CANCER VACCINES: THE CHALLENGE OF DEVELOPING AN IDEAL TUMOR KILLING SYSTEM

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1. ABSTRACT

Despite the evidence that the immune system plays a significant role in controlling tumor growth in natural conditions and in response to therapeutic vaccination, cancer cells can survive their attack as the disease progresses and no vaccination regimen should be currently proposed to patients outside experimental clinical trials. Clinical results show that the immune system can be actively polarized against malignant cells by means of a variety of vaccination strategies, and that in some cases this is associated with tumor regression. This implies that under some unique circumstances, the naturally "dormant" immune effectors can actually be put at work and used as endogenous weapons against malignant cells. Consequently, the main challenge of tumor immunologists appears to lie on the ability of reproducing those conditions in a larger set of patients. The complexity of the immune network and the still enigmatic host-tumor interactions make these tasks at the same time challenging and fascinating. Recent tumor immunology findings are giving new impetus to the development of more effective vaccination strategies and might revolutionize the way of designing the next generation of cancer vaccines. In the near future, the implementation of these insights in the clinical setting and the completion/conduction of comparative randomized phase III trials will allow oncologists to define the actual role of cancer vaccines in the fight against malignancy.

Cancer Vaccines

Table 1. Anti-tumor immunotherapy strategies

Non-specific immunotherapy	Exogenous immunostimulants	BCG, OK-432	
	Cytokines	IL-2, IFN-α, IFN-γ	
Antigen-specific immunotherapy	Passive	mAb[1]	Naked/conjugated (radioisotope, toxin)
			Partially/fully humanized
	Active (vaccines)	Undefined TAA	Whole tumor cells
			Modified tumor cells[2]
			Shed antigens
			Heat shock proteins
		Defined TAA	Recombinant protein
			Ganglioside
			Ab idiotype[3]
			Anti-TAA Ab[4]
			Peptide (± IL-2)
			DNA plasmids[5]
			Viral vectors[5]
		Dendritic cells	Pulsed with peptides/tumor cells
			Transfected with TAA
			Fused with tumor cells (hybrid)
Adoptive transfer	CTL (± IL-2)		
	NK cells (± IL-2)		

Ab: antibody, BCG: bacillus Calmette-Guérin, CTL: cytotoxic T lymphocytes, IFN-α: interferon-alpha, IFN-γ: interferon-gamma, IL-2: interleukin-2, NK: natural killer, TAA: tumor-associated antigens. [1] Monoclonal antibodies to TAA, [2] Tumor cells can be haptenized or genetically engineered to secrete cytokines such as IL-2, IFN-α, etc, [3] The idiotype of Ab expressed by some B-lymphomas can be considered and used as highly specific TAA, [4] Using murine Ab to human TAA it is possible to induce in patients the production of anti-idiotype Ab that resemble the original TAA, [5] Coding for TAA ± cytokines

2. INTRODUCTION

Despite the continuous advances in tumor biology and the refinements of conventional therapeutic approaches (i.e. surgery, chemotherapy and radiation therapy), cancer-related mortality rates have not strikingly changed over the last few decades. Therefore, the development of newer molecularly targeted treatments is urgently needed in order to translate our knowledge of the molecular mechanisms underlying cancer development/progression into effective therapeutic strategies (1).

Cancer vaccines embody the ideal tumor-killing system for three main reasons: 1) unlike chemotherapy, which follows a log-kill kinetics, immune system mediators can theoretically hunt-down single malignant cells; 2) its potentially extreme tumor specificity, which has so far no equals among anticancer agents/strategies, guarantees minimal toxicity (2); 3) once appropriately trained, the immune system can mount a "cytotoxic memory" against the targeted tumor, ensuring further protection against disease recurrence.

The concept of immune surveillance, proposed by Burnet in the 1950s, holds that a physiologic function of the immune system is to recognize and destroy clones of transformed cells (3). Although the importance and even the existence of immune surveillance has been questioned, it is now clear that the immune system can react and sometime play an important role in tumor control in humans (4). Both cytotoxic T-lymphocytes (CTL) and antibodies specific for tumor-associated antigens (TAA) have been found in tumor-bearing patients (5-8). In addition, CTL isolated from patients affected with cancercan lyse autologous and HLA-matched tumor cells *in vitro* (9). Immunosuppressed individuals, such as organ transplant recipients or patients affected with primary or acquired immunodeficiency disorders, have an increased risk for developing malignancy (10,11).

