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Tumor antigen targets and tumor immunotherapy

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1. ABSTRACT

Tumor immunology and immunotherapy attempt to use the exquisite specificity and lytic capability of the immune system to treat malignant disease with a minimum of damage to normal tissue. Increasing knowledge of the identity of tumor antigens should point the way to effective therapeutic vaccines or more specific immunotherapeutic strategies. Tumors, however, have evolved mechanisms to inactivate cytolytic T-cells and other immune responses targeting tumor antigens. The current goal of immunotherapy research is to use contemporary advances in cellular and molecular immunology to develop strategies to overcome the disabling effects of the tumor microenvironment on the immune system attack against tumor antigen targets. This review will summarize our current knowledge of the spectrum of tumor antigen targets available for immune recognition in cancer, the obstacles to tumor immunotherapy, the use of adoptive immunotherapy to overcome some of these obstacles, the use of monoclonal antibodies to target tumor antigens for immunotherapy, and finally the potential use of heat shock proteins as targets for cancer immunotherapy.

2. INTRODUCTION

The ultimate goal of cancer therapy is the development of therapeutic modalities that have the specificity to target and destroy tumors cells, while sparing their normal counterparts. In theory, the immune system has the required exquisite specificity to accomplish this. Active immunotherapy has proven effective against pathogens that normally cause acute self limiting infectious disease, followed by a long lasting immune response. However, attempts at active immunotherapy against human tumors have not proven effective. The major difference between the pathogens responsible for infectious diseases and tumor cells is that tumors cells are host derived , and as such most of their macromolecules are self antigens also present on normal cells. The first definitive evidence that the adaptive and innate immune systems can recognize spontaneous and induced tumors arises from studies of the role of interferon γ (IFNγ) in tumor immunosurveillance. IFNγ deficient mice had an increased rate of spontaneous and chemically induced tumors (1). In addition, studies in RAG2/ IFNγ receptor/ STAT-1 knock out mice showed a similarly enhanced susceptibility to chemical carcinogenesis, with all the resulting sarcomas showing

similar histology (2). These studies have been extended to spontaneous tumors arising in mice lacking lymphocytes (3). In such mice, alone or in combination with a defect in IFNγ signaling, there is a greatly increased susceptibility to the development of carcinomas . Together these studies suggested that T cells, B cells, natural killer cells, and NK T cells can play a significant role in suppressing tumor growth and development (1-3).

3. TUMOR CELL ANTIGENS AS TARGETS FOR IMMUNOTHERAPY

A key recent advance in immunology has been the increased understanding of the antigenic basis for tumor cell recognition and destruction. The ultimate "killer cell" that mediates the rejection of transplanted tissues or organs is the $CD8^+$ T-cell or cytotoxic T-lymphocyte (CTL). Although, as discussed below, monoclonal antibodies have been effective in inducing tumor lysis, most vaccine strategies focus on CTL lysis of tumors. In effect, the tumor is viewed as a foreign tissue in the host. Each CTL expresses a clonotypically unique T cell antigen receptor (TCR), that specifically recognizes a particular tumor antigen bound within the cleft of an HLA class I molecule (4,5). The HLA system (designated MHC in mice) allows the detection of intracellular proteins by CTL. Identifying tumor antigens with respect to their class I HLA presentation allows the targeting of both intracellular and extracellular antigens by the immune system. Cytoplasmic proteosomes degrade the panoply of intracellular proteins into short peptides that are transported to the endoplasmic reticulum. This organelle loads the peptides onto nascent class I MHC molecules (HLA-A,B,C) such that the peptides are recognized by the T-cell receptors on CTL. This increases the utility of CTL by increasing the variety of antigens available as targets for immune attack against tumors. Further, the $CD8^+$ T-cells, active in the rejection of foreign tissue or transplanted organs, may recognize tumor cells as similarly foreign through this mechanism Thus, although monoclonal antibodies have been successful in targeting selected tumors for immune destruction, most tumor vaccine strategies have chosen to take advantage of activation of CTLs for tumor cell lysis (4,5).

