Molecular Pathways

Clinical Cancer Research

Molecular Pathways: Targeted α-Particle Radiation Therapy

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Abstract

An α-particle, a ^{4}He 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. A burgeoning interest in the development of TAT 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, hematologic 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 DNA breaks without full examination of the actual molecular pathways and targets that are activated that directly affect 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. *Clin Cancer Res; 19(3); 530–7. ©2012 AACR.*

Background

An α-particle is a naked ^{4}He 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 cytocidal 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 radiotherapy. The α-emitting radionuclides that are medically 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). Although there are several isotopes of radium, ^{223}Ra (Alpharadin) has recently moved to the forefront for clinical translation to treat bone metastases (*vide infra*; ref. 2), whereas ^{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 conduct 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 using 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

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management of patients with minimal disease such as micrometastases or residual tumor after surgical debulking, hematologic 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 was 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 about 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; for example, the optimal targeting time profile versus radionuclide physical half-life can dictate choice of an α-emitting radionuclide or render it nonfeasible. Thus, 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 about 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 and Hassfjell very clearly showed this wherein the physical cellular path taken by an α-particle through cells defined cytotoxicity (9). Traversal through the cytoplasm failed to be cytotoxic whereas traversal through the nucleus as well as the actual distance traveled through the nucleus was correlated to cytotoxicity. In addition, 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 breaks (DSB), 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 mitochrondrial 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, complex multiple molecular pathways are involved in the therapeutic application of targeted α-particle radiation.

The therapeutic benefit of α-radiation 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 is depicted in Fig. 1, which outlines some of the participating genes and complexes. DNA damage is possibly sensed by the ATM/ATR system, which activates downstream 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, and this group includes MAPK8. Cell-cycle checkpoints are generally observed and are associated with arrests to permit the performance of repair through various mechanisms, such as homologous and nonhomologous end joining followed by progression or initiation of the apoptotic process. The pathway taken is dependent upon the degree of damage; higher percentages of unrejoined DSBs remain after high-LET radiation due to the more complex nature of these breaks being more difficult to repair. A recent authoritative review provides an in-depth assembly of information about DNA DSBs due to ionizing radiation and coordination between cell-cycle progression and the relevant repair mechanisms; however, the aspects of the pathways that are applicable to TAT remain unclear at this time (13).

Investigation of TAT mechanisms *in vitro*

A limited number of reports of mechanistic investigations into TAT provide specific detail of the involvement of the various repair proteins. Many of the investigations have been conducted using *in vitro* cell culture systems. Petrich and colleagues described a TAT study of a ^{211}At-labeled anti-CD33 monoclonal antibody (mAb; ∼1:1,000 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 DSBs resulted (14). Dilution of the toxin conjugate to the 1:1,000 activity, use of unlabeled control mAb, or control "free" ^{211}At, resulted in no DNA DSBs in HL-60 cells. The degree of DSBs from the ^{211}At-labeled mAb was shown to be dose dependent. Induction of radioactivity-dependent apoptosis related to caspase activation was also observed. Taken as a whole, this study shows 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 though when it is compared with other toxin or drug conjugates.

