

Adjuvant Therapy in High Risk Patients with Primary Cutaneous Melanoma: Past, Present and Future

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In spite of all the preventive measures, the incidence of melanoma of the skin continues to rise according to World Health Organization 2014. It is estimated that 132,000 patients are diagnosed globally each year. Therefore, cutaneous melanoma remains to be a worldwide health threat.

Surgery remains to be the most effective therapeutic modality in the management of primary lesions. However patient survival depends on the stage of the disease at the time of diagnosis [1]. In general, superficial primary melanoma lesions, less than 0.75 mm depth of invasion and without other risk factors can potentially be cured by wide local excision only. On the other hand, high risk patients include those with deeply invasive primary lesions over 0.75 mm depth of invasion, as well as those with ulceration or high mitotic rate at the primary site even without palpable regional lymph nodes or distant metastases. They should undergo sentinel lymph node(s) identification and biopsy to rule out occult regional metastases. Furthermore, patients with regional lymph node metastases, whether gross or occult, have very guarded prognosis and should undergo regional lymph node dissection followed by an effective adjuvant therapy to prevent recurrences and

metastases. Similarly, patients with resected distant metastases also need an effective adjuvant therapy.

As melanoma patients succumb to distant metastases, adjuvant therapy was administered systemically after excision of the primary (with and without regional lymph node dissection) to prevent or at least delay disease recurrence and metastases with the hope of prolonging survival. Adjuvant therapy with postoperative systemic administration of chemotherapy failed to show any benefit [2]. In addition, systemic administration of multiple chemotherapeutic agents with cytokines in patients with metastatic melanoma resulted in high toxicity and no benefit [3]. Therefore, melanoma was considered a chemo resistant tumor. However, hyperthermic isolated limb perfusion with melphalan or melphalan and tumor necrosis factor had resulted in over 60% response rate, mostly partial responses, with 25-26% of the patients having complete tumor response at 6 months [4]. This invasive approach is restricted to in-transit metastases of the extremities with limited success as most of the responders do develop recurrences and metastases within a year.

On the other hand, melanoma is an immunogenic tumor as it expresses various melanoma-specific antigens, a variety of peptides, gangliosides and has different genetic profiles. In addition, some adjuvant immunotherapeutic approaches had been supported by several factors: 1. the biology of melanoma, as regression of normal skin pigment in

the form of vitiligo has been noted in patients with melanomas as well as those receiving immunotherapy [5-7]. 2. Clinical responses are noted in melanoma lesions injected with microbial immune stimulators and monoclonal antibodies as well as with systemic administration of immune modulators and vaccines [8-10]. 3. The presence of mononuclear cell infiltrate at some primary and metastatic lesions may indicate the presence of cellular immune response [11, 12]. 4. Spontaneous tumor regression has been noted on rare occasion in patients with wide spread metastatic melanoma [13].

For the aforementioned reasons, adjuvant immunotherapy became the primary target for the research. These immunotherapeutic approaches include two main forms: biotherapy and tumor vaccines. Either approach was administered systemically by the intravenous route or subcutaneously.

Biotherapy with Cytokines

Postoperative administration of interferon alfa-2b is the most common adjuvant therapy used today. It is a cytokine administered intravenously at high dose for one year [14]. While the statistical analysis in this study showed modest benefit, other studies showed no benefit or only benefit in disease-free survival but not in overall survival. Statistics do not lie, but it depends on how to look at it. Looking at the survival curves of this study, it shows that 60% of the treated patients had recurrences and metastases and 50% died of their disease in the first 5 years of therapy. Therefore, it is very expensive and toxic with very limited benefit.

Another cytokine that was tested as adjuvant therapy is Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) [15]. It is administered subcutaneously daily for 14 consecutive days and repeated every 28 days for one year. The duration of therapy has been extended to 3 years. This program is less toxic and included patients with resected distant metastases, and gave almost similar results as interferon therapy.

In addition, it is of interest to mention InterLeukin-2 (IL-2) a cytokine approved for the treatment of metastatic melanoma and renal cell carcinoma.

Cytokines and Pathway Blockades

Recent advances in the treatment of metastatic melanoma have established a role for CTLA-4 blockade and BRAF inhibition, nurturing optimism that these agents may have a plausible role in the adjuvant setting. Combinations of immune modulators and pathway inhibitors are anticipated to be promising, and several Phase III clinical trials are being undertaken. Pegylated interferon- α 2b has recently been approved in 2011 on the basis of the results of the European Organization for Research and Treatment of Cancer (EORTC) 18991 Phase III trial demonstrating a sustained impact on disease-free survival in patients with metastases to the regional lymph nodes. In addition, the effectiveness of adjuvant therapy with ipilimumab (anti-CTLA-4) is assessed in the fully accrued EORTC 18071 trial. Adjuvant trials with BRAF and MEK inhibitors are in the planning phase. Furthermore, new schedules are also being explored with high-dose interferon- α and ipilimumab alone or combined with tyrosine kinase inhibitors. At present, several trials investigating combinations of novel agents with existing immune modulators are also underway [16].