To utilize the specificity of the immune system for cancer therapy, it is necessary to define the human tumor antigens that may be recognized by CTL. Major advances in the biochemical and molecular identification of tumor associated antigens including overexpressed or selectively expressed molecules, viral proteins, and mutated proteins has led to the classification of these molecular targets into six major groups. It is particularly important to categorize these antigens with respect to the class I HLA allele that restricts their recognition by T-cells as well as their tissue distribution in order to define their usefulness as targets in immunotherapy.

The first group are HLA class I restricted cancer/testis antigens. Among these antigens are the MAGE, BAGE, and CAGE families of genes and the NY-ESo-1 antigens. These antigens are called cancer/testis antigens, because they are expressed in histologically different human tumors, and in the spermatocytes/spermatogonia of testis. They are also occasionally expressed in placenta. These tumor antigens result from reactivation of the transcription of genes in tumors that are normally silent in adult tissues. Their expression in testis does not provide targets for immune attack, because testis cells do not express class I HLA. Despite the fact that these antigens are highly characterized, the physiological function of these proteins remains obscure (6).

The second major class are the class I HLA-restricted differentiation antigens. These antigens are expressed on both the normal tissue and the tumors arising from these tissues. Most of these antigens have been identified on normal melanocytes or melanoma cells. Melanosome proteins can give rise to epitopes that are recognized by both $CD4^+$ and $CD8^+$ T-cells (6,7). There are two archetypes for this antigen group. First, there are the carbohydrates antigens (particularly gangliosides) that can be dramatically overexpressed following malignant transformation. Secondly, there are the melanosome associated proteins themselves. Melanosomes are membrane bound organelles that specifically synthesize the melanin pigment in melanocytes. The first differentiation associated antigen recognized as an autoantigen was tyrosinase related protein I (TRP-1) also known as gp75. Other melanosomal autoantigens are tyrosinase, TRP-2, gp 100/p MOL 17 and MART-1/MelanA. These differentiation antigens are often recognized by autoantibodies and autoreactive CTL in patients with melanoma (7).

A third group of class I HLA restricted antigens are those that are widely expressed among a variety of normal tissue types. There is no preferential expression of these antigens on any particular tumor type. Some of these antigens can generate T-cell defined epitopes through alterations in gene transcription or translation. Examples of these antigens are Her2/Neu that is widely expressed on epithelial cells, MUC-2, which is expressed in the colon, small intestine, bronchia, cervix, and gall bladder, and SART-3 expressed on testis and fetal liver (6).

A fourth group of class I HLA restricted antigens are relatively tumor specific. These unique tumor antigens arise from point mutations that accompany neoplastic transformation in normal genes. Examples of such proteins are β-catenin, CDK- 4, and ras. These antigens are thus expressed only in the tumors in which they were discovered. It is unlikely that the same mutation would occur in two different neoplasms, unless it occurs in a gene absolutely required for cell transformation in that tissue type. In mouse models, such unique antigens have been demonstrated to be more immunogenic than other groups of shared antigens. However, as they can only induce an immune response against the tumor of origin, these antigens are of limited clinical utility (6).

The last group of antigens to be considered are class II HLA restricted antigens. The first class II restricted epitope identified that was capable of inducing a $CD4^+$ T-

cell response was the enzyme tyrosinase in melanoma. The discovery of class II restricted epitopes was a slow process limited by technological and methodological problems for identifying $CD4^+$ T-cell epitopes in tumor antigens. Since 1998, new technologies including immunized transgenic mice and improved biochemical technniques have allowed the identification of at least 27 new class II HLA restricted epitopes from 14 antigens, including MUC-1, Gp100, CDC27/m and TPI/m (6).

