



Intratumoral Therapy I: Association of Immunotherapy with Permanent Long Term Cure of Metastatic Cancer

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Abstract

We have demonstrated in a randomized prospective study the superiority of intratumoral (intralesional) dinitrochlorobenzene (DNCB) compared to intratumoral (intralesional) bacillus Calmette-Guerin (BCG) in the treatment of progressive metastatic melanoma. The metastatic melanoma was in the form of satellitosis and/or in-transit metastases. We now demonstrate the ability of intralesional DNCB to permanently cure a selected group of patients with the same clinical criteria, and describe their clinical characteristics and treatment regimens. The described cured patients were followed for their remaining lifetimes, for up to 30 years, after being immunotherapeutically rendered free of metastatic cancer. They were each female, between the ages of 51 and 56 when intratumoral treatments were begun. Treatment was for progressive cutaneous and subcutaneous metastatic disease in the leg in two cases and for rapidly spreading scalp and forehead metastases in another. In each case the disease was not surgically controllable. Treatments were continued for 6 to 26 months. Subsequently the patients survived tumor free for either 18 years, dying at age 83; or for 24 years, dying at age 89; or for 30 years, dying at age 97.

There has been a significant interest in recent years in immunotherapy for advanced cancer. The absence of systemic toxicity in the cured patients reported herein provides a basis for consideration of combining intratumoral treatments with current effective but systemically more toxic immunotherapeutic approaches to some metastatic cancers. Intratumoral treatments may be particularly applicable in cases of uncontrollable melanoma with cutaneous metastases, without evidence of distant spread, as in the current report.

Keywords: Immunotherapy; Intralesional; Long term cure; Long term follow-up; Metastatic melanoma

Introduction

Generally speaking, whereas autochthonous antibodies to human cancer cells may have been high in specificity, such antibodies were frequently found to be too low in potency to be clinically effective. More recently, induction of cell mediated immunity has been more

promising, though technically difficult to establish. In any event, no immunologic treatment of cancer has heretofore been demonstrably able to permanently cure a group of patients, where cure is defined as long term (over ten year) freedom from recurrence, combined with demonstration in such follow-up that there is no recurrence for the rest of the patients' lifetimes.

Our previous randomized study did not answer the question of whether we could render any patients with melanoma satellitosis and/or in-transit metastases 'functionally cured' of melanoma with intralesional dinitrochlorobenzene (DNCB), where 'functionally cured' for this purpose refers to rendering a patient free of detectable melanoma during one to three decades of follow-up with no apparent melanoma during the post-treatment years of life. The long term follow-up and the special circumstances of the three selected patients reported herein help shed light on the issue of the ability of intralesional DNCB to be associated with regression and long term control of injected, as well as uninjected metastatic melanoma deposits. Furthermore the circumstances presented by these patients helped address the question of whether the injections are associated with developing immunity to melanoma. In addition, future potential therapeutic options are mentioned in light of recent advances in the treatment of metastatic melanoma.

Materials and Methods

Eligibility for treatment with intralesional DNCB in the selected patients reported herein and for the previous randomized trial comparing intralesional DNCB with intralesional BCG [1] was determined by the following criteria: 1) The patients had each undergone resection of a primary cutaneous malignant melanoma followed by local recurrences. The recurrences were in the form of multiple, discrete, progressive intradermal and/or subcutaneous and/or in-transit metastases, no longer surgically controllable. The term "intralesional" disease referred, in general, to metastases that were raised on the skin, and frequently pigmented. In the case of "subcutaneous" recurrences, areas of apparently normal skin were elevated over palpable melanoma deposits, giving the impression of solid lesions located beneath otherwise normal skin. In-transit metastases were a series of metastases apparently following a lymphatic channel toward regional lymph nodes. 2) Each patient had undergone multiple surgical procedures culminating in the opinion of the treating physicians that the local disease could not be controlled by surgery. 3) There must have been no prior immunotherapy, chemotherapy or radiation therapy. 4) The patient had to be less than 70 years of age, free of active infection, and accessible for follow-up. 5) The patient must have no evidence of spread past the regional lymph nodes; no patient with evidence of visceral or hematogenously disseminated metastasis was included. Radiologic evaluations of lung and liver, as well as blood chemistry panels were normal prior to initiation of intralesional (intratumoral) injections. In patients presenting with the above-mentioned clinical findings of apparently uncontrollable, progressive, locally metastatic melanoma, lethality was usually the result of progressive metastatic disease to the liver, lungs and/or brain.