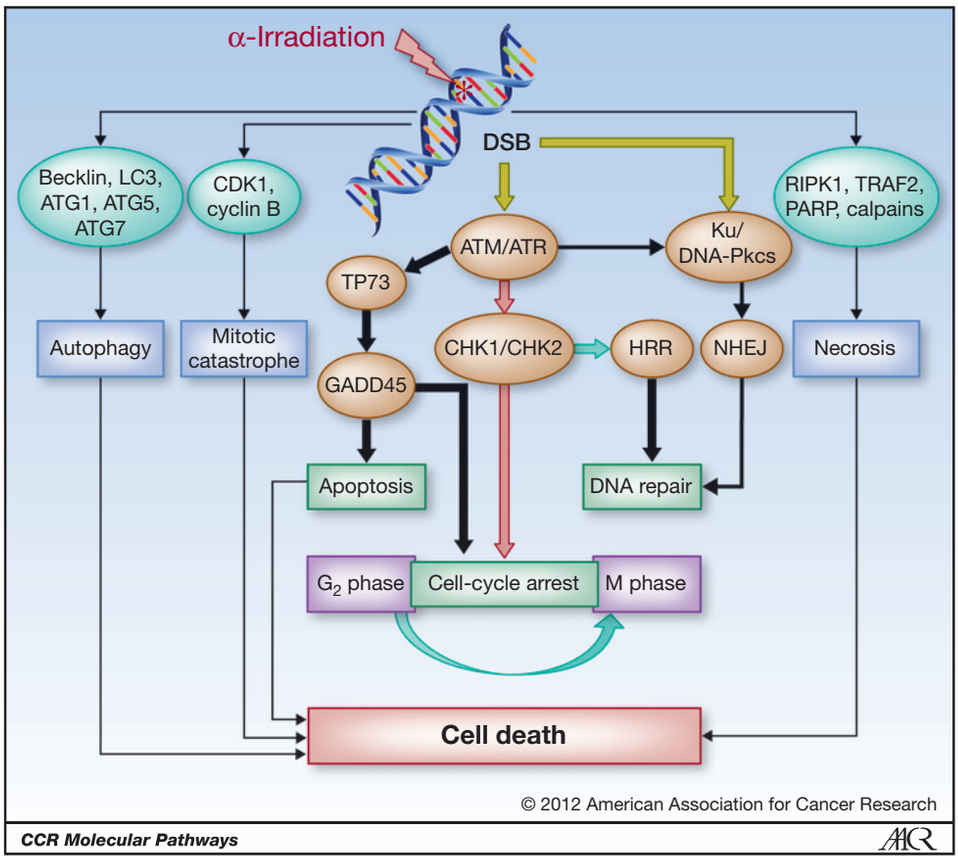

Figure 1. Mechanisms of cell death by α-radiation. Irradiation of cancer cells by α-radiation produces DSBs that evoke a myriad of cellular responses and pathways that include apoptosis, mitotic catastrophe, autophagy, necrosis, cell-cycle arrest, and DNA repair. Many genes and proteins are involved in these pathways, some of which are depicted here. When cell death occurs by autophagy, Becklin, LC3, ATG1, ATG5, and ATG7 are involved. CDK1 and cyclin B are involved in mitotic catastrophe, whereas 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 nonhomologous end joining or homologous repair.

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* (IFN- γ), *ISG20L1* (IFN-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* (TNF superfamily member 9), and *TRAF4* (TNF receptor–associated factor 4). The objective of the study was to evaluate the potential of the genes as measures of α-particle biodosimetry. Although it is 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 upregulated over various ranges of exposure; *GRAP*, linked to transmission of extracellular stimuli for induction of proliferation, differentiation, or apoptosis, was significantly downregulated independent of dose.

Several studies about the molecular mechanisms of ^{213}Bi (^{212}Bi) TAT have been reported. Macklis and colleagues 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 and colleagues 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, although pretreatment with either drug resulted in G_2–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. Although radiosensitization from combination therapy was noted, involvement of apoptosis was ruled out as a mechanism for cell death. Seidl and colleagues have executed far more extensive studies targeting d9-E-cadherin with ^{213}Bi-labeled d9mAb using human gastric cancer cells (HSC45-M2; ref. 18). These studies showed 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, and the mode of cell death was therefore concluded to be different