Molecularly defined or undefined TAA can be administered to cancer patients in an attempt to induce a systemic immune response ultimately leading to malignant cell destruction. Like vaccine development for infectious diseases, this procedure is defined as active specific immunotherapy (ASI) or vaccination, as the host immune system is activated *ex novo* or re-stimulated to mount an effective tumor-specific immune reaction against malignant cells. Other cancer immunotherapy types have been proposed, which are not the subject of this review (Table 1). Following a variety of ASI modalities, objective tumor responses have been obtained in up to 20-40% of patients with different types of solid and hematological malignancies (12,13). However, the results of anticancer vaccines appear to have reached a plateau of results in the clinical setting, and currently no vaccination regimen is indicated as the standard therapy for any type of malignancy.

Overall, despite the large body of data available on this subject, we still do not know in detail the cascade of molecular events leading to an effective immune response seen in some cancer patients following vaccination: only the dissection of these mechanisms will allow us to identify better strategies to polarize the immune system against tumors.

In this work, the principles, challenges and results of anticancer vaccination are reviewed. To this aim, PubMed searches of the National Library of Medicine were performed. Where appropriate, cited references from

Table 2. Tumor associated antigens (TAA)

Category	TAA	Tumor
Unique antigens	β-catenin	Melanoma
	CDK-4	Melanoma
	Gn-TV	Melanoma
	SYT-SSX (fusion protein)	Soft tissue sarcoma
	p53	Several carcinomas
	K-RAS	Several carcinomas
	APC	Colorectal carcinoma
	TGFβ receptor-II	Colorectal carcinoma
	Caspase-8	Head & neck tumors
Differentiation antigens	gp100 (pmel117)	Melanoma
	MART-1 (Melan-A)	Melanoma
	Tyrosinase	Melanoma
	TRP-1	Melanoma
	TRP-2	Melanoma
	PSA	Prostate carcinoma
Shared antigens	MAGE family	Several tumor types
	GAGE family	Several tumor types
	BAGE-1	Several tumor types
	SSX-2	Several tumor types
	SAGE	Several tumor types
	LAGE-1/CAMEL	Several tumor types
	NY-ESO-1/LAGE-2	Several tumor types
Overexpressed antigens	CEA	Several carcinomas
	HER-2/neu (erbB-2, p185)	Several carcinomas
	CO17-1A (Ep-Cam, GA733-2)	Several carcinomas
	MUC-1	Several carcinomas
	Survivin	Several carcinomas
	Telomerase	Several carcinomas
	gp72/CD55	Several carcinomas
	PRAME	Several carcinomas
	β-hCG	Several carcinomas
	α-fetoprotein	Hepatocellular carcinoma
	globo-H, TF-alpha, sialyl-Tn	Several carcinomas
	Gangliosides (e.g. GM1, GM2)	Melanoma
Viral antigens	E6, E7 (human papilloma virus)	Cervical carcinoma

Tumor associated antigens (TAA) are generally classified into five categories: *a) unique antigens* (or tumor-specific antigens) are specific to an individual tumor and result from tumor genetic instability phenomena (e.g. mutations, translocations); *b) differentiation antigens* (or tissue-specific antigens) are expressed by the tumor and the normal tissue from which it derives; *c) shared antigens* (or cancer-testis antigens) are expressed by a variety of tumor types and not by normal tissues, with the exception of spermatogonia; *d) overexpressed antigens* can be found in normal tissues but are massively overexpressed by malignant cells; *e) viral antigens* are coded by the genome of viruses implicated in the genesis of some malignancies.

selected articles were also reviewed. The search topics included cancer vaccines, immunotherapy, and clinical trials, the only restriction criterion being English language. For ongoing clinical trials, the dedicated National Cancer Institute website (http://www.cancer.gov/clinicaltrials) was also searched.

3. TUMOR IMMUNOLOGY

3.1. Tumor associated antigens

For a long time it was thought that tumors are ignored by the immune system and that TAA do not exist. After the milestone experience of chemically or virally induced tumors rejected by syngeneic mice as a result of the *ex novo* generation of highly immunogenic tumor-specific transplant antigens (14), investigators could demonstrate that several naturally occurring TAA can be the target of humoral and/or cellular adaptive immune response (15). In particular, in the 1980s investigators

recognized that tumor-infiltrating lymphocytes (TIL) obtained from different HLA-matched patients with melanoma were capable of lysing HLA-matched melanoma cell lines (16). This proved that melanoma TAA can be shared and led to the characterization of their gene sequences and the immunogenic amino-acid sequences presented by HLA molecules on the cell surface. Since then, the number of TAA has increased rapidly. The molecular identification of TAA recognized by T-cells has opened new possibilities for the development of effective immunotherapies for patients with cancer (17). Although some TAA derive from mutated genes (18), most of them are products of non-mutated genes encoding intracellular proteins that are commonly expressed by autologous cancer cells (19) (Table 2).

