CANCER IMMUNOTHERAPY PRINCIPLES AND PRACTICE

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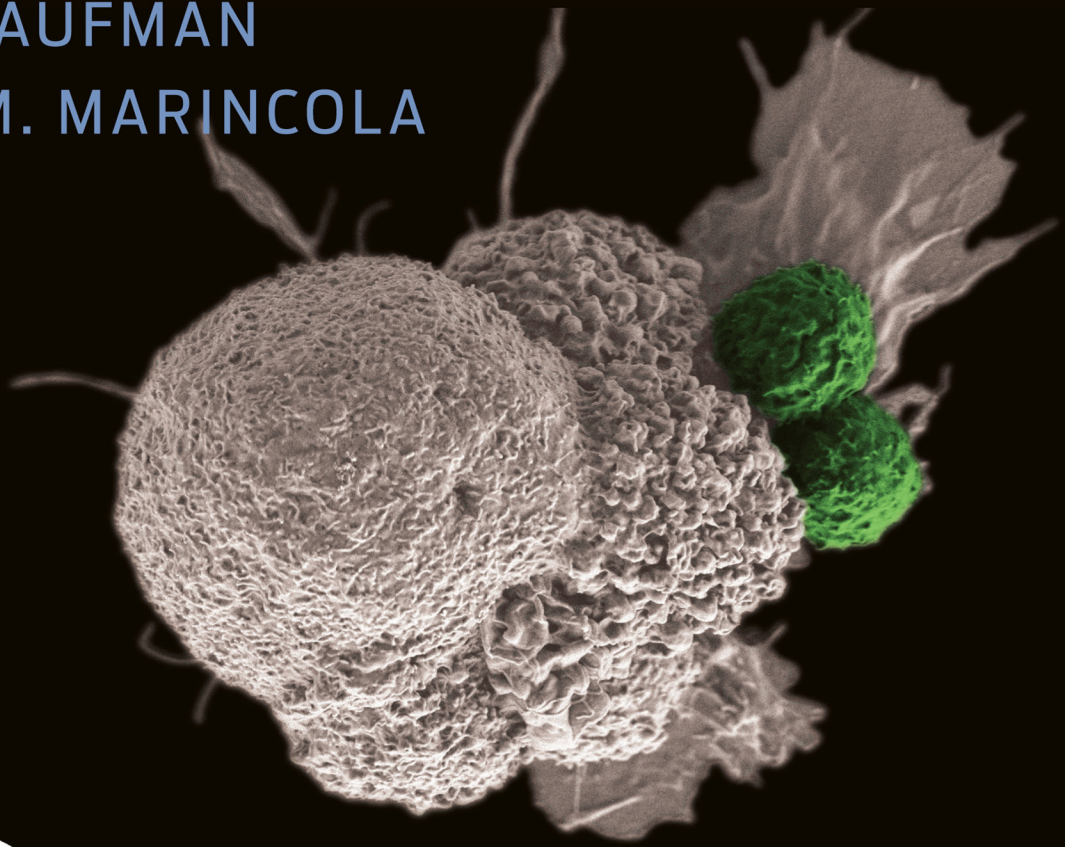

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Cancer Immunotherapy
Principles and Practice

Cancer Immunotherapy Principles and Practice

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Preface

After a prolonged germination phase, anticancer immunotherapy has blossomed and is producing a plentiful harvest. Just a decade ago, the field consisted of a passionate group of immunologists and a handful of oncologists and surgeons interested in a peculiar phenomenon: the occasional disappearance of advanced cancer in response to immune stimulation. It was reproducible enough to transcend the threshold of anecdotal insignificance and impart sufficient legitimacy to the field to sustain a miniature ecosystem. We were inspired by rare but concrete successes and we pursued the treatment of cancer patients in experimental settings when all other options had failed. There was no need for a textbook then, because we were a selected group of connoisseurs exchanging information at the Annual Meeting of the Society for Immunotherapy of Cancer (SITC, then called the Society for the Biological Therapy) or similar gatherings. And we held a primer at the Annual Meeting to introduce a handful of neophytes to the intricacies of immunologic responses against a tissue that was self and nonself at the same time.

Things have changed recently, with rapid developments in terms of scientific understanding and clinical outcomes. The identification of cancer-specific antigens recognized by immune cells and the mechanistic characterization of the interactions that modulate the cross-talk between neoplastic and immune cells gave molecular precision to a phenomenological description of cancer regression in mice, and less frequently in humans. The increasing occurrence of clinical responses with the new immunotherapy agents, whether checkpoint inhibitors or adoptively transferred immune effector cells, and the corresponding survival benefit for patients with advanced cancer, have awakened the interest of skeptics, whether scientists, clinicians, or industry partners. Hordes of oncologists, who were never trained in clinical immunology, are embracing this new modality of treatment and they need comprehensive training to deal with the unique pharmacodynamic profile and toxicity management of immunotherapy agents, which are distinct from standard chemotherapy. In addition, a young generation of basic scientists now perceives tumor immunology as a concrete opportunity to pursue a fruitful career bridging their knowledge with the tangible opportunity of impacting people's lives.

Moreover, the ever-growing speed of biomedical discovery relevant to anticancer immunotherapy unrelentingly spawns a wealth of candidate therapeutics requiring efficient clinical testing as single agents or in combination. Industry faces an unrealistic challenge, as it is hampered by the exponentially growing pipeline of candidate products that target not only cancer cells directly, but also their interactions with the host's immune environment. Drug development and respective clinical testing should be prioritized to optimize patient selection and reduce costs by enhancing the probability of successful outcomes. Nowadays, a wealth of candidate targets, resulting from high-throughput biomedical discovery, exacerbates the demand, particularly when innumerable combinations for the treatment of complex disorders such as cancer are contemplated; hence the need to identify evidence-based tools for prioritization based on discovery of useful concepts that could feed the development of novel precision-guided therapeutics. At the same time, a strategy to identify useful predictive and surrogate biomarkers is needed. The optimization of evidence-based study design will help manage the extraordinary cost of clinical testing by guiding the selection of optimally informed choices. In association with high-quality prospective correlative studies, this strategy will improve the design of novel, second-generation precision-guided therapeutics. In accord with the rapid development within the field, regulatory and payer agencies also need to keep pace so that more rapid approval of promising drugs and patient access to high-quality delivery of such agents is possible. Finally, the ultimate beneficiaries of these efforts, the patients and their families, are becoming increasingly empowered to make their own choices—but they will need guidance and a reference to make the best-informed decisions.

SITC is trying to respond to the exponential growth of educational needs from all these sectors by providing primers at the Annual Meeting, itinerant courses to clinicians throughout the United States (and abroad in the near future), expanding with topical meetings addressing specific questions related to the field, providing practical guidelines for patient management and policy

development, and informing on other themes as they emerge through the SITC portal to include as many up-to-date educational activities as possible. In this context, the SITC leadership decided to collate into an authoritative compendium as much information as possible, primarily targeting young basic and clinical investigators but open to all other constituencies.

It made sense that the current presidents of SITC, supported by the SITC staff, should take on the initiative. We tried to include many of them as contributors and we cannot thank them enough for their enthusiastic response. Chapters for textbooks can be painstakingly overbearing, but all contributors managed to complete their part, areas in which they are recognized worldwide as experts, to bring together cutting-edge insight that every translational investigator and practicing clinician needs to know about tumor immunology and immunotherapy. The textbook is divided into five sections: Basic Principles of Tumor Immunology, Cancer Immunotherapy Targets and Classes, Immune Function in Cancer Patients, Disease-Specific Treatments and Outcomes, and Regulatory Aspects of Cancer Immunotherapy. Each section has its own introduction and we will not dwell on the details here. Suffice to say that we tried to cover in these sections the continuum from basic principles to practical and clinically relevant information that could allow a critical understanding of the development and testing of novel therapeutics, companion diagnostics, or useful biomarkers, and could inform about the regulatory processes that support safe yet efficient commercialization.

In addition, a chapter on the history of immunotherapy was devoted to the recognition of those who pioneered and championed the field when it did not enjoy its current popularity to provide the reader with a better appreciation of its evolution.