4. OBSTACLES TO USING ANTIGENIC TARGETS FOR TUMOR IMMUNOTHERAPY

Two major obstacles block the development of vaccines utilizing newly described target antigens. The first is the need to develop methods to induce a sufficiently strong immune response to erradicate a tumor. This is especially a problem for many target antigens to which the immune system has developed tolerance, because of their expression in normal tissues. The second obstacle arises from the fact that tumors have developed evasion mechanisms to overcome or evade the host immune response. Efforts to increase the effectiveness of vaccines have featured increasing the function of antigen presenting cells (APCs), as well as enhancing the activation of CTLs.

Optimal activation of T-cells requires two sets of signals. One signal is generated by the interaction of antigenic peptides bound to HLA with the TCR. Many of the antigens potentially useful in immunotherapy have very low affinity for their HLA molecules. The effectiveness of these antigens in immunotherapy has been enhanced by molecular engineering of the important epitopes. This involves altering the amino acid sequence of the antigen to increase its affinity for HLA molecules by utilizing known sequence motifs for peptide binding to the anchor residues required for HLA binding . A potential technique to accomplish this increased affinity for HLA anchoring is to screen combinatorial libraries to screen sequences for improved HLA binding (11). The second signal arises from the interaction of co-stimulatory molecules expressed on antigen presenting cells with counter recognition receptors on T-cells. An alternative strategy to improve the immunotherapeutic potential of antigens is to focus on the positive costimulatory signals provided by the B7 and TNF family members expressed on the cell membrane of antigen presenting cells (8). A classic example of such molecules is CD40 expressed on the surface of a number of antigen presenting cells. Such a tack is based on the assumption that T-cell stimulation is primarily occurring through antigen presenting cells rather than the tumor cells themselves. Finally, it should be noted that CD137, a member of the TNF receptor family expressed on monocytes, dendritic cells, NK cells and T-cells, induces cytotoxic T-cell immunity to tumors previously thought to be non immunogenic (8,12).

5. ADOPTIVE CELL TRANSFER FOR IMMUNOTHERAPY OF TUMORS

Although large numbers of circulating tumor reactive CTL can be generated in cancer patients using peptides emulsified with adjuvants, only rare regressions of established tumors have been obtained that meet the rigorous criteria required to document a clinical response. Even in transgenic mice engineered such that every T-cell expressed a tumor reactive T-cell receptor, tumors still grew progressively. This is probably due to the fact that tumors have developed methods to escape CTL destruction. Several mechanisms have been proposed whereby tumors expressing a self antigen may evade host T-cell recognition: a.) low numbers of antigens reactive T-cells are present in the CTL repertoire, b) anergy or immune tolerance occurring through T-cell depletion or through induction by the tumor microenvironment that may include host regulatory cells, c) naive CTL against self peptide fail to recognize antigen positive tumor cells due to low affinity of self peptide for host HLA molecules, and d) tumor secretion of factors into the microenviroment (e.g. IL-10, TGFβ, and VEGF) that inhibit dendritic cell activation. The disappointing data on tumor cell evasion of CTL attack has spurred the development of strategies to enhance CTL based immunotherapy.

One of the most successful strategies to enhance CTL immunotherapy effectiveness is adoptive cell transfer (13). Using this technique effector CTLs with optimal characteristics, such as high tumor cell antigen avidity and specificity are selected. The selected CTL are then expanded and activated *in vitro*, bypassing *in vivo* host immune modulatory mechanisms and any potentially CTL suppressive activity in the tumor microenvironment. The optimal methods for expanding antigen specific CTL are still being developed. In general, however dendritic cells presenting the specific antigen are used to activate reactive CTL, which can then be selected and stimulated with antibodies to CD3 and additional cytokines such as IL-2 to support lymphocyte proliferation, differentiation, and survival. Using these techniques reduces any potential