In the previously reported randomized trial each patient had been randomly assigned to intralesional BCG or DNCB according to the last digit of a randomly assigned chart number. After we found in the previous study that intralesional DNCB was as effective as intralesional BCG, but with greater safety, and with an absence of serious side

effects, the use of intralesional BCG for this purpose discontinued. With regard to treatment with intralesional DNCB, although the randomized trial had showed that patients could be at least temporarily cleared of apparent melanoma metastases with intralesional injections, the question remained as to whether patients so treated, could be cured. The three patients reported herein were selected from a large cohort of intralesional treated DNCB patients, screened and qualified as mentioned above, with variations in technique and methodology, as will be mentioned below. They were selected based on their good outcomes, their availability for long term follow up and their demonstration of long term tumor free survivals, as measured in decades and lifetimes.

As in the randomized trial, because injections of DNCB in acetone were painful, they were preceded by injecting lidocaine 0.5% with 1:100,000 epinephrine into the anticipated intralesional injection site. We previously reported that patients receiving intralesional DNCB in the randomized trial were sensitized to the chemical two weeks prior to beginning the intralesional injections. This presensitization had been accomplished by the topical application of 2000 ug DNCB and of 50 ug DNCB, each dissolved in 0.1 cc of acetone and each applied simultaneously onto a site on the surface of the skin of the forearm. Two weeks later these presensitization applications elicited raised erythematous reactions at one or both of the application sites indicating sensitization to DNCB. However while involved with intralesional DNCB treatments in non-randomized patients, we found that the local efficacy of intralesional DNCB injections was apparently not diminished by eliminating the presensitization step. Accordingly we did not use presensitization in the three long term survivors described in this article, unlike our procedure in the randomized patients we previously reported. We continued to observe that the superficial intradermal metastatic lesions were easier to locally obliterate than the more deeply seated and subcutaneous deposits, as was true in the randomized patients. In the currently reported non-randomized patients it also became apparent that the best results with superficial metastases were achieved by administering the DNCB injection as superficially as possible within the tumor metastasis, ideally by creating a 'bubble'. The bubble was created by inserting the injected fluid just barely within the skin, even if such superficial injections were partly superficial to the gross metastatic deposits. Methodology was otherwise as previously described [1].

Case Reports

Patient 1

Patient #1 was 66 years old when she presented for treatment with progressive satellitosis and in-transit metastases in her right leg. Four years previously she had undergone elsewhere a wide excision and split thickness skin grafting for a Clark Level IV melanoma of her right calf. At that time, four years before presentation, she had also undergone a superficial right inguinal lymphadenectomy which had revealed no evidence of microscopic positivity in 14 lymph nodes that had been removed prophylactically. One month prior to her presentation with satellitosis, recurrence had been noticed in the skin of the right popliteal fossa, and slightly higher. Her surgeon had planned to do a local excision in the region but had desisted when, at the time of the skin incision, multiple metastatic deposits were grossly visible in the deep dermal and subcutaneous regions. Metastatic workup at the time of presentation revealed no evidence of distant metastasis. Two weeks after presentation, at the time of the first round of intralesional

injections, the patient was noted to have 14 metastatic lesions in the posterior thigh region. Each lesion was infiltrated with 0.5% lidocaine containing 1:100,000 epinephrine, followed in a few minutes with intralesional DNCB. Each lesion was individually marked and followed. It was later estimated that 13 of the 14 lesions injected on this round ultimately regressed. A month after the first round of injections the patient underwent a second round of injections into five lesions on the posterior thigh.

Four weeks after her second round of injections, a third round of injections was carried out for 10 lesions which had newly appeared. In addition, it was noted that the patient had a three centimeter mass surrounding the upper saphenous vein region anteriorly in the right groin. Accordingly the patient was taken to the operating room and an excision was carried out. There was extensive permeation of the soft tissue with residual melanoma, which was left in place at the termination of the gross excision. Seven of the 10 lesions that had been injected on the third round of injections were no longer apparent.

