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The potential complementary role of targeted alpha therapy in the management of metastatic melanoma

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Practice points

- New kinase inhibitors and immune checkpoint inhibitory antibodies may improve survival of metastatic melanoma patients on a population-wide basis. Not all patients benefit, however, and other approaches such as targeted alpha therapy (TAT) should be considered.
- Molecular profiling of melanoma now dictates its treatment. Most cutaneous metastatic melanomas have constitutive activation of MAPK signaling, with 40% having activating mutations of *BRAF*. Selective *BRAF* inhibitors target the mutant oncoprotein. Adding a MEK inhibitor further improves patient survival, but complete remission rates remain at 5–10%, and relapse of drug resistant disease is virtually inevitable.
- Immunotherapy will come to dominate metastatic melanoma treatment. Antibodies that inhibit the immune checkpoint molecules, CTLA4 and PD1, produce durable survival in up to 20 and 40% of treated patients, respectively.
- Although a compelling scientific and clinical rationale exists for combining immunotherapy with MAPK inhibitors to overcome the limitations of each therapy, no clinical data yet confirm a safe and effective combination.
- Melanoma brain metastases are common and must be controlled to cure melanoma. Dabrafenib and ipilimumab are similarly active against both intracranial and extracranial disease, thus ensuring overall disease control and providing a backbone for other therapies.
- Treatment of both local and metastatic uveal melanoma remains an unmet medical need. Although surgery, external beam radiotherapy and brachytherapy provide effective local treatment, local and distant recurrence rates are too high, and local complications of treatment still represent a significant risk to sight.
- TAT is a promising new treatment. Alpha-particles produce a high density of lethal DNA lesions within 6–10 cell diameters of the point of emission, thus maximizing killing of targeted cancer cells and minimizing damage to surrounding normal cells. Hence, targeting of an alpha-emitting radioisotope by its conjugation to a cancer-specific monoclonal antibody (mAb) may confer distinct therapeutic advantages.
- In preclinical and clinical TAT studies, the alpha-emitting radioisotope, bismuth-213, was conjugated to the 9.2.27 mAb to form the ²¹³Bi-alpha-immunoconjugate (AIC). This mAb is specific for human melanoma chondroitin sulphate proteoglycan, also known as NG2. NG2 is expressed in most cutaneous and uveal melanomas. Bi-213 emits a high-energy alpha-particle but with a half-life of 46 min.
- The antigen-specific cytotoxicity of ²¹³Bi-AIC against human melanoma cells *in vitro* was recapitulated through *in vivo* studies of human melanoma xenografts using both intratumoral and intravenous injections of ²¹³Bi-AIC.
- In a Phase I clinical trial of intralesional TAT, 16 patients were enrolled with inoperable and NG2-positive cutaneous melanoma metastases. Intralesional TAT was associated with posttreatment biopsy evidence of massive tumor cell death and reduced tumor cell proliferation. Some tumor shrinkage was observed without evident ill effects.

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Practice points (cont.)

- A Phase I trial of systemic TAT, 22 metastatic melanoma patients treated with a single, ascending doses of intravenous ^{213}Bi -AIC. All patients had cutaneous or subcutaneous lesions and 50% had intransit disease. A maximum tolerated dose was not reached. One patient had a confirmed complete response; three patients had confirmed partial responses; two patients had transient partial responses; and four patients had stable disease for at least 6 weeks. The median survival time among the final study population of 38 treated patients was 266 days. Among patients with stable disease or partial responses attributable to treatment, median survival time was significantly longer at 612 days, although favorable prognostic factors and poststudy treatments may also account for this result.
- The therapeutic advantages of TAT has certain may be exploited to complement standard therapies: when MAPK pathway inhibitor therapy induces partial regression; for loco-regionally recurrent melanoma; as adjunctive therapy to surgery, radiotherapy, or both in uveal melanoma; as intralesional TAT to eradicate intransit micrometastases or isolated cancer cells lying within lymphatic vessels proximal to injected lesions.
- In conclusion, the anti-melanoma activity of ^{213}Bi -AIC in vivo is unexpected, and may be explained by the targeting of NG2 on tumor endothelial cells and their covering pericytes. The promising clinical results of ^{213}Bi -AIC therapy warrant Phase I and II clinical investigation of intralesional and systemic TAT.

Standard treatments for metastatic melanoma have recently extended survival although many patients still succumb. Targeted alpha therapy (TAT) is a new therapeutic approach in which a cancer-targeting vector is labeled with an alpha-emitting radioisotope. Alpha-particles have the shortest range and highest energy transfer, and produce localized, high-density and lethal ionization damage to DNA. Thus, the targeted radiation can kill isolated cancer cells circulating in blood and lymphatic vessels, regress metastatic cancer cell clusters, and disrupt the vasculature of solid tumors. Preclinical and clinical studies of TAT for metastatic melanoma demonstrate its safety and anti-tumor activity. We recommend ways in which TAT can be used to treat small-volume disease sometimes in conjunction with cytoreductive anti-melanoma therapies.

