[Frontiers in Bioscience 13, 249-257, January 1, 2008]

Chemotherapy and tumor immunity: an unexpected collaboration

Leisha A. Emens

Department of Oncology, The Johns Hopkins University and the Sidney Kimmel Comprehensive Cancer Center, 1650 Orleans Street, Room 4M90, Bunting Blaustein Cancer Research Building, Baltimore, MD 21231-1000 USA

TABLE OF CONTENTS

1. ABSTRACT

Chemotherapy directly targets the transformed tumor cell, and has long been a key component of therapy for most early and advanced cancers. However, its utility is ultimately limited by unavoidable toxicity to normal tissues, and by drug resistance pathways deeply embedded within the biology of the tumor cell itself. These limitations strongly argue for innovative strategies to treat and manage cancer. Engaging the power of the patient's own immune system is a highly attractive way to complement the activity of standard cancer treatment. Tumor vaccines offer the potential for preventing cancer in those at high risk for disease development, preventing relapse in those diagnosed with early cancer, and treating advanced disease. Notably, the barriers to tumor vaccine efficacy are distinct from the limitations of combination chemotherapy. The ability of vaccines to induce a response robust enough to mediate tumor rejection is limited by the extent of disease burden, the suppressive effect of the local tumor micronenvironment, and multiple layers of systemic immune tolerance established to keep the immune response turned off. Chemotherapy can be used with tumor vaccines in unexpected ways, breaking down these barriers and unleashing the full potential of the antitumor immune response.

2. INTRODUCTION

Current cancer therapies are limited either by local modes of action that fail to eradicate distant disease, or by inherent drug resistance integral to the biology of the transformed tumor cell. Many years of refining traditional modes of therapy have maximized their efficacy, but further therapeutic gains of significance are unlikely. More recently, biologically targeted drugs that precisely interrupt signaling pathways indispensable for tumor growth and progression have produced substantial improvement. As examples, the use of Trastuzumab to treat HER-2/*neu*-overexpressing breast cancer (1) and Imatinib to manage chronic myelogenous leukemia (2) greatly improve patient outcomes. Despite their promise, even precisely targeted therapeutics will ultimately be limited by drug resistance, resulting in disease relapse. This limitation of therapies that target the tumor cell itself is a call for the development of cancer therapies that work in a fundamentally different way.

Tumor vaccines can recruit the power of the patient's own immune system to actively seek out and destroy transformed tumor cells, and represent a potentially potent complement to standard cancer therapies. Immune-based therapy has a higher level target than the tumor cell

itself, re-tooling the host-tumor interaction to induce host cells that favor tumor rejection. Additional unique features of immune-based therapy make it even more attractive. Tumor vaccines can potentially induce a durable antitumor effect by virtue of the immunologic memory response, even in the absence of continued therapy. This memory response also defines immunotherapy as a particularly appropriate strategy for cancer prevention. Despite these unique features, a number of factors pose substantial barriers to cancer vaccine efficacy. First, the extent of the tumor burden frequently overcomes the magnitude of the immune response induced by vaccination. Second, in advanced disease, the tumor mass is a harsh environment for immune effectors to enter and function effectively. Finally, systemic layers of immune tolerance act to keep immune effectors shut down. Integrating immune-based therapy with standard cancer therapies represents one approach for breaking down these barriers.

3. IMMUNE TOLERANCE: A MANIFESTATION OF THE HOST-TUMOR INTERACTION

3.1. Systemic Immune Tolerance

Immune tolerance results from the integration of multiple, overlapping systemic regulatory mechanisms that together prevent the development of immunity to antigens perceived as self (3). The vaccine-mediated induction of immunity to foreign antigens compared to tumor antigens results in T cell responses that are distinct in both quantity and quality. Responses to foreign antigens are routinely of high avidity, with antigen-specific T cell frequencies on the order of 10% or greater. In contrast, responses to self antigens are more typically of very low avidity, with antigen-specific T cell frequencies of 1% or less. These tepid responses to tissue-derived antigens result from distinct mechanisms that control the immune response globally. Additional regulatory mechanisms that map to the tumor microenvironment further conspire to keep antitumor immune responses shut down. At the global level, T cells with the highest avidity for self antigens are deleted either in the thymus, or in the periphery. This process of deletion establishes a peripheral T cell repertoire with lower avidity for antigens that might be perceived as self (3). Moreover, a fail-safe mechanism exists to ensure that any potentially self-reactive T cells that escape the deletion process remain suppressed. A unique T cell population defined in part by expression of CD4, CD25, and the transcription factor FoxP3 (regulatory T cells or Treg) normally function to prevent immune-mediated tissue destruction, and to curtail the normal immune response to foreign antigens (4). This negative feedback is so important in maintaining immunologic homeostasis that Treg activity is complemented by the activity of myeloid suppressor cells and tolerizing dendritic cells to ensure that the system remains under control (3). Notably, progressive tumor growth recruits and expands each of these negative regulatory populations, co-opting the normal host response to facilitate tumor growth and progression (4, 5).

3.2. Local Immune Suppression

When tumors begin to grow, they are so small that they do not access the peripheral lymphoid tissues, thereby "sneaking through" immune surveillance (6). With further growth, the tumor and the immune system begin to influence one another, beginning the process of immunoediting (7). At this point, the potency of immune-mediated tumor rejection is determined by the relative balance between the growth kinetics and physical burden of tumor cells compared to the intensity and diversity of the effector T cells (8, 9). Further limiting the antitumor immune response, the tumor microenvironment is not conducive to the activity of immune effectors that do manage to reach the tumor site. Tumor antigens are presented in a suboptimal fashion, often in the absence of positive co-stimulatory molecules or in the presence of negative accessory molecules for costimulation (10). These alternative contexts for antigen presentation result in weak T cell activation, nonresponsive T cells, or even overt T cell apoptosis (11). The level of tumor antigen itself may be so low that the tumor may simply come to harbor T cells that are functional, but simply fail to see their target and become activated (3).