Five weeks after her third round of injections, the patient underwent her fourth round of intralesional injections. Eleven lesions were injected at that time. The lesions were new, and the patient's disease was felt to be progressive. Nevertheless, the 11 lesions that were injected were all felt to regress with follow-up. Four weeks after her fourth round of injections, the patient was felt to have only minimal progression in the preceding interval, and two lesions were each injected. Two months after her fifth round of injections, the patient was found to have three new subcutaneous nodules, two in the popliteal space and one in the right inguinal incision. These were each injected.

Following this sixth round of injections at age 67, six months after her presentation, the patient's disease progression halted, with no subsequent apparent local or metastatic melanoma. In the intervening 30 years between her last injections and her death at age 97, she remained apparently disease free with regard to her melanoma history: At age 70, approximately 3 years into this apparently tumor-free 30 year period, she suffered a mild expressive aphasia which later improved to the point where she could speak on the telephone and reactivate her driver's license. No apparent relationship was detected between her cerebral event and her melanoma history. A year after her cerebral event, at age 71, she broke her right hip, related to entrapment involving a closing automobile door. Nine years later, at age 80 she entered an independent living facility, where she later broke her left hip following an accidental slip and fall. She later suffered complications of her diabetes mellitus culminating in bilateral lower limb amputations. She developed progressive dementia, stopped eating, and ultimately died 10 days after her 97th birthday.

Patient 2

Patient #2 was 65 years old when she first presented with multiple melanoma satellite lesions on her left leg. She had a history of having noted a skin lesion on her left distal lateral leg for several years which had become raised. Three years before her presentation to us her primary melanoma had been excised, and she had undergone a prophylactic lymph node dissection, with removal of her superficial inguinal lymph nodes, which were reportedly free of apparent metastatic tumor. However following that left inguinal node dissection the patient had pain and dysesthesia in the left leg, presumably related to trauma to the cutaneous branches of the left femoral nerve. In the months prior to our seeing her, several lesions appeared including one on her left buttock, and two on her left thigh, each of which was shown by biopsy to contain metastatic melanoma. During the four months

prior to her presentation to us, several additional lesions had appeared, so that at the time of presentation, she had 13 lesions distributed in her left anterior thigh. After evaluation she was treated with intralesional DNCB, undergoing injections of 6 lesions in the left thigh region.

Ten weeks after presentation, 14 lesions were injected. Two weeks later, 7 more were injected. Four weeks later, and two weeks after that, 9 and 16 lesions were injected respectively. Three weeks later, 4 lesions were injected on the thigh, and one near the ankle. This was the first time that a metastasis had been detected in the vicinity of her primary site on her lower leg; with the other lesions all having been in-transit metastases proximal to the knee.

Two weeks later, five weeks after that, and 3 weeks after that, 6, 7, and 3 lesions were injected respectively, with the injections then ending some 7 months from the time of presentation. Subsequently, no further apparent lesions developed, and the patient remained well for the next 12 years. During that period of follow up there was no evidence of metastasis on repeated physical and radiologic examinations. She eventually died at age 89, with no presumed residual melanoma. At the time of her death it had been some 24 ½ years since the beginning of her intralesional injections.