We want to emphasize that the book is not meant to cover all aspects of tumor immunology. Indeed, the field is a compound science that includes two overlapping disciplines: immunology and cancer biology. Plenty of textbooks cover more basic concepts relevant to each of the two areas; in this textbook we tried to focus on converging concepts and peculiarities relevant to the relationship between the host and the neoplastic tissue.

Furthermore, we were concerned about producing a contemporary textbook as close as possible to the current status of the field. However, considering the rapid evolution of anticancer immunotherapy, particularly in the clinics, it is impossible to claim absolute success: The number of successful clinical trials and corresponding regulatory licensing initiatives are growing at an accelerating pace. Thus, this textbook aims at guiding the neophyte through a critical interpretation of upcoming results based on a solid understanding of anticancer immunotherapy concepts within the context of alternative treatments and the potential for their combinations. Because some areas are likely to progress more rapidly than others, we are planning to periodically publish ad hoc updates either as reviews in the SITC official journal—the *Journal for the Immunotherapy of Cancer* (*JITC*)—or more formal and detailed chapter updates and new editions through this publisher.

We hope that the readers, especially the young ones, will enjoy this book and find useful information to complement other SITC activities and that they will be inspired to become active members of the tumor immunotherapy community.

Lisa H. Butterfield, Howard L. Kaufman,
and Francesco M. Marincola

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Thank you all,

Lisa H. Butterfield, Howard L. Kaufman, and Francesco M. Marincola

Share
Cancer Immunotherapy Principles and Practice

1

History of Cancer Immunotherapy

William Kelly

Michael T. Lotze

Michael B. Atkins

KEY POINTS

- The first cancer immunotherapies used nonspecific immunostimulants with then-unknown mechanisms of action that rarely limited tumor growth but provided impetus for creation of the Biologic Response Modifiers Program of the National Cancer Institute (NCI; prior to 1980s).
- The second generation of immunotherapies utilized well-characterized recombinant cytokines including interleukin-2 (IL-2) and interferon alpha (IFNα). These agents were associated with substantial toxicity when utilized at effective doses, but demonstrated deep responses in less than 10% of patients (prior to 1990s). Other cytokines, including IFNγ, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, IL-25, and so on, failed to provide substantive benefit, although anecdotal responses were observed. These were the first effective immunotherapies.
- The third generation of immunotherapies utilized humanized and human monoclonal antibodies (mAbs) to cell surface receptor proteins present on tumor cells (human epidermal growth factor receptor 2 [HER2]/Neu, epidermal growth factor receptor [EGFR], etc) and were integrated into cancer care (prior to 2000s).
- Vaccination strategies using the available peptide, whole tumor, recombinant proteins, dendritic cells (DCs), and adjuvants were only modestly successful (heteroclitic glycoprotein [gp]100 vaccine with IL-2, anti-idiotype vaccines following effective chemotherapy, and long peptides for human papillomavirus [HPV] E6, E7 in precancerous lesions) (prior to 2000s).
- The modern era of immunotherapy launched with the extraordinary novel efficacy (and toxicities) of mAbs to immune checkpoints in patients with lung cancer, melanoma, renal cancer, bladder cancer, and head and neck cancer (2005–current).
- Current application of cellular therapies including chimeric antigen receptor (CAR) T cells and expanded tumor-infiltrating lymphocytes (TILs) engages the highly evolved communication network of immunity within the host and represents early emergent therapies that have been or shortly will be realized after substantial steadfast cellular engineering (1990s–current).
- The modern treatment of patients with cancer will integrate immunotherapy with conventional surgical, chemotherapeutic, and radiation oncologic strategies (the future).

The relationship between cancer regression and infection dates back to the 18th century, and perhaps earlier. Recognition of the relationship between erysipelas due to *Streptococcus pyogenes* and remission of tumors was first credited to W. Busch in 1866 and F. Fehleisen confirmed these results in 1882. In subsequent clinical work by Coley, the injection of toxins derived from bacteria (from *Serratia marcescens* and *Streptococcus*, Coley's Toxin) into cancer patients to induce systemic inflammation led to tumor regression in rare patients, many of them with what were called sarcomas and possibly melanomas

(also known as melanosarcomas in the past). The ability to capitalize on this observation was hindered by its rarity and the lack of understanding of the underlying biologic and immunologic processes. As a consequence, the field of cancer immunotherapy lay largely dormant for decades.

In the 1960s and 1970s, the reemergence of interest in cancer immune therapy was focused on the intratumoral and systemic administration of bacterial products or extracts such as Bacillus Calmette–Guérin (BCG) and *Corynebacterium parvum* (*C. parvum*), to nonspecifically enhance overall immune function. Although the activity of these intratumoral injections was well documented, further development was hindered by limited insight into the concepts of unique or shared tumor antigens, the nature of antigen presentation and T cell recognition, the biology of dendritic cells (DCs) and the factors involved in stimulating, expanding, and maintaining an immune response. Likewise, the critical alterations in cancer cells with either primary genomic instability (pediatric tumors, glioma, sarcomas) or secondary genomic instability (most adult tumors) subsequent to chronic inflammation were not fully recognized (1). Indeed, we now recognize that the *pas de deux* between the tumor and the immune response has been carried out for more than 7 to 10 years before the tumor is recognized clinically. Individual loss of major histocompatibility complex (MHC) molecules, immune editing of particular oncogene mutations (eg, p53), and immune infiltrate characterized by tumor-infiltrating lymphocytes (TILs) and tumor-infiltrating dendritic cells (TIDCs) are evidence of this ongoing dialogue. Indeed, the T cell response to tumor arises to recognize individual neoepitopes within the tumor, something abundantly apparent now (2), but shrouded in the past by lack of a deep understanding of either tumor immunology or cancer biology.

In the 1970s and 1980s, lymphocyte subsets and cytokines such as the interferons (IFNs) (3) and interleukin-2 (IL-2) (4) that induced activation and proliferation of T cells and natural killer (NK) cells when administered to patients were explored. Further, the advent of recombinant DNA technology enabled these cytokines to be produced in quantities sufficient to deliver supra-physiologic doses to patients with cancer (5). Studies with high doses of recombinant IL-2 (6) produced durable responses in a small subset of patients with either metastatic melanoma or renal cell carcinoma (RCC). Administration of recombinant IFNα prevented tumor recurrence in a subset of patients with high-risk melanoma (7), prompting Food and Drug Administration (FDA) approval of these agents—the first for immunotherapeutics.

These nonspecific immune activators were associated with significant toxicities and in many patients induced the activation of countervailing immunosuppressive properties, greatly limiting their therapeutic index and overall applicability. Efforts to expand on this early success, through the development of combination regimens, investigation of other cytokines (IL-1, IL-3, IL-4, IL-10, IL-12, IL-18, IL-25, etc), and application in individual other tumor types, were largely unsuccessful. Nonetheless, the initial studies with cytokines served as "proof of principle" that the immune system, if properly activated, could produce durable cancer control or "cure" in select individuals with specific tumor types, thus leading to sustained interest in the immunotherapy field.

The first evaluation of T cells as opposed to antibodies or cytokines was performed by Fefer, Cheever, and Greenberg (8) in the friend virus-induced leukemia (FBL3) murine lymphoma model. In late 1987, the results of adoptive transfer of lymphokine-activated killer (LAK) cells with IL-2 in 157 patients with advanced melanoma were first presented. LAK activity mediated by IL-2-activated NK cells and T cells has potent in vitro activity against non cultured fresh tumor. Combinations of IL-2 and LAK were found to have at best only modest advantage over IL-2 alone, with about 10% deep complete responses.

Identification of tumor antigens and insights into the biology of antigen presentation also yielded a large number of diverse and novel cancer vaccine trials. Sadly, most of the trials in patients with advanced disease produced low levels of objective responses, and most adjuvant trials eventually showed no meaningful benefit (9). Studies with TILs confirmed that immune cells, if isolated from the tumor microenvironment and expanded in IL-2, could recognize tumor-specific antigens and when readministered to patients with melanoma following lymphodepleting chemotherapy could induce tumor regressions in many patients whose disease was refractory to high-dose (HD) IL-2. The more specific application of TILs allowed substantial evolution in our understanding of the requirements for T cell therapy. The approach of using adoptive transfer of TIL involved the sequential identification of: (a) use of tumor fragments to allow egress of TILs, (b) specialized culture flasks to allow more effective gas exchange, (c) application of nonmyeloablative chemotherapy to enhance homeostatic proliferation of the adoptively transferred cells with concomitant ablation of the residual immunosuppressive cells, (d) more rapid expansion of cells early in culture (young TIL), and (e) identification and selection of neoepitope reactive T cells. In the setting of melanoma and anecdotally other tumors, objective response rates increased to as much as 56%. Together, these insights suggested that effective immunotherapy was frequently being limited by regulatory mechanisms for controlling immune activation and a potent immunosuppressive tumor microenvironment.

Subsequent research identified a raft of immunosuppressive factors, including cells, cytokines, and proteins (checkpoints), within the tumor microenvironment that

dampened an ongoing or induced immune response. Many of these factors, particularly the immune checkpoints, were presented on the cell surface and therefore were targetable with specific monoclonal antibodies (mAbs). Remarkably, targeting of these checkpoints, particularly the programmed death 1 (PD-1)/programmed death ligand-1 (PD-L1) checkpoint, led to unleashing of the tumor-directed immune response in the tumor microenvironment and producing tumor regressions in a variety of tumor types with manageable toxicity. These observations provided both an explanation for the previously limited effects of immunotherapy and a path on which to move forward. This chapter describes in more detail the history of immunotherapy, which laid the foundation for the current clinical promise of this approach and sets the stage for the more in-depth discussions throughout this textbook.

EARLY DAYS OF IMMUNOTHERAPY

Bacterial Toxins

If one were to trace the genesis of immunotherapy, the most appropriate place in the United States to begin might be in 1891, with William B. Coley (10). Dr. Coley was a bone sarcoma surgeon at the Bone Tumor Service of Memorial Hospital in New York City (10). In 1890, Dr. Coley lost a young patient to Ewing's sarcoma and had sought to discover a more therapeutic course of action. He learned of a patient seen at New York Hospital 7 years ago with a nonoperable neck tumor who had apparent resolution of his tumor following the development of erysipelas. Dr. Coley personally sought out this patient, a German immigrant in Lower Manhattan by the name of Stein. Years later, the patient still showed no signs of cancer recurrence. (A remarkably similar story launched the career of Steven Rosenberg, who had a patient with an inoperable gastric cancer at the Roxbury Veterans Administration [VA] who many years later, was found to have had a spontaneous regression.) Erysipelas due to *S. pyogenes* and remission of tumors had first credited to a German physician, W. Busch in 1866. Other physicians such as Diedier, Paget, Bush, and Burns had noted a similar relationship between infection and tumor regression (11). Armed with these examples, Coley began to experiment with bacterial mixtures and devised what was to be labeled Coley's Toxin, a preparation of chopped meat bouillon inoculated with *Streptococci* and *Serratia*, incubated for several weeks and then thermally sterilized (11). He injected his first patient with this mixture in 1891 and observed shrinkage in the tumor soon thereafter. Spurred on by this initial success, Coley continued his injections and by 1895 had treated 84 patients with some additional successes (11).

Despite this apparent promise, there was considerable suspicion in the scientific community about these observations for a variety of reasons, including: (a) Dr. Coley had poorly controlled and poorly documented patient follow-up (10) and (b) his toxins varied in preparation, effectiveness, and route of administration. As a consequence, he met with fierce resistance from many, including the head of Memorial Hospital, the famed pathologist Dr. James Ewing, who espoused the more modern application of radiation therapy. Interestingly, some even questioned if Coley's patients had actually had a diagnosis of cancer to begin with. Nevertheless, Coley's Toxin made its way into production in 1899 and was widely used for more than 30 years. Gradually, this toxin became less accepted as a useful treatment and in 1952 production was finally halted. Ten years later, the FDA denied recognition of Coley's Toxin as a proven drug, rendering its use illegal.

Though Coley's Toxin never regained its prior standing, his work was carried on by his children. His son, an orthopedic surgeon, continued to advocate use of the toxin as adjuvant therapy for patients with resected cancers. His daughter, Helen Nauts, a cancer researcher, tabulated hundreds of cases showing near-complete tumor regression and garnered enough support to found the Cancer Research Institute, which remains in existence today. Even a one-time rival, Dr. Codman, conducted a controlled study in 1962 showing dramatic response in 20 of 92 cancer patients. Coley, for all the scientific community's disbelief, was on to something and as a consequence has come to be considered by many the honorary "Father of Cancer Immunotherapy."

Ehrlich's Magic Bullet

Paul Ehrlich was a Jewish internist practicing in Berlin in the late 19th century (11). Early on he made contributions to the fields of histology characterizing granulocytes and mast cells. In 1885, Ehrlich began publishing his thoughts on the nature of cellular receptors (12). He argued that the uptake of oxygen and other molecules required specific receptors and that harmful compounds took advantage by binding to these receptors. In 1897, 3 years after Hermann Emil Fischer postulated his "lock and key" model for enzymes, Ehrlich proposed his "side chain theory" (13). This stated that cells expressed side chains on their surface and that these side chains had the ability to recognize and bind specific molecules, which he called "antigens." Side chain binding of antigens promoted the creation of additional side chains that were released into the extracellular fluid to counter the antigen (13). In 1900, Ehrlich renamed his receptive side chains "receptors" (13). Ehrlich began exploring the creation of chemicals that could target these receptors and how these chemicals could be made less toxic. He envisioned a

Zauberkugel, a "magic bullet" chemical that would bind only with its target and therefore have no toxic effects. Ehrlich's "magic bullet" concept was primarily focused on infectious etiologies in his time. His laboratory was able to synthesize the antisyphilis agent, Salvarsan, the first "synthetic chemotherapy" (13). Salvarsan and its derivatives were the preferential treatment of syphilis until the arrival of penicillin in the early 1940s (13). Years later, his idea of a chemical able to specifically bind cellular structures would have profound ramifications in targeted oncological treatment and presaged the discovery of antibodies that could specifically block receptor–ligand interactions.

MONOCLONAL ANTIBODIES

The ability of the immune system to recognize foreign compounds and living organisms, and to produce proteins (antibodies) that react with them, is one of the crucial means by which the body defends itself against disease. Although each antibody targets a specific antigenic target, in a typical immune response to an illness, many different types of antibodies are produced in response to the variety of antigenic targets presented to the immune system. The ability to administer large numbers of antibodies targeting a single antigen presented on a particular cell was seen as a potentially valuable approach to cancer treatment—the realization of the "magic bullet" proposed by Ehrlich.

The use of polyclonal antisera was limited by the inability to reproducibly obtain high-titer antisera to tumors, even though antigen expression was identified with several serological techniques in the early 1960s, including the first identification of carcinoembryonic antigen, alpha-fetoprotein, and even p53 using these techniques as cancer- "specific" antigens. Cell surface antigens expressed by human cancers also included altered glycosylation of molecules such as Muc1 (14) and a variety of targets that are overexpressed, mutant, or variably expressed compared with other tissues. A transformative technology was the development of mAbs championed by the Nobel Prize–winning Brit and Argentinian, George Köhler and Cesar Milstein (15). This was accomplished by fusing myeloma cells with antibody-secreting mouse spleen cells that had been immunized against specific antigens, this technique, called *somatic cell hybridization*, produced a series of fused cells called *hybridomas*, each of which was immortal and secreted a limitless supply of a single mAb. This pioneering research by Köhler and Milstein received the 1984 Nobel Prize in physiology or medicine and launched the era of antibody therapy. Interestingly, they promoted use of mAbs to help mankind and it was Croce and Koprowski (16) who demonstrated their therapeutic use, leading to several modern biotech companies developing these strategies for clinical use (Centocor, Amgen, Immunex, and Genentech).

Early efforts used murine monoclonal antbodies (17) that could bind and image tumor, but were limited in their effectiveness by their immunogenicity. Subsequent work was able to create mAbs that were either chimeric or fully human, enabling them to be administered to patients without themselves triggering an immune response against the antibody that produced both toxicity and their prompt elimination. The steps in transforming mAbs into agents for human use, the challenges in target and construct selection, and the crucial role of the immune system in antibody therapy were developed through efforts to target individual tumor molecules.

mAbs have proven to be powerful additions to the therapeutic armamentarium for a wide range of human diseases, including many types of cancer. The class of antibody most frequently used clinically is immunoglobin G (IgG). IgG is further divided into subclasses, each with unique and sometimes overlapping properties, including the ability not only to target and interfere with cell signaling but also to induce cell death through properties of their Fc domains (18).

The initial focus of antibody-mediated cancer therapy was to target cell surface proteins that are aberrantly expressed on tumor cells. Trastuzumab, pertuzumab, and cetuximab, mAbs that target the receptors of the epidermal growth factor family, have been FDA approved for the treatment of subsets of patients with breast or colon cancer. By directly binding to these membrane-bound receptors, these antibodies inhibit the receptors' activity, resulting in dampened function of the downstream signaling cascades. However, in addition to signaling blockade, some members of this family of antibodies can also mediate antibody-dependent cell-mediated cytotoxicity (ADCC) of tumor cells (19,20).

Other antibodies such as rituximab, targeting CD20 expressed on B cell malignancies, are also capable of inducing a signaling-mediated death. However, a growing body of work has demonstrated that both the variable and constant regions mediate the effects of rituximab by inducing complement-dependent cytotoxicity (CDC) and ADCC (21).

This information has led to the development of novel anti-CD20 antibodies selected for their superiority in inducing CDC and ADCC based on their physical properties that may alter binding with Fc receptors on immune effector cells (22). Other clinically useful direct targets of mAbs in cancer therapy include vascular endothelial growth factor (VEGF; bevacizumab) and receptor activator of nuclear factor kappa-B ligand (RANK-L; denosumab); however, efforts to target other proteins such as Smadc and

the insulin-like growth factor receptor have been unsuccessful.

The company IDEC was initially charged with developing anti-idiotypic antibodies to tumor-specific antibody molecules expressed on the cell surface (23). This was only possible in about a quarter of the patients with lymphoma, and Nabil Hanna reasoned that perhaps an antibody to all B cells might be useful, leading to the development and approval by the FDA of rituximab for certain B cell non-Hodgkin lymphomas, the first mAb therapy approved for treating cancer patients. Subsequent successes using similar approaches for targeting HER2/Neu (24) and the EGFR (25) required humanization of previously murine antibodies. A variety of toxin-conjugated antibodies, so-called bytes using antibodies to CD3 crosslinked to antibodies to other molecules such as CD19, and most recently the remarkable success of the checkpoint inhibitors targeting cytotoxic T lymphocyte–associated protein 4 (CTLA-4), PD-1, and PD-L1, make these the most versatile and effective anticancer agents. These checkpoint inhibitor antibodies function by essentially taking the physiologic brakes off of T cells, immune cells with innate cytolytic properties, enabling restoration of effective antitumor tumor activity. Checkpoint inhibitor antibodies have revitalized interest in immunotherapy and are proving to be the backbone for the lion share of immunotherapy research.

BCG and *C. Parvum*

The next advance in immunotherapy came during the 1970s from an unlikely source. The scourge of the 19th century, tuberculosis, would prove a bellwether for immunotherapy. In 1929, Raymond Pearl first reported an inverse association between cancer and tuberculosis in autopsy patients (26). Subsequently, in 1935, Holgren reported successful treatment of some gastric cancer patients with BCG (26). Years later, Rosenthal noted that the reticuloendothelial system could be activated by BCG (27). Armed with this information, Old et al in the 1960s showed that BCG had activity against experimental tumors in mouse models (28). Then, in 1974, Morton treated 151 patients with melanoma by direct intratumoral injection of BCG, and noted that a fourth of those patients remained free of disease for 1 to 6 years (29). According to Morton's initial report, 91% of melanoma nodules injected had complete regression and 70% of uninjected melanoma nodules had regression (29). These observations led Morton and colleagues to include BCG as a vaccine adjuvant administered to patients with resected stage III and IV melanoma, an approach that continued to be investigated into the early 21st century before it was ultimately shown to be inferior to BCG alone in phase III trials (29). These results, while ending the study of canvaxin, an allogeneic irradiated melanoma vaccine (see subsequently), left open the possibility that BCG injection, by itself, may produce beneficial effects.

Another area where BCG immunotherapy was actively investigated was in the treatment of non-muscle-invasive bladder cancer. Coe and Feldman (30) first described a delayed hypersensitivity reaction in the bladder following injection of live BCG in 1966 (26). Then, in 1976, Morales (31) reported on a clinical trial of nine patients with recurrent superficial bladder cancer given six weekly treatments of intravesical BCG. He observed a 12-fold reduction in bladder tumor recurrence compared with historical controls. A follow-up controlled study by Lamm et al (32) confirmed reduction in tumor recurrence with intravesical administration, leading to its FDA approval for treatment of carcinoma in situ of the bladder in 1989 (31), perhaps the longest period between the development of a drug (1920s) and its approval. This approval was expanded to include Ta or T1 papillary tumors of the bladder in 1998. Intravesical BCG continues to be used for the treatment of patients with recurrent superficial bladder cancer and although its precise mechanism of action is not fully understood, BCG continues to be a component of combination immunotherapy regimens for this disease.

Alongside BCG, *C. parvum* injection also generated interest as a potential cancer immunotherapy. Currie showed that intradermal *C. parvum* following cyclophosphamide produced complete and lasting regression in murine models (33). The time from chemotherapy to immunotherapy was noted to be critical, with a 12-day interval curing 70% of the mice (33). Despite this early data, a clinical trial in patients with melanoma comparing surgical excision with and without adjuvant *C. parvum* injections showed no survival difference at 3 years (34). However, a subset analysis of patients with melanomas greater than 3 mm in thickness showed a 73% 3-year disease-free survival rate in the *C. parvum* group versus 33% in the operation alone control group (34), suggesting a possible value of this approach in patients with higher-risk melanomas. This observation was never pursued in a subsequent trial and therefore remains to be validated.

CYTOKINES AND NONSPECIFIC IMMUNE ACTIVATORS

Cytokines, literally "cell movers," are secreted proteins that have pleiotropic effects, including regulation of innate immunity, adaptive immunity, and hematopoiesis. Distinct cytokines often have overlapping effects

providing a level of redundancy to the immune system; indeed, the characteristics of cytokines include redundancy, pleiotropism, synergy, and antagonism. The first cytokines identified for cancer treatment were the IFNs. The name *interferon* was adopted based on the ability of these agents to "interfere" with viral infection of cells. Subsequently, many characterized cytokines were referred to as interleukins because they were principally produced by and acted on leukocytes, primarily released following folding and posttranslational modification in the Golgi, and released following clipping of a leader sequence. They are joined by the tumor necrosis factor (TNF) family of cytokines as well as the "leaderless" cytokines, the transforming growth factor-beta (TGFβ) family of cytokines, high mobility group Box 1 (HMGB1), and the extended IL-1 family (IL-1α and β, IL-18, IL-33, and IL-37 and IL-38).

Cytokines play a critical role in the recognition, persistence, and elimination of malignancies by the immune system. Mice that are deficient in IFNγ, the type I or type II IFN receptors, or elements of their downstream signal transduction intermediates have a higher frequency of tumors compared to control mice (35,36). Thus, cytokines play a critical role in immune surveillance, further promoting consideration of their use as cancer immunotherapeutics. The development of recombinant DNA technology allowed production of cytokines in sufficient quantities to enable their utility as antitumor agents to be tested in the clinic.

Interferon

IFNα, initially referred to as leukocyte IFN, is comprised of a group of at least 12 distinct proteins (37) encoded by 13 distinct genes. These are IFNα1, IFNα2, IFNα4, IFNα5, IFNα6, IFNα7, IFNα8, IFNα10, IFNα13, IFNα14, IFNα16, IFNα17, and IFNα21. Only single copies of the other type I IFNs, IFN-beta (IFNB), IFN-epsilon, IFN-kappa, and IFN-omega genes are found, with the human IFNα gene family sharing 70% to 80% amino acid homology, and 35% with IFNB. Recombinant IFNα2a, IFNα2b, and IFNα2c differ by one to two amino acids and are the forms of IFNα that have been tested clinically (37). In the United States, IFNα2a is sold under the trade name Roferon (Hoffmann-La Roche, Nutley, NJ) and IFNα2b is available as Intron A (Shering, Kenilworth, NJ). IFNα2c is available in Europe as Berofor (Bender, Vienna, Austria). These compounds have never been compared in a randomized fashion; however, their spectrum of activity is likely to be quite similar. Soon after their development, these agents were tested in virtually every cancer type and showed reproducible efficacy in a few diverse settings. The antitumor effects were sufficient to support FDA approval for IFN in patients with hairy cell leukemia (HCL), chronic myelogenous leukemia (CML), RCC (in combination with bevacizumab), and as adjuvant therapy for patients with resected high-risk melanoma (38). Subsequently, IFNαs conjugated to polymer polyethylene glycol (PEG)–IFN, to increase the half-life and allow for longer dosing intervals and long exposure times, have been introduced (39). Pegylated IFNα2a (Pegasys, Roche) and pegylated IFNα2b (PegIntron, Merck) are the two forms of PEG–IFN that are available in the United States (40,41). These agents are widely used in combination with ribavirin in the treatment of hepatitis C and have recently gained approval as adjuvant treatment for patients with stage III melanoma (42).

IFNs have a pleiotropic mechanism of action. In HCL, CML, and RCC, IFN's mechanism of action appears to be more antiproliferative or antiangiogenic rather than immune based, as continued administration appeared to be necessary to maintain benefit. Although IFNs were initially viewed as breakthrough therapies in these three diseases, their use was soon superseded by agents that more directly inhibit relevant tumor cell pathways in each malignancy (eg, pentostatin, imatinib, and the VEGF pathway inhibitors).

In contrast, IFNs exhibited more typical immune effects in patients with melanoma. Responses to single-agent IFNs in patients with metastatic melanoma were observed in approximately 15% of patients, with those with low metastatic tumor burden responding best, perhaps presaging its clinical activity in the adjuvant setting (43). A maximally tolerated dose regimen of IFNα2B involving a 4-week intravenous induction followed by a year of subsequent maintenance was developed by Kirkwood et al and tested in the adjuvant setting in a series of Eastern Cooperative Oncology Group coordinated trials (44). A similar set of trials was carried out in Europe, largely involving lower or intermediate doses of IFNα2A (45–47). In the aggregate, these studies showed an approximately 1-year delay in median relapse-free survival, a 20% relative reduction in relapse, and an 11% relative reduction in death in patients with high-risk melanoma (48). This high dose (HD) IFN regimen showed the most robust impact on overall survival, leading to its FDA approval in the United States in 1996. This survival benefit, though small, was associated with autoimmune phenomena such as vitiligo, thyroid dysfunction, and increased titers of autoantibodies (49), suggesting a T cell–mediated immune mechanism of action and establishing these factors as hallmarks of effective immune therapy, at least in patients with melanoma. It is unclear whether a survival advantage or similar immune effects occur with lower or intermediate-dose IFN or PEG–IFN in patients with advanced melanoma, suggesting that the HD bolus induction period might

be essential to activating the critical components of the immune system.

The toxicities of IFN therapy can be broken down into five major categories: constitutional, neuropyschiatric, gastrointestinal, hematologic, and autoimmune. Constitutional symptoms are the most common, with more than 80% of patients in the HD IFN trials reporting fever and fatigue (50). Additionally, more than half report headache and myalgias (50). The majority of these symptoms can be controlled with nonsteroidal anti-inflammatory drugs (NSAIDs); however, severe fatigue often requires a treatment hiatus with a subsequent dose reduction for amelioration. This considerable toxicity for HD IFN, together with its marginal survival benefit, has increasingly limited its use even in the adjuvant setting and highlighted the need for more effective adjuvant treatments for patients with high-risk melanoma.

High-Dose IL-2

In 1976, Morgan et al demonstrated the existence of a growth factor present in the conditioned medium of lectin-stimulated human peripheral blood mononuclear cells that could indefinitely sustain the ex vivo proliferation of human T cells (51). Interestingly, this factor capable of expanding T cells was discarded because it did not expand leukemic cells, as the Gallo lab was seeking. A visiting Israeli scientist, Isaac Witz, was surprised that they could grow T cells and encouraged them to report their findings. This initial report was followed in short order by the isolation, biochemical characterization, and ultimately, the cloning of what was then termed T cell growth factor (TCGF) (52). Subsequently designated IL-2, this factor was shown to be a 15 kDa polypeptide made up of 153 amino acids, the first 20 of which form a signal sequence that undergoes proteolytic cleavage during secretion (as a member of the "leadered" group of cytokines). The molecule has cysteine residues at positions 58, 105, and 125, the first two of which form an intramolecular disulfide bridge. The third cysteine is not essential for biological activity and can be replaced with alternative amino acids to minimize polymerization and increase shelf life—an approach taken in the development of the recombinant human IL-2, aldesleukin (Proleukin) initially developed by Cetus, then Chiron, then Novartis, and now distributed by Prometheus/Nestle.

In addition to its proliferative effects, IL-2 induces a capillary leak syndrome allowing tissue entry of immune cells as well as the synthesis of an array of secondary cytokines, including IL-1β, IFNγ, TNF, IL-5, IL-6, and lymphotoxin (53). Several of these secondary cytokines are detectable in the circulation of patients with cancer receiving IL-2 immunotherapy (see subsequently) and thought by many investigators to contribute to the side effects of IL-2 (54). The biological effect of IL-2 arguably most pertinent to its use as an antitumor agent may be its ability to enhance the cytolytic activity of antigen-specific cytotoxic T cells (CTLs) and NK cells (55), promoting the increase in perforin and granzyme B.

Based on these in vitro studies, IL-2 underwent extensive evaluation as an antitumor agent in a variety of murine tumor models. In these models, IL-2—used either alone, in combination with other cytokines, or in conjunction with the adoptive transfer of various ex vivo–activated lymphoid preparations—was shown to be able to eradicate a wide range of local and metastatic tumors. Early studies demonstrated that IL-2 used alone could reduce or eliminate pulmonary metastases from methylcholanthrene-induced sarcoma and melanoma cell lines and that this antitumor effect was strictly dependent on the dose of IL-2 administered (56). In some animal models, tumor eradication by IL-2 administration resulted in immunization against the tumor. In other studies in which mice were immunized with DCs pulsed with tumor lysates, the concurrent systemic administration of IL-2 enhanced the efficacy of the vaccine (57). In several studies, the effects of IL-2 could be enhanced by the concurrent administration of LAK cells generated by culturing splenocytes ex vivo in media containing IL-2 (58). Mice bearing hepatic micrometastases from poorly immunogenic mouse colon adenocarcinoma-105 (MCA-105) or MCA-102 sarcomas or MCA-38 adenocarcinoma cells, for example, were highly responsive to treatment with the combination of IL-2 and LAK cells, but unresponsive to LAK cells alone and only partially responsive to IL-2. In pulmonary metastases models, NK cells could be eliminated at day (D) 7 following tumor injection, but this partially abrogated the effects of IL-2 therapy if eliminated on D3. Furthermore, when TILs were isolated, activated with IL-2, and tested in vitro for cytolytic activity against autologous tumor cells, they were shown to be 50- to 100-fold more potent than IL-2-activated splenocytes (LAK cells) (59).