Anticancer vaccination is based on the assumption that the immune system can recognize and react to TAA. Only short peptide sequences of the entire tumor

antigen protein are immunogenic. These peptide sequences (called epitopes) are presented by HLA molecules according to a set of rules derived from the proteasome cleavage sites, the affinity of transporters associated with antigen processing (TAP), and the anchoring pockets in the peptide-binding groove of the HLA molecule (20). Within the so-called immune synapse, the HLA-peptide complex presented by the antigen-presenting cell (APC) is recognized by the T-cell receptor complex (TCR) expressed on the surface of the T-cell (21).

Cancer cells frequently have derangements of gene expression, but only a minority of tumors expresses truly foreign (non-self) proteins (22). The recognition of non-mutated self-antigens on tumor cells indicates that the immune system has the adequate T-cell repertoire to generate antitumor responses that are in fact anti-self responses. The self-reactive T-cells that escaped central tolerance in the thymus circulate in the periphery of normal individuals, but can be maintained in a tolerant or ignorant state by the lack of recognizable antigen (cryptic or subdominant) or by homeostatic processes in what is called peripheral tolerance (20,23). To initiate an immune response and overcome peripheral tolerance the TAA - whether self or non-self - has to be presented with the appropriate immune activating signals (20,23).

3.2. Two-signal model and TAA cross-presentation

Immunologists have long recognized that two signals are necessary for the initial activation of naive T-cells (24). The two-signal model predicts that when an antigen is presented by an HLA molecule (signal-1) together with the costimulatory molecules B7.1/CD80 and B7.2/CD86 (signal-2), an immune response will be generated. Conversely, if only signal-1 is presented, which is the case for the majority of tumor cells, an immune response will not be initiated because signal-2 is lacking, which helps maintain peripheral tolerance or ignorance of TAA (25,26).

Because cancer cells only have signal-1 and usually lack non-self epitopes that would generate a danger signal (27), TAA need to be presented to T-cells by other types of cells, i.e. APC. This can be achieved by a process known as cross-presentation, which is a general mechanism for the induction of T-cells. TAA released by malignant cells are taken up by APC, which process and present them on their surface in a HLA class-I or -II restricted fashion. APC (e.g. dendritic cells, DC) can efficiently prime T-cells when they display HLA-antigen complexes (signal-1) together with costimulatory molecules (signal-2), which activates naive T-cells in a process known as cross-priming. However, under particular circumstances, cross-presentation can induce T-cell tolerance, a process known as cross-tolerance, which may have an important role in maintaining tolerance to self antigens (28).

3.3. Dendritic cells

DC have emerged as the most powerful APC to stimulate naive T-cells (29). These infrequent bone marrow-derived leukocytes are optimally prepared for antigen presentation and stimulation of immune cells because they have the greatest surface density of HLA and costimulatory molecules, together with a high motility (which allows them to traffic from the periphery, where they take-up antigens, to lymph nodes, where they cross-present antigens to T-cells), and have the ability to produce immunostimulatory cytokines (e.g. interleukin-12, IL-12) and chemokines. In the majority of other cell types, the HLA class-I pathway only presents endogenous antigens and is therefore unable to cross-present exogenous antigens. DC are specialized in antigen presentation to and stimulation of both the innate and adaptive immune system because of their ability to interact with CD4^{+} and CD8^{+} T-cells (adaptive immunity) as well as natural killer (NK) and natural killer T (NKT) cells (innate immunity) (30).

3.4. Immune response autoregulation

A balance exists between immune response and tolerance or ignorance of TAA. DC can tip this balance by guiding the immune system towards acceptance or rejection of tumor cells, thus making a judgment of what should be presented and recognized as non-dangerous self, dangerous self, or non-self (31). Once T-cells are activated, the immune system makes a great effort to keep them under control. Uncontrolled exponential expansion of lymphocytes after antigen stimulation would quickly overwhelm the lymphoid organs, and unchecked cytokine production and cytotoxic activity may lead to autoimmunity. An understanding of these control mechanisms of immune activation will undoubtedly help optimize the design of immunotherapy interventions and cancer vaccine development (32,33).