Tumor cell biology further sets the stage for immune evasion. Tumor cells secrete inhibitory cytokines such as interleukin-10, transforming growth factor-beta, and prostaglandin E2 that serve to further shut down infiltrating T lymphocytes (12). Tumor cells may also express surface fas ligand (fasL or CD95L) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), thereby inducing T cell death. This situation becomes even worse as the result of the inherent genetic instability of tumors, which can produce a dynamic plasticity of its antigen expression profile. This promotes the downregulation of tumor antigens either spontaneously, or as the result of a targeted immune-mediated intervention (13, 14). Alternatively, tumors may downregulate various components of the antigen processing machinery (MHC Class I, MHC Class II, proteasome subunits, and the TAP transporter) (12). These mechanisms together provide a means for the outgrowth of antigen loss variant tumors that are resistant to antigen-specific immunotherapy. Even in the absence of immune-based therapy, this phenotype has been correlated with poor clinical outcome (15).

4. CHEMOTHERAPY AUGMENTS TUMOR IMMUNITY

Given the multiplicity of regulatory pathways that shut off tumor-specific immune responses, it is not surprising that most clinical trials testing cancer vaccines as a single intervention in patients with advanced, treatment refractory cancers have failed to demonstrate clinically relevant bioactivity. Since some combination of surgery, radiation, and chemotherapy is routinely used to treat most established cancers, it seems obvious that integrating tumor vaccines with these other modalities could be additive or even synergistic. It is critical to consider the appropriate dose and timing of standard cancer therapy in relation to cancer vaccines, including chemotherapy. First, appropriately sequencing tumor vaccines after these therapies achieve maximal cytoreduction can shift the balance of disease burden and the T cell response in favor of the T cell. Second, chemotherapy can be used to groom

the tumor microenvironment to better support a productive antitumor immune response. Third, chemotherapy can be used to alter systemic immunoregulatory pathways to optimize immune priming and/or effector T cell expansion.

4.1. Systemic Chemotherapy and Cytoreduction

Studies reveal conflicting results when testing tumor vaccines in close proximity with standard dose cytotoxic chemotherapy, with chemotherapy having no impact, a detrimental effect, or a promoting influence on tumor immunity. Moreover, other studies show that treatment with a tumor vaccine can increase the response to subsequent chemotherapy. One study tested a prime/boost vaccination regimen that primed the antitumor immune response with recombinant vaccinia virus (rVV)-expressing prostate-specific antigen (PSA) admixed with rVV-expressing B7.1, followed by boosting the immune response with recombinant fowlpox virus (rF)-expressing PSA with and without concurrent Docetaxel therapy in patients with hormone-refractory metastatic prostate cancer (16). Vaccination produced a 3.33-fold increase in PSA-specific T cell precusors by ELISPOT after three months, regardless of concomitant Docetaxel treatment. Median progression-free survival for vaccinated patients on Docetaxel was 6.1 months compared to 3.7 months for a historical cohort treated with Docetaxel alone. Another study tested 3 cycles of standard Irinotecan/high dose 5-Fluorouracil/Leucovorin and concurrent vaccination with a carcinoembryonic antigen (CEA)-derived peptide, followed by weekly vaccination alone in patients with newly diagnosed metastatic colorectal cancer (17). Recall antigen-specific $CD8^+$ T cells decreased about 14% in these patients, but almost half demonstrated the induction of CEA-specific immune responses by intracellular cytokine assays. A third study evaluated 17 patients with advanced cancer vaccinated with a CYP1B1 plasmid DNA vaccine encapsulated in biodegradable poly-DL-lactide-coglycolide microparticles (18). Five patients who developed CYP1B1-specific immunity and then went on to receive chemotherapy had a greater, more durable response to salvage chemotherapy than similarly treated patients who did not develop CYP1B1-specific immunity. Together, these studies show that vaccine-induced T cell responses may not be inhibited by chemotherapy, and that previously vaccinated patients may benefit more from subsequent standard dose chemotherapy than those who have never been vaccinated.

At least two studies suggest that standard dose chemotherapy may inhibit vaccine-induced immune responses. In one, the canary pox vaccine ALVAC-CEA B7.1 was given to patients with advanced carcinoembryonic antigen (CEA)-expressing colorectal cancer, and CEA-specific immune responses were measured (19). The number of vaccine-induced CEA-specific T cells was lower in patients who had received a greater number of prior chemotherapy regimens, and in those who had received chemotherapy most recently. A distinct study evaluated vaccination with a granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting vaccine as part of adjuvant therapy in 14 patients with high risk Stage II and III pancreatic cancer (20, 21). Three of these patients developed vaccine-induced mesothelin-specific $CD8^+$ T cells (as measure by ELISPOT) with one vaccine given after pancreaticoduodenectomy. This immune response became undetectable after the patients completed six months of 5-Fluorouracil (5-FU)-based chemoradiation, and was restored only with three additional monthly vaccinations given after chemoradiation was completed.