Dendritic cells are the archetype APC. They mature through a stepwise process requiring hematopoietic cytokines (i.e. GM-CSF, flt-3 ligand, and IL-4), and stimulation through toll like receptors or TNF family receptors (8). Dendritic cells also secrete IL-12 and IL-15 that contribute to CTL activation and memory. Complete differentiation of dendritic cells is required for these cells to effectively present antigen associated with HLA molecules. IFN γ and lipopolysaccharide can be used to help generate mature dendritic cells. Both these molecules induce expression of the α subunit of the IL-15 receptor and IL-15 itself , in monocytes and dendritic cells (8) . Interestingly, the IL-15 receptor α subunit presents IL-15 to adjacent cells including CTLs. The presented IL-15 can then activate the IL-2 receptor common β and γ chains on the T-cells facilitating memory $CD8^+$ T-cell expression that is critical for a sustained immunological response to vaccination with tumor antigens (9). Another approach to improving immunotherapy involves incorporating into vaccines cytokines such as GM-CSF, which induce dendritic cell differentiation. In some strategies, the tumor antigen is targeted to specific receptors on dendritic cells by using anitgen GM-CSF fusion proteins (8,10).

effector suppressive activity in the tumor microenvironment. Tumor reactive CTL can be expanded to enormous numbers *in vitro* allowing multiples of 10^9 specific T-cells to be infused. This achieves *in vivo* CTL frequencies unobtainable with current vaccine strategies (13).

The patient can also be given chemotherapy before the adoptive cell transfer. This avoids toxicity to the transferred CTLs, and allows regeneration of a host immune cell mircroenvironment conducive to the activity of the expanded effector cells. In practice, adoptive cell transfer immunotherapy has been difficult to implement in patients, because tumor antigen specific effector cells are difficult to isolate. However, several methods for improving the production and selection of specific CTL for immunotherapy have been devised (14,15). One approach involved vaccinating patients with autologous tumor cells, harvesting cells from the draining lymph nodes, and stimulating the resulting CTL *in vitro*. There remains, however, the need for improving the expansion of tumor antigen specific CTL from immunized patients. The molecular identification of tumor antigens has enabled the investigation of methods to produce such cell cultures. To achieve this goal in melanoma patients, dendritic cells pulsed with peptides derived from melanoma antigens are used to stimulate peripheral blood lymphocytes (13).

The importance of helper $CD4^+$ T-cells for tumor immunotherapy has also recently been recognized. Recent reports of $CD4^+$ T-cells increasing the persistence of CTL after an immune response is elicited, underscores the importance of including these cells in adoptive cellular therapy protocols . Trials with adoptive immunotherapy for viral infections have shown increased *in vivo* proliferation and persistence of CTL if specific $CD4^+$ helper T-cells are also present (16, 17). In order for infused lymphocytes to be effective in tumor immunotherapy, they must also persist in the tumor microenvironment (13,14, 18). $CD4^+$ T-cells may provide critical cytokines and additional APC, activation as well as effector function against infected or tumor cells. Identifying and characterizing the specificity of tumor reactive $CD4^+$ cells has proven more difficult than characterizing the tumor reactive CTL. $CD4^+$ helper function is currently provided by substitute exogenous cytokines. Infusion of IL-2 has been clearly shown to enhance the persistence of $CD8^+$ T-cells in melanoma patients. Additional methods to enhance CTL survival include manipulating the cytokine content of the *in vitro* culture medium to maintain a central memory T-cell phenotype, and genetically engineering $CD8^+$ T-cells to autonomously secrete anti apoptotic cytokines.

Molecular genetic and immunological techniques can also be used to interfere with signaling cascades that negatively impinge on T-cell receptor signal strength. For example, interfering with the transcription or translation of the Cbl-b gene reduces the activation threshold in T-cell lines and primary human T-cells (15). Cytotoxic T lymphocyte antigen -4 (CTLA-4) is an important negative regulator of T-cell activation. Administration of blocking antibodies to CTLA-4,in preclinical murine models, as well as recent trials in patients, has shown dramatic effects on CTL infiltration into tumors and significant tumor regression (19-21). CTLA-4 blockade *in vivo* of course can lead to significant autoimmunity, as well as the desired anti-tumor immune response. This autoimmunity must be managed by the therapist. These studies, however, emphasize the potentially impressive activity of anti-tumor CTL, when their lytic activity is appropriately modulated.