3.5. Cytokine profile

On the basis of the pattern of cytokine production, CD4^{+} T-cell clones can be subdivided into two distinct populations that influence and are at the same time affected by DC activity (34). T-helper-1 (Th1) clones produce interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor (TNF) (type-1 cytokines), whereas Th2 clones produce IL-4, IL-5, IL-6, and IL-10 (type-2 cytokines). Th1 clones mediate cytotoxic and delayed-type hypersensitivity (DTH) reactions, whereas Th2 cells are more potent helpers for antibody production (humoral response). Deviation from a type-1 to a type-2 cytokine profile has been associated with decreased protection to tumors and this phenomenon has been mainly linked to depressed DC function (35-38). Remarkably, the production of helper cytokines is not a function restricted to CD4^{+} T-helper cells. Other immune cells are able to produce polarized type-1 and type-2 cytokines, including CD8^{+}/NK/NKT cells and DC themselves. In particular, the latter cells can also produce IL-12, which is emerging as a pivotal cytokine in determining an efficient adaptive immune response against cancer (39,40).

3.6. Dendritic cell subsets

DC have been reported to have not only stimulatory but also inhibitory effects on the immune system (41,42). The nature, maturation process and activation state of DC may determine the type of immune response (protective/tolerogenic) generated towards a given antigen. The so-called myeloid DC and plasmacytoid DC

cells are the two principal subpopulations of human DC. These two lineages of DC have been shown to derive from a common committed hematopoietic precursor, without either one having a clearly distinct role in immune activation or tolerance. However, the maturation status of the DC, although not clearly defined, does have an impact on their ability to generate immunity or tolerance towards TAA, as demonstrated not only in preclinical models but also in humans (43-46). In order to drive DC maturation and elicit an anti-TAA immune response of therapeutic value, several agents have been evaluated (e.g. all-trans-retinoic acid; heat shock proteins (HSP); toll-like receptor (TLR) agonists, such as CpG motif-containing oligodesoxynucleotides (ODN) and double-strand RNA analogs; cytokines, such as IL-12) and have been shown to increase antitumor ASI effectiveness in preclinical models (40,47-56), while only some of them (e.g. HSP, CpG-ODN, IL-12) have already entered the clinical phase of experimentation (12,13).

3.7. Regulatory T-cells

The existence of peripheral lymphocytes with a professional suppressor function has been suspected for many years, but better characterization of these cells is relatively recent (57). A distinct population of 5% to 10% of the total $CD4^+$ T-cells constitutively express CD25 (the alpha chain of the IL-2 receptor), which is only expressed by other $CD4^+$ T-cells after TCR activation. These $CD4^+/CD25^+$ T-cells (also called T-regulatory cells) actively suppress auto-reactivity because in their presence other lymphocytes specific for self-antigens fail to react to them. The depletion of these cells leads to the development of autoimmune diseases, such as colitis or encephalitis and the potentiation of antitumor responses. These cells have been remarkably conserved in evolution, with similar properties in different species. These properties include a limited ability to proliferate, constitutive expression of intracellular and surface CTLA-4, and an ability to produce immune-suppressive cytokines, such as IL-10 and TGF-β (58). Remarkably, the presence of T-regulatory cells (particularly those expressing Foxp3, the forkhead/winged helix transcription factor) has been repeatedly correlated with immunosuppression and dismal clinical outcome in cancer-bearing patients (59-61). An intense cross-talk exists between DC and T-regulatory cells. In particular, accumulating evidence indicates that subsets of human DC play a critical role in the induction of peripheral tolerance not only by directly anergizing effector $CD4^+$ and/or $CD8^+$ T-cells but also inducing the differentiation of naive T-cells into T-regulatory cells (21,62,63). Human DC subsets with the property of suppressing an antigen-specific T-cell response include plasmacytoid DC, which are either in an immature state or in a mature state induced by CD40-ligand stimulation, and monocyte-derived DC, which are either in an immature state or have had their state modulated by treatment with IL-10. These "tolerogenic" DC may be relevant to explain how the human immune system can remain in a dormant mode though in the presence of TAA-specific T-cells (64,65). Accordingly, the modulation of T-regulatory cells activity directly or by redirecting DC function on their generation might drastically improve the efficacy of therapeutic cancer vaccines, as already

suggested in animal models (66-68) and advocated in humans (69).