KEYWORDS

- Bi-213 alpha emitter
- complementary therapies
- immunotherapy
- intralesional • kinase inhibitors • metastatic melanoma • Phase I clinical trials • radioisotopes
- systemic • targeted alpha therapy

Background

Although surgery usually cures melanoma of the skin, it is a tumor with high metastatic potential relating to the extent of tumor thickness as well as the degree of tumor ulceration and mitotic activity [1]. Once melanoma metastasizes to lymph nodes or other organs, survival rates worsen. For example, 10% or fewer patients with the most advanced disease survive 5 years. Recently, the US FDA has approved new drugs for metastatic melanoma because randomized clinical trial data have demonstrated improved survival for use of these drugs [2–4] compared with standard chemotherapy. These treatments have now become the new standard of care. Together with other treatment advances (to be discussed below), population-wide improvements in survival of patients with metastatic disease are expected.

While the results for these new therapies are promising and increased survival is achieved, patients still succumb to the disease. Since adverse events may limit the therapeutic dose to be administered or drug resistant

clones can develop, alternative and complementary therapeutic approaches are needed. Radioimmunotherapy is one such approach where cancer cells are targeted by vectors labeled with a radioisotope. The radiation emitted in the decay of the radioisotope then kills the targeted cancer cells. Alpha radiation has the shortest range and highest energy transfer, resulting in localized but significant ionization damage (e.g., to DNA). Alpha-emitting radioisotopes can be used to kill isolated cells, small cell clusters and to regress tumors. This approach is called targeted alpha therapy (TAT).

Preclinical studies and clinical trials of TAT for many cancers have been reported. In particular, in Phase I trials of intralesional and systemic therapy in metastatic melanoma patients, TAT has been found to be safe and has demonstrated some evidence of antitumor activity. Consequently, in the clinical setting of small-volume disease, TAT may usefully complement current, cytoreductive, anti-melanoma therapies.

This review examines the scientific basis for recently approved therapies and gives an

overview of clinical results in an endeavor to recommend whether and how TAT can be integrated into the therapeutic armamentarium for metastatic melanoma.

Current therapeutic approaches for metastatic melanoma

• Molecular lesions & use of targeted kinase inhibitors

The molecular classification of advanced melanoma dictates its treatment. The MAPK signaling pathway is activated in over 90% of cutaneous melanoma through activating mutations of the receptor tyrosine kinase, KIT [5], or the downstream kinase molecules, NRAS [6] and BRAF [7]. Most BRAF mutations are of the V600E type [7], and occur in up to 40% of metastatic melanoma. Activating mutations of these key MAPK genes convert this signal transduction pathway to a dominant driver of cancer cell proliferation and survival; a cellular state described as oncogene addiction [8]. The consequent constitutive activation of the MAPK signaling is manifested as phosphorylation of ERK [7]. As a corollary, oncogene addiction makes cancer cells acutely susceptible to pathway blockade using signal transduction inhibitors, which can abrogate its kinase-mediated activation.

Knowing the BRAF mutation status of unresectable metastatic melanoma decides the medical treatment of patients. If a patient's melanoma has a V600 *BRAF* gene mutation then standard treatment includes the selective BRAF inhibitors, dabrafenib (Tafinlar®), or vemurafenib (Zelboraf®). The addition of the MEK inhibitor, trametinib (Mekinist®) to dabrafenib significantly improves progression-free survival (PFS) thus indicating that greater suppression of the MAPK pathway seems to delay emergence of drug resistance. Initial dose-finding clinical studies of the selective BRAF inhibitors indicated that suppression of MAPK pathway signaling, which was manifest as at least 80 or 90% inhibition of phosphoERK in tumor biopsies, was associated with tumor regression [9,10]. The clinical activity of dabrafenib [11] is almost indistinguishable from that of vemurafenib [12] with objective tumor response rates of around 50% and median PFS intervals of 6–7 months.

In the BRIM3 Phase III registration trial of 675 patients with previously untreated, metastatic melanoma containing the V600E *BRAF* mutation, patients were randomized to standard

dacarbazine versus vemurafenib. The co-primary study end points of superior PFS and overall survival (OS) were met [2]. At 6 months, OS was 84% (95% CI: 78–89%) in the vemurafenib group and 64% (95% CI: 56–73%) in the dacarbazine group. In the interim analysis for OS and final analysis for PFS, vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared with dacarbazine ($p < 0.001$ for both comparisons). In the BREAK3 Phase III study [3] of patients with untreated, advanced *BRAF*-mutant melanoma, the median PFS of dabrafenib-treated patients was 5.1 months (cf. 2.7 months in the dacarbazine arm). The updated median OS (mOS) data from BREAK3 showed mOS of 18.2 months for dabrafenib-treated patients versus 15.6 months for dacarbazine-treated patients, which may have resulted from patient cross-over between treatment arms and the treatment beyond progression of patients receiving dabrafenib [13]. In contrast, in the BRIM3 registration trial of vemurafenib, in which the comparator arm was also dacarbazine, mOS of vemurafenib-treated patients was 13.6 months (cf. 10 months in the dacarbazine arm) [14]. These small-molecule kinase inhibitors are generally well tolerated in most patients.

The clinical studies of BRAF inhibitors show that most tumor responses occur within 8 weeks of commencing treatment. Although most patients achieve partial tumor responses, complete responses are uncommon (~5%). The most common clinical scenario is persistent disease. Indeed, despite the retention of the oncogenic BRAF mutation drug target in virtually all tumors, most patients relapse as various mechanisms of drug resistance evolve. Reactivation of MAPK signaling via various molecular lesions is most common (~70% of cases) [15,16] but MAPK-independent 'oncogene bypass' mechanisms are also very important [17–20].