The differences between the results obtained across these studies are likely related to significant differences in the extent of tumor burden in treated patients, the sequence in which standard dose chemotherapy and vaccine were given, and the chemotherapy drugs given to the patients. Interestingly, the enhanced clinical response to chemotherapy given to vaccine responders may be due to the triggering of pre-existing immunity by treatment-related apoptosis. Here, chemotherapy-induced cell death may function to release tumor antigens, thereby cross-priming the immune response. This mechanism has been shown in animal models with Gemcitabine (22-24), Doxorubicin (25, 26), Cyclophosphamide (27), and Paclitaxel (28). At least one study provides evidence of this in humans. Neoadjuvant Paclitaxel therapy given to locally advanced breast cancer patients induced tumor infiltrating T lymphocytes (TIL), with the extent of TIL present correlating with clinical response (0% in nonresponsive disease, 25% in disease that partially responded, and 67% in disease characterized by a complete clinical response but residual pathologic disease) (29). Importantly, the magnitude of tumor cell apoptosis with the first neoadjuvant Paclitaxel treatment predicted subsequent TIL and clinical benefit.

4.2. Systemic Chemotherapy and the Tumor Microenvironment

Chemotherapy can modify the tumor microenvironment to promote an effective antitumor response (Table 1). As discussed above, one way that chemotherapy can modulate the tumor microenvironment is to induce frank apoptosis in tumor cells themselves, thus potentially augmenting antigen processing and presentation through cross-priming. Chemotherapy may also augment antitumor immunity in diverse ways by altering the tumor microenvironment to favor a more productive tumor-specific immune response. For example, many chemotherapy drugs have dose- and sequence-specific anti-angiogenic activity. Cyclophosphamide, Paclitaxel, Doxorubicin, and Vinblastine given at regular intervals preferentially targets the tumor-associated vasculature (30). This may augment tumor immunity both by normalizing the tumor-associated vasculature early in treatment, thereby facilitating the delivery of drugs and T lymphocytes. It may also induce tumor cell apoptosis in those areas of the tumor that do not undergo vascular normalization, thereby enhancing antigen presentation.

Accumulating data suggests that the way that cancer cells die can render them immunogenic (31, 32). Doxorubicin (and other anthracyclines) activate caspases and thereby augment tumor immunity (25, 26). Interestingly, this enhancement is not through increased

Table 1. Chemotherapy and the immunobiology of the tumor microenvironment

Tumor Effect	Immunologic Effect
Debulk tumor cells	• Achieve a favorable balance between tumor cells and immune effectors
Tumor cell apoptosis	• Enhance antigen presentation
Vascular normalization	• Increased delivery of immune effectors
Modulation of gene expression	• Increased expression of tumor antigens • Increased expression of costimulatory molecules • Decreased expression of counterregulatory molecules • Increased display of cell-surface calreticulin

Table 2. Manipulating immune tolerance with chemotherapy

Immunologic Effect	Chemotherapy Drugs
Abrogate suppressive influence of Treg	Cyclophosphamide, Fludarabine
Relieve inhibition mediated by myeloid suppressor cells	Gemcitabine
Enhance dendritic cell migration/maturation	Paclitaxel, Docetaxel
Reversal of immunologic skew	Cyclophosphamide, Paclitaxel, Bleomycin, Melphalan
Promote evolution of the memory response	Cyclophosphamide
Promote homeostatic T cell proliferation	High dose chemotherapy

tumor apoptosis, but rather through the efficient induction of the intracellular chaperone calreticulin to the cell surface prior to apoptosis (26). This was necessary but not sufficient for immunogenicity, suggesting that additional changes in the tumor cell are also necessary to augment antitumor immunity.

Chemotherapy can also be used to reverse epigenetic changes, re-inducing the expression of both tumor antigens themselves, and molecules that play a role in antigen processing and presentation. 5'-Aza-2'-deoxycytidine (33) and 5-Fluorouracil (34) can render some tumor cell lines sensitive to lysis by cytotoxic T lymphocytes (CTL). Melphalan and Mitomycin-C can upregulate the expression of positive regulatory molecules for T cell co-stimulation like B7, thereby enabling tumor cells themselves to effectively present antigen to TIL (35, 36). Additionally, in a murine model of leukemia, cytosine arabinoside can increase expression of the positive co-regulators B7-1/B7-2 and decrease expression of the negative co-regulator B7-H1, thereby promoting T cell-mediated killing (37). Similar effects of cytosine arabinoside were demonstrated on a panel of human leukemia cell lines *in vitro*. Finally, multiple cytotoxic drugs can sensitize tumor cells to T cell-mediated apoptosis through Fas-or perforin-granzyme-mediated pathways (38). Most recently, cisplatin was shown to sensitize tumor cells to CTL-mediated lysis in a murine model of papillomavirus-driven malignancy, although the precise mechanism remains undefined (39).

4.3. Chemotherapy and the Host Immunologic Milieu

Cytotoxic drugs can be used in unexpected ways to mitigate systemic mechanisms of immune tolerance, or to alter the host environment in which the antitumor immune response develops (Table 2). Many chemotherapy drugs can have both positive and negative immune-modulating activity, with the type of influence dependent on both the drug dose, and the relative timing of drug and vaccination (40). For example, Cyclophosphamide given at the same time or after antigen exposure (vaccination) established antigen-specific immune tolerance. In contrast, Cyclophosphamide when given at low doses one to three days prior to antigen exposure can abrogate immune tolerance, augmenting both humoral and cellular immunity. Consistent with these observations, a number of groups have demonstrated that Cyclophosphamide can relieve the suppressive influence of $CD4^+CD25^+$ Treg, thereby allowing immunity to develop and mature (41-44). Treatment with low dose Cyclophosphamide prior to vaccination promotes the evolution of the T helper type I phenotype, thus reversing the immunologic skew typically associated with established tumor burdens (45). Furthermore, mitigating the influence of Treg with Cyclophosphamide pre-treatment enabled the vaccine-mediated recruitment of high avidity $CD8^+$ T cells to the antitumor response in tolerized *neu* transgenic mice (41). Importantly, these findings correlated with tumor rejection, an outcome never seen with vaccine alone in this tolerized setting. Cyclophosphamide can also upregulate type I interferons, thereby promoting the evolution of the $CD44^{hi}$ memory response (46). Together, these three distinct effects of Cyclophosphamide result in a higher quality tumor-specific immune response. Further studies have shown that Cyclophosphamide can modulate immune responses by inhibiting the induction of inducible nitric oxide synthase (47).

