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Intralesional Immunotherapy for Metastatic Melanoma: The Oldest and Newest Treatment in Oncology

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Abstract

The last few years have yielded exciting developments in immunotherapy for cancer. The promise of cancer immunotherapy has been well known for many years, but had generally produced limited or inconsistent benefit to patients. Intralesional therapies, which are in fact one of the oldest forms of immunotherapy, are also demonstrating benefits in the modern age. This review discusses the origins of intralesional immunotherapy and its underlying rationale. It also discusses the reemergence of this mode of therapy into the modern era, which is where Donald L. Morton, subject of this edition of the journal, plays a major role. The review also discusses current areas of investigation. Given the intuitive advantages of this strategy and the demonstrated, expanding areas of clinical responses, it is likely that intralesional immunotherapy will remain a useful component of cancer treatment into the future.

Keywords

melanoma; immunotherapy; intralesional

I. INTRODUCTION

For much of the last century, immunotherapy was considered “promising” by many oncologists and researchers. However, at the same time, it was actually used in almost no cancer patients. Over the last several years, that has changed dramatically with the approval of several new therapies and the likely approval of others. As with earlier, less widely accepted immune treatments, the newly developed treatments can lead to long-lasting or perhaps even life-long responses and control of even widespread metastatic disease. The initial areas of breakthroughs have come in tumors such as melanoma that have been resistant to cytotoxic treatments and have lagged behind advances seen in other solid tumors. These recent discoveries, however, are being applied successfully to other tumor types and immunotherapy may become the dominant form of systemic treatment for cancer in even the most common tumor types.

Although it is part of a modern revolution in treatment, immunotherapy has deep roots in oncology, dating back well over a century. These began with clinical observations of anecdotal tumor regressions and led to modest, but intriguing attempts at therapy. Intralesional therapies, in which an immune stimulus is applied directly to the tumor, were the first areas for initial therapy. A combination of the lack of knowledge of tumor immunology (or of the immune system in any form) and the lack of modern clinical

investigation doomed these early efforts to failure. However, in the modern era, interest in immunotherapies persisted and has now been borne out by more sophisticated preclinical and clinical studies.

II. RATIONALE FOR INTRALESIONAL TREATMENT

The attraction of immunotherapy for cancer is related to the power, specificity, and persistence of responses. It is now very apparent that the immune system can naturally recognize tumors as abnormal and mount immune responses to them and that these responses are clinically important. Two lines of evidence for this are naturally occurring immune responses that can be seen and measured in a number of tumor types and the effectiveness of treatments that work by boosting preexisting immune responses.

There is now ample evidence in multiple tumor types, not only that natural recognition of tumors occur, but also that strong immunologic recognition and response are associated with favorable clinical outcomes.¹⁻³ Examples include the presence of tumor-infiltrating lymphocytes in primary melanomas. Such infiltration to the edge, particularly within melanomas, is associated with lower metastasis rates and improved survival. Similarly, in ovarian cancer, large retrospective studies have shown clearly that patients with tumors containing lymphocytic infiltrates are associated with markedly improved outcomes relative to those patients without such responses. Perhaps the most striking, revolutionary change will be in colorectal cancer. In this disease, which has always been staged using the anatomic tumor-node-metastasis system, an analysis of immune infiltration by CD3- and CD8-positive lymphocytes to the edge and within the tumor is highly related to outcome.⁴ In fact, this relationship is so strong that, in the initial studies, this “immunoscore” overwhelmed and displaced the effects of almost all other anatomic staging variables from prognostic models. Therefore, this type of endogenous response is clearly important, but it is also apparent that, by definition, this natural immune recognition of tumors is insufficient in all patients with progressive, metastatic disease.

It is possible to strengthen these preexisting immune responses and establish clinical regression of established metastases. This is likely to play a role in clinical responses derived from intralesional therapies, including intralesional Bacillus Calmette-Guérin (BCG), but it is also critical to the effectiveness of many of the approved immunotherapies for cancer. These include interleukin-2 (IL-2), which serves as a growth factor for lymphocytes, and checkpoint inhibitors such as those blocking Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and Programmed Death-1 receptor (PD-1). These therapies have no direct effect on the tumor, but work through the patients' own lymphocytes, which must initiate the antitumor recognition on their own. Similarly, adoptive immunotherapy using tumor-infiltrating lymphocytes removes immune cells from the body and places them in *ex vivo* conditions that favor the growth of tumor-killing cells. Intralesional therapies work, at least in part, by inducing such immune-favoring conditions *in vivo*.