The most clinically successful approach to managing the emergence of drug-resistant clones thus far has been further suppression of the MAPK pathway by the combination of a BRAF inhibitor and a MEK inhibitor. The COMBI-D Phase III trial of dabrafenib and placebo versus dabrafenib and the MEK inhibitor, trametinib, in patients with V600 *BRAF*-mutant metastatic melanoma, has met its primary PFS end point. The median PFS was 8.8 months and 9.3 months in the dabrafenib + placebo versus

dabrafenib + trametinib arms, respectively. At this interim analysis, the OS data remain very immature [21]. Interestingly, the complete response rate in this study and in the CoBRIM study of a BRAF/MEK inhibitor combination therapy (vemurafenib + placebo versus vemurafenib + cobimetinib) had increased from about 5–10% [22]. Recent preliminary data indicate that the predominant mechanism of resistance to combination therapy is still MAPK pathway reactivation [23]. These results further indicate the importance of urgent and ongoing investigation of combinations of MAPK pathway inhibitors with other, noncross-resistant, therapeutics such as immunotherapy.

• Approved immunotherapy

The monoclonal antibody (mAb), ipilimumab (Yervoy®), is a worldwide-approved treatment for patients with unresectable or metastatic melanoma. This mAb binds specifically to CTLA4, which is a T-cell surface molecule expressed after T-cell activation. Ipilimumab blocks the interaction of CTLA4 with CD80 and CD86 molecules, which are expressed on the surface of antigen presenting cells. CTLA4 binding of CD80/CD86 terminates T-cell activation and clonal expansion. Therefore, by blocking the interaction of CTLA4 with CD80/CD86, ipilimumab, in effect, ‘releases the brake’ on T-cell signaling and potentiates T-cell activation and proliferation. Ipilimumab is the first in a class of therapeutic agents called immune checkpoint inhibitors.

In a Phase III randomized controlled trial of pretreated patients with metastatic melanoma (MDX-020), ipilimumab was compared with a gp100 vaccine, which was considered a relatively inactive control arm. A landmark survival analysis at 2 years showed that 23.5% of patients receiving ipilimumab alone were alive compared with only 13.7% of patients who did not receive ipilimumab [4].

A second class of immune checkpoint inhibitory mAbs, pembrolizumab (Keytruda®) and nivolumab (Opdivo®), which block another T-cell negative regulatory molecule called PD1, have now been FDA approved for the treatment of metastatic melanoma after failure of ipilimumab. Associated objective response rates of up to about 30% were observed in pretreated patients including with ipilimumab [24,25]. In one study, 62 and 43% of treated patients were alive at 1 and 2 years, respectively [25].

• Combination therapies

Once patients progress on treatment with inhibitors of BRAF and MEK, disease progression may be rapid and lead to early death [2,12,26]. Retrospective data indicate a significant proportion of patients (up to 50%) may not complete second-line ipilimumab treatment because disease progression is too rapid to permit delivery of more than one or two doses, which are too few to have efficacy [27,28]. Hence, there is a rationale for testing combinations of BRAF inhibitor therapy and immunotherapy to mitigate the risk of disease progression. Although a compelling scientific rationale exists for combining immunotherapy with BRAF and MEK inhibitors [29,30], early clinical data have indicated difficulties. A Phase I trial of ipilimumab and vemurafenib was closed early due to hepatotoxicity although the mechanism was not clear [31]. In an ongoing Phase I trial of ipilimumab with or without dabrafenib and/or trametinib in patients with metastatic melanoma (NCT01940809), an interim finding of several cases of severe colitis has prevented further recruitment to the ipilimumab and dabrafenib/trametinib arm while recruitment to the ipilimumab and dabrafenib arm continues [32].

Rapid disease progression preventing up to 50% of patients with *BRAF*-mutant melanoma obtaining benefit from ipilimumab justifies exploration of other treatment modalities, which may consolidate treatment responses with combination BRAF/MEK inhibitors and which may thus induce higher rates of complete remission.

• Control of brain metastases

Brain metastases are a common feature of melanoma. Cerebral metastases are present at diagnosis in 20% of patients, occur during its disease course in almost 50% of patients, and are found in up to 75% of patients at autopsy [33,34]. The median survival of patients with untreated cerebral metastases is 4 months. Hence, for any systemic treatment to have a favorable impact on survival of metastatic melanoma patients, cerebral metastases must be managed as effectively as extracranial disease.

Two systemic treatments have been shown to be active against brain metastases. In a Phase II study of ipilimumab, 51 patients with asymptomatic cerebral metastases (cohort A) and 21 patients with symptomatic, steroid-requiring cerebral metastases (cohort B) were enrolled. The primary study end point was disease control, which was defined as complete

response, partial response, or stable disease at both intracranial and extracranial sites of disease at 12 weeks after commencement of ipilimumab 10 mg/kg for four doses. Nine out of 51 (18%) patients in cohort A, and 1 out of 21 (5%) patients in cohort B, exhibited disease control. Median OS for patients in cohorts A and B was 7.0 and 3.7 months, respectively. Importantly, these data demonstrate that ipilimumab via activation of anti-melanoma lymphocytes may provide useful treatment for cerebral metastases of melanoma, particularly if they are asymptomatic and small [34].