Similarly, the suppressive effects of $CD4^+$, $CD25^+$ T-regulatory cells in suppressing autoimmune responses indicates that they may also have a suppressive effect on CTL anti-tumor responses (22,23). Additional research on the depletion of these cells from patients to enhance the effectiveness of CTL tumor cell killing during adoptive immunotherapy is required.

Another approach to improving this immunotherapy involves genetic engineering of T-cells to express a defined antigen specificity (15). A library of effective TCR and β chains could be cloned *in vitro* into appropriate vectors, and transferred into selected cancer cells. There are several reports of the transfer of CTLs, engineered to express a gene coding for a tumor antigen specific T-cell receptor enabling such engineered CTL to recognize tumor cells expressing the cognate antigen. Finally, other aspects of CTL physiology, such as improving CTL cytokine cocktails or engineering CTL to produce autocrine cytokines that would enhance their *in vivo* survival, may also improve the effectiveness of CTL in adoptive immunotherapy (15).

6. HUMORAL TARGETING OF TUMOR ANTIGENS

Activation of B lymphocytes results in antibody synthesis and secretion. Antibodies directed against tumor cell antigens can find proteins on the tumor cell surface and mediate complement dependent cell lysis or initiate antibody dependent cell mediated cytotoxicity (ADCC), with NK cells or macrophages. Cell surface bound antibody may also inhibit cell growth, induce apoptotic signals, or increase immunogenicity. Enhancing antibody production from B-cells *in vivo* or infusing large amounts of *in vitro* derived antibody has the potential to enhance tumor cell cytolysis. The development of monoclonal antibodies in the mid 1970's actually allowed the development of large quantities of specific antibodies for passive antibody transfer therapy. Initial attempts at using monoclonal antibodies therapeutically utilized murine monoclonal antibodies against the IL-2 receptor expressed in many T-cell leukemias and lymphomas. This early approach , however, had low therapeutic efficacy. Native mouse monoclonal antibodies were found to be immunogenic in humans. In addition, they have a short *in vivo* half lives, and generally do not fix human complement or elicit ADCC with human macrophages or NK cells (8). With improved understanding of the reasons for the early failure of monoclonal antibody therapy, strategies were developed to molecularly modify the antibodies to remove immunogenic murine sequences while

retaining the 5-10% of the mouse component involved in the antibody antigen recognition. This also improved the pharmacokinetics of these antibodies (24,25).

Such strategies have led to increasing numbers of monoclonal antibodies being used in tumor therapy regimens. One of the most widely used is rituximab, which binds CD 20, an antigen involved in B-cell activation. When administered alone or in combination with chemotherapy, high rates of response were observed in patients with low grade non-Hodgkins lymphoma (26). Rituximab appears to work through an ADCC mechanism, whereas other monoclonal antibodies directly induce apoptosis or block growth signals in the target tumor cell. A humanized monoclonal antibody against the Her-2/Neu tyrosine kinase receptor on mammary tumor cells and antibodies against an IL-2 receptor submit on HTLV-1 associated T-cell leukemia have also shown regression responses in patients (27,28). The tumor cell lytic capacity of monoclonal antibodies can be enhanced by engineering recombinant bispecific antibodies that simultaneously bind tumor cells, while activating surface receptors such as CD3 or Fc γ receptor on immune effector cells. In fact, it has been suggested that engagement of Fcγ receptors in effector cells is the major component of *in vivo* monoclonal antibody anti tumor activity (29,30). Experiments with Fcγ receptor knock out mice, that do not express Fcγ receptor III, have shown a reduced anti tumor responsiveness with rituximab against mouse tumor xenografts. It was further shown that the inhibitory receptor Fc-γII B can be an important negative regulator of *in vivo* ADCC. Thus, engineering monoclonal antibodies to enhance binding to appropriate Fc receptors while reducing interaction with inhibitory Fc receptors could facilitate the use of monoclonal antibodies against tumor antigen targets (8, 30).