Patient 3

Patient #3 was 62 years old when she saw her local physician in upstate New York because of a pigmented posterior scalp lesion. Biopsy at that time was originally read as a (benign) blue nevus. The diagnosis was later felt to be incorrect after a recurrent lesion at the same location continued to advance, becoming larger and bleeding upon combing her hair. The reoccurrence led to a rebiopsy, now one year after the original biopsy which had been read as benign. The rebiopsy revealed a diagnosis of melanoma, now with satellite lesions. A re-review of the slides from the first biopsy was then carried out. This reevaluation resulted in a change in diagnosis of the first biopsy from benign blue nevus to melanoma. The patient was referred to the Memorial Sloane Kettering Hospital in New York City and was prepared for urgent wide excision and skin grafting of her posterior scalp lesions. Upon anesthetizing the patient and shaving her scalp, she was found to have numerous melanoma satellite lesions as far anteriorly as her forehead. The operative procedure was cancelled and the patient was referred. Our initial radiographic evaluation for distant disease was negative, and remained so. She received intralesional DNCB injections of 2-10 satellite lesions every 4-6 weeks. On several occasions histologic or fine needle aspiration biopsy was carried out to continue to confirm the malignant nature of the lesions before intralesional injection. Biopsies were also carried out in order to confirm the absence of malignant cells in some post-injection inflammatory sites. These post-injection sites frequently demonstrated macrophages, granulomatous changes and inflammatory cells. Four months after her presentation to us, she was noted to have fewer than usual malignant lesions for injection, and received only 2 DNCB injections. However 1 month later she underwent 10 DNCB injections. One month after that she underwent DNCB injections of 8 malignant scalp lesions. Another month later she underwent intralesional DNCB injection of 10 malignant satellite scalp lesions. One and two months later she underwent DNCB injection of 8 lesions on each of the two visits. Five weeks later and 5 weeks after that, 2 lesions were injected on each visit. Chest x-ray and distant evaluations remained negative. The injection sites were being routinely pre-anesthetized with 0.5% lidocaine injections containing 1:100,000 epinephrine a few minutes before the injections with DNCB in acetone. Nevertheless the DNCB

injection sites were uncomfortable for 4-6 hours after the lidocaine wore off, frequently with slight residual "soreness" the next day. Her last-mentioned round of two injections took place 11 months after the beginning of her injections. No new lesions were apparent for the next 2 months. The patient reported that after this last mentioned round of two injections, there was residual scalp discomfort for 8 days. However there were no constitutional symptoms such as fatigue, fever, or malaise. Her strength and activity remained normal, including frequent vigorous dancing with her husband. She often brought photographs of her scalp taken between visits. She occasionally reported sensations in uninjected areas in the scalp, ear or mastoid regions. No new lesions were apparent for 2 months.

After a vigorous dancing episode some 9 weeks after her last-mentioned round of 2 injections, her left knee locked up, after which she was evaluated by her local orthopedist in Florida, to where she had moved from New York. He diagnosed her with sinovitis in her knee and treated her with steroid injections, followed later by a Medrol pack with decreasing oral steroid dosage. When she returned to us a week or so later, 2 new lesions on her scalp were biopsied, revealing melanoma satellitosis. Five weeks later, and five weeks after that, 2 and 3 more satellite lesions respectively were identified and injected. After the last-mentioned round of injections, the patient reported that there was mild facial swelling for a few days. No new malignant scalp lesions were apparent 5 weeks later or for a year after that, when she had a waxing and waning lesion at the vertex of the scalp. Fine needle aspiration biopsy was carried out in order to preserve the lesion for intralesional treatment. Following a cytological positive diagnosis of melanoma, the lesion was injected a week later, the last such lesion to be so injected, some 2 years and five months since the beginning of the intralesional injections.

On one of her subsequent return visits we performed a fine needle aspiration biopsy of a "subsuspicious" or "not very concerning" thickening in her left breast, which resulted in a cytological diagnosis of carcinoma of the breast. Near her then residence in Florida she subsequently underwent therapeutically successful excision and radiation therapy. Eighteen years after her last melanoma intralesional injection she died following complications of a fall. She was 83 years old. There was no apparent melanoma during the 18 year period following her last intralesional injection.

Discussion

The three functionally cured long term survivors reported herein were each female, and between the ages of 51 and 66 at the time intralesional injections began. The intralesional injections in the patients persisted for periods of 6 and 7 months, respectively, in the two patients with metastatic extremity melanoma (patients #1 and #2), and for 29 months in patient #3 with metastatic scalp melanoma. Patients #1 and #2 each had a primary lesion on the leg, below the knee. They each had undergone prophylactic groin dissection yielding pathologically negative lymph nodes.