In the Phase II BREAK-MB study, the anti-melanoma activity of dabrafenib in cerebral metastatic disease mirrored its effects on extracranial disease [33]. Patients with V600E or V600K *BRAF*-mutant melanoma and at least one asymptomatic brain metastasis (≥ 5 mm and ≤ 40 mm in diameter) were enrolled into either cohort A (had not received previous local treatment for brain metastases) or cohort B (had progressive brain metastases after previous local treatments). The study end point was overall intracranial response (i.e., complete response and partial response) as the best response in patients with V600E *BRAF*-mutant melanoma.

Among 172 patients in the total study population, 139 (81%) had V600E *BRAF*-mutant melanoma. Among the 139 patients with V600E *BRAF*-mutant melanoma, the overall intracranial response rates were 39.2 and 30.8% in cohorts A and B, respectively. Similarly, overall response rates (intracranial and extracranial complete response and partial response) were 37.8 and 30.8% in cohorts A and B, respectively. Among the patients with V600E *BRAF*-mutant melanoma, PFS was 16.1 and 16.6 weeks in cohorts A and B, respectively, and OS was 33.1 and 31.4 weeks in cohorts A and B, respectively [34].

These Phase II clinical data indicate that these new modalities of anti-melanoma treatment, both targeted therapy and immunotherapy, provide effective disease control in the brain, establishing the principle that these therapies can provide a backbone for the addition of other therapies.

• Uveal melanoma

Uveal melanoma (UM) is the most common primary malignancy of the adult eye. However, it is less common than cutaneous melanoma with an incidence of 6–8 per million per year [35–37]

compared with 33 per million per year for the incidence of cutaneous melanoma in Australia, which has the world's highest incidence. UM can cause visual impairment, loss of the eye, distant metastases and death. Eye-preserving treatments include radiotherapy, local resection, transpupillary thermotherapy, laser photocoagulation or a combination of these [36,37]. Radiotherapy can be applied as plaque brachytherapy, stereotactic irradiation or proton beam radiotherapy and all are effective in achieving local tumor control in most cases [36–42]. But radiation-associated normal tissue complications can also persist and be severe. Consequently, enucleation may be required in the event of locally advanced disease, local recurrence or treatment complications. Despite effective local treatment, however, approximately 40% of patients develop liver-predominant metastases within 5 years of enucleation [43]. Metastatic UM is almost uniformly fatal although some encouraging activity of ipilimumab has been observed [44]. So far, there has been a relative dearth of therapeutic targets in UM.

Plaque brachytherapy is generally the treatment of choice for UM. Low-range or low-energy radiation from radioisotopes in the form of low-energy photon seeds (e.g., I-125, Pd-103, and Cs-131) or solid beta-emitting plaques (e.g., Ru-106 and Sr-90) are used to minimize normal tissue complications [45]. Plaques are generally sutured to the eye and may have to be replaced regularly depending on the lifetime of radioisotope. Thus, the treated volume is irradiated inhomogeneously, with the tumor base receiving several times the dose of the tumor apex.

Despite the widespread use of plaque brachytherapy for UM, 'there exist no prospective randomized or case-matched clinical trials comparing the clinical effectiveness or side effects related to these radionuclides.' Moreover, 'there exist no data to allow a meta-analysis comparing relative tumor size and location' [42]. The 12-year long Collaborative Ocular Melanoma Study (COMS) of patients with medium-sized choroidal melanoma, which is the commonest form of UM, demonstrated the relative equivalence of I-125 plaque brachytherapy compared with enucleation in prolonging survival of metastatic UM patients [46]. Furthermore, these data indicate that plaque brachytherapy may not be an appropriate treatment choice if the estimate of local tumor recurrence exceeds 5%,

or if the tumor has an irregular shape, extends close to optic disc, or has a thickness exceeding 45 mm [46].

Stereotactic irradiation using linear accelerator technology, which is available at most radiation oncology facilities, or more specialized apparatus like Gamma knife, is a relatively new UM treatment ([40] and references within). Overall, local tumor control rates of at least 80–90% can be achieved and are comparable to those of plaque brachytherapy [38]. The treatment can be delivered in a single high-dose fraction (of up to 35 Gy) as stereotactic radiosurgery (SRS), or in several fractions (usually 4–5 at total doses between 50 and 70 Gy) as stereotactic radiotherapy (SRT). Both SRS and SRT require techniques for ocular immobilization, ranging from retrobulbar anesthesia, bridle sutures to eye muscles, to computer-assisted eye tracking systems with radiation beam gating [40].

In a retrospective study of linear accelerator-based SRS at a tumor radiation dose of 25 Gy in 78 UM patients not suitable for ruthenium plaque brachytherapy, patients received either SRS monotherapy (group 1, 60 patients) or SRS combined with tumor resection (group 2, 18 patients) [37]. With a median follow-up of 33.7 months (range: 0.13–81.13 months), there were no statistically significant differences between treatment groups for both local control (group 1: 85% vs group 2: 100%; $p = 0.22$) or eye preservation rates at 3 years (group 1: 77% vs group 2: 77%; $p = 0.82$). There was also no significant difference in the development of metastases ($p = 0.33$).