Other cytotoxic drugs can also influence Treg. Standard dose Fludarabine can decrease the number and function of Treg in patients with B cell chronic lymphocytic leukemia (48). When patients with metastatic colorectal carcinoma were treated with the combination of Gemcitabine and FOLFOX 4 (Oxaliplatin, 5-Fluorouracil, and Folinic acid) followed by subcutaneous GM-CSF and interleukin-2, there was a significant reduction in Treg in about 69% of patients, with an overall objective response rate of about 70% (49).

There has been little investigation of the impact of chemotherapy on myeloid suppressor cells. However, in addition to promoting cross-priming and T helper type 1 immunity, Gemcitabine can reduce myeloid suppressor cells in mice, thereby enhancing the antitumor activity of $CD8^+$ T cells and natural killer cells (50).

The taxanes, Paclitaxel and Docetaxel, also have an unexpected and multi-faceted immunomodulatory activity. As discussed previously, they effectively induce tumor cell apoptosis, and can thus enhance the T cell response by cross-priming. The combination of Docetaxel and vaccination with a GM-CSF-secreting vaccine has been shown to be effective in murine models of B16 melanoma and Lewis lung carcinoma (51). These drugs also modulate immunity by other mechanisms. Like Cyclophosphamide, Paclitaxel given one day prior to vaccination in tumor-bearing, tolerant *neu* transgenic mice augments tumor immunity to levels that facilitate tumor rejection (45). This

effect is in part due to reversal of immunologic skew, with conversion of the tumor-specific immune response from the T helper type 2 to the T helper type 1 phenotype. Paclitaxel has a lipopolysaccharide (LPS)-mimetic effect, binding to toll-like receptor 4 (TLR-4) expressed by murine dendritic cells (DCs) and promoting the secretion of proinflammatory cytokines (52, 53). In humans the LPS-like effect of Paclitaxel is independent of TLR-4 but remains dependent on Myd88, suggesting the involvement of an alternative TLR (54). Consistent with these data, Paclitaxel can augment vaccine-induced immunity when given prior to a HER-2/*neu*-specific virally-derived vaccine or a genomically-modified fibroblast vaccine in murine models of breast cancer (55, 56).

Doxorubicin also has a dose- and sequence-specific immunomodulatory activity. In tolerant *neu* transgenic mice, giving Doxorubicin one week after vaccination augments vaccine activity (45). Similarly, Doxorubicin given one week after vaccination in the CT26 model of colon cancer augmented antitumor immunity, resulting in a higher $CD8^+$ T cell response (57). These effects may be due to modulation of tumor cells themselves as discussed previously, with caspase and calreticulin induction rendering them more immunogenic. Alternatively, other mechanisms may also be in play. Supporting this, Doxorubicin could enhance vaccine activity when given prior to vaccination in other systems (56).

The time dependence of the immunomodulatory activity of these chemotherapeutics is striking, and argues for synergy at the time of immune priming for Cyclophosphamide (Treg modulation) and Paclitaxel (DC modulation), and at the time of effector T cell activity for Doxorubicin (apoptosis and cross-priming). Multiple clinical trials testing the ability of low dose chemotherapy to modulate immunity are underway or have just been completed. Two small clinical trials involving patients with metastatic pancreatic cancer (58) or non-small cell lung cancer (59) tested low doses of Cyclophosphamide given one day prior to vaccination with a cell-based GM-CSF-secreting vaccine. These trials showed a trend toward increased vaccine-activated immunity and clinical benefit with Cyclophosphamide-modulated vaccination compared to vaccination alone. There was a transient decrease in Treg numbers with time after Cyclophosphamide treatment in the patients with non-small cell lung cancer. Current clinical trials actively testing these concepts include a Phase I clinical trial of a GM-CSF-secreting breast tumor vaccine given in a specifically timed sequence with Cyclophosphamide and Doxorubicin in patients with stable metastatic breast cancer (60), and a Phase III clinical trial testing a GM-CSF-secreting prostate cancer vaccine with Docetaxel in men with advanced prostate cancer.