Melanoma Vaccines

Most of melanoma antigens that are recognized by T lymphocytes remain unknown. Therefore, tumor cell itself is the best source for immunization. Several vaccines have been tried as adjuvant therapy:

Allogenic vaccines: These are prepared and easily available from banked tumor cells, cell cultures or tumor derived proteins. A) Active specific immunotherapy with Polyvalent Vaccine that consisted of 3 cultured cell lines and 18 melanoma associated antigens given initially with BCG as adjunct agent, then the vaccination was continued for 5 years [17]. This was administered postoperatively to patients with stage III disease (lymph node metastases) and resected stage IV disease (distant metastases). The results showed no benefit. B) Gangliosides Vaccines: Gangliosides are carbohydrate antigens formed of sialic acid containing glycolipid molecules and have increased surface membrane expression on cancers of neuro-ectodermal origin including melanoma. Those vaccines were

inferior to interferon alfa-2b [18]. C) Allogenic Melanoma Lysates: there are 3 types; viral, vaccinia and melacine: all failed to show any benefit and one of the authors suggested that further trials are not justified [19-21]. D) Peptide Vaccines: The best way to create an immune response is to induce tumor antigens into Antigen Presenting Cells (APCs) to process such antigens and present their product to cytotoxic T-lymphocytes. Tumor derived proteins, RNA or synthetically generated peptides, epitopes and DNA were used as antigen-specific for immunization. These were utilized in two ways; single peptide [22-24] or multi-peptides vaccines [25, 26]. These resulted in an immune response in the form of delayed hypersensitivity skin reaction or a rise in CD8+ cell markers in the peripheral blood to the given antigen, which has no bearing on the management of melanoma as there was no survival advantage. It was of interest that Doctor Rosenberg and his team reported that tumor progression can occur despite the induction of very high levels of CD8+ cytotoxic T-cells. However, the results of these trials revealed an important observation in that melanoma patients are immune competent as they responded to the administered antigen(s) and there is no role for general immune stimulation.

Autologous Cell Vaccine

This is prepared from patient own tumor. Therefore, only patients with large regional lymph node metastases or resected distant metastases could be treated on this program. Large tumor deposit is needed to cultivate enough tumor cells for the vaccine. We conducted a small feasibility study of 22 patients which resulted in 68% overall survival [27]. A larger study by Berd and his colleagues of 214 patients gave 58% overall survival [28]. In either study, patients who develop metastases, their metastases were resected and the patient was revaccinated by tumor cells from the newly resected metastases. Therefore, it seems that autologous vaccines are superior to any allogenic ones. However, over one third of the patients still succumb to their disease. Furthermore, this approach is only applicable to patients with large tumor deposit and requires special facilities to dissect tumor cells, irradiate and store these tumor cells in deep freeze until these are ready to be use.

In addition, the route of antigen or vaccine administration can be critical variable in determining the outcome of an immune response. In an animal model, when a vaccine with naked antigen-encoding RNA was administered in the skin, subcutaneous tissue or near a lymph node, no significant immune response was noted. However, when this vaccine was given in a lymph node, it elicited potent prophylactic and therapeutic antitumor immunity [29].

While melanoma is proven to be an immunogenic tumor, it seems to be very heterogeneous group of tumors that expresses various levels of a variety of antigens and peptides with different genetic profiles among different patients. To overcome such heterogeneity, autogenetic and tumor specific approaches in the form of autoimmunization is needed for each patient. This can be established by utilizing the melanoma site as a source for tumor-specific antigen for each patient and prior to its excision.

Intralesional Immunotherapy

Preoperative intralesional (intratumoral) administration of low nontoxic doses of two cytokines can induce autologous immune competent cells at the tumor site. These cytokines are Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and InterLeukin-2 (IL-2).

GM-CSF is a cytokine with multi-functional molecule. It is a hematopoietic growth factor produced commercially by recombinant DNA technology. Clinically, its intralesional administration can activate and stimulate autologous dendritic cells in vivo and increase their number. Dendritic cells are APCs that are efficient in processing tumor antigens and crosstalk to cytotoxic T-lymphocytes in the context of both Major Histo Compatibility (MHC) class I and II molecules [30, 31]. In addition, dendritic cells are rich in co-stimulatory factors such as B7-1 and B7-2 which are needed to complete the second immunological signal to T-lymphocytes which become committed to specific immune response. Dendritic cells can also increase the expression of interleukin-2 receptors on some subset of T cells. It has also been shown that local administration of GM-CSF increases the number and activation of dendritic cells and CD8+ T-cells in the sentinel lymph nodes

[32, 33]. When administered in in-transit metastases at 400-500 microgram /day for 4-5 consecutive days and repeated every 21-28 days, it gave high response rate but with some side effects in the form of leukocytosis and fatigue [34, 35].