In two case-series of 19 [39] and 60 UM patients [38] in which SRS with a gamma knife was used, long-term results indicated tumor control rates around 90%, although 3- and 5-year OS rates over a 40-month mean follow-up period were 86 and 55%, respectively [38]. Serious side effects of stereotactic irradiation such as radiation retinopathy and neovascular glaucoma can occur. Neovascular glaucoma, in particular, can complicate SRS. Reducing the SRS dose to 40 Gy or less as well as excluding treatment to tumors near the ciliary body can decrease the rate of glaucoma without affecting the rate of tumor control [39]. In another retrospective study of UM patients treated by SRS with and without tumor resection, it was concluded that SRS in combination with tumor resection might be associated with increased tumor control and fewer radiation complications than SRS alone [41].

Proton beam radiotherapy (PBT) can be considered superior to stereotactic irradiation because the dose can be shaped more accurately to ocular structures while limiting dose to healthy tissues. However, PBT is associated with high capital cost and is not readily available [47]. In a recent review, PBT resulted in approximately 90% eye preservation rates and 50% vision preservation after 5 years, and the clinical benefit accrued mainly to patients with larger tumors [47]. In a retrospective study of 147 UM patients with mostly medium and large size tumors treated with PBT, enucleation post-PBT occurred in 22.4% of patients because of suspected recurrence (48%) and neovascular glaucoma (42%). The 5-year eye preservation rate was 71.3% and disease-specific survival rate was 87.7% [36]. Hence, these data indicate that surgical resection may be used with PBT to mitigate the risk of morbidity.

In all, the clinical data suggest that while external beam radiotherapy and brachytherapy can provide effective local treatment, local and distant recurrence rates are too high and local complications of treatment still represent a significant risk to sight. Therefore, novel approaches such as internal targeted radiation therapies may improve local control of UM while reducing the risk of visual impairment.

Clinical targeted alpha therapy

• Background

Since the development of hybridoma technology [48], many mAbs have been raised against antigens overexpressed by different cancers [49]. Some of these mAbs exhibit direct antitumor effects via surface antigen binding and others may exert antibody-dependent cell-mediated cytotoxicity (ADCC) [50]. However, emerging clinical evidence shows that the potency of anticancer mAbs can be enhanced further through arming with either a cytotoxic payload (as an antibody drug conjugate; ADC) or a radioisotope (as a radioimmunoconjugate; RIC). Two ADCs have recently been FDA-approved for the treatment of lymphoma and breast cancer [51,52]. A RIC directed toward the surface CD20 antigen of B-lymphoid malignancies and bearing the beta particle-emitting radioisotope, yttrium-90, has demonstrated clinically meaningful activity [53].

Radioisotopes have a wide range of half-lives and radiation decay properties to suit different applications, and alpha-particles, in particular, may offer distinct therapeutic advantages

because of their short range, high linear energy transfer (LET), and correspondingly high radiobiological effectiveness [54]. These properties mean that a greater portion of the energy is deposited in the cancer cell and thus fewer hits are required to kill the targeted cell.

Recently, alpha radiation from intravenously administered $^{223}\text{RaCl}_2$ (Xofigo®) has been shown to extend life and reduce pain in prostate cancer patients with bone metastases because the calcium-mimetic effect of ^{223}Ra enables incorporation of the alpha-emitter in the mineralizing front of bone close to prostate cancer cells [55]. Nevertheless, alpha radiation is most effective when delivered directly to cancer cells by alpha-immunoconjugates (AIC) using cancer-specific antibodies or peptides [56]. Over the past 20 years the development of AICs has enabled TAT to progress from *in vitro* studies, through to preclinical *in vivo* experiments and clinical trials [54]. The dose to normal tissues always provides a limitation to the injected dose and to that received by the tumor. However, TAT can achieve cancer regression within the maximum tolerated dose (MTD) for normal tissues. TAT was originally thought to be an ideal therapy for ‘liquid’ cancers, for example, leukemia [57] and micrometastases [58], because the short half-lives of the radioisotopes were sufficient to target blood-borne cancer cells. In addition, the short range of alpha-particles ensures that the targeted cancer cells received the highest radiation dose, thus minimizing the dose to surrounding normal cells.

With energies from 2–8 MeV and ranges of 20–80 μm , the high LET radiation of alpha-emitting radioisotopes produces increased rates of DNA double-strand breaks that can induce cellular apoptosis within the alpha-particle range. In particular, the lethality of alpha-particles strictly depends on the number of nuclear traversals [59]. The LET is typically approximately 100 keV/ μm , giving a higher probability of causing DSBs (the ratio SSB/DSB ~20 compared with 60 for low LET radiation) [60]. However, if mismatch DNA repair occurs, then alpha radiation has a much higher probability than photons ($\times 20$) of causing genetic damage and carcinogenesis. Consequently, we hypothesize that targeted alpha radiation is ideal for the killing of isolated cancer cells in transit in the vascular and lymphatic systems and of tumor capillary endothelial cells, thus disrupting tumor capillary networks and shrinking tumors. This

latter effect has been termed tumor antivascular alpha therapy (TAVAT) [61], and it may have been responsible for tumor regressions observed in a Phase I trial of TAT for metastatic melanoma targeting the melanoma-associated chondroitin sulfate proteoglycan [62] as discussed below.

Although the difficulty of proving efficacy against micrometastatic disease cannot be underestimated [63], first clinical proof of concept of systemic TAT was demonstrated in the Phase I trial of a ^{213}Bi -AIC of an anti-CD33 mAb in 18 patients with relapsed/refractory acute or chronic myeloid leukemia [57]. Myelosuppression was observed but a maximum tolerated dose was not reached at the top dose of 37 MBq/kg given in 4–7 doses over several days. Clinical evidence of antileukemic efficacy was obtained with 93 and 78% of patients experiencing reductions in peripheral blood and bone marrow leukemic blast counts, respectively. Current and projected experience with clinical TAT trials is further reviewed by Elgqvist *et al.* [64] and Dadachova *et al.* [65].

