch81C6	18 pts treated. 14 pts survived 12 mo	27, 28
Andersson et al.	Ovarian cancer	^{211}At -MX35-F(ab') ₂	9 pts. Treated. No significant toxicity	29
The Scheinberg group	AML	^{225}Ac -HuM195 (^{225}Ac - lintuzumab)	18 pts treated. Trial expanded to multicenter Phase I/II	31, 32
Heeger et al.	B-cell non-Hodgkin's lymphoma	^{213}Bi -labeled anti-CD19 and anti-CD20-CHX-A''-DTPA	9 pts Treated. 2 pts showed response. Limited toxicity in 2 pts.	33
The Allen group	Melanoma	^{213}Bi -mAb 9.2.27	22 pts treated 6 % CR, 14% PR, 50% stable disease	34
The Scheinberg group	AML	^{213}Bi -HuM195 (^{213}Bi - lintuzumab)	18 pts treated. 14 pts had reductions in marrow blasts.	35
Jurcic et al	AML	^{213}Bi -HuM195 (^{213}Bi - lintuzumab)	31 pts treated. marrow blasts reductions observed at all dose levels	36
The Merlo group	Glioblastoma	^{213}Bi -substance P	5 pts treated. Barthel Index improved for 2pts.	37, 38
Areva Med LLC	Ovarian	^{212}Pb -TCMC-Trastuzumab	3 pts treated. Study ongoing. No further information available.	39
*Parker et al.	Castration-resistant prostate cancer and bone metastases	Alpharadin (^{223}Ra chloride)	292 pts treated. median overall survival increased by 4.5 months compared to placebo group	40

* Note: This is strictly speaking not a TAT trial per se but uses $^{223}\text{Ra}^{2+}$. Alpharadin is not an immunoconjugate but is included here because ^{223}Ra is an α -emitter.