Patient #1 eventually had metastasis as high as a 3cm mass in the femoral triangle, undergoing the only resection we carried out in these three patients, although her resection was grossly incomplete because of residual unresected malignant permeation of surrounding tissue. She also had uninjected subcutaneous disease behind her knee exposed during the aborted surgical procedure that led to her referral.

Patient #2 had biopsy confirmed metastasis from below her knee to as high as the soft tissue in the region of her ipsilateral buttock.

Patient #3 had satellitosis involving most of her scalp, and her upper forehead. She may have experienced a systemic side effect of the injections, consisting of a mild allergic reaction on one of the repeat injections of DNCB, as she reported that after returning from Bethesda, Maryland to Florida after a round of injections, she felt she had mild facial swelling for a few days. It is unclear whether this was a true allergic reaction or the settlement of edema associated with an inflammatory reaction into the loose tissues of her face. This was the only indication of a systemic reaction to the injections in these three patients, a finding consistent with our observation in the randomized trial of a lack of systemic side effects of intralesional DNCB, in marked contrast, for example, to the frequent and sometime serious systemic side effects from intralesional BCG, as we and others have reported [1-6], and in contrast as well to fatalities reported with BCG administration [7-9].

Toward the end of her intralesional DNCB injections, patient #3 also was clear of apparent metastases for about two months. Interestingly, around this time corticosteroids were administered to her in Florida by injections into her knee and by oral administration, after which, on her return to Bethesda, we found her to have clinically apparent scalp metastases during her next three visits, treated by intralesional injections, followed by a year of no further apparent metastases.

The three patients reported herein are highly selected, and their results, including long term survival free of apparent metastatic disease, are not typical. The substantial majority of patients with progressive melanoma satellitosis and/or in-transit metastases succumb to metastatic disease. Additionally, because melanoma is a tumor whose progression is not uniformly predictable, the question may be asked whether the good results reported in these three patients was a result of the intralesional injections or simply the result of the sometimes capricious nature of melanoma. Indeed, although melanoma accounts for only 2% of the invasive malignancies, it accounted in one study for approximately 11% of the cases of reported spontaneous regression of malignancy [10]. This suggests that melanoma is relatively prone to spontaneous regression compared to other malignancies. However, although melanoma may be more prone to spontaneous regression than most other tumors, long term spontaneous complete regression of biopsy proven metastatic melanoma is itself a relatively rare event; such rarity was illustrated in our experience, for example, by the fact that our report of such a case of documented long term spontaneous regression of biopsy proven hepatic metastases was, to our knowledge, the first such case to be reported [11]. There are also indications that we are not observing merely temporary partial spontaneous regressions in the patients treated with intralesional DNCB in this and our previous randomized study. Beside the rarity, in absolute terms, of spontaneous regression of melanoma in individual patients, we observed that patients treated with intralesional DNCB had a greater than usual incidence of pauses or halts in progressive cutaneous metastasis. Such halting was rarely observed in other contemporaneous patients who were not so treated. This leads us to believe that a degree of systemic antitumor immunization is achievable in some metastatic melanoma cases undergoing intralesional treatments, since the intralesionally treated patients had more frequent halting of disease progression than individuals not so treated. It is to this degree of systemic antitumor immunization that we generally attribute halting of disease progression when we have observed it.

In our report on the randomized trial we mentioned the ability of the intralesional injections to achieve local control in patients with intradermal, as opposed to subcutaneous melanoma metastases. However distant metastases nevertheless ultimately occurred in all the randomized patients, thus emphasizing the desirability of long term follow-up in order to make definitive outcome conclusions. Additionally, full local control was not accomplished in the randomized trial in cases where there was subcutaneous disease, emphasizing the usefulness of distinguishing intradermal from subcutaneous local metastases. In that regard it is noteworthy however that in our current patient #1, clearing of all apparent subcutaneous disease was accomplished.

Patient #1 demonstrated the achievement of control of three types of unremoved subcutaneous metastasis: Firstly she demonstrated the most commonly observed type of local subcutaneous metastasis, namely a series of easily palpable and typically visible subcutaneous lesions in her right leg and thigh. These were clinically apparent at the time of her presentation. Unlike the patients in the previously reported randomized trial, we were able to clear these clinically apparent metastases, which is to say that the metastases were no longer clinically apparent after intralesional injections. In this regard, it should be noted that we have previously reported, based on random biopsies in this post-intralesional injection setting, that residual melanoma was almost always present microscopically in a nodule when it was felt to be present on clinical examination; conversely microscopic disease was absent about three-fourths of the time when it was felt on clinical examination that there was no further melanoma in a nodule after intralesional injection [1].