• TAT for metastatic melanoma

In the preclinical and clinical TAT studies to be discussed below, the alpha-particle emitting radioisotope Bi-213 (from the Ac-225/Bi-213 generator) was conjugated to the 9.2.27 murine monoclonal antibody (mAb) via a bifunctional chelator (cDTPA) to form the AIC ^{213}Bi -cDTPA-9.2.27 [66].

The 9.2.27mAb is specific for the 250 kDa cell-surface antigen, human melanoma chondroitin sulphate proteoglycan (MCSP), which is also known as human NG2. NG2 is expressed in over 90% of melanoma cells lines [67,68], in more than 90% of cutaneous melanomas [69], and in 18 of 19 (95%) of primary uveal melanomas [70]. Radiolocalization of the 9.2.27mAb has been shown in high MCSP-expressing human melanoma xenografts of nude mice [71,72].

Expression of MCSP or NG2 has been found on other tumors including chondrosarcomas, glioblastomas and some leukemia's [73–75]. NG2 immunoreactivity has been detected in human microglia [76], and in the new vessels associated with normal developing tissues, wound healing and tumors [77] where it can be expressed by both pericytes [78,79] and endothelial cells [74]. Thus, NG2 has a functional role in both angiogenesis and in tumor development including the growth and metastatic properties of melanoma cells [80].

• Preclinical data

The 9.2.27 mAb does not display any evident antitumor activity as a naked antibody. However, as an AIC by labeling with the Bi-213 alpha-emitting radioisotope, this mAb is transformed to a cytotoxic agent, as first synthesized and reported by Rizvi [80]. The ^{213}Bi -AIC was found to be one hundred times more cytotoxic to cells of the mm138 human melanoma cell line than a beta-emitting immunoconjugate [81]. *In vitro* studies testing the specific cytotoxicity of the ^{213}Bi -cDTPA-9.2.27 AIC against UM cell lines were also performed. Several UM cell lines displayed strong NG2 immunoreactivity, and a UM cell line lacking NG2 expression was used as a negative control. There was direct, concentration-dependent cytotoxic effect of this AIC on human NG2-expressing UM cell lines. On the hand, significant cytotoxicity was not observed using the ^{213}Bi -cDTPA-9.2.27 AIC with the NG2-negative UM cell line, nor using the naked 9.2.27mAb, its cold conjugate, or an AIC of irrelevant specificity [43].

Allen [82] established an *in vivo* mm138 xenograft model and tested the inhibition of tumorigenesis by the local and systemic administration of the AIC. At 2 days after subcutaneous inoculation of 10^6 MM138 cells, the lesion comprised isolated cells and cell clusters. Solid tumors began to appear at 5–7 days postinoculation. The acute maximum tolerated activity of the ^{213}Bi -AIC in nude mice was approximately 10 mCi (370 MBq)/kg at the end point of 15% weight loss.

Subcutaneous injection of 25 μCi of AIC at the site of tumor inoculation 2 days postinoculation prevented tumor formation. This effect was antigen-specific as a nonspecific AIC did not demonstrate anti-melanoma activity. In established tumors (20–300 mm^3 in size), dose dependence of intralesional TAT was established. A 25 μCi dose produced regression of small tumors whereas this dose was ineffective for larger tumors. In contrast, multiple intralesional injections of 100 μCi caused complete regression in 100–300 mm^3 subcutaneous melanomas over a 30–60-day period [81,82].

In conclusion, these results were consistent with the expected advantages of ^{213}Bi including cytotoxicity of targeted and nearest neighbor cells and sparing of stem cells in normal tissues [58]. These results were encouraging for the potential clinical utility of TAT for melanoma.

• Phase I trial of intralesional therapy

The safety of the ^{213}Bi -AIC Phase I dose was investigated in a dose escalation study in patients with cutaneous metastases of melanoma to facilitate the observation of antitumor effects of intralesional TAT [66]. In addition to the evaluation of safety, the pharmacokinetics of the AIC was measured to derive the biologically effective dose administered to the injected tumor and to nontargeted tissues and organs.

All 16 enrolled patients demonstrated positivity for immunohistochemical staining with the 9.2.27mAb. AIC doses from 150 to 1350 μCi were injected into lesions of different sizes. Intralesional TAT for melanoma was found to be safe up to the maximum dose of 1350 μCi (50 MBq). Antitumor efficacy was first observed at a dose of 200 μCi . Posttreatment biopsy evidence of massive tumor cell death and apoptosis and reductions in the Ki67 proliferation marker were observed. These effects depended on arming of the 9.2.27 mAb because intralesional injection of the unconjugated mAb did not have these effects. A human antimouse-antibody response was not evident. These results indicate that intralesional TAT may be useful for the control of inoperable secondary melanoma or primary ocular melanoma.