3.8. Tumor immune escape

Despite the evidence that the immune system plays a significant role in controlling tumor growth in natural conditions or in response to therapeutic manipulation, cancer cells can survive their attack as the disease progresses. Several mechanisms underlying immune escape have been proposed (25,70,71) (Figure 1). Cancer genetic instability can lead to TAA/HLA down-regulation as well as to the disruption of the TAA processing/presenting machinery (e.g. proteasome, TAP-1), which in turn allows malignant cells to elude the surveillance of immune sentinels (72,73). The production of immunosuppressive cytokines (e.g. transforming-growth-factor-beta, TGF-β; interleukin-10, IL-10) (74) and the expression of lymphotoxic molecules (i.e. FAS ligand) (75) by malignant cells suggest that cancer can actively inhibit the activity of tumor-specific T-cells. To take another example, tumor cells (along with most normal cells) generally lack costimulatory molecules, such as B7.1/CD80 and B7.2/CD86, which are physiologically expressed on professional APC (76,77). In the absence of costimulation, T-cells tend to become anergic (78). In the non tumor-bearing setting, the absence of B7 molecule expression has been hypothesized to protect normal cells against autoreactivity (79). Transfection of tumor cells with both isoforms has been used successfully to trigger their immune-mediated rejection of experimental mouse tumors with some inherent immunogenicity (80).

Nevertheless, these mechanisms cannot explain all cases of immunotherapy failure and their *in vivo* relevance has been partially questioned. In humans, TAA/HLA expression is not always downregulated in progressing metastases of patients undergoing vaccination (81), and FAS-L is not expressed in most melanoma specimens (82). The immunosuppressive role of some cancer-derived molecules (e.g. IL-10) has been revisited and at least in part confuted (83,84). Yet, in animal models tumor rejection is not observed when B7 molecules are inserted into poorly immunogenic tumors (85). Non-immunogenicity is a category into which most, if not all, human tumors would fall: thus, a lack of expression of the CD80 and CD86 costimulatory molecules is unlikely to be a global explanation for immune escape.

Overall, despite the significant advances in our understanding of tumor-host relationship, the cascade of molecular events leading to tumor rejection/tolerance by the immune system is still incompletely elucidated, especially in humans. However, several recent insights in tumor immunology might help oncologists break the immune tolerance towards malignant cells in the next future.

3.9. Tumor microenvironment and immune responsiveness

Different tumor types respond to immune manipulation with a dramatically different level of sensitivity. Among cancers, melanoma and renal cell
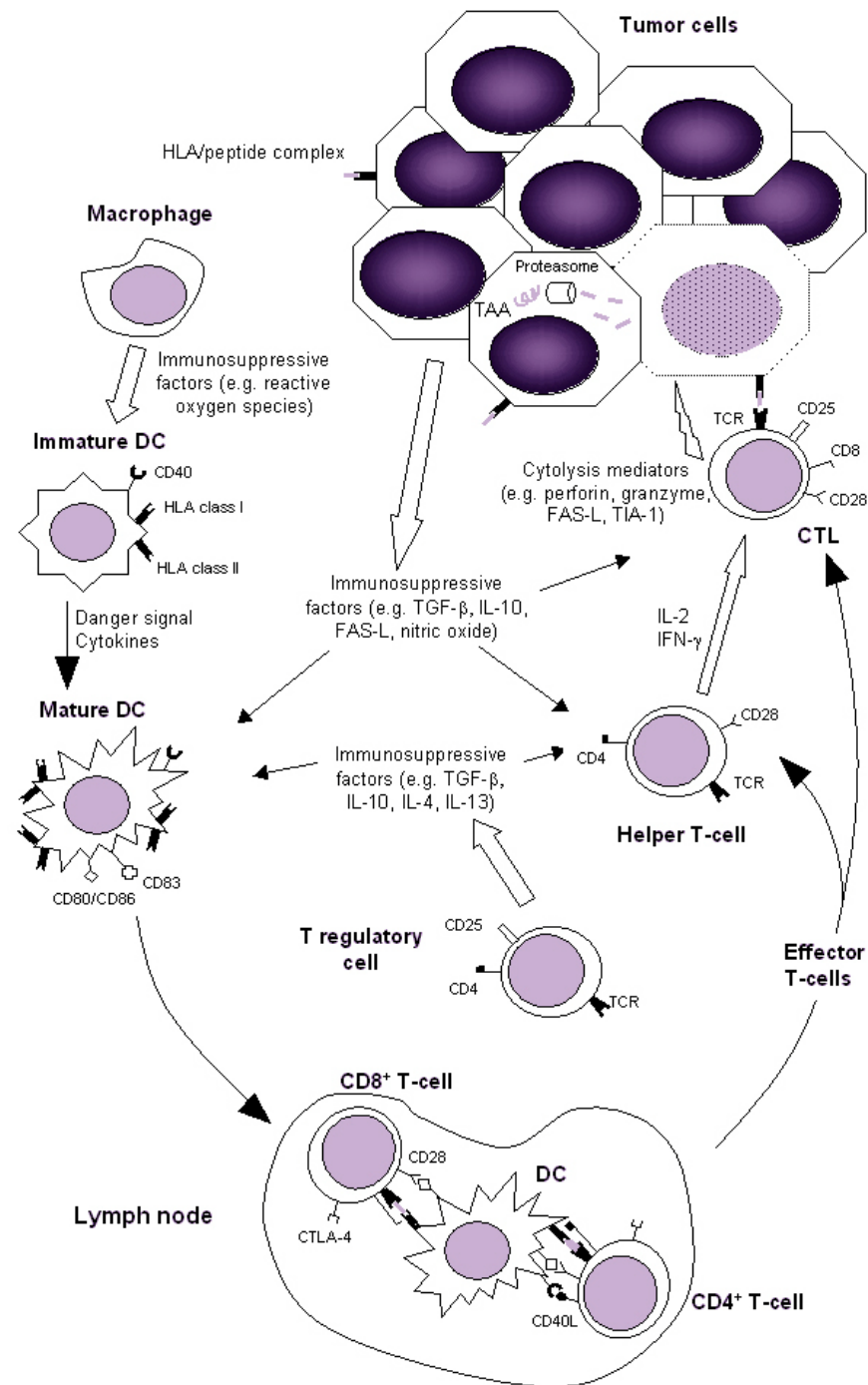
Figure 1. Immune response towards malignant cells: key cell players (for details, see main text).