High dose chemotherapy can also synergize with tumor vaccines. It is highly effective in debulking established tumor burdens, establishing a state of minimal residual disease. It can eliminate Treg, and sets the stage for lymphopenia-induced homeostatic T cell proliferation (61, 62). Notably, the adoptive transfer of lymphocytes or active vaccination during homeostatic T cell proliferation presents an opportunity to skew the re-established T cell repertoire toward a desired antigenic specificity (63). For example, a highly active population of melanoma-specific T cells developed when lymphopenic, melanoma-bearing RAG-1 deficient mice were vaccinated with a GM-CSF-secreting vaccine, resulting in significant tumor regressions (64). Additionally, vaccine-induced antitumor immunity was increased when tumor-bearing mice were immunized with GM-CSF-secreting vaccines during early engraftment after syngeneic or allogeneic T cell-depleted bone marrow transplantation (65, 66), and can be enhanced by donor leukocyte infusion from vaccinated donor mice (67). In a related strategy, treating tumor-bearing mice with surgical resection followed by nonmyeloablative allogeneic stem cell transplantation and donor leukocyte infusions plus vaccination with a GM-CSF-secreting vaccine developed immune responses capable of rejecting metastatic 4T-1 breast tumors (68). These concepts have been evaluated in clinical trials employing the adoptive transfer of T lymphocytes, and in clinical studies testing immunization combined with the adoptive transfer of primed lymphocytes during immune reconstitution subsequent to myeloablative chemotherapy (69, 70). Significant levels of tumor-specific T cells in the setting of tumor regression were documents in these studies of adoptive cellular therapy. While these approaches have promise, characterizing the kinetics, persistence, and functional quality of tumor-antigen-specific T cells after immune reconstitution will be essential to bring these treatment strategies to routine cancer care.

5. CONCLUSIONS AND PERSPECTIVES

Accumulating data suggests that it will be imperative to combine active immunotherapy with chemotherapy in a rational fashion, capitalizing on additive or even synergistic activity. The mechanisms by which distinct chemotherapeutics can enhance vaccine-induced tumor immunity are highly disparate, and quite dependent on chemotherapy dose, schedule, and perhaps tumor burden. Careful preclinical modeling using the most clinical relevant murine models will be essential for designing the most informative clinical trials, ensuring that tumor vaccines are effectively integrated with standard cancer care to improve clinical outcomes.

6. ACKNOWLEDGEMENTS

This work was supported by funding from the Department of Defense (W81XWH-04-1-0595), the Breast SPORE program (U19CA72108), the National Institutes of Health (1K23 CA098498), the AVON Foundation, and the Cancer Treatment Research Foundation. This review describes work using granulocyte-macrophage colony-stimulating factor-secreting tumor vaccines. Under a licensing agreement between Cell Genesys and Johns Hopkins University, the university is entitled to a share of royalty received from sales of products described in this review. The terms of this agreement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

7. REFERENCES

1. Emens, L.: Trastuzumab: targeted therapy for the management of HER-2/*neu*-overexpressing metastatic breast cancer. *Amer J Ther* 12, 243-253 (2005)

2. Giles, F., J. Cortes & H. Kantarjian: Targeting the kinase activity of the BCR-ABL fusion protein in patients with chronic myeloid leukemia. *Curr Mol Med* 5, 615-623 (2005)

3. Walker, L. & A. Abbas: The enemy within: keeping self-reactive T cells at bay in the periphery. *Nature Rev Immunol* 21, 11-19 (2001)

4. McHugh, R. & E. Shevach: The role of suppressor T cells in regulation of immune responses. *J Aller Clin Immunol* 110, 693-702 (2002)

5. Serafini, P., I. Borrello & V. Bronte: Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. *Sem Cancer Biol* 16, 53-65 (2006)

6. Old, L., E. Boyse & D. Clarke: Antigenic properties of chemically induced tumors. *Ann NY Acad Sci* 101, 80-106 (1962)

7. Dunn, G., L. Old & R. Schreiber: The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 21, 137-148 (2004)

8. Ochsenbein, A., P. Klenerman, U. Karrer, B. Ludewig, M. Pericin, H. Hengartner & R. Zinkernagel: Immune surveillance against a solid tumor fails because of immunological ignorance. *Proc Natl Acad Sci* 96, 2233-2238 (1999)

9. Perez-Diaz, A., P. Spiess, N. Restifo, P. Matzinger & F. Marincola: Intensity of the vaccine-elicited immune response determines tumor clearance. *J Immunol* 168, 338-347 (2002)

10. Pardoll, D.: Spinning molecular immunology into successful immunotherapy. *Nature Rev Immunol* 2, 227-238 (2002)

11. Khoury, S. & M. Sayegh: The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* 20, 529-538 (2004)

12. Marincola, F., E. Jaffee, D. Hicklin & S. Ferrone: Escape of human solid tumors from T cell recognition: molecular mechanisms and functional significance. *Adv Immunol* 74, 181-273 (2000)

13. Davis, T., D. Czerwinski & R. Levy: Therapy of B-cell lymphoma with anti-CD20 antibodies can result in the loss of CD20 antigen expression. *Clin Cancer Res* 5, 611-615 (1999)

14. Knutson, K., B. Almand, Y. Dang & M. Disis: *Neu* antigen-negative variants can be generated after neu-specific antibody therapy in *neu* transgenic mice. *Cancer Res* 64, 1146-1151 (2004)

15. Bangia, N. & S. Ferrone: Antigen presentation machinery (APM) modulation and soluble HLA molecules in the tumor microenvironment :do they provide tumor cells with escape mechanisms from cytotoxic T lymphocytes? *Immunol Invest*, 35, 485-503 (2006)

16. Arlen, P., J. P. Gulley, C, L. Skarupa, M. Pazdur, D. Panicali, P. Beetham, K. Tsang, D. Grosenbach, J. Feldman, S. Steinberg, E. Jones, C. Chen, J. Marte, J. Schlom & W. Dahut: A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. *Clin Cancer Res* 12, 1260-1269 (2006)

17. Weihrauch, M., S. Ansen, E. Jurkiewicz, C. Geisen, Z. Xia, K. Anderson, E. Gracien, M. Schmidt, B. Wittig, V. Diehl, J. Wolf, H. Bohlen & L. Nadler: Phase I/II combined chemoimmunotherapy with carcinoembryonic antigen-derived HLA-A2-restricted CAP-1 peptide and irinotecan, 5-fluorouracil, and leucovorin in patients with primary metastatic colorectal cancer. *Clin Cancer Res* 15, 5993-6001 (2005)