A. Autologous Vaccination

Although tumor vaccines have not met with consistent success to date, some clinical successes have proven the principal of enhancing antitumor immune responses as a means of

improving patient outcomes.⁵ One difficult question in vaccine development has been determining the optimal source of tumor antigen for vaccination. Approaches have varied from using peptide antigens, which are relatively simple to prepare but offer a very narrow range of targets, to autologous, whole-cell vaccines, which are quite difficult to produce and administer. Autologous, whole-cell vaccines are theoretically ideal because they offer a broad array of antigens that are certainly well matched to targets relevant to the patient.⁶ Intralesional treatments theoretically offer the advantages of autologous vaccines prepared *in vitro* while eliminating the need for complex manufacturing requirements. With this approach, immune adjuvants, which are given as a component of externally prepared vaccines, are applied directly to the tumor. This approach has a long history in oncology, as described below, and will likely have an ongoing role into the future.

III. HISTORY

The history of cancer immunotherapy began with direct local application of immune modulators. This concept is often attributed to William B. Coley, a New York surgeon, and the history includes the story of another figure central to current oncology in multiple ways. One of Coley's first patients, Bessie Dashiell, was a 17-year-old woman who died of metastatic sarcoma despite treatment by amputation.⁷ Coley was deeply distressed by her death and began his quest to develop curative therapies for cancer. Coincidentally, Dashiell was also a friend of John D. Rockefeller, Jr., who was also profoundly affected by her death, and its impact was part of the inspiration for his family's support for cancer research and the founding of both the Sloan-Kettering and Rockefeller University research programs. Coley sought clues from the literature and found examples of patients with unresectable tumors that regressed completely and permanently in the setting of severe infection.⁸ Such observations had also been made in the past and did not seem to incite surprise in earlier medical correspondence, including that of Anton Chekov. Chekov noted tumor regression in the relative of a colleague and stated "It has long been noted that the growth of malignant tumors halts for a time when this disease is present."⁹ This suggests that the phenomenon of infection-mediated tumor regression was recognized in the era before antibiotics and before any potentially effective cancer therapy was known.

Coley took the observation further by proposing the introduction of bacterial toxins to the tumor in an effort to induce the same type of response. He first described the process of application of such toxins in 1893.^{10–12} In that instance, a patient with an unresectable sarcoma underwent a series of injections of "Coley's toxins" (a mixture of killed *Serratia marcescens* and *Streptococcus pyogenes*), with subsequent regression over a period of several months. His research focused on improving methods of growing and purifying the most effective bacterial cultures to produce the toxins. Dosing was varied based on the intensity of inflammatory reaction induced by the toxins. Coley favored titration of the dose to induce a fever of 102–104°F.⁸ It appears that cultures resulting from severe or even fatal infections were favored.

Coley faced substantial opposition to the acceptance of this therapy. In fact, within a few years of his first report, the *Journal of the American Medical Association* published an editorial declaring the treatment a failure.¹³ Although Coley himself had ongoing success in

some, but not all, cases, the inability to standardize the manufacture of the toxins and the lack of prospective clinical trials to document its efficacy prevented the treatment from extending much beyond his own clinic. In hindsight, it is now clear that Coley's treatment almost certainly worked, providing long-term remissions in selected patients, but it has taken over a century to come to that realization.

After his death, Coley's daughter, Helen Nauts, continued to work for the development of the treatment, but the advent of other therapies, including radiation and chemotherapy, which produced more consistent—although generally temporary—benefits, made it difficult or impossible for her to succeed in promoting this earliest immunotherapy.

IV. THE MODERN ERA BEGINS

Although immunotherapy fell into nearly total disuse after Coley, interest in harnessing the power of the immune system continued in some research circles. Belisario and Milton investigated vaccinia virus injections in the 1960s and 1970s.¹⁴ Tumor vaccines in particular were an active area of research. The ability to induce tumor protection through transplantation or vaccination in animal models led some to consider that approach for human patients as well. One such investigator was Dr. Donald L. Morton (Figure 1), who was conducting research at the National Cancer Institute (NCI). Morton had developed a model of tumor vaccination using allogeneic whole cells administered with BCG as an immune adjuvant. His development reached the point of human clinical testing and he applied to the institute's ethics committee for approval to begin protocols. However, at about the same time, a controversy had emerged in New York after Chester Southam, a physician at the Sloan-Kettering Institute, administered live cancer cells to residents of a chronic disease hospital without their informed consent.¹⁵ This controversy generated a great deal of negative publicity and, perhaps partly due to that cloud of controversy, the NCI ethics committee was unwilling to approve Morton's vaccination protocol.