carcinoma appear to be most sensitive to immune therapy, though the reason for this immune sensitivity is not known. Among these patients, only a limited number appear to respond to various types of immunotherapy and, also in this case, the reason for this heterogeneity is not clear. Finally, in the same patient it is possible to observe that some tumor nodules regress or disappear while others progress (mixed response). By identifying the unique features that govern tumor immune responsiveness/resistance by comparing responding patients/lesions with those non-responding may help understand the mechanisms governing tumor rejection and design newer and more effective immunotherapeutic regimens (86). Adopting this strategy, it was found that melanoma metastases undergoing complete regression in response to peptide/IL-2 based immunotherapy are characterized by a gene profile different from that of

progressing lesions (87,88). Using high-throughput cDNA microarray technology and quantitative real time PCR to analyze transcript levels of FNA material (89-91), a set of immune-related genes including TIA-1 and IL-10 was found to be over-expressed in melanoma metastases responding to vaccination. TIA-1 codes for a 15 kd cytotoxicity-related protein expressed by CTL and NK cells and is characterized by pro-apoptotic properties (92,93). IL-10 is generally considered an immunosuppressive molecule that can anergize CTL, acting both directly on these cells (94) and through its inhibitory effects on DC (74,95). However, several pre-clinical models have shown that IL-10 can also mediate tumor regression by stimulating NK cells activity (96-101). Furthermore, IL-10-conditioned NK cells (but not CTL) increase both TIA-1 expression and cytotoxic activity (84,102). These observations led to hypothesize that, in the presence of high levels of IL-10 in the tumor microenvironment, NK cells might be stimulated to lyse cancer cells, thus increasing the availability of TAA and the delivery of biologically active molecules (e.g. HSP, dsDNA) required by DC to be activated and effectively prime CTL against TAA (103,104). The idea that an inflammatory status can lead to a better TAA presentation has been recently strengthened in a preclinical model, in which intradermal injection of plasmid DNA encoding a transcriptionally targeted cytotoxic gene along with HSP70 not only promoted tissue-specific, inflammatory killing of normal melanocytes, but also induced a CD8$^+$ T-cell-dependent, antigen-specific response in mice that eradicated systemically established B16 tumors (50).

It is believed that NK cells play a crucial role in the early phase of adaptive immunity engagement against both infectious agents and tumor cells, thus providing a key link between innate and adaptive immunity (105). Moreover, it has been postulated that NK cell activity within the tumor microenvironment may be suppressed by some factors, including reactive oxygen species produced by tumor infiltrating monocytes and macrophages (106). Agents that inhibit the generation of reactive oxygen species (ROS), such as IL-10 (107,108) and histamine (109), might abrogate this suppression, thus offering potential therapeutic benefit as immunological adjuvants, as suggested by a recent phase III clinical trial (110). Other findings support the idea that – beside the development of TAA-specific immune cells - tumor microenvironment features play also a crucial role in determining an effective immune response against cancer. For instance, the tumor barrier comprised of non-antigenic stromal cells may contribute to the failure of tumor rejection. Forced expression of LIGHT, a member of the TNF superfamily (111), in the tumor environment induces a massive infiltration and activation of naive T-cells that ultimately leads to the rejection of established, highly progressive tumors at local and distal sites (112).