IL-2 is an immune modulator cytokine that can stimulate the proliferation and function of T- lymphocytes, augment Natural Killer (NK) cells proliferation and activate cytotoxic T-cells triggering the release of various cytokines [36]. When given intravenously at high dose, it can induce Lymphokine Activated Killer (LAK) cells which are cytotoxic T-cells. Its intralesional administration can activate Tumor Infiltrating Lymphocytes (TILs) which are more cytotoxic to the tumor than LAK cells. When give at 0.6-18 million IU two to three times a week, it resulted in over 60% response rate but with 58% systemic side effects with the higher doses [37-39]. These two cytokines, each has different biological mechanism of action that can complement one another. The intralesional administration of GM-CSF can activate autologous dendritic cells, while IL-2 administration can activate cytotoxic T cells at the patient's own tumor site and its antigen. This could be an ideal approach.

Initially, we evaluated the response rate to each of these two cytokines in patients with in-transit metastases who had very guarded prognosis. All the patients were failure to previous therapy for their metastases with different therapeutic modalities that included repeated multiple excisions, intralesional BCG, hyperthermic isolated limb perfusion and combinations of these modalities. We chose reduced weekly doses of GM-CSE and IL-2 to avoid toxicity and identify any clinical benefit. The studied metastases involved the skin and subcutaneous tissues. All the patients received intralesional GM-CSF 500 µg in as many lesions once per week. If no complete clinical response was obtained in 4-6 weeks of therapy, intralesional IL-2 was substituted at 11 million IU weekly for the same length of time. 127 small size lesions, ranging from few mm up to 1 cm, were treated with GM-CSF and 110 had complete tumor response in 4 weeks. 17 lesions failed GM-CSF therapy and were successfully treated with IL-2 with complete response in 4 weeks. Most of the treated sites

were biopsied 6-8 weeks after cessation of the therapy and the pathological examination revealed no residual tumors or mononuclear cells infiltrate. Three patients developed new limited in-transit metastases in areas that were not previously injected. These were managed by local excisions only without any additional therapy. The survival of this group of patients ranged from 31-48 months. These data suggested that some melanoma lesions need the activation of dendritic cells, while other lesions require the activation of cytotoxic T-cell.

Therefore, we felt that a combination therapy of both cytokines could be more beneficial than either of them alone. A patient with deeply invasive cutaneous melanoma of over 1.9 mm depth of invasion and a 2mm satellite lesion located about 1 cm from the primary site with regional lymph node metastasis, received both cytokines sequentially at the primary site and the satellite: 500 µg of GM-CSF on day # 1, followed by 11 million IU of IL-2 on day # 2. A week later, the patient underwent the standard surgical procedure which included wide excision of the primary site including the satellite and regional lymph node dissection. The pathological examination of the resected tissue revealed complete tumor necrosis with massive histiocytes infiltrate at the primary site and the satellite. In addition, immunohistochemical studies showed over expression of CD8+ cells (cytotoxic T-lymphocytes) and CD4+ cells (helper T-lymphocytes) at the sites of injection as well as in some regional lymph nodes, compared to tissues from biopsy site (before intralesional therapy) of the same patient, and to lymph node tissues of a patient who did not receive preoperative intralesional therapy [40]. There were no side effects except for moderate skin reaction at the injection sites. This patient is alive free of disease for over 4½ years. The long term survival of the treated patients suggests a systemic therapeutic effect. These results strongly suggested an immense antitumor immune response at the primary site and satellite that seemed to be transmitted initially via the lymphatics.

As mentioned before, the induction of high levels of immune cells in the peripheral blood of patients with melanoma do not correlate to tumor response in the adjuvant setting [26]. It has also been shown that patients with resected metastatic

melanoma whose tumors have melanoma-specific tumor TIL cells had statistically better survival compared to those who have melanoma-specific T-cells in their peripheral blood, and at no time these immune cells are detected in the tumor and in the peripheral blood at the same time in any given patient [41]. This clearly indicates the questionable value of inducing immune cells in the peripheral blood. It has also been reported that the amount of TIL cells at the primary site can be an independent prognostic factor that reflects on the status of sentinel lymph nodes and patients' prognosis [42]. While intralesional administration of GM-CSF can activate preexisting autologous dendritic cells, the intralesional administration of IL-2 can activate autologous T-cells and stimulate preexisting TIL cells in vivo. Such combination can create specific antitumor autologous cytotoxic T-cells.

Intralesional administration of low nontoxic doses of GM-CSF followed by IL-2 prior to the surgical resection of small melanoma lesions could be an effective adjuvant therapy in high risk melanoma patients regardless to their tumor antigenic or genetic characteristics. This autologous approach utilizes patient own tumor, own dendritic cells and own cytotoxic T-cells in vivo can overcome tumor heterogeneity. It seems also to be more effective and cheaper than any other therapeutic approaches.

Finally, there is a need for prospective clinical trials for this new approach of adjuvant therapy.

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