7. USE OF HEAT SHOCK PROTEINS AS NON SPECIFIED TARGETS FOR IMMUNOTHERAPY

Heat Shock proteins are chaperones aiding in the intracelluar transport of peptides throughout the cell (31). The observation that heat shock proteins isolated from murine tumors elicited immunity to the tumors from which they were derived led to the finding that these chaperone proteins were associated with antigenic peptides (31). These heat shock protein bound peptides induced strong and specific T-cell immunity. Peptides stripped from the accompanying heat shock proteins were composed of peptides from a large variety of proteins, including CTL epitopes from the tumor of origin. The heat shock proteins themselves, however, were not immunogenic. Thus, the specific antitumor immunogenicity generated by heat shock protein vaccination resides in the unique mixture of antigenic peptides that are present in the different tumors the heat shock proteins are derived from. The unique mix of peptide sequence is a function of the mutations in the tumor genome. As mutations occur randomly, the antigen repertoire is likely to be highly tumor specific. The heat shock protein peptide complex confers specific immunity against the tumor that the complex was derived from (31,32).

A striking aspect of the heat shock protein complex resides in the fact that although the heat shock proteins themselves are not immunogenic, they are different from other peptide binding proteins in the endoplasmic reticulum. Other such peptide binding proteins are loaded with much larger amounts of peptide, but they do not induce immunity. The immunogenicity of the gp96 heat shock protein is the result of a distinct receptor (CD91) present on dendritic cells that recognizes gp96 (33). Sequencing studies have shown that the gp96 receptor is the same as the α_2 macroglobulin receptor, and α_2 macroglobulin itself can block gp96 uptake by APCs. This receptor probably facilitates the uptake of antigen by these APC's, and their subsequent activation (32-34). Recently, however, other receptors for heat shock proteins have been recognized, including the Toll like receptor pathway and the lipopolysaccharide pathway (35). This is consistent with the finding that the immunity elicited by heat shock protein vaccination requires APCs and CTLs, as deletion of either cell type abrogates specific protection (35, 36). The effectiveness of exogenously administered heat shock protein peptide complexes in priming CTL suggests that peptides gain access to the HLA class I (murine MHC) antigen processing pathway in the vaccinated host's dendritic cells and macrophages. Observations of purified heat shock proteins with a variety of immunocytes shows that these proteins elicit a variety of innate responses that are peptide independent. Such responses include cytokine and chemokine release by lymphocytes and antigen presenting cells, differentiation of dendritic cells, and migration of dendritic cells to draining lymph nodes. These studies suggest that the induction of these innate immune responses contribute to the induced tumor immunity (32,35,36). Autologous heat shock protein vaccines have the advantage of being able to deliver a tumor unique antigen mix to patients, and the unique tumor antigens do not need to be identified to create the vaccine. The heat shock proteins constitute one of the most highly conserved families in biology and it should be possible to transfer the vaccine development techniques from laboratory to laboratory with a high degree of success.

8. REFERENCES

1. Dighie, A. Richards, E., Old, L., and R. Schreiber.: Enhanced *in vivo* growth and resistance to rejection of tumors expressing dominant negative interferon γ receptors. *Immunity* 1, 447-456 (1994)

2. Shankaran, V., Ikeda, H., Bruce, A. White, M., Swanson, P., Old, L., and , R. Schreiber : IFN δ and lymphocytes prevent primary tumor development and shape tumor immunogenicity.
Nature 410, 1107-1111 (2001)

3. Dupont, B. : Introduction: Current concepts in immunity to human cancer and therapeutic anti cancer vaccines. *Immunological Reviews* 188, 5-8 (2002)

4. Germain, R., and D. Margulies:The biochemistry and cell biology of antigen processing and presentation. *Ann. Rev. Immunol*. 11, 403-450 (1993)