4. CLINICAL IMPLEMENTATION OF CANCER VACCINES

4.1. Cancer vaccine clinical trial design

It is perhaps useful to briefly discuss how the design of cancer vaccine trials differs from the features of traditional chemotherapy trials (113). In the development of conventional antineoplastic agents, a basic assumption is that the maximum tolerated dose (MTD) is the most effective one, which is the fundament of standard phase I studies. In developing antitumor vaccines, investigators are not usually interested in determining the MTD for at least a couple of reasons: first, most vaccines are well tolerated at dosages clinically active; second, there is wide experience with preclinical models to suggest that high vaccine doses can be less immunogenic, a phenomenon known as high-zone tolerance (114). Therefore, initial clinical trials with a new vaccine typically seek to define the tolerability (general toxicity, induction of autoimmune disease) and immunogenicity of a narrow range of relatively low dosages of vaccine. Unlike conventional chemotherapeutic drugs, the activity of vaccines is not directly proportional to the dose and depends upon several other variables, such as route of administration (intradermal, subcutaneous, intravenous, intramuscular, intranodal, intratumoral), schedule of administration and use of immunological adjuvants, as the antitumor effect of vaccines is not direct but mediated by the stimulation of a highly complex and complexly integrated cellular/molecular network, such as the immune system. Therefore, the main objective of phase I ASI trials is to identify an immunogenic formulation eliciting an appropriate immune response (surrogate marker of response to vaccination) against the target (molecularly defined or undefined TAA). Then, larger clinical trials in the therapeutic (presence of measurable, clinically evident disease) or adjuvant (absence of clinically evident disease, e.g. after radical surgery or complete remission following systemic chemotherapy) setting can be designed with phase II type objectives, such as tumor response rate or time to relapse/overall survival, respectively. As surrogate parameters of ASI efficacy (immune response) can be deceiving (i.e. immune response without clinical benefit) (64,115-117), most initial ASI studies should be designed as phase I-II trials in order to identify the optimal vaccine preparation not only on the basis of surrogate endpoints but also considering the ultimate goal of anticancer vaccination (evidence of antitumor effect).

The final test for ASI studies, as for chemotherapeutic agents, is represented by phase III randomized trials comparing immunotherapy with standard treatments.

4.2. Immune response monitoring

Like any other antineoplastic treatment, the aim of cancer vaccines is to induce tumor regression/disappearance and ultimately prolong patients' survival. The antitumor effectiveness of a given vaccine may require months (therapeutic setting) or even years (adjuvant setting) of observation to be demonstrated. A surrogate endpoint of anticancer ASI could be considered the induction of an effective immune response against malignant cells, as this objective can be yielded much more quickly than the tumor control rate by comparing the immune reactivity against TAA before and after vaccination (118-122). However, a major issue in immunological monitoring of vaccinated cancer patients is whether or not the measured immune response correlates

with the antitumor activity/effectiveness of a given ASI strategy. In fact, while the immune response (mainly antibody production) to anti-infectious disease vaccines well correlates with the degree of protection towards the targeted infectious agent, no similar conclusions can be currently drawn in the field of anticancer ASI, particularly in the case of T-cell-mediated immune responses.

4.2.1. Humoral response

Plasma antibody titers are generally measured by ELISA-based methods, which allow not only the measurement of the overall antibody response to a particular TAA but also the identification of the antibody class (IgM and/or IgG) and the avidity induced by the vaccination. These parameters have been useful to determine both the most antigenic TAA and the most appropriate immunological adjuvant to induce immunological memory as reflected by the IgG production. Cancer vaccine trials that seek to immunize patients with a carbohydrate-based antigen, which predominantly induces a humoral response, often measure tumor-specific antibody generation as a primary immunological endpoint. By contrast, many clinical studies of cancer vaccines targeting non carbohydrate-based TAA do not include analysis of humoral response. Antibody levels in some circumstances can reflect T-cell responses, as demonstrated in infectious disease and cancer models. Moreover, in some clinical studies a positive correlation between the generation of anti-TAA antibodies and patients' clinical outcome (123-128). Therefore, a larger use of humoral response monitoring in cancer vaccine trials has been advocated (119).

4.2.2. T-cell response

The two most commonly used in vitro methods for measuring TAA-specific T-cell responses include: 1) the assessment of T-cell clonal expansion following incubation of T-cells with the TAA in the presence of a radio-labeled nucleotide (e.g. ^{3}H-thymidine), and 2) the measurement of T-cell ability to lyse TAA expressing target cells labeled with radioactive (e.g. chromium release assay) or chromogenic (e.g. tetrazolium salt or MTT assay) substances. T-cell proliferation assays have distinct limitations, such as the inability to determine what T-cell type (i.e. CD8$^+$ or CD4$^+$ T-cells) is responding, poor reproducibility and a non-quantitative readout. Furthermore, the evaluation of the blastogenic response requires that antigens used are exceptionally pure. This is a challenging issue as many recombinant proteins are made in *E. Coli* systems and even small amounts of endotoxin can result in T-cell proliferation.

T-cell cytotoxicity assays, which are appropriate to for detecting CTL lytic activity, are limited by the low reproducibility (due to inconsistencies in choice and labeling of targets) and the scarce availability of autologous tumor cells (particularly in the adjuvant setting and in case of visceral tumors/metastases). The use of HLA-matching TAP-deficient T2 cells loaded with the relevant peptide can be a useful tool to overcome the latter issue.

No correlation between the immune response as measured by these two methods and clinical outcome has been reported, yet.

Enzyme-linked immunospot (ELISPOT) assays is based on the principle of the ELISA method. A 96-well nitrocellulose-bottomed microtiter plate is coated with an antibody that binds the cytokine of interest (most often IFN-γ). To detect TAA-specific T-cells, either unseparated peripheral blood mononuclear cells (PBMC) or isolated CD8$^+$ or CD4$^+$ T-cells are incubated along with the TAA. In response to the TAA, T-cells release the cytokine of interest, which is bound to the antibody-coated well and revealed by the following enzyme-labeled detection antibody/chromogenic substrate system. The detection limit of ELISPOT assays was calculated to be 10-200 times lower than ELISA performed on culture supernatants, which is generally considered the lowest limit among all methods for immunological monitoring. Correlation with clinical outcome has shown conflicting results (12).

Compared to ELISPOT, intracellular fluorescence-activated cell sorting (intracellular-FACS) offers the advantages of speed and the ability of identifying subsets of reactive T-cells (e.g. cytotoxic versus helper T-cells; memory versus effector T-cells); however, its detection limit is higher. The application of this immunological tool in cancer vaccine trials is still in its infancy.

Soluble recombinant HLA-peptide tetramers are being increasingly used to identify and quantify TAA-specific T-cells. Fluorescent HLA/peptide tetramers, upon incubation with a polyclonal mixture of T-cells, bind those bearing the corresponding specific TCR and can be detected by flow cytometry. While most published information has been gathered using HLA class I/peptide tetramers for CD8$^+$ T-cell screening, HLA class II/peptide tetramers have also been developed for the assessment of CD4$^+$ T-cell-mediated immune responses. A limitation of this technology is that many clinically important epitopes bind HLA molecules with low affinity that precludes the production of the corresponding tetramers. Moreover, when T-cells are stimulated with the relevant epitope, a high level of TCR downregulation can occur that does not allow for the identification of all T-cells expanded by vaccination. Finally, it must be remembered that the information provided by this method is purely quantitative, whereas the functional status of T-cells identified with this technology has been found to be depressed (129,130). Although some investigators could correlate the presence/increase of CTL as assessed by tetramer technology with a favorable clinical outcome (131,132), others did not (130,133).

Finally, quantitative real-time polymerase chain reaction (qrt-PCR) has been recently proposed for the monitoring of vaccine trials (89,134).

Overall, several monitoring strategies have been implemented to evaluate the T-cell response to anticancer ASI in the peripheral circulation as well as the tumor site, including limiting dilution, *in vitro* sensitization and ELISPOT, which have been so far the most widely utilized methods. These assays, though demonstrating that antigen-specific vaccination can elicit immune responses detectable in the peripheral blood of immunized patients, have been

faulted by their limited sensitivity that often requires in vitro expansion of patient's lymphocytes. More recently, the use of soluble HLA/peptide complex tetramers, intracellular (FACS) analysis, and quantitative real-time polymerase chain reaction (qrt-PCR) has been proposed for the monitoring of vaccine trials. When combined, these methods have the appeal of allowing not only direct enumeration of T-cells specific for a particular epitope within relevant samples (e.g. PBMC; secondary lymphoid organs such as lymph nodes; tumor biopsies) but also to determine the lymphocyte activation status with high sensitivity.

4.3. CURRENT VACCINATION STRATEGIES

4.3.1. Polyvalent vaccines
4.3.1.1. Whole cell polyvalent vaccines
Living whole tumor cells inactivated by irradiation are the earