YALE JOURNAL OF BIOLOGY AND MEDICINE 84 (2011), pp.371-380.

FOCUS: IMMUNOLOGY AND IMMUNOTHERAPEUTICS

Cancer Immunotherapy Takes a Multi-Faceted Approach to Kick the Immune System into Gear

Peniel M. Dimberu and Ralf M. Leonhardt

Department of Immunobiology, Yale School of Medicine, New Haven, Connecticut

Cancer accounts for about every fourth death in the United States, with approximately 1,500 people dying each day as a result of this disease. Despite some progress in the last decades, these numbers alone undoubtedly demonstrate the urgent need for new and more efficient treatments. Immunotherapy aims to activate an efficient immune response against tumors or even prevent cancers from occurring in the first place. It is a growing field currently flourishing with several successful trials, some of which have led to the recent approval of new anti-cancer drugs by the Food and Drug Administration (FDA†). This review addresses the manifold strategies that immunotherapy has taken in the past and discusses the most recent achievements in the field.

INTRODUCTION

With about 7.6 million cancer deaths worldwide in 2008 [1] and more than 570,000 cancer deaths projected to occur in 2011 in the United States alone (>1500 deaths per day) [2], cancer is clearly one of the most pressing health problems we face today. Although surgery, radiation therapy, and chemotherapy have been significantly improved over the past years [3], metastatic disease can rarely be controlled by these treatments and cures remain scarce [4].

To whom all correspondence should be addressed: Peniel M. Dimberu and Ralf M. Leonhardt, Department of Immunobiology, Yale School of Medicine, 300 Cedar Street, Rm. S670, New Haven, CT 06519; Tele: 203-737-2452; E-mail: peniel.dimberu@yale.edu, ralf.leonhardt@yale.edu.

†Abbreviations: FDA, Food and Drug Administration; ER, endoplasmic reticulum; MHC I, major histocompatibility complex I; CTL, cytotoxic T lymphocytes; Tregs, regulatory T cells; IL-2, interleukin-2; ACT, adoptive cell therapy; HBV, hepatitis B virus; HPV, human papillomavirus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus 1; APC, antigen-presenting cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; ADCC, antibody-dependent cell-mediated cytotoxicity; MART1, melanoma antigen recognized by T-cells; IDO, indoleamine-pyrrole 2,3-dioxygenase; PD-1, programmed death 1; CTLA-4, cytotoxic T lymphocyte antigen 4; NK cells, natural killer cells; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor.

Keywords: immunotherapy, cancer vaccine, monoclonal antibody, immune response

PMD is supported by an NIH Fellowship. RML is supported by a Cancer Research Institute Fellowship.

Promising recent developments suggest that cancer immunotherapy — modulating the immune system to target the cancer — may become a powerful new weapon in the arsenal of treatments that oncologists can offer patients. Immunotherapy offers several advantages to today's standard treatments. Activated and tumor-specific immune cells can reach areas that a surgeon cannot, and the immune system may, when appropriately stimulated, target even microscopic disease and disseminated metastases. Further, immunotherapy does not preferentially attack dividing tumor cells, as chemotherapy and radiation therapy usually do. Thus, cancer cells that are slowly dividing or quiescent — properties many believe are associated with cancer stem cells [5] — might be more efficiently targeted by immunotherapy. Depending on the approach, immunotherapy might strike more specifically against the tumor, thus lowering the damage to surrounding healthy tissue and preventing debilitating side effects that are nearly unavoidable with radiation and chemotherapy. It should nevertheless be noted that severe toxicity can be associated with some particular immunotherapies, such as systemic cytokine treatment [6] or immunoregulatory therapy using anti-CTLA4 antibodies [7] (as discussed later). Finally, memory cells can suppress the re-emergence of the cancer. Long-term control or even complete eradication of the disease is possibly the biggest promise that immunotherapy holds for the future, as induced anti-tumor responses have sometimes proven durable over many years, at least in a subset of patients [8]. This contrasts sharply with what is all too frequently observed with chemotherapy and radiation therapy, whose effect is often only temporary and eventually results in multidrug resistance [9].

The origins of immunotherapy may date back as far as 1774, when a Parisian physician injected pus into the leg of a patient with advanced breast cancer and subsequently observed tumor regression as the infection worsened [4]. Today, novel immunotherapies strike far more specifically and in a more sophisticated manner against cancers by targeting tumors through individual tumor antigens or disarming the tumor's defense strategies. Several recent immunotherapeutic trials demonstrate the impressive clinical benefit of many of these new treatments even for end-stage patients, raising hopes that the intensive research of immunologists worldwide is eventually paying off by delivering substantial progress in the fight against cancer.

MHC CLASS I-MEDIATED ANTIGEN PRESENTATION — HOW TUMORS BETRAY THEIR PRESENCE

Human cells constantly break down a fraction of their protein content, and some of the resulting peptides get translocated into the endoplasmic reticulum (ER) via the peptide transporter TAP. There they are loaded onto MHC class I molecules (MHC I) within the peptide-loading complex organized by the chaperone tapasin [10]. Upon binding a suitable ligand, MHC I dissociates from tapasin and migrates to the plasma membrane for display of its antigen to cytotoxic T cells (CTLs). Along the secretory route, some peptide exchange may occur, if the original cargo proves suboptimal [11]. A typical human cell might well present >10,000 peptides to CTLs at steady-state [12], thus reflecting a representative sample of the current cellular repertoire of proteins. If CTLs detect antigens of non-self origin displayed in the context of MHC I, they will attack and kill the presenting cell and secrete cytokines like interferon-γ that further augment MHC I-mediated antigen presentation. Killing by CTLs is typically achieved through release of perforin/granzyme-containing cytolytic granules or through the Fas-FasL pathway — processes that induce target cell apoptosis [13]. Critical tumor antigens include cancer testis antigens (e.g., MAGE family genes or NY-ESO-1), antigens derived from melanocyte differentiation factors (e.g., gp100, MART1 or tyrosinase), antigens encoded by mutated genes (e.g., oncogene-derived antigens), or antigens derived from proteins that are overexpressed in the tumor (e.g., HER2/Neu)

[14]. Cancer testis antigens are normally only expressed in male germline cells, which lack surface MHC I. Hence, their presentation in tumor cells, typically resulting from altered DNA methylation in various cancers, is truly tumor specific. This makes them not only useful as promising biomarkers [15], but this property also may be critical in the context of immunotherapy. Thus, induction of autoimmunity may be avoided by vaccination with peptides whose expression is strictly confined to cancer cells. Nevertheless, some of the most successful peptide vaccines are derived from melanocyte differentiation factors (g209-2M, derived from gp100, is discussed below). Mutated antigens typically have the disadvantage that they are unique to a particular patient and thus cannot be broadly applied in immunotherapy.

A comprehensive database providing information on tumor antigens presented by MHC I or MHC II can be found at http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm.

COMMON MECHANISMS OF IMMUNE EVASION BY CANCERS — HOW THE CANCER STRIKES BACK

Not surprisingly, tumor cells block the MHC I pathway at practically every possible step in order to evade an efficient immune response. For instance, down-regulation or even complete elimination of expression of TAP and tapasin are very frequently observed in tumors of diverse tissue origins [16]. Partial or complete loss of individual MHC I alleles, whole HLA haplotypes, or β_2-microglobulin (β_2m), an essential structural component of the MHC I molecule, are also sometimes found. Moreover, the interferon-γ induction pathway is often impaired in cancer cells [17]. All these measures cause a drop or even a complete collapse of MHC I surface levels and thus render specific CTLs unable to identify and attack their targets. As a backup mechanism, the immune system may then utilize natural killer (NK) cells, which are particularly designed to track down and kill surface MHC I-deficient tumors. Cancer cells, however, frequently attempt to abrogate NK cell-mediated killing in a variety of ways, including upregulation of molecules related to MHC I, such as HLA-E [18], shedding of NK cell activating ligands, or inhibitory cytokine release [19]. Many tumors also induce factors that directly or indirectly block potentially attacking T cells and NK cells in the tumor microenvironment like IDO or TGF-β [20]. IDO is a particularly interesting molecule, since it breaks down tryptophan into kynurenines and thereby counteracts T cells by starving them for this essential amino acid [21], while kynurenines additionally cause downregulation of activating NK cell receptors [22] and further inhibit T cells. Further, tumors frequently recruit regulatory T cells (Tregs) or myeloid suppressor cells that abrogate efficient T cell responses and induce a niche of tolerance allowing the cancer to grow unimpeded [20]. TGF-β might be one of the factors that play a key role in establishing a favorable protective tumor microenvironment, as this cytokine can induce the differentiation of Tregs. TGF-β may additionally have a direct suppressive effect on CTLs, by downregulating critical effector molecules such as perforin, granzymes, FasL or interferon-γ [20].

TUMOR IMMUNE EVASION AND THE OUTCOME OF ANTI-CANCER IMMUNOTHERAPY

The particular immune evasion strategy that an individual tumor adopts may determine whether and how it is going to respond to immunotherapy. Heterogeneity within a tumor or among different metastases can even lead to disparate responses within the same patient, with some lesions disappearing while at other sites disease progresses. Cancers displaying irreversible structural defects like HLA-haplotype loss or deleterious mutations in β_2m may be more difficult or impossible to target by some immunotherapies, while tumors with reversible defects like TAP downregulation, which can often be restored by cytokines, may be more sensitive to these approaches [17]. Future

therapies will have to take this into account, and efficient immunotherapy treatments may have to be tailored according to specific tumor phenotypes rather than to offer "one size fits all" solutions.

HIGH-DOSE IL-2 TREATMENT AND ITS INTEGRATION WITH VACCINATION AND ADOPTIVE T CELL TRANSFER THERAPY

Melanoma is a particularly immunogenic cancer, and many of its typical tumor antigens are known and have been extensively characterized. Also, melanoma is one of the deadliest types of cancer with grim 5-year survival rates of about 16 percent for patients with metastasis [2]. While chemotherapy gives very poor results [23], immunotherapy has been emerging as a promising novel approach. In order to induce or enhance a patient's anti-tumor immune response, immunostimulatory cytokine treatment was considered early on, although severe toxicity often posed limits to this strategy. Interleukin-2 (IL-2), a growth factor for lymphocytes like T cells and NK cells, proved particularly interesting and its toxicity appears to be manageable in many patients [24]. Although high-dose IL-2 produced tumor regressions qualified as an objective response in only about 13 percent to 17 percent of patients, unlike what is typically observed with chemotherapy, these responses proved durable in a subset of cases [8]. Based on this remarkable property, in 1998, the Food and Drug Administration (FDA) approved this cytokine as the first immunotherapy for metastatic melanoma.

Researchers have since sought to enhance the effect of IL-2, for instance by combining cytokine treatment with vaccination against tumor antigens. A number of different vaccines and vaccination strategies have been tested in patients, including DNA vaccination, peptide vaccination, vaccination with tumor lysates, viral transfer of antigens, or antigen delivery via dendritic cells [8]. A vaccine that proved particularly potent and superior over other vaccines in this context is g209-2M [24], a peptide based on an immunodominant epitope derived from the tumor antigen gp100. Strikingly, in a recent phase III trial, roughly three times more objective responses were observed in metastatic melanoma patients who had received this vaccine plus IL-2 versus those who had received IL-2 alone. Also progression-free survival was extended, and there was a trend toward longer overall survival in vaccine-treated patients [25].

Another powerful new tool in the repertoire of immunotherapeutic strategies is Adoptive Cell Therapy (ACT). In ACT, T cells are isolated from cancer patients and stimulated with tumor-antigen presenting cells *ex vivo*, before clones selected for strong anti-tumor immunity are massively expanded and infused back into the same individual [8]. Before transfer of the cells, patients typically undergo non-myeloablative, lymphodepleting preconditioning in order to eliminate immunosuppressive regulatory T cells and non-tumor specific bystander T cells, which might otherwise compete with the freshly transferred cells for cytokines. A further recent development of ACT even includes the option of genetically modifying T cells before transfer in order to drive the expression of selected high-affinity T cell receptors (TCRs). This might be particularly helpful if patients have no pre-existing tumor-reactive T cells. In 2006, researchers engineered patients' T cells to express a TCR targeted against the tumor antigen MART1. After reinfusion of those cells, some individuals indeed demonstrated sustained objective regressions of their melanoma [26]. Another alternative is to specifically isolate tumor-infiltrating lymphocytes and use those for ACT. Rates of objective tumor regression with this protocol have reached impressive percentages of 49 percent to 72 percent, with some patients achieving complete and durable responses [27].

TARGETING IMMUNOMODULATORY MOLECULES TO ENHANCE ANTI-TUMOR RESPONSES

The new "rising star" in melanoma therapy, however, is an antibody called ipil-

imumab (Yervoy®), directed against the inhibitory receptor CTLA-4 on T cells. CTLA-4 counteracts the activation of T cells, blocks their proliferation and IL-2 release, and thereby suppresses immune responses [28]. The molecule is critical for establishing peripheral self-tolerance and avoiding autoreactivity, but tumors exploit this characteristic relentlessly in order to fend off immune attack. Ipilimumab binds and blocks CTLA-4, and thereby augments T-cell mediated immunity. In a recent phase III trial, median overall survival was 6.4 months in patients receiving the gp100 vaccine alone, but it was 10 months in patients receiving ipilimumab along with the vaccine and 10.1 months in patients receiving treatment with ipilimumab alone [29]. In fact, it was the first randomized clinical trial demonstrating a statistically significant benefit on overall survival in metastatic melanoma patients [29]. This remarkable success prompted the FDA in 2011 to approve ipilimumab for treatment of metastatic melanoma — more than a decade after the last "new" drug for this cancer, IL-2, had been approved. Most importantly, a recent phase III trial comparing ipilimumab in conjunction with chemotherapy against chemotherapy plus placebo corroborated the striking clinical benefit of CTLA-4 blockade by significantly extending overall survival and causing higher survival rates in the group of ipilimumab-treated patients. In fact, after 3 years, almost twice as many patients in the ipilimumab-treated group were alive than in the control group [30]. Moreover, a combination of ipilimumab and IL-2 therapy may prove synergistic in achieving an even higher complete response rate [31]. Besides CTLA-4, there are other immunoregulatory molecules that future therapies might target like PD-1 or IDO. PD-1 is also an inhibitory receptor expressed on T cells, and tumors frequently express its ligand, PD-L1, in order to abrogate cytotoxic T cell activity. Recent trials suggest that targeting PD-1 might have very similar clinical potential like targeting CTLA-4 [32]. Judging from all the above, it might be a safe guess that disarming defense strategies of tumors will be a cornerstone of future anti-cancer immunotherapies.

STAYING AHEAD OF CANCER — STRIKING WITH PREVENTIVE VACCINES

Attempts to create vaccinations against cancer have taken many forms. One approach has been to vaccinate against viruses that increase the chances of developing cancer. The very first such vaccine was the hepatitis B virus (HBV) vaccine approved by the FDA in 1981 and is now part of the schedule of vaccines given to infants. The widespread use of this vaccine has dramatically reduced the rates of HBV infection and hepatocellular carcinoma (HCC). The most compelling data comes from a comparison of vaccinated and unvaccinated birth cohorts in a 20-year follow-up study of a universal vaccination program in Taiwan [33]. The study found an incidence rate of 0.57 per 100,000 person-years in those born before the start of the vaccination program but an incidence rate of 0.17 in those born after its start. Further, it was found that this dramatic decrease in HCC rates continued well into the adulthood of those vaccinated at an early age [33]. Another such preventive vaccine has been developed against human papillomavirus (HPV), which is responsible for causing virtually all cases of cervical cancer. Two well-known HPV vaccines that have made it to market in recent years are Merck's Gardasil® and GlaxoSmithKline's Cervarix®. Both vaccines target HPV types 16 and 18, which are responsible for more than 70 percent of cervical cancer cases (Gardasil® also targets types 6 and 11, which contribute to virtually all cases of genital warts) [34].

Today, researchers have identified several other viruses that are classified as being carcinogenic [35]. This includes hepatitis C virus (HCV), which, similar to HBV, can lead to hepatocellular carcinoma. Epstein-Barr virus is also implicated in several types of lymphomas and human immunodeficiency virus 1 (HIV-1) infection that can lead to Kaposi sarcoma. Currently, there are

no vaccines against these pathogens. However, it is likely that the development of an effective vaccine would indeed lower rates of cancer associated with these viruses.

NOVEL THERAPEUTIC VACCINES AIM TO ACTIVATE IMMUNE RESPONSES AGAINST ESTABLISHED TUMORS

In contrast to preventive vaccines, therapeutic vaccines attempt to coax the patient's immune system to respond to an existing cancer. The most promising new vaccines in this area are autologous tumor vaccines, in which antigen-presenting cells (APCs) are isolated from a patient and then cultured in the presence of a cancer antigen before being infused back into the patient. At this point, the APCs can present the antigen to cytotoxic T cells and activate them to attack the tumor. The first such vaccine to come to market is Sipuleucel-T (Provenge®), which targets advanced, metastatic prostate cancer and was approved by the FDA in April 2010. Sipuleucel-T is created by exposing a patient's dendritic cells to a fusion protein that is composed of prostatic acid phosphatase (an antigen found in 95 percent of prostate cancers) and granulocyte-macrophage colony-stimulating factor (GM-CSF) before reinfusion. Clinical trials in men who were unresponsive to androgen-deprivation therapy demonstrated an increase in median survival of 4.1 months compared to placebo (25.8 vs. 21.7 months). Further, 36-month survival rates for the Sipuleucel-T group were 31.7 percent compared to 23.0 percent in the control group [36].

Another autologous vaccine that has had strong Phase III clinical trial results is BiovaxID®, created against follicular non-Hodgkin lymphoma. BiovaxID® is created using a novel approach known as rescue fusion hybridization, where a tumor cell is fused to an antibody-secreting cell. This hybridoma then secretes a unique idiotype antigen that is unique to the tumor and can be injected into the patient to elicit an immune response. In patients who had experienced chemotherapy-induced remission of longer than 6 months, vaccination with BiovaxID® resulted in maintenance of remission for 44.2 months compared to the 30.6 months in the control group [37].

Despite the success of these vaccines, caveats that need to be addressed remain. One aspect that currently limits the further development of autologous tumor vaccines is selection of an appropriate antigen to target. While some antigens are unique to a tumor, often they are similar to those found on healthy cells and thus could result in adverse side effects. Further, some tumors exhibit great diversity in the antigens that could be targeted, rendering ineffective any vaccine that only targets one or two antigens. And the more antigens that need to be targeted, the more complex and difficult it becomes to develop a truly efficacious treatment. Therefore, cancers that express a unique and limited number of antigens are likely to be more susceptible to this approach.

MONOCLONAL ANTIBODY TREATMENTS DESIGNED TO TARGET AND BLOCK MOLECULES ESSENTIAL FOR TUMOR DEVELOPMENT/SURVIVAL

Eleven years after Georges Köhler and César Milstein developed hybridoma technology to produce monoclonal antibodies, the first therapeutic product was approved to treat transplant rejection in 1986. It took another 11 years before a monoclonal antibody therapy against cancer was developed. Rituximab (Rituxan®), which targets the B-cell marker CD20, was approved in the United States in 1997 and has found widespread use in not only treating B cell malignancies, but also B cell-mediated autoimmune disorders. This is particularly the case with rheumatoid arthritis, for which rituximab was FDA-approved in 2006 [38]. Although rituximab targets both normal and dysfunctional B cells, studies have found that patients treated with it do not exhibit an increased susceptibility to infection [39].

Today, monoclonal antibody treatments for cancer represent some of the most successful cancer immunotherapies. These

drugs function by targeting various proteins that cancer cells utilize for their survival and proliferation. For example, trastuzumab (Herceptin®), used in HER2-expressing breast cancer, facilitates downregulation of the HER2 receptor (overexpressed in about 30 percent of breast cancer cases) and induces antibody-dependent cell-mediated cytotoxicity (ADCC). Further, bevacizumab (Avastin®) binds and prevents the function of the angiogenic vascular endothelial growth factor (VEGF), whereas cetuximab (Erbitux®) binds to the epidermal growth factor receptor (EGFR). Despite their various mechanisms of action, all of these drugs have become part of the standard treatment protocol, in combination with chemotherapy and/or radiation [40], and for good reason as these drugs have been demonstrated to be effective in patients. For instance, clinical studies that compared chemotherapy alone with a combination of chemotherapy and trastuzumab found that combination therapy slowed disease progression, increased response rates, and increased median survival rates by 25 percent [41].

Bevacizumab was initially investigated and approved for use against colorectal cancer after clinical studies demonstrated an increase in median survival of 20.3 months compared to 15.6 months in the control group [42]. However, it has recently been found to be effective against several other cancers, including recurring glioblastoma multiforme, for which it received FDA approval in 2009 [43]. Cetuximab is another antibody that was initially approved for use against metastatic colorectal cancers, in which EGFR overexpression is found in as much as 80 percent of cases [44]. Another mutation common in colorectal cancers is that of the proto-oncogene KRAS, found in approximately 40 percent of cases. Clinical studies involving cetuximab found that patients with the KRAS mutation did not respond to this antibody treatment and current guidelines call for cetuximab's use in KRAS-wild type patients only [45]. More recently, cetuximab was approved for use in squamous cell carcinoma of the head and neck. Long-term follow-up studies have found a 45.6 percent 5-year survival rate when cetuximab is combined with radiation therapy, compared with a rate of 36.4 percent when radiation is used alone [46].

RADIOIMMUNOTHERAPY — SEEK AND DESTROY

Radioimmunotherapy is a treatment that involves conjugating a radionuclide to an antibody that targets cancer antigens. The specificity of the antibody targets the toxic radionuclide to the cancer cells with minimal damage to healthy cells. Currently, there are two FDA-approved drugs on the market, ^{90}Y-ibritumomab tiuxetan (Zevalin®) and ^{131}I-tositumomab, both of which are used against B-cell malignancies [47]. Ibritumomab tiuxetan is an IgG1 anti-CD20 antibody conjugated with ^{90}Y and is used to mainly treat non-Hodgkin lymphoma. In clinical trials that compared it to rituximab, it was demonstrated that ibritumomab elicited a higher response rate compared to rituximab alone (80 percent vs. 56 percent) [48]. Ibritumomab has also been shown to slow the progression of disease in patients experiencing a relapse, including those who had a strong initial response to rituximab treatment [48].

Similarly, ^{131}I-tositumomab is an IgG2a anti-CD20 antibody used to treat refractory or relapsed, low-grade lymphoma [49]. In clinical studies comparing it to ^{90}Y-ibritumomab tiuxetan, ^{131}I-tositumomab was demonstrated to be nearly as efficacious as ^{90}Y-ibritumomab tiuxetan, but with a significantly less severe decline in a patient's platelet counts [49]. As these two drugs were approved between 2002 and 2003, it remains to be seen if more promising therapies using this approach will make it to the clinic in the near future.

CONCLUDING REMARKS

Recent promising clinical trials justify hopes that immunotherapy could become a keystone of future cancer treatments. Nevertheless, there can be no doubt that much work lies ahead for immunologists to optimize the existing approaches and also to assess new strategies in this growing field. Future research should, for instance, allow predicting

which patient populations are likely to respond best to which kind of therapy. With this information, treatment protocols may become more specifically tailored to individual patient groups. Moreover, it is necessary that we better understand why some vaccines work better than others, which route and particular protocol of vaccine delivery is the most efficient, and which combinations of therapies might synergize most effectively. Unfortunately in the past, cancer has proven an ever-creative disease, often eventually overcoming natural or induced immune responses. Thus, better understanding how tumors evade immune attack may lead to novel therapies against which cancers can mount less or no resistance. For some immunotherapies, like ACT, it will also be crucial to make them available for larger patient groups. Hence, even though the list of future challenges is long, current progress in the field is already impressive. And notably, this progress coincides with successes in other areas of cancer therapies, for instance the treatment with kinase inhibitors like Gleevec® [50] or the BRAF inhibitor PLX4032 [51]. Already, researchers are trying to integrate these achievements into powerful novel therapies. Bristol-Myers Squibb and Roche recently announced a clinical collaboration in order to assess the potential of combination therapy using PLX4032 and ipilimumab against melanoma. And this is only one of many current trials designed to discover possible synergies between either two or more simultaneously applied immunotherapies or testing the integration of immunotherapy with standard approaches. Driven by promising preliminary data, these combination treatments are getting more and more attention by oncologists. In this context, it is interesting to note that it was the combination of individually insufficient drugs that turned HIV infection from an invariably fatal disease into a condition that is controllable in the long-term in most patients [52]. Perhaps future progress can make cancer a similarly manageable disease for many.

Acknowledgments: The authors wish to thank Dr. Amit Kunte for helpful comments on the manuscript.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.
2. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011;61(4):212-36.
3. Urruticoechea A, Alemany R, Balart J, Villanueva A, Vinals F, Capella G. Recent advances in cancer therapy: an overview. Curr Pharm Des. 2010;16(1):3-10.
4. Davis ID. An overview of cancer immunotherapy. Immunol Cell Biol. 2000;78(3):179-95.
5. Moore N, Lyle S. Quiescent, slow-cycling stem cell populations in cancer: a review of the evidence and discussion of significance. J Oncol. Epub 2010 Sep 29.
6. Li CY, Huang Q, Kung HF. Cytokine and immuno-gene therapy for solid tumors. Cell Mol Immunol. 2005;2(2):81-91.
7. Bakacs T, Mehrishi JN, Moss RW. Ipilimumab (Yervoy) and the TGN1412 catastrophe. Immunobiology. Epub 2011 Jul 7.
8. Fang L, Lonsdorf AS, Hwang ST. Immunotherapy for advanced melanoma. J Invest Dermatol. 2008;128 (11):2596-605.
9. Harris AL, Hochhauser D. Mechanisms of multidrug resistance in cancer treatment. Acta Oncol. 1992;31(2):205-13.
10. Rufer E, Leonhardt RM, Knittler MR. Molecular architecture of the TAP-associated MHC class I peptide-loading complex. J Immunol. 2007;179(9):5717-27.
11. Leonhardt RM, Fiegl D, Rufer E, Karger A, Bettin B, Knittler MR. Post-endoplasmic reticulum rescue of unstable MHC class I requires proprotein convertase PC7. J Immunol. 2010;184(6):2985-98.
12. Engelhard VH. Structure of peptides associated with class I and class II MHC molecules. Annu Rev Immunol. 1994;12:181-207.
13. Chavez-Galan L, Arenas-Del Angel MC, Zenteno E, Chavez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. Cell Mol Immunol. 2009;6(1):15-25.
14. Vigneron N, Van den Eynde BJ. Insights into the processing of MHC class I ligands gained from the study of human tumor epitopes. Cell Mol Life Sci. 2011;68(9):1503-20.
15. Grizzi F, Franceschini B, Hamrick C, Frezza EE, Cobos E, Chiriva-Internati M. Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. J Transl Med. 2007;5:3.
16. Romero JM, Jimenez P, Cabrera T, Cozar JM, Pedrinaci S, Tallada M, et al. Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. Int J Cancer. 2005;113(4):605-10.

17. Garrido F, Cabrera T, Aptsiauri N. "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. Int J Cancer. 2010;127(2):249-56.
18. Wischhusen J, Friese MA, Mittelbronn M, Meyermann R, Weller M. HLA-E protects glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo. J Neuropathol Exp Neurol. 2005;64(6):523-8.
19. Groth A, Kloss S, Pogge von Strandmann E, Koehl U, Koch J. Mechanisms of tumor and viral immune escape from natural killer cell-mediated surveillance. J Innate Immun. 2011;3(4):344-54.
20. Adler AJ. Mechanisms of T cell tolerance and suppression in cancer mediated by tumor-associated antigens and hormones. Curr Cancer Drug Targets. 2007;7(1):3-14.
21. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9(10):1269-74.
22. Della Chiesa M, Carlomagno S, Frumento G, Balsamo M, Cantoni C, Conte R, et al. The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. Blood. 2006;108(13):4118-25.
23. Mouawad R, Sebert M, Michels J, Bloch J, Spano JP, Khayat D. Treatment for metastatic malignant melanoma: old drugs and new strategies. Crit Rev Oncol Hematol. 2010;74(1):27-39.
24. Smith FO, Downey SG, Klapper JA, Yang JC, Sherry RM, Royal RE, et al. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res. 2008;14(17):5610-8.
25. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364(22):2119-27.
26. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314(5796):126-9.
27. Goff SL, Smith FO, Klapper JA, Sherry R, Wunderlich JR, Steinberg SM, et al. Tumor infiltrating lymphocyte therapy for metastatic melanoma: analysis of tumors resected for TIL. J Immunother. 2010;33(8):840-7.
28. Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/ B7 family. Immunol Rev. 2011;241(1):180-205.
29. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23.
30. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517-26.
31. Prieto PA, Yang JC, Sherry RM, Hughes MS, Kammula US, White DE, et al. The combination of ipilimumab and IL-2 appears to have an increased complete response rate. J Clin Oncol. 2010;28(15 Suppl): 8544.
32. Sznol M, Powderly JD, Smith DC, Brahmer JR, Drake CG, McDermott DF, et al. Safety and antitumor activity of biweekly MDX-1106 (Anti-PD-1, BMS-936558/ONO-4538) in patients with advanced refractory malignancies. J Clin Oncol. 2010;28(15 Suppl): 2506.
33. Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. J Natl Cancer Inst. 2009;101(19):1348-55.
34. Lowy DR, Schiller JT. Prophylactic human papillomavirus vaccines. J Clin Invest. 2006;116(5):1167-73.
35. Frazer IH, Lowy DR, Schiller JT. Prevention of cancer through immunization: Prospects and challenges for the 21st century. Eur J Immunol. 2007;37(Suppl 1):S148-55.
36. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411-22.
37. Schuster SJ, Neelapu SS, Gause BL, Muggia FM, Gockerman JP, Sotomayor EM, et al. Idiotype vaccine therapy (BiovaxID) in follicular lymphoma in first complete remission: Phase III clinical trial results. J Clin Oncol. 2009;27:2A.
38. Jacobi AM, Dorner T. Current aspects of anti-CD20 therapy in rheumatoid arthritis. Curr Opin Pharmacol. 2010;10(3):316-21.
39. Lanini S, Molloy AC, Fine PE, Prentice AG, Ippolito G, Kibbler CC. Risk of infection in patients with lymphoma receiving rituximab: systematic review and meta-analysis. BMC Med. 2011;9:36.
40. Stern M, Herrmann R. Overview of monoclonal antibodies in cancer therapy: present and promise. Crit Rev Oncol Hematol. 2005;54(1):11-29.
41. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344(11):783-92.
42. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350(23):2335-42.

43. Norden AD, Young GS, Setayesh K, Muzikansky A, Klufas R, Ross GL, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. Neurology. 2008;70(10):779-87.
44. Spano JP, Lagorce C, Atlan D, Milano G, Domont J, Benamouzig R, et al. Impact of EGFR expression on colorectal cancer patient prognosis and survival. Ann Oncol. 2005;16(1):102-8.
45. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66(8):3992-5.
46. Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol. 2010;11(1):21-8.
47. Waldmann TA. Immunotherapy: past, present and future. Nat Med. 2003;9(3):269-77.
48. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. Nat Rev Drug Discov. 2004;3(6):488-99.
49. Jacene HA, Filice R, Kasecamp W, Wahl RL. Comparison of 90Y-ibritumomab tiuxetan and 131I-tositumomab in clinical practice. J Nucl Med. 2007;48(11):1767-76.
50. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994-1004.
51. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364(26):2507-16.
52. De Clercq E. Antiretroviral drugs. Curr Opin Pharmacol. 2010;10(5):507-15.