5. Berzofsky, J. Terabe, M., Oh, S., Belyakov, I., Ahlers, J., Janik, J., and J. Morris.: Progress on new vaccine strategies for the immunotherapy of cancer. *J. Clin. Invest.* 11, 1515-1525 (2004)

6. Renkvist, N., Castelli, C., Robbins, P., and G. Pariniani.: A listing of human tumor antigens recognized by T-cells. *Cancer Immunol. Immunother*. 50, 3-15 (2001)

7. Houghton, A., Gold, J., and N. Blachere.: Immunity against cancer: lessons learned from melanoma. *Curr. Opin. Immunol*. 3, 134-140 (2001)

8. Waldmann, T.: Immunotherapy: past, present, and future. *Nature Med*. 9, 269-277 (2003)

9. Dubois, S. Mariner, J., Waldmann, T. and Y. Tagaya.: IL-15 receptor α recycles and presents IL-15 in trans to neighboring cells. *Immunity* 17, 537-547 (2002)

10. Dranoff, G., Jaffee, E. Lazenbera, A., Golumbek, P., Levitsky, H., Brose, K. Jackson, V., Hamada, H., Pardoll, D., and R. Mulligan: Vaccination with irradiated tumor cells engineered to secrete murine GM-CSF stimulates potent specific and long lasting anti tumor immunity.*Proc Nat. Acad. Sci. USA* 90, 3539-3543 (1993)

11. LaRosa, C., Krishnan, R., Markel, S. Schneck, J., Houghton, R., Pinilla, C., and D. Diamond : Enhanced immune activity of cytotoxic lymphocyte epitope analogs derived from positional scanning of combinatorial libraries. *Blood* 97, 1776-1786 (2001)

12. Melero, I., Shuford, W., Newby, S. Aruffo, A., Ledbetter, J., Hellstrom, K., Mittler, R.,and L. Chen: Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicates established tumors, *Nature Med*. 3, 682-688 (1997)

13. Dudley, M., and S. Rosenberg, : Adoptive cell transfer therapy for the treatment of patients with cancer. *Nature Med*. 3, 682-688 (1997)

14. Yee, C., Thompson, J., Byrd, D., Riddell S., Rodie, P., Celis, E., and P. Greenberg.: Adoptive T-cell therapy using antigen specific CD8+ T-cell clones for the treatment of patients with metastatic melanoma: *In vivo* persistence, migration, and anti tumor effect of transferred T-cells. *Proc. Nat Acad.. Sci. USA* 99, 16168-16173 (2002)

15. Ho, W., Blattman, J., Dossett, M., Yee, C., and P. Greenberg.: Adoptive immunotherapy: engineering weapons for tumor mass destruction. *Cancer Cell*, 3, 431-437 (2003)

16. Sun, J., and M. Bevan: Defective $CD8^+$ T cell memory following acute infection without CD4+ help *Science* 300, 339- 342 (2003)

17. Shedlock, D., and H. Shen: Requirement for $CD4^+$ T cell help in generating functional $CD8^+$ T cell memory, *Science* 300, 337-339 (2003)

18. Blattman, J., and P. Greenberg: Cancer Immunotherapy: a treatment for the masses. *Science* 305, 200-205 (2004)

19. van Elsas, A., Sutmuller, R., Hurwitz, A., Ziskin, J., Villsenor, J., Medina, J., Overwyk, W., Restifo, N., Melif, C., Offringa, R., and J. Allsion.: Elucidating the autoimmune and anti tumor effector mechanisms of a treatment based on cytotoxic lymphocyte antigen 4 blockade in combination with a B-16 melanoma vaccine: comparison of prophylaxis and therapy. *J.Exp. Med.* 194, 481-489 (2001)

20. Phan, G., Yang, J., Sherry, R., Hwu, P., Topalian, S., Schwartezenruber, D., Restifo, N., Haworth, L., Seipp, C. Freezer, L., Morton, K., Mauronakis, S., Duray, P., Steinberg, S., Allison, J., Davis, T., and S. Rosenberg.: Cancer regression and autoimmunity induced by cytotoxic T-lymphocyte associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Nat. Acad. Sci*. 100, 8372-8377 (2003)