Patient #1 also had a second type of subcutaneous metastatic disease controlled, in her case. She had uninjected, clinically occult subcutaneous lesions which became apparent behind her right knee only at the time of an incision for her surgical exploration, prior to her referral. As noted, the impetus for her transfer by her referring surgeon was his discovery of numerous metastatic deposits at the time he made the incision behind her right knee to remove a palpable mass.

As mentioned, the patient's numerous subcutaneous metastatic deposits behind her right knee were not clinically apparent to the referring surgeon before making the skin incision. After he aborted the planned procedure and referred the patient, we began her intralesional DNCB injections to other clinically apparent subcutaneous and intradermal lesions more proximal in her affected leg. Indeed no further attempted resections were carried out in the area behind her right knee and no injections were carried out in that area. Thus these clinically occult, surgically exposed metastases behind her knee represented uninjected metastatic disease that was functionally permanently controlled, if not obliterated, coincident with intralesional injections elsewhere in her leg.

A third type of metastatic disease in patient #1 was also managed without its direct injection, also coincident with intralesional injections elsewhere in her right leg. Specifically, as we were resecting a 3 cm mass of melanoma in the femoral triangle region of the right upper thigh, we left grossly apparent black residual metastatic disease in the deep soft tissue within the operated site. As with the surgically exposed uninjected metastatic subcutaneous disease behind her right knee, there was, during the decades of her long term follow-up, no clinically apparent consequence related to this uninjected unresected deep soft tissue metastatic disease in the region of the groin, although she did require later injection of a cutaneous lesion in the groin operative incision, followed by its subsequent disappearance.

We have reported that DNCB is capable of acting as a general enhancer of immunologic capability in non-tumor bearing mice, guinea pigs, monkeys and humans [12]. However for reasons already mentioned, it is nevertheless our belief that immunologic benefit derived following intratumoral injections of the type described herein is the result of enhancement of melanoma specific immune capability, rather than the result of general enhancement of immune capability. We also base this belief in part on the fact that as we performed serial immune testing in patients undergoing intralesional injections we found that of the eighteen patients in our randomized study, six patients who did not have reactivity to melanoma extracts before intralesional treatment, nevertheless, on retest after intralesional treatment, each had reactivity to inactivated melanoma extracts (1). To the extent that intralesional treatment is associated with specific antimelanoma activity, it may be useful to combine such tumor specific antitumor activity with treatments containing immune checkpoint inhibitors such as the anti-CTLA-4 monoclonal antibody ipilimumab, and/or the anti-PD-1 monoclonal antibody pembrolizumab, or similar agents. Also, intralesional treatment has historically been used primarily on surfaces such as skin and bladder lining; however with sophisticated radiological imaging and injection capability, potential metastatic targets within the body may become more feasible and accessible intralesional administration sites, although the consequences of a possibly intense local inflammatory reaction at the injection site must be taken into account.

Conclusion

In conclusion, we administered DNCB differently to the patients described in this report, compared to the administration methods described in our randomized trial. The patients described in this report did not undergo preimmunization with DNCB, and the DNCB injection fluid was administered as superficially as possible during the injection of intradermal melanoma nodules. Three patients were selected for reporting in detail herein, based on their unusual combinations of good results, long term follow up, and up to 30 years of apparent functional cure by immunotherapy. Such 30 year tumor free survival has not been previously reported to our knowledge in patients with metastatic solid cancer treated essentially with immunotherapy alone. Intralesional DNCB, without associated systemic toxicity, but with signs of antitumor efficacy, could be useful in combination with current effective but potentially toxic treatments associated with serious autoimmune side effects. Finally, because of the permanency that may be obtainable with an intralesional approach, other intralesional agents should continue to be subjected to investigation, as we [13] and others have reported.

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