18. Gribben, J., D. Ryan, R. Boyajian, R. Urban, M. Hedley, K. Beach, P. Nealon, U. Matulonis, S. Campos, T. Gilligan, P. Richardson, B. Marshall, D. Neuberg & L. Nadler: Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. *Clin Cancer Res* 11, 4430-4436 (2005)

19. von Mehren, M., P. Arlen, J. Gulley, A. Rogatko, H. Cooper, N. Meropol, R. Alpaugh, M. Davey, S. McLaughlin, M. Beard, K. Tsang, J. Schlom & L. Weiner: The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 7, 1181-1191 (2001)

20. Jaffee, E., R. Hruban, B. Biedrzycki, D. Laheru, K. Schepers, P. Sauter, M. Goemann, J. Coleman, L. Grochow, R. Donehower, K. Lillemoe, S. O'Reilly, R. Abrams, D. Pardoll, J. Cameron & C. Yeo: Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 19, 145-156 (2001)

21. Thomas, A., L. Santarsiero, E. Lutz, T. Armstrong, Y. Chen, L. Huang, D. Laheru, M. Goggins, R. Hruban & E. Jaffee: Mesothelin-specific $CD8^+$ T cell responses provide evidence of *in vivo* cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J Exp Med* 200, 297-306 (2004)

22. Nowak, A., R. Lake, A. Marzo, B. Scott, W. Heath, E. Collins, J. Frelinger & B. Robinson: Induction of tumor cell apoptosis *in vivo* increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. *J Immunol* 170, 4905-4913 (2003)

23. Nowak, A., B. Robinson & R. Lake: Gemcitabine exerts a selective effect on the humoral immune response: implications for combination chemo-immunotherapy. *Cancer Res* 62, 2353-2358 (2002)

24. Nowak, A., B. Robinson & R. Lake: Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. *Cancer Res* 63, 4490-4496 (2003)

25. Casares, N., N. Pequignot, A. Tesniere, F. Ghiringhelli, S. Roux, N. Chaput, E. Schmitt, A. Hamai, S. Hervas-Stubbs, M. Obeid, F. Coutant, D. Metivier, E. Pichard, P. Aucouturier, G. Pierron, C. Garrido, L. Zitvogel & G. Kroemer: Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 202, 1691-1701 (2006)

26. Obeid, M., A. Tesniere, F. Ghiringhelli, G. Fimia, L. Apetoh, J.-L. Perfettini, M. Castedo, G. Mignot, T. Panaretakis, N. Casares, D. Metivier, N. Larochette, P. van Endert, F. Ciccosanti, M. Piacentini, L. Zitvogel & G. Kroemer: Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nature Med* 13, 54-61 (2007)

27. Tong, Y., W. Song & R. Crystal: Combined intratumoral injection of bone-marrow-derived dendritic cells and systemic chemotherapy to treat pre-existing murine tumors. *Cancer Res* 61, 7530-7535 (2001)

28. Yu, B., S. Kusmartsev, F. Cheng, M. Paolini, Y. Nefedova, E. Sotomayor & D. Gabrilovich: Effective combination of chemotherapy and dendritic cell administration for the treatment of advanced stage experimental breast cancer *Clin Cancer Res* 9, 285-294 (2003)

29. Demaria, S., M. Volm, R. Shapiro, H. Yee, R. Oratz, S. Formenti, F. Muggia & W. Symmans: Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. *Clin Cancer Res* 7, 3025-303 (2001)

30. Bocci, G., K. Nicolaou & R. Kerbel: Protracted low-dose effects on human endothelial cell proliferation and survival *in vitro* reveal a selective antiangiogenic window for various chemotherapeutic agents. *Cancer Res* 62, 6938-6943(2002)

31. Lake, R. & R. van der Most: A better way for a cancer cell to die. *N Engl J Med* 354, 2503-2504 (2006)

32. Storkus, W. & L. J. Falo: A 'good death' for tumor immunology. *Nature Med* 13, 28-30 (2007)

33. Coral, S., L. Sigalotti, M. Altomonte, A. Engelsberg, F. Colizzi, I. Cattarossi, E. Maraskowsky, E. Jager, B. Seliger & M. Maio: 5-aza-2'-Deoxycytidine-induced expression of functional cancer testis antigens in human renal cell carcinoma: immunotherapeutic implications. *Clin Cancer Res* 8, 2690-2695 (2002)

34. Correale, P., A. Aquino, A. Giuliani, M. Pellegrini, L. Micheli, M. Cusi, C. Nencini, R. Petrioli, S. Prete, L. De Vecchis, M. Turriziani, G. Giorgi, E. Bonmassar & G. Francini: Treatment of colon and breast carcinoma cells with 5-fluorouracil enhances expression of carcinoembryonic antigen and susceptibility to HLA-A*02/01 restricted, CEA-peptide-specific cytotoxic T cells *in vitro*. *Int J Cancer* 104, 437-445 (2003)

35. Donepudi, M., P. Raychaudhuri, J. Bluestone & M. Mokyr: Mechanism of Melphalan-induced B7-1 gene expression in P815 tumor cells. *J Immunol* 166, 6491-6499 (2001)

36. Sojka, D., M. Donepudi, J. Bluestone & M. Mokyr: Melphalan and other anticancer modalities up-regulate B7-1 gene expression in tumor cells. *J Immunol* 164, 6230-6236 (2000)