However, a patient presented for treatment at the NCI Surgery Branch with extensive in-transit melanoma metastases of her arm. This patient's other arm had been paralyzed by polio, and the proposed treatment of a forequarter amputation was not acceptable to her. Morton saw the tumor nodules in place and recognized that the situation was analogous to his proposed experimental vaccination model, although the patient lacked sufficient local immune stimulation to generate an effective response. Much like Coley, he hypothesized that application of an immune adjuvant, such as the BCG he had been using in his preclinical research, might convert those in-transit tumor deposits using an effective autologous vaccine. He began injections of BCG into the dermal and subcutaneous tumor nodules in that patient, who experienced a complete and durable response lasting many years. That successful case led to the use of BCG in many more patients. In the initial publication reporting results of this therapy, 91% of injected dermal metastases were seen to regress and, in 21% of patients, noninjected tumors regressed as well, suggesting a broader immunological effect.¹⁶ The striking nature of these responses and their durability led to a new wave of enthusiasm for immune treatments, especially BCG.

Several additional reports of successful application of BCG followed Morton's initial publication.^{17,18} Similar frequencies of response in injected lesions were demonstrated and regression of even distant metastatic disease was observed in the setting of injection of superficial metastases.¹⁹ It is fascinating, in the era of ipilimumab, to note that the regression of a lung metastasis after BCG injection into skin metastases was observed after initial progression of the distant site. The time course of this delayed response parallels almost exactly that observed in delayed ipilimumab responses that are seen frequently with current checkpoint blockade.

Unfortunately, these initial successes were quickly followed by significant problems. At that time, very high doses of BCG were injected, on the order of 1×10^9 colony-forming units (cfu), even in patients who were previously sensitized to the agent.²⁰⁻²² In retrospect, it is not surprising that that type of dosing would be accompanied by the risk of severe toxicity. Several reports of such toxicity followed, including reports of anaphylaxis, disseminated intravascular coagulation, and death. These dangers tempered the enthusiasm for BCG. Other agents were explored as alternatives, including injection of methanol extraction products of BCG and chemical irritants including dinitrochlorobenzene.²³

The combination of the development of BCG alternatives and the reported adverse effects led to general disuse of BCG. However, Morton, his trainees, and a few others continued to use and refine intralesional BCG regimens. These treatments have evolved toward using much lower doses of BCG and, in some cases, combination with other immunomodulators. The current regimen consists of initial sensitization of the patient to BCG at distant intradermal sites (lateral chest and lower abdomen). For patients who have a negative tuberculosis skin test, the total initial sensitization dose is 3×10^6 cfu. Additional sensitization may be performed before or with intralesional injections at lower doses ($0.15\text{-}1 \times 10^6$ cfu/mL, with ~ 0.1 mL per typical dermal in-transit metastasis; Figure 2).

It may be fair to say that intralesional BCG was impeded by being too far ahead of its time. Understanding of tumor immunology at the time of its development was rudimentary and clinical research had not yet evolved to the point where this treatment would have undergone the kind of clinical trials that might have led to its widespread adoption. However, for those clinicians who have followed Morton's example and continue to use BCG, it is an extremely cost-effective therapy that provides excellent clinical effectiveness in appropriately selected patients. Ironically, though, its low cost may be a major obstacle to more widespread adoption of the treatment: there is almost no potential for substantial cost recovery for pharmaceutical development of an agent that is inexpensive enough to be used as a vaccine in millions of patients worldwide.

V. CURRENT INTRALESIONAL THERAPIES

Even if BCG is only used by a select group of physicians now, the concept of intralesional immunotherapy is alive and well. Alternatives to BCG now include numerous cytokines, cytotoxic agents, oncolytic viruses, and other immunomodulators. Combination of these local immunotherapies with systemic treatments such as checkpoint blockade antibodies is of particular investigational interest.

A. Cytokines

Two cytokines are approved for the treatment of melanoma: interferon- α 2b (IFN- α 2b) and IL-2. Both of these agents have been examined in the intralesional setting. IFN- α 2b was used by von Wussow et al. in 51 patients with metastatic melanoma and at least one injectable lesion.²⁴ Highly purified or recombinant IFN was injected at 6 or 10 million units, respectively, 3 times per week. Forty-five percent of the patients had response at the injected sites and 18% had systemic responses. Toxicities were typical of the systemic administration of the cytokine, with about one in four patients requiring dose reduction. IFN- β has also been examined by Paul et al.,²⁵ who treated 20 patients with inoperable melanoma with a combination of the cytokine (3–5 million units, 5 days/week) and external beam radiation. Seventeen of the 20 patients had a response, seven of which were complete and often durable. Notably, several of these patients had relatively bulky disease, which is often less responsive to this type of intralesional treatment.