Taken all together, this preclinical work laid the foundation for clinical studies with IL-2. Although initial studies with purified IL-2 showed limited efficacy, studies with recombinant human IL-2 (aldesleukin) enabled dose escalation to maximally tolerated doses, which when combined with LAK cells produced groundbreaking antitumor efficacy. In initial studies performed by Rosenberg et al at the National Cancer Institute (NCI) Surgery Branch (NCI SB), tumor responses were seen in more than 30% of patients with advanced melanoma and kidney cancer (56). This remarkable result generated substantial enthusiasm for the promise of immunotherapy, leading the Director of the NCI, Vincent Devita, to fund formation of the NCI Biologic Response Modifiers Program,

the creation of the NCI-sponsored Extramural IL-2 LAK Working Group, which subsequently became the Cytokine Working Group (CWG), and the formation of the Society for Biologic Therapy (SBT), which subsequently became the Society for the Immunotherapy of Cancer (SITC). The Extramural IL-2/LAK Working Group validated the initial results of the NCI SB with IL-2 and LAK and subsequently showed, in conjunction with the NCI SB investigators, that in contrast to mouse models, LAK cells added no significant clinical benefit. A compilation of phase II studies with HD IL-2 alone showed that it produced responses in approximately 15% of patients with advanced melanoma or RCC, with the responses, although infrequent, being extremely durable (29,34,60,61). In fact, long-term follow-up of responders on these initial studies established that patients still responding after 30 months rarely experienced disease progression, suggesting that they were likely "cured." These early studies led to the FDA approval of HD IL-2 for patients with metastatic RCC in 1992 and advanced melanoma in 1998.

HD IL-2, like IFN, is a nonspecific immune activator, and therefore its administration was associated with significant side effects (62,63). This toxicity was related to the release of secondary cytokines from activated immune cells that produced a sepsis-like cytokine release associated with fever, rigors, capillary leak syndrome, hypotension, and multiorgan failure. This has been called a systemic autophagic syndrome (64,65), as it is associated with reversible organ dysfunction rather than true parenchymal damage. Though this toxicity was quickly reversed by withholding treatment and, therefore, was manageable, it limited the use of HD IL-2 to patients with intact organ function treated at select centers skilled in the management of these side effects and prevented its broad application in malignancies besides melanoma and RCC. Currently, we presume that HDs are needed to exceed the capacity of T regulatory cells to consume the IL-2 and limit its access to T effector cells.

Attempts throughout the 1990s to develop a cytokine-based regimen with a better therapeutic index, through use of lower doses of IL-2, combinations of IL-2 with IFN, chemotherapy or peptide vaccines, efforts to dissociate the antitumor effects of HD IL-2 from its toxicity, or the study of more T cell selective cytokines such as IL-4, 6, 12, 18, and 21, were all largely unsuccessful (66–71).

Consequently, efforts in the early 21st century turned to trying to identify those patients most likely to respond, in order to limit the needless exposure to IL-2 toxicities of patients not destined to benefit. Studies in melanoma suggested that those most likely to benefit had a normal lactic dehydrogenase (LDH) (perhaps indicating the absence of tumor necrosis) (72) and an inflamed tumor microenvironment (perhaps indicating preexisting tumor-specific T cells). Further preliminary data suggested that in many patients, IL-2 tended to activate suppressor cells rather than effector cells (73) and that some genetically determined immune regulatory component, as evidenced by the frequent association of IL-2 response with autoimmune conditions such as vitiligo and thyroid dysfunction, was necessary to achieve benefit (74,75).

As in mouse models, TILs when isolated and expanded in vitro with IL-2 were shown to recognize melanoma cells. Re-administration of these TILs following lymphodepleting chemotherapy (presumably to eliminate immunosuppressive factors in the tumor microenvironment) produced antitumor responses even in patients whose disease had progressed following HD IL-2 (76). TIL/IL-2 therapy was labor intensive and required inpatient therapy, and thus was not readily translatable to most academic medical centers; nevertheless, it succeeded in revealing the addressable barriers for effective cancer immunotherapy and in identifying the critical role for reactivated tumor-specific T cells in the process.

Taken together, aggregate findings suggested that the immune system, when properly activated in the right host and tumor, could produce durable responses, and that immune cells within the tumor microenvironment recognize the tumor, although their tumor lytic activity was being thwarted by suppressive factors. These critical observations both kept the immunotherapy field alive and prompted the search for targetable immunosuppressive factors within the tumor microenvironment as a way of enhancing or unleashing antitumor immunity.

VACCINES

Prophylactic vaccines have changed the natural history of many infectious diseases; consequently, the development of vaccines that could stimulate the immune system to recognize and destroy cancer cells has been a major focus of immunotherapy research for decades. Many vaccine approaches have been tried, and several have shown promise in early phase trials compared to historical controls. However, with a few notable exceptions, randomized controlled phase III trials have failed to confirm the benefit of these vaccines relative to standard therapies, observation, or placebo. Nonetheless, examination of these approaches in the light of our recent understanding of tumor immunology provides insight into the likely limitations of previous vaccine approaches that can inform current and future cancer vaccine development.

Early approaches focused on whole tumor cell vaccines using autologous tumor cells. Although this approach had the advantage of exposing the immune system to all tumor-associated antigens, it meant that

each vaccine had to be individually made, increasing the cost and time needed to prepare these samples (77). Autologous whole tumor cell vaccines have universally failed to show benefit in phase III trials, including studies with GVAX (a granulocyte macrophage colony-stimulating factor [GM-CSF]-transduced autologous tumor cell vaccine) given alone or in combination with other agents (78,79).

In order to overcome these limitations inherent in autologous vaccination strategies, researchers have explored vaccines that induced immunity to shared antigens that are common on specific tumor lineages. These shared antigens included tissue differentiation antigens (eg, cancer testes antigens) that were reexpressed on dedifferentiated cancer cells from tumors of the same tissue or origin and less commonly on differentiated normal tissues and overexpressed or aberrantly expressed antigens. The latter include proteins such as HER2/neu and the epidermal growth factor receptor (EGFR), which are overexpressed in breast cancers and many epithelial cancers, and MUC-1, a heavily glycosylated protein that is expressed on the luminal surface of normal glandular cells, but is aberrantly expressed on the surface of many types of adenocarcinomas and thus conceivably not subject to immune tolerance. These latter antigens came to be known collectively as *cancer antigens*. Approaches to vaccinating against shared antigens included the use of mixtures of allogeneic tumor cells together with an immune adjuvant, or the direct vaccination against putative cancer antigens administered as whole proteins. Allogeneic tumor cell vaccines that showed some benefit in phase II trials but not in phase III trials include Canvaxin (a allogeneic whole-cell vaccine combining melanoma lines with BCG), Melacine (a similar polyvalent melanoma vaccine), as well as belagenpumatucel-l (an allogeneic tumor vaccine modified to limit secretion of TGFβ2) (77,80,81). As noted previously, Canvaxin showed exciting results compared with historical controls in patients with resected stage IV melanoma, with as many as 40% of patients remaining alive 5 years. However, in randomized phase III trials comparing Canvaxin to BCG alone in patients with resected stage III or Stage IV melanoma, the BCG alone control arm was found to produce superior survival (29). Taken together, these studies illustrate some of the pitfalls of using historical controls to assess the efficacy of adjuvant therapies, including, but not limited to, stage migration over time related to improved imaging techniques and patient selection, particularly the requirement for patients to be disease free on post-resection imaging. They also raised the possibility that certain vaccinations, in the absence of co-stimulation, might induce immune suppression or immune tolerance, rather than enhanced antitumor effects.

With the discovery of DCs and their ability to present processed proteins or peptides on MHC molecules to specific T cell populations (82), vaccination approaches shifted to the use of specific peptides (usually human leukocyte antigen [HLA]-0201 restricted) either alone or pulsed onto HLA-restricted autologous DCs or the direct injection of tumor DNA or RNA into DCs, enabling them to process and present either the shared antigens or all of the tumor antigens. Further, these 9 to 15 amino acid peptides were sometimes mutated to produce a substitution of an amino acid in order to enhance their antigenicity. Although vaccination strategies using these approaches have largely been equally disappointing, a couple of approaches have produced more promising results.