• Phase I trial of systemic TAT

The safety of systemic TAT administered via a single intravenous bolus injection was studied in a first in human Phase I trial. In all, 38 evaluable patients with metastatic melanoma were treated up to a dose of 25 mCi (925 MBq) [83]. Patients were enrolled in a conventional 3 + 3 design at dose levels of 1.25, 2.5, 4, 5.5, 7.5, 12.5, 17.5, 23 and 25 mCi (46–925 MBq). The starting dose was based on the highest dose reached in the Phase I intralesional study. Treatment responses in skin and subcutaneous tissues were evaluated by RECIST v1.0 at 0, 2, 4, 8 and 12 weeks posttreatment. In some patients, a CT scan, which was performed at baseline and at 8 weeks posttreatment, was also evaluated by RECIST v1.0.

In an interim report of 22 evaluable patients, all had cutaneous or subcutaneous lesions and 50% had intransit disease. Toxicity was not reported except for one patient who had grade 1 nausea posttreatment. A MTD was not reached. In all, one patient had a confirmed complete response. Three patients had confirmed partial responses. Two patients had transient partial

responses. Four patients had stable disease for at least 6 weeks. One patient, whose cutaneous metastases of the leg responded to TAT so that 20 of 21 tumors completely regressed, was later retreated because there was no evidence of a human antimouse-antibody reaction [62].

In the follow-up study report of 38 treated patients, for those patients in whom stable disease or partial response were attributed to treatment, the median survival time was 612 days, which was significantly higher than the median survival time of 266 days among the overall study population. However, known prognostic factors and poststudy treatment may confound interpretation of this survival analysis. The treatment effect was compared with other prognostic variables in a Cox regression analysis. The combination of M1a and M1b disease (skin, soft tissue and pulmonary metastases) had a significantly lower hazard ratio (HR) of 0.230 (95% CI: 0.054–0.969; $p = 0.045$), whereas serum LDH as a continuous variable had a significantly elevated HR of 1.002 (95% CI: 1.000–1.003; $p = 0.018$). Hence, these prognostic variables may be sufficient to account for the apparent difference in survival between patients progressing and not progressing post-TAT. Moreover, after receiving TAT, some patients received surgery, chemotherapy, or radiotherapy and these treatments may have affected OS. The injected dose did not have a statistically significant effect on survival in this study [83].

In conclusion, this degree of systemically mediated antitumor effect was unexpected given that a single dose of a very short-lived radioisotope with a very short range had been administered. In particular, the lack of a dose response on survival suggests either that the treatment effects were stochastic or that an important therapeutic factor was not being controlled. In an attempt to explain this paradoxical result, it was hypothesized that the therapeutic effect of the ^{213}Bi -AIC depended on vascular targeting or ‘tumor antivascular alpha therapy (TAVAT)’ [61]. The authors ascribed the effects of TAVAT to the variable permeability of the tumor capillary vasculature. This would significantly affect the delivery of the AIC to the perivascular space and to the antigens expressed by the contiguous melanoma cells and pericytes. Alpha radiation from the Bi-213 decay can hit the capillary endothelial cells within the 80 μm range, causing high linear energy transfer to the cell nuclei and inducing apoptosis, closing the capillary. If enough

capillaries close down, the tumor regresses by oxygen and nutrient starvation [61].

Complementary therapeutic advantages of TAT & potential clinical applications

The unique properties of high LET and short path length confer significant therapeutic advantages on TAT that may complement the activity of current anti-melanoma therapies. For example, the small-molecule kinase inhibitors of MEK and of oncogenic V600 mutant *BRAF* usually induce partial regression and stabilization of disease before disease progression occurs in most patients. This state of reduced burden of disease may be at a sufficiently low volume as to be amenable to a therapeutic approach such as TAT. TAT directed toward melanoma cell surface antigens will first produce a ‘self-dose,’ which may be sufficient to cause lethal or sublethal damage in the targeted cell. Despite the short path length of alpha-particles, however, some radiation ‘cross-dose’ is likely to be delivered to surrounding cells that may not express the cell surface target antigen. This cross-fire effect may be potent enough to elicit killing or, at least, sublethal damage in the third-party cells [84].

In addition, to possible beneficial effects of TAT after a first step of cytoreductive treatment, emerging data indicate that immunogenic cell death mediated by DNA-damaging agents may enhance the antitumor activity of immune checkpoint inhibitory antibodies [85]. For example, TAT is known to cause DNA double strand breaks in tumor cells and to induce production of cytokines and diffusible second messengers [86]. These mediators can have deleterious bystander effects on untargeted tumor cells, and activation of damage-associated molecular pattern (DAMP) receptors may augment functions of tumor-infiltrating lymphocytes, which are a necessary accompaniment of the antimelanoma activity of immune checkpoint inhibitory antibodies [87].

Preclinical and clinical data indicate that large macromolecules such as radiolabeled mAb can access the brain if the blood–brain barrier is disrupted by a tumor or if neoangiogenesis occurs [88,89]. Therefore, TAT may also be used to treat small-volume metastatic disease in the brain with minimal risk to surrounding normal brain tissue because of the short path length of alpha particles.

Convincing data support the clinical utility of ionizing radiation in the treatment of

localized uveal melanoma. Our preclinical and clinical studies of TAT directed toward the NG2 molecule, which is strongly expressed by UM cells, suggest that intralesional therapy using the ^{213}Bi -cDTPA-9.2.27 AIC may complement plaque or external beam radiotherapy in the treatment of localized uveal melanoma. Again, the properties of high LET and short path length increase the probability that TAT could increase local radiation dose without toxicity to nearby normal structures. Consequently, TAT could enable reduction of the radiation dose delivered by the other radiotherapeutic modalities, and thus limit the associated toxicity, the need for surgery, or both. Furthermore, systemic or regional TAT could be considered for the metastatic complications of localized uveal melanoma. In particular, the propensity for liver metastases may allow for clinical trials of ^{213}Bi -cDTPA-9.2.27 AIC via hepatic arterial infusion, which has provided a successful route for other internal radiotherapy [90].