37. Vereecque, R., A. Saudemont & B. Quesnel: Cytosine arabinoside induces costimulatory molecule expression in acute myeloid leukemia cells. *Leukemia* 18, 1223-1230 (2004)

38. Yang, F. & F. Haluska: Treatment of melanoma with 5-fluorouracil or dacarbazine *in vitro* sensitizes cells to antigen-specific CTL lysis through perforin/granzyme- and Fas-mediated pathways. *J Immunol* 172, 4599-4608 (2004)

39. Bae, S., Y.-J. Park, J.-B. Park, Y. Choi, M. Kim & J.-I. Sin: Therapeutic synergy of human papillomavirus E7 subunit vaccines plus cisplatin in an animal tumor model: causal involvement of increased sensitivity to cisplatin-treated tumors to CTL-mediated killing in therapeutic synergy. *Clin Cancer Res* 13, 341-349 (2007)

40. Emens, L., J. Machiels, R. Reilly & E. Jaffee: Chemotherapy: friend or foe to cancer vaccines? *Curr Opin Mol Ther* 3, 77-84 (2001)

41. Ercolini, A., B. Ladle, E. Manning, L. Pfannenstiel, T. Armstrong, J. Machiels, J. Bieler, L. Emens, R. Reilly & E. Jaffee: Recruitment of latent pools of high-avidity $CD8^+$ T cells to the antitumor immune response. *J Exp Med* 201, 1591-602 (2005)

42. Ghiringhelli, F., N. Larmonier, E. Schmitt, A. Parcellier, D. Cathelin, C. Garrido, B. Chauffert, E. Solary, B. Bonnotte & F. Martin: $CD4^+CD25^+$ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol* 34, 336-344 (2004)

43. Lutsiak, M., R. Semnani, R. De Pascalis, S. Kashmiri, J. Schlom & H. Sabzevari: Inhibition of $CD4^+CD25^+$ T

regulatory cell function implicated in enhanced immune response by low dose Cyclophosphamide. *Blood* 105, 2862-2868 (2005)

44. Taieb, J., N. Chaput, N. Schartz, S. Roux, S. Novault, C. Menard, F. Ghiringhelli, M. Terme, A. Carpentier, G. Darrasse-Jeze, F. Lemonnier & L. Zitvogel: Chemoimmunotherapy of tumors: cyclophosphamide synergizes with exosome based vaccines. *J Immunol* 176, 2722-2729 (2006)

45. Machiels, J., R. Reilly, L. Emens, A. Ercolini, R. Lei, D. Weintraub, F. Okoye & E. Jaffee: Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/*neu* tolerized mice. *Cancer Res* 61, 3689-97 (2001)

46. Proietti, E., G. Greco, B. Garrone, S. Baccarini, C. Mauri, M. Venditti, D. Cariel & F. Belardelli: Importance of Cyclophosphamide-induced bystander effect on T cells for a successful tumor eradication in response to adoptive immunotherapy in mice. *J Clin Invest* 101, 429-441 (1998)

47. Loeffler, M., J. Kruger & R. Reisfeld: Immunostimulatory effects of low-dose Cyclophosphamide are controlled by inducible nitric oxide synthase. *Cancer Res* 65, 5027-5030 (2005)

48. Beyer, M., M. Kochanek, K. Darabi, A. Popov, M. Jensen, E. Endl, P. Knolle, R. Thomas, M. von Bergwelt-Baildon, S. Debey, M. Hallek & J. Schultze: Reduced frequencies and suppressive function of $CD4^{+}CD25^{hi}$ regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood* 106, 2018-2025 (2005)

49. Correale, P., M. Cusi, K. Tsang, M. Del Vecchio, S. Marsili, M. Placa, C. Intrivici, A. Aquino, L. Micheli, C. Nencini, F. Ferrari, G. Giorgi, E. Bonmassar & G. Francini: Chemo-immunotherapy of metastatic colorectal carcinoma with gemcitabine plus FOLFOX 4 followed by subcutaneous granulocyte macrophage colony stimualting factor and interleukin-2 induces strong immunologic and antitumor activity in metastatic colon cancer patients. *J Clin Oncol* 23, 8950-8958 (2005)

50. Suzuki, E., V. Kapoor, A. Jassar, L. Kaiser & S. Albelda: Gemcitabine selectively eliminates splenic Gr-$1/Cd11b^{+}$ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res* 11, 6713-6721 (2005)

51. Prell, R., L. Gearin, A. Simmons, M. Vanroey & K. Jooss: The anti-tumor efficacy of a GM-CSF-secreting tumor cell vaccine is not inhibited by docetaxel administration. *Cancer Immunol Immunother* 12, 1-9 (2006)

52. Byrd-Leifer, C. A., E. F. Block, K. Takeda, S. Akira & A. Ding: The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur J Immunol* 31, 2448-2457 (2001)

53. Kawasaki, K., S. Akashi, R. Shimazu, S. Yoshida, K. Miyake & M. Nishijima: Mouse toll-like receptor 4.MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. *J Biol Chem* 275, 2251-2254 (2000)

54. Wang, J., M. Kobayashi, M. Han, S. Choi, M. Takano, S. Hashino, J. Tanaka, T. Kondoh, K. Kawamura & M. Hosokawa: MyD88 is involved in the signalling pathway for Taxol-induced apoptosis and TNF-alpha expression in human myelomonocytic cells. *Br J Hemtol* 11, 638-645 (2002)

55. Chopra, A., T. Kim, I. O-Sullivan, D. Martinez & E. Cohen: Combined therapy of an established, highly aggressive breast cancer in mice with paclitaxel and a unique DNA-based vaccine. *Intl J Cancer* 118, 2888-2898 (2006)