In 1996, Murphy published a phase I clinical trial showing that autologous DCs pulsed with prostate-specific membrane antigen resulted in cellular immune response and decreased prostate-specific antigen (PSA) in patients with advanced prostate cancer (83). Building on this approach, the sipileucel-T vaccine strategy was developed, which involved the use of an autologous antigen-presenting cell cultured with prostatic acid phosphatase linked to GM-CSF. A phase III placebo-controlled trial in patients with advanced prostate cancer treated with sipileucel-T showed a 4.5-month improvement in overall survival (84). Subsequent trials confirmed this overall survival benefit (85), leading to the FDA approval of sipuleucel-T for men with castration-resistant prostate cancer (86). Despite this reproducible survival benefit, the absence of efficacy surrogates such as clinical response, PSA decline, or laboratory correlates, together with the expense of producing an individualized autologous product, have limited use of this agent and hindered its further development.

A phase II trial conducted at the NCI SB involving HD IL-2 and a mutated gp-100 peptide vaccine in HLA $A2^+$ patients with advanced melanoma showed an overall response rate of 42%, significantly higher than what would have been expected with HD IL-2 alone (87). This result led to a multicenter randomized phase III trial of HD IL-2 with or without the gp100 peptide vaccine in a similar patient population, which showed an improved response rate (22.1% vs 9.7%), improved progression-free survival (PFS), and a trend toward improved overall survival (17.6 vs 12.8 months, $P = .01$) for the vaccine-containing arm (88). Although this result suggested that the immune response could be enhanced through specific peptide vaccination, the lower-than-anticipated response rate in the IL-2 alone arm and the failure of this same peptide to enhance the efficacy of checkpoint inhibitors such as ipilimumab (89) or nivolumab (90) has called into question the validity or at least the generalizability of this observation. Further, although many studies with peptide vaccines were successful at inducing high levels of antigen-specific T cells, this immune activation rarely

correlated with clinical benefit, suggesting that either the vaccine-specific T cells did not recognize these antigens in the context of the tumor, did not travel to the tumor, or were stymied once they reached the tumor microenvironment.

Recently it has become apparent that the immune system typically recognizes neoantigens/neoepitopes rather than shared antigens on tumors, and that tumors, survive by using a variety of means of evading the resultant immune response generated against these neoantigens. Taken together, this suggested that for vaccines to be effective, they must involve the autologous tumor cells and likely be combined with factors that sustain any induced response. This has led to strategies involving fusions of autologous tumor cells with DCs (91), direct tumor injection of substances that enhance tumor neoantigen expression (eg, genetically engineered oncolytic viruses) genes coding for Toll-like receptor agonists and IFN-gamma inducers (eg, stimulator of interferon genes [STING]; 92) or more recently neoantigen vaccines (93). It is noteworthy that a phase III trial (Oncovex Pivotal Trial in Melanoma [OPTiM]) involving intralesional administration of talimogene laherparepvec, T-VEC, a genetically engineered oncolytic herpes simplex virus expressing GM-CSF, to patients with metastatic melanoma, showed improved response rates and median overall survival compared to GM-CSF injections alone, resulting in its FDA approval in 2015. Furthermore, studies combining T-VEC with checkpoint inhibitors, either ipilimumab (94) or pembrolizumab (95), in patients with melanoma showed apparent improvements in antitumor efficacy relative to the single agents, suggesting a possible way forward for tumor vaccine therapy.

OVERCOMING IMMUNOSUPPRESSIVE FACTORS

Because of the largely disappointing data with vaccines and cytokines, increased attention was given during the 1990s to the concept that effective immunotherapy was limited by regulatory mechanisms for controlling immune activation and an immunosuppressive tumor microenvironment. Several factors were implicated, including inhibitory ligand–receptor interactions, which limited T cell activation and function (CTLA-4); immunosuppressive cytokines (eg, TGFβ, IL-4, IL-6, and IL-10); immunosuppressive cells (eg, Tregs, myeloid-derived suppressor cells [MDSCs]); and cell signaling disruption (via class 1 antigen loss, down modulation of T cell receptor [TCR] zeta chain expression and indoleamine 2,3-dioxygenase [IDO] secretion). Together or separately, these factors constrained immune-activating signals, contributed to tumor-induced immune suppression, and likely inhibited the antitumor immune response. In retrospect, and in view of the many known mechanisms for counteracting the stimulatory effects of cytokines and vaccines and for suppressing immune responses in the tumor microenvironment, it seems surprising that a subset of patients with advanced disease could respond so well to IL-2 and occasionally to a cancer vaccine.

Two clinical approaches that entered the clinic in the early part of the 21st century heralded the new era of cancer immunotherapy: immune checkpoint inhibitors and cumulative advances in adoptive cellular therapy.

Immune Checkpoint Inhibitors

The first breakthrough came from preclinical and clinical studies, which demonstrated the substantial antitumor effects of blocking inhibitory ligand–receptor interactions that served as physiologic brakes on the immune system. The first of these "immune checkpoints" to be targeted for clinical development was CTLA-4. Administration of antagonist antibodies to CTLA-4 (ipilimumab or tremelimumab) to patients with advanced melanoma led to tumor responses in 10% to 20% of patients (89,96). Ipilimumab prolonged median survival of patients with metastatic melanoma in randomized phase III trials (89), and like HD IL-2, the responses were sufficiently durable to produce a tail (20%–24% alive at 3 or more years) on the overall survival curve (97). This result led to the FDA approval, in 2011, of ipilimumab for the treatment of patients with advanced melanoma.

Observations made in clinical studies of anti-CTLA-4 generated new paradigms for safe management of patients and interpretation of clinical results from immunotherapy trials. Administration of CTLA-4 antibodies was associated with reactivated T cell immunity against many normal tissues, leading to a raft of immune-related adverse events beyond the thyroid dysfunction and vitiligo observed in patients receiving IL-2 or HD IFN (98). These included dermatitis, colitis, hepatitis, and hypophysitis. Algorithms were developed for successful management of these adverse events with corticosteroids and other immune modulatory agents which—interestingly, in contrast to their use in the context of cytokine therapy—did not appear to interfere with the antitumor immune response once it was established (99). Unconventional tumor response patterns were also observed, including initial progression of disease, so-called pseudo-progression, followed by clear-cut tumor regression, which in many cases persisted following treatment cessation. In addition, in a subset of patients who responded to anti-CTLA-4 and subsequently developed disease progression, a second "re-induction" course of anti-CTLA-4 could produce additional antitumor activity (100). These observations contributed to the finding that the

survival benefit from ipilimumab greatly exceeded what would have been anticipated based on its response rate or median PFS and suggested that landmark survival or "treatment free survival" might be better indicators of ipilimumab efficacy.

Efforts were subsequently focused on the discovery and targeting of other immune checkpoints that might be relevant to cancers in addition to melanoma. The discovery that PD-L1 on tumor cells served to suppress the function of PD-1 expressing activated CTLs in the tumor microenvironment provided a target that could be inhibited in a more selective way than CTLA-4. Targeting the PD-1–PD-L1 interaction provided a means of unleashing the suppressed T cell response in situ. Remarkably, as described in various sections of this book, antagonist antibodies against this single immune inhibitory pathway demonstrated unprecedented and clinically relevant anticancer activity in a subset of patients across at least 20 different types of malignancy (101,102). From late 2014 until the end of 2016, the anti-PD-1 agents nivolumab and pembrolizumab and the anti-PD-L1 agent atezolizumab received FDA approval for the treatment of patients with advanced melanoma, non-small cell lung cancer, kidney cancer, urothelial cancer, head and neck cancer, and Hodgkin disease, and many more approvals are anticipated in the coming years. Because PD-1 pathway blockers, as noted previously for ipilimumab, have their most profound impact on overall survival, virtually all randomized phase III trials conducted to date have shown superior survival related to standard therapies. Further, in contrast to CTLA-4 antibody therapy, the toxicities associated with anti-PD-1 blockade appear to be fairly minor, with less than 15% of patients typically experiencing any type of grade 3 immune-related toxicities. The tolerability of these agents has made it possible to consider combinations of anti-PD-1/PD-L1 with practically any type of therapy and their study in the adjuvant setting.