IL-2, when administered systemically, can induce long-term durable regressions of advanced metastatic disease. However, it is accompanied by substantial toxicity and can only be tolerated by relatively healthy individuals. High local concentrations can be achieved through direct administration into tumors while avoiding substantial systemic exposure and much of the typical toxicity. Radny et al. reported a series of 24 patients injected with between 0.6 and 6 million international units 2–3 times per week, with the dose varying by the tumor volume being injected²⁶; 15 (62.5%) patients had complete responses, with 91% of lesions responding. Toxicity was greatly reduced from that seen with systemic administration: only grade 1 or 2 in most cases. Weide et al. updated the series in 2010, with a total of 72 treated patients.²⁷ Complete response rates remained high (66.7%), as was overall survival at 2 years (stage IIIB, 95%; stage IIIC, 72%; stage IV M1A, 66.7%; and stage IV M1B/C, 9.1%). Interestingly, response rates to systemic chemotherapy among those who failed IL-2 were higher than expected at 36.7%.

Other cytokines, including GM-CSF,²⁸ IL-12, and IL-21,²⁹ have been examined in the intralesional setting. Although the response rates to these agents do not appear to be as high as with the above treatments, local administration of agents and combinations will enable exploration of multiple combinations and sequences in *in vitro* experiments with limited toxicity and little systemic exposure. In addition, there are other local immunomodulators that can be used, including the tolllike receptor agonist imiquimod.^{30,31} Imiquimod has been used as a single agent and in combination with both IL-2 and BCG, with very promising results demonstrated.^{32,33}

B. Oncolytics

Two oncolytic agents in particular exhibit a combination of the ability to kill tumor directly and to engender immune responses. One of these is Rose Bengal or PV-10.³⁴ This dye was evaluated in a phase 2 study among 80 patients with refractory stage III and IV melanoma. The treatment yielded a 51% response rate, with half of the responses complete. Both injected and noninjected lesions were seen to regress and toxicity was quite limited, mainly consisting of injection site reaction. The potential interaction of this local ablative treatment

with the immune system is suggested by the regression of distant sites of disease and is being investigated in translational studies now.

Another oncolytic therapy that is perhaps furthest along in clinical development is an oncolytic virus called *Talimogene laherparepvec* (T-VEC).^{35,36} T-VEC is a herpes simplex virus modified to attenuate its ability to replicate in non-malignant cells and for the production of GM-CSF. It was evaluated in a phase III trial that randomized patients to either T-VEC or subcutaneous GM-CSF. The trial met its primary end point of improved durable response rate and also demonstrated a strong trend ($p = 0.051$) for benefit in terms of overall survival.³⁷ Among the subgroup of subjects with stage IIIB and IIIC metastatic melanoma, the improvement in survival was marked and statistically significant. Combination trials of this agent with other immunomodulators are ongoing and may demonstrate synergy. Several other oncolytic viruses are also in development and clinical trial evaluation.

VI. CONCLUSION

During this remarkably exciting time of advances in immune treatments for cancer, it is important to remember that it all began over 100 years ago with intralesional therapy. Although this modality has waxed and waned in popularity and use over the intervening years, it appears to have a solid place in current and future treatment options. It is in part thanks to the initiative, efforts, and tenacity of Dr. Donald L. Morton that this strategy remains a part of our armamentarium. The theoretical advantages include direct application of an agent to the tumor site, which enables high local concentrations with limited systemic exposure and accessibility of tumor for biopsy and study. These characteristics make it possible to investigate numerous combinations and sequences of treatment with feedback both in terms of clinical response and translational correlative findings. Immunotherapy may be among the best routes forward to optimal treatments.

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ABBREVIATIONS

BCG	Bacillus Calmette-Guérin
cfu	colony-forming units
NCI	National Cancer Institute

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FIG. 1.
Donald L. Morton, MD.

FIG. 2.
Typical BCG patient and response to therapy.
(A) Pretreatment recurrence with adjacent skin graft.
(B) Inflammatory response to injection at 4 months.
(C) Resolved metastases with scars at injection sites at 10 months.