from apoptosis. Seidl and colleagues also conducted gene expression profiling and a time course microarray for the whole genome (19). Of the 682 to 1,125 genes that showed upregulation and 666 to 1,278 genes that showed downregulation at one time point each, 8 genes appeared to be upregulated and 12 genes were downregulated throughout the course of study. Of those that were upregulated, *COL4A2*, *NEDD9*, and *C3* had not been previously found to be linked to high-LET radiation response; complementarily, this observation held for the downregulated *WWP2*, *RFX3*, *HIST4H4*, and *JADE1*. This discovery process also yielded genes that were not previously associated with any biologic process or molecular function; *ITM2C*, *FLJ11000*, and *MSMB* were consistently upregulated, whereas *HCG9*, *GAS2L3*, and *FLJ21439* were complementarily downregulated. 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 conducted *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 conduct. Recent studies by Yong and colleagues 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 with several controls. Significant apoptosis induction and DNA DSBs were observed after 24 hours. In addition, Rad51 protein expression was found to be downregulated, indicating delayed DNA double-strand damage repair as compared with controls. The cell cycle was also affected, resulting in G_2–M arrest, depression of the S-phase fraction, and depressed DNA synthesis that persisted beyond 120 hours whereas 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 and colleagues examined the impact of TAT combination therapy wherein gemcitabine, a standard-of-care therapeutic for pancreatic cancer and a well-defined radiosensitizer, was administered before 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 pretreatment with gemcitabine abrogated G_2–M arrest, which was associated with inhibition of Chk1 phosphorylation and increased apoptosis. This combination therapy also resulted in reduced DNA synthesis, enhanced DNA double-strand breaks, accumulation of unrepaired DNA, and with downregulation of Rad51, all correlating with 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 double-strand DNA destruction as a result of high-LET α-particle traversal of the nucleus included significant interference with the homologous repair mechanism through the downregulation 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 ravening horde of "molecular targeted" drugs. In cancer therapy, the simple facts are that $\geq$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, and 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 time frame 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; locoregional or compartmentalized diseases of similar presentation; and readily accessible diseases such as leukemia and lymphoma. Furthermore, because of the limited range of the α-particle, normal tissue toxicity is expected to be quite low when a TAT strategy is used. Finally, although it is 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 and colleagues provide a study on induced resistant clones of HL-60 cells to high-LET radiation (26). Although this study did not concern TAT *per se* with the α-emission originating from an ^{241}Am source, it showed that it is possible to have a population of tumor cells that might be refractory to TAT (26). Regardless of these attributes, a very limited number of clinical trials have been executed to date evaluating TAT (Table 1). However, there has been an increase in this activity recently, particularly spurred by the

Table 1. Clinical trials using α-particle emitters

Trial	Cancer type	Radioimmunoconjugate	Outcome	Reference
Zalutsky and colleagues	Glioblastoma	^{211}At-ch81C6	18 patients treated; 14 patients survived 12 mo	27, 28
Andersson and colleagues	Ovarian cancer	^{211}At-MX35-F(ab')$_2$	9 patients treated; no significant toxicity	29
The Scheinberg group	AML	^{225}Ac-HuM195 (^{225}Ac-lintuzumab)	18 patients treated; trial expanded to multicenter phase I/II	31, 32
Heeger and colleagues	B-cell non-Hodgkin lymphoma	^{213}Bi-labeled anti-CD19 and anti-CD20-CHX-A"-DTPA	9 patients treated; 2 patients showed response; limited toxicity in 2 patients	33
The Allen group	Melanoma	^{213}Bi-mAb 9.2.27	22 patients treated; 6% CR; 14% PR; 50% stable disease	34
The Scheinberg group	AML	^{213}Bi-HuM195 (^{213}Bi-lintuzumab)	18 patients treated; 14 patients had reductions in marrow blasts	35
Jurcic and colleagues	AML	^{213}Bi-HuM195 (^{213}Bi-lintuzumab)	31 patients treated; marrow blast reductions observed at all dose levels	36
The Merlo group	Glioblastoma	^{213}Bi-substance P	5 patients treated; Barthel index improved for 2 patients	37, 38
Areva Med LLC	Ovarian	^{212}Pb-TCMC-trastuzumab	3 patients treated; study ongoing; no further information available	39
Parker and colleagues	Castration-resistant prostate cancer and bone metastases	Alpharadin (^{223}Ra chloride)	292 patients treated; median overall survival increased by 4.5 mo compared with placebo group	40

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

Abbreviations: AML, advanced myeloid leukemia; CR, complete response; PR, partial response.

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 conducted 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 and colleagues 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 overexpressed 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 III 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 show 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 in Sweden. Andersson and colleagues 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, 9 patients have been infused with ^{211}At-TAT via a peritoneal catheter to assess the

strategy of intracavitary administration. Results have shown 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, only one clinical trial has used ^{225}Ac. The targeting vector for this trial is a humanized antibody, lintuzumab, that targets CD33 on acute myeloid leukemia (AML) 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 cynomologus 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 (New York, NY) with a primary goal to define both safety and the maximum tolerated dose of ^{225}Ac TAT in patients with advanced AML through a dose-escalation series (31). The initial dose of 0.5 μCi/kg, which is several orders of magnitude less than doses routinely used in RIT with β^--emitters, shows 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 showing that ^{225}Ac TAT targeted by lintuzumab had antileukemic 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 patients with AML at Memorial Sloan-Kettering and the Fred Hutchinson Cancer Research Center (Seattle, WA). 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 colleagues at the German Cancer Research Center in Heidelberg 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 2 patients with 2 patients responding to the therapy. This trial has been continued at the University Hospital Düsseldorf, in 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. A total of 22 patients with stage IV/in-transit metastasis were treated (34). Patients showed disease reduction at 8 weeks based on the tumor marker melanoma-inhibitory protein activity; 6% showed 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). Rosenblat and colleagues conducted a follow-up study with ^{213}Bi TAT wherein 13 newly diagnosed patients and 18 patients with relapsed/refractory AML were first treated with continuous cytarabine infusion for 5 days (36). Myelosuppression was the primary toxicity, and 2 of 21 patients treated with the maximum tolerated dose died. At all dose levels, marrow blast reductions were observed and CD33 sites were found to be saturated by ^{213}Bi TAT lintuzumab.

The Merlo group conducted 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 overexpressed in grade 2, 3, and 4 gliomas (37, 38). In this pilot study, 5 patients were enrolled, and treatments were administered with an implanted catheter system (intratumoral injection) Four patients received 1 therapeutic cycle, and 1 patient received 4 therapeutic cycles. Again, the ^{213}Bi TAT agent was retained at the target site without local or systemic toxicity being observed. Pretherapeutic functional scores (Barthel index) for the 2 patients with glioblastoma multiforme were 75 and 80, which after TAT improved to 90 of 100. Radiation-induced necrosis and demarcation of the tumors were detected by MRI (38).

The first phase I clinical trial using ^{212}Pb TAT, sponsored by Areva Med LLC, opened at the University of Alabama in Birmingham in 2011. As one might expect, this trial is designed to determine dose-limiting toxicities and antitumor efficacy for treating intraperitoneal cancers, specifically, primarily ovarian cancer. Adverse events and immune response monitoring, as well as 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. Although 3 patients have completed treatment in 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 onward into phase III trials. Of 292 patients treated with ^{233}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 white blood cells, 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 shown 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 acces-

sibility and delivery based upon physical considerations is a key criterion to their success; however, development and growth of clinical TAT have 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. Additional *in vivo* studies of the molecular mechanisms of response, repair, and cell death resulting from TAT treatment are clearly needed. A host of additional genetic pathways appear to be activated *in vivo* that simply have not been recognized or studied until relatively recently (41). The studies need to be conducted in relevant *in vivo* tumor models in which 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 conducted 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 use and integration with other therapies. A full mechanistic understanding of the therapy will accelerate its development and clinical translation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.E. Baidoo, M.W. Brechbiel
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Yong

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