More than any other development in this field, the broad clinical activity of anti-PD-1 and anti-PD-L1 antibodies energized the pharmaceutical and biotech industry to initiate or expand preclinical and clinical research programs for cancer immunotherapy agents. As a result, several new immune modulatory agents advanced into the clinic, and a large number of combination trials were initiated, most based on blockade of the PD-1/PD-L1 pathway. The first combination to be tested involved nivolumab with ipilimumab. In phase I studies involving patients with melanoma, the combination produced rapid and deep tumor responses in more than 50% of patients (103), and phase II and III trials suggested that the combination was superior in terms of tumor response and median PFS to either single agent. Similar enhanced efficacy was suggested for the combination in patients with non-small cell lung cancer, kidney cancer, and bladder cancer (104,105).

Although overall survival data comparing nivolumab plus ipilimumab to nivolumab alone have yet to be reported, the current data serve as "proof of principle" that combination immunotherapy is superior to anti-PD-1 alone, thereby opening the floodgates for the study of various combinations in a multitude of diseases and settings. In 2016, there were 20 PD-1 pathway blockers in development, in 803 clinical trials slated to accrue 166,736 patients (Cancer Letter October 7, 2016). These staggering numbers aptly indicate both the excitement and promise attached to current immunotherapy research involving immune checkpoint inhibitors.

Adoptive Cellular Therapy

An alternative approach to overcoming the suppressive tumor microenvironment involved the administration of genetically modified autologous T cells targeting specific cancer-related antigens (106,107). This could be done in the form of TILs in which TCRs for a shared tumor antigen (eg, NY ESO1) or a tumor-specific neoantigen were inserted before being expanded in vitro in IL-2 and re-administered in large numbers following lymphodepleting chemotherapy. The function of these genetically modified T cells or tumor infiltrating lymphocytes could be enhanced by the cotransduction of stimulatory cytokines such as IL-12 or sustained through the coadministration of PD-1 pathway blockers. These approaches have produced dramatic responses in a few patients with a variety of individual tumor types (108,109), suggesting that this might be a key to treating patients with solid tumors in which the immunosuppressive factors in the tumor microenvironment could not be identified and/or blocked.

An alternative to modified T cell therapy is the adoptive cell transfer with T cells modified to express chimeric antigen receptors (CARs). CARs are receptors combining tumor-specific binding domains (single-chain variable fragment from a mAb) fused with T cell intracellular signaling domains (110). In 1989, Gross generated a chimeric TCR from a T cell constant domain fused to a 2,4,6-trinitrophenyl (TNP) antibody variable domain. He transfected these into a CTL hybridoma and observed functioning of this TCR. He also observed that the transfected cells expanded through IL-2 production and were cytolytic against TNP-bearing cells of different strains and species (111). This indicated that these T cells killed target-bearing cells in an HLA unrestricted fashion. Initial studies in mouse models showed promise, but the first clinical trials showed no reduction of tumor burden, likely due to lack of exogenous co-stimulation (110). Second-generational CARs were developed that included co-stimulatory molecules such as CD28,

4-1BB, OX40, CD27, and ICOS (110) within the chimeric transgene.

These second-generation CAR T cells have been created targeting a variety of tumor-related shared antigens such as CD19, CD22, and mesothelin, and studied in clinical trials. The most success has been seen with CD19 CAR T cells targeting CD19 expressing hematological malignancies. In 2010, Kochenderfer treated a patient who had heavily pretreated advanced follicular lymphoma with autologous CAR T cells engineered to target CD19 (112). The patient experienced a partial remission lasting 32 weeks with absent B cells and low immunoglobulins following treatment (112). In 2015, Porter et al reported on a series of 14 patients with relapsed or refractory chronic lymphocytic leukemia who were treated with autologous T cells transfected with the anti-CD19 lentivirus (113). They observed an overall response rate of 57%, with four complete and persistent remissions (113). Perhaps the most promising results, though, were observed in patients with acute lymphoblastic leukemia (ALL). Maude et al observed complete remission in 90% of patients, primarily children with ALL, treated with the anti-CD19 CAR T cells (114). Similar phase 1 studies at other institutions have confirmed this antitumor activity with overall response rates of 88% (115). CAR T cells have also been tested in patients with chemotherapy-refractory multiple myeloma targeting CD138, with four of the five patients having stable disease for longer than 3 months (116).

Treatment of patients with solid tumors using CAR T cells has been less fruitful. One study using Epstein Barr-specific T cells, engineered to express neuroblastoma disialoganglioside GD2, showed complete remission in 3 of 11 patients with neuroblastoma (117). Another phase I/II study used anti-HER2 CAR T cells on HER2-positive sarcoma (118). Of the 19 patients treated this way, four had stable disease from 3 to 14 months (118). Recently similar studies have been reported targeting the tumor-associated antigen IL-13 receptor alpha 2 (IL13Rα2) chain (119).

It has been hypothesized that many different factors could be contributing to the decreased efficacy of CAR T cells in solid compared to hematologic malignancies. First, T cells must travel to the tumor site, and this can be impaired if there are mismatches in chemokine or adhesion mechanisms (120). There is a concern that to enhance specificity and avoid off-tumor/on-target toxicity, CAR T cells must be directed against tumor-specific antigens (121). To date, most studies have focused on "self" antigens rather than tumor neoantigens (121). Once the T cells infiltrate the tumor, they must overcome oxidative stress, nutritional depletion, acidic pH, and hypoxia of the tumor environment (120). Soluble factors and suppressive cytokines secreted by tumor cells may also inhibit the T cells (120). Additionally, the tumor microenvironment may contain immunosuppressive immune cells such as regulatory T cells and MDSCs, as well as tumor-associated macrophages, mast cells, plasmacytoid DCs, and neutrophils (120). T cell activation-induced surface molecules, such as PD-1, might be expressed and negatively regulate the antitumor response (120). Given the nearly limitless possibilities for engineering the T cells ex vivo, a number of these potential obstacles will likely be addressed through additional modifications of the T cell product prior to therapy.

CONCLUSION

It has been a long and storied road to our current understanding of immunology. Initial observations by Coley and others that bacterial preparations could induce tumor regression were noted, but then fell out of favor for decades. Ehrlich's idea of a magic bullet for cellular receptors eventually led to the concept of employing mAbs to target specific surface proteins on tumor cells and eventually immune cells. The first substantial clinical benefits of immune inducing agents were seen with the local administration of BCG in the treatment of patients with skin metastases from melanoma or superficial bladder cancer. These results spurred interest in identifying approaches to activate anticancer immunity in a more systemic fashion.

Investigations with early immunotherapies, such as HD IL-2 performed within the NCI SB and the CWG (121,122), established that activated T cells could produce durable clinical responses and cures in a subset of patients with metastatic melanoma or kidney cancer. Subsequent research, also spearheaded by the NCI SB, determined that many tumors contained immune cells that, when reactivated and expanded ex vivo and readministered following lymphodepleting chemotherapy, could eradicate melanoma in 20% of patients not responsive to HD IL-2. These seminal observations sustained interest in cancer immunotherapy through a long period of frustration spanning a quarter of a century between 1985 and 2010 and spawned efforts to reactivate TILs in situ. These efforts were rewarded by the discovery and targeting of immune checkpoints, such as PD-1 and its ligand (PD-L1) and thereby unleash effective antitumor immunity. Antibodies against the PD-1/PD-L1 pathway have produced antitumor responses—with little toxicity—not only in patients with advanced melanoma and kidney cancer, but also in at least 20 other tumor types, revolutionizing both immunotherapy and the broader field of cancer therapy.

Combining CTLA-4 and anti-PD-1 antibodies produced antitumor activity superior to anti-PD-1 monotherapy in melanoma, establishing proof of principle that these immunosuppressive mechanisms were not

redundant, paving the way for further exploration of this approach and other combination strategies. The existence and power of these immunoregulatory checkpoints served to highlight why early efforts with immunotherapies were largely ineffective, while at the same time providing a means to potentially improve their efficacy. Further, the demonstrated power of unleashed antitumor immunity has fueled efforts to generate or expand TILs in less inflamed or non-PD-1 pathway-protected tumors or, failing that, to manufacture ex vivo antitumor immune cells that can be adoptively transferred into patients to aggressively attack tumor-associated antigens.

Therefore, despite its long and checkered past, the future of cancer immunotherapy looks incredibly bright. The current and proposed investigations with cancer immunotherapy promise to forever change not only the way we treat many (if not most) cancers, but also oncology in general.

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