56. Eralp, Y., X. Wang, J. Wang, M. Maughan, J. Polo & L. Lachman: Doxorubicin and paclitaxel enhance the antitumor efficacy of vaccines directed against HER-2/*neu* in a murine mammary carcinoma model. *Breast Cancer Res* 6, R275-R283 (2004)

57. Nigam, A., R. Yacavone, M. Zahurak, C. Johns, D. Pardoll, S. Piantadosi, H. Levitsky & W. Nelson: Immunomodulatory properties of antineoplastic drugs administered in conjunction with GM-CSF-secreting cancer cell vaccines. *Int J Cancer* 12, 161-170 (1998)

58. Laheru, D., J. Nemunaitis & B. B: A feasibility study of a GM-CSF-secreting irradiated whole cell allogeneic vaccine (GVAX) alone or in sequence wtih Cytoxan for patients with locally advanced or metastatic pancreatic cancer. Presented at the Proc AACR: Pancreatic Cancer 2005--Advances and Challenges (2005)

59. Schiller, J., J. Nemunaitis & H. Ross: A phase 2 randomized study of GM-CSF gene-modified autologous tumor vaccine (CG8123) with and without low dose Cyclophosphamide in advanced stage non-small cell lung cancer (NSCLC). Presented at the International Association for the Study of Lung Cancer 2005 (2005)

60. Emens, L., D. Armstrong, B. Biedrzycki, N. Davidson, J. Davis-Sproul, J. Fetting, E. Jaffee, B. Onners, S. Piantadosi, R. Reilly, V. Stearns, I. Tartakovsky, K. Visvanathan & A. Wolff: A phase I vaccine safety and chemotherapy dose-finding trial of an allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin. *Hum Gene Ther* 15, 313-37 (2004)

61. Cho, B., V. Rao, Q. Ge, H. Eisen & J. Chen: Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. *J Exp Med* 192, 549-556 (2000)

62. Goldrath, A., L. Bogatzki & M. Bevan: Naive T cells transiently aquire a memory-like phenotype during

homeostasis-driven proliferation. *J Exp Med* 192, 557-564 (2000)

63. Mackall, C., C. Bare, L. Granger, S. Sharrow, J. Titus & R. Gress: Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J Immunol* 156, 4609-4616 (1996)

64. Hu, H., C. Poehlein, W. Urba & B. Fox: Development of antitumor immune responses in reconstituted lymphopenic hosts. *Cancer Res* 62, 3914-3919 (2002)

65. Borrello, I., E. Sotomayor, F.-M. Rattis, S. Cooke, L. Gu & H. Levitsky: Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood* 95, 3011-3019 (2000)

66. Teshima, T., N. Mach, G. Hill, L. Pan, S. Gillessen, G. Dranoff & J. Ferrara: Tumor cell vaccine elicits potent antitumor immunity after allogeneic T-cell-depleted bone marrow transplantation. *Cancer Res* 61, 162-171 (2001)

67. Teshima, T., C. Liu, K. Lowler, G. Dranoff & J. Ferrara: Donor leukocyte infusion from immunized donors increases tumor vaccine efficacy after allogeneic bone marrow transplantation. *Cancer Res* 62, 796-800 (2002)

68. Luznik, L., J. Slansky, S. Jalla, I. Borrello, H. Levitsky, D. Pardoll & E. Fuchs: Successful therapy of metastatic cancer using tumor vaccines in mixed allogeneic bone marrow chimeras. *Blood* 101, 1645-1652 (2003)

69. Dudley, M., J. Wunderlich, P. Robbins, J. Yang, P. Hwu, D. Schwartwentruber, S. Topalian, R. Sherry, N. Restifo, A. Hibicki, M. Robinson, M. Raffeld, P. Duray, C. Seipp, L. Roger-Freezer, K. Morton, S. Mavroukakis, D. White & S. Rosenberg: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 298, 850-854 (2002)

70. Dudley, M., J. Wunderlich, J. Yang, R. Sherry, S. Topalian, N. Restifo, R. Royal, U. Kammula, D. White, S. Mavroukakis, L. Rogers, G. Gracia, S. Jones, D. Mangiameli, M. Pelletier, J. Gea-Banacloche, M. Robinson, D. Berman, A. Filie, A. Abati & S. Rosenberg: Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastastic melanoma. *J Clin Oncol* 23, 2346-2357 (2005)

Abbreviations: TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; MHC: major histocompatibility complex; TAP: transporter of antigen processing; rVV: recombinant vaccinia virus; PSA: prostate specific antigen; ELISPOT: enzyme-linked immunosorbent assay; CEA: carcinoembryonic antigen; GM-CSF: granulocyte-macrophage colony-stimulating factor; TIL: tumor infiltrating lymphocytes; CTL: cytotoxic T lymphocytes; TLR: toll-like receptor; LPS: lipopolysaccharide

Key Words: TRAIL, CEA, rVV, PSA, GM-CSF, CTL, TLR, LPS, Tumor Immunity, Chemotherapy, Cancer Vaccines, Immune Tolerance, Review

Send correspondence to: Leisha A. Emens, MD, PhD, Tumor Immunology and Breast Cancer Research Programs, Department of Oncology, Johns Hopkins University, Bunting Blaustein Cancer Research Building, 1650 Orleans Street, Room 4M90, Baltimore, MD 21231-1000, Tel: 410-502-7051, Fax: 410-614-8216, E-mail: emensle@jhmi.edu

http://www.bioscience.org/current/vol12.htm