Recently, the identification of perivascular invasion or angiotropism as an important mechanism for the development of melanoma metastases, particularly in high-risk ulcerated primary melanomas [91], indicates that TAVAT could provide a useful therapeutic rejoinder through targeting of the melanoma cells *per se* as well as pericytes.

Future clinical studies using an AIC of the 9.2.27 mAb that employs longer lived alpha-emitting radioisotopes such as At-211, Pb-212, Ac-225 and Th-227 may permit more sustained delivery of TAT than is possible with the 46-min half-life of Bi-213. Such new AICs would enable fresh clinical studies of systemic TAT using single and repeated doses and, in particular, more complete evaluation of the TAVAT hypothesis.

There remains an unmet medical need for the effective treatment of extensive and highly morbid loco-regional recurrences of cutaneous melanoma, particularly of the leg, which could be met by TAT. However, for patients with less extensive intransit metastases, carefully designed clinical studies of TAT might test the hypothesis that intralesional TAT eradicates proximal intransit metastases that could lie in the same lymphatic vessels as the injected lesion. If successful, these studies could be extended to use of perilesional TAT injections at the time that sentinel lymph node mapping with lymphoscintigraphy is indicated.

Many alpha-emitting radioisotopes also emit measurable gamma rays or beta particles during their radioactive decay, and any cytotoxic effects of TAT on lymph node metastases could be evaluated pathologically at the time of the subsequent sentinel lymph node biopsy (SLNB). Indeed, the risk of nonsentinel lymph node involvement escalates according to the extent of adverse clinicopathological factors present at the sentinel lymph node biopsy and has recently been expressed as a Non-Sentinel Node Risk Score (N-SNORE) [92]. The N-SNORE is used to decide the need for completion lymph node dissection (CLND), which has its own risks of significant morbidity. The accuracy of the N-SNORE has been validated in an independent and retrospective study [93]. This risk stratification scheme could be also used to define the clinical utility of TAT by assessing the cytotoxic effects at CLND of different TAT dose-escalation regimens in future clinical trials of perilesional TAT administered at the time of SNLB.

Finally, depending on the MCSP/NG2 expression profiles of certain melanoma subtypes, and the anticipated increase in activity with longer lived radioisotopes, other clinical trial opportunities may emerge. For example, 9.2.27-AICs could be considered for peri- and intralesional therapy of lentigo maligna melanoma and desmoplastic melanoma, both of which are radio-responsive and associated with high local recurrence rates [94,95].

Future perspective

This review describes the current status of anti-melanoma therapies and the potential value of systemic TAT. Based on its tumor localization, high cytotoxic potency and clinical evidence of antitumor activity, we expect that TAT might find a place in the future clinical management of this disease. Moreover, targeting alpha therapy to NG2 on tumor vessels may extend the opportunities for effective anti-melanoma therapy via direct effects on the tumor-feeding vasculature.

Future clinical exploration of melanoma TAT could focus on niche therapeutic indications. These include a role in the treatment of some kinds of metastases such as locoregional, liver, and cerebral, particularly once cytoreductive therapies have produced a smaller volume of disease. In this respect, TAT may be useful for melanoma cerebral metastases because the short range of alpha-particles minimizes normal tissue

toxicity. Intransit skin metastases may be susceptible to TAT alone if lesions are directly injected or if TAT can eradicate small clusters of melanoma cells in draining lymphatic vessels. TAT may be useful as intralesional therapy for uveal melanoma, and thus may complement current therapeutic modalities like radiotherapy or surgery. Some evidence that alpha-particles produce immunogenic cell death suggest that TAT could be used to augment the anti-melanoma activity of immune checkpoint inhibitory antibodies.

Conclusion

The primary objectives of alpha-immunotherapy are to kill targeted cancer cells, limit tumor progression and where possible eradicate the cancer. This is achieved by improved delivery and specificity of the targeting vector. Delivery is improved by selecting antibodies with the highest specificity, which in turn limits damage to normal tissues. The new generation of targeted therapies have advanced survival but relatively few patients have long-term survival. TAT offers a complementary approach that may further enhance survival. Published pre-clinical and clinical data suggest that alpha-radioimmunotherapy may best be suited to the management of isolated cancer cells circulating in the lymphatic and vascular systems, the regression of metastatic cancer cell clusters, and for disrupting the vasculature of solid tumors.

Another advantage of TAT worthy of further preclinical and clinical exploration is potential therapeutic benefits that may result from favorable interactions between immune modulatory properties of TAT and lymphocyte-mediated antitumor effects of immune checkpoint inhibitory antibodies.

Given that the maximum tolerated dose had not been yet reached in our studies, intralesional or loco-regional TAT for locally advanced melanoma or its loco-regional metastases offers the prospect of tumor control with minimal toxicity. Systemic TAT offers significant potential as a metastatic melanoma treatment that complements current cytoreductive therapies irrespective of the location of metastatic disease. Accordingly, we believe that further Phase I and II clinical trials of this promising therapeutic approach are warranted.

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