21. Hodi, F., Mihm, M., Soiffer, R., Haluska, F., Butler, M., Seiden, M., Davis, T., Spries, R., MacRae, S., Willman, A., Padera, R., Jaklitsch, M., Shankar, S., Chen, T., Korman, A., Allison, J., and G. Dranoff.: Biologic Activity of cytotoxic T-lymphocyte associated antigen 4 antibody blockade in previously vaccinated melanoma and ovarian carcinoma patients. *Proc. Nat. Acad. Sci.* 100, 4712-4717 (2003)

22. O'Garra, A., and P. Viera: Regulatory T cells and mechanisms of immune system control, *Nature Med.*, 801-805 (2004)

23. Sakaguchi, S.: Naturally arising CD4+ regulatory T cells for immunologic self tolerance and negative control of immune responses, *Annu. Rev. Immunol*. 22, 531-562 (2004)

24. Jones, P., Dear, P., Foote, J., Newberger, M., and G. Winter, Replacing the complementarity determining regions in a human antibody with those from a mouse. *Nature* 321, 522-525 (1986)

25. Queen, C., Schneider, W., Selick,H., Payne, P., Landolfi, N., Duncan, J., Andolovic, N., Levitt, M., Junglians, R., and T. Waldmann.: A humanized antibody that binds to the human IL-2 receptor. *Proc. Nat. Acad. Sci. USA* 86, 10029-10033, (1989)

26. Mc Laughlin, P., Grillon-Lopez, A., Link, B., Levy, R., Czuczman, M., Williams, M., Heyman, M., Bence-Bruckler, I., White C., Cabanillas, F., Jani, V., Lister, J., Wey, K., Shin, D., B. Dallaire: Rituximab chimeric anti CD 20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to four dose treatment program. *J Clin. Oncol*. 16, 2825-2633 (1998)

27. Baselga, J., Norton, L., Albarell, J., Kim, Y. and J. Mendelsohn.: Recombinant humanized anti HER-2 antibody (Herceptin) enhances the anti tumor activity of

paclitaxel and doxorubicin against Her-2 neu expressing human breast cancer xenografts. *Cancer Res*. 58, 2825-2831 (1998)

28. Waldmann, T. White, J., Goldman, C., Top, L., Grant, A., Baniford, R., Roessler, E., Horak, I., Zaknoen, S., and C. Kasten-Sportes: The interleukin-2 receptor: a target for monoclonal antibody treatment of human T-cell leukemia. *Blood* 82, 1701-1712 (1993)

29. Clynes, R., Takechi, Y., Moroi, Y., Houghton, A., and J. Ravetch.: Fc receptors are required in passive and active immunity to melanoma. *Proc. Nat. Acad. Sci. USA* 95, 6252-6256 (1998)

30. Clynes, R. Towers, T., Presta, L., and J. Ravetch: Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. *Nature Med*. 6, 443-446 (2000)

31. Srivastava, P., Menaret, A., Bash, S., Binder, R., and K. McQuade : Heat shock proteins come of age; primitive functions acquire new roles in the adaptive world. *Immunity* 8, 657-665 (1998)

32. Srivastava, P.: Immunotherapy of human cancer: lessons from mice, *Nature Immunol*. 5, 363-366 (2000)

33. Binder, R., Han, D., and P. Srivastava.: CD91: a receptor for heat shock protein gp96 *Nature Immunol.* 2, 151-155 (2000)

34. Schild, H., and H-G. Rammensee: gp96- The immune system's Swiss knife, *Nature Immun*. 2, 100-101 (2000)

35. Oki, Y. and A. Younes: Heat shock protein based cancer vaccines, *Expert Rev. Immunol*. 3, 403-411 (2004)

36. Lewis, J.: Therapeutic cancer vaccines: using unique antigens, *Proc.Nat.Acad.Sci.USA* 101, suppl.2 14653-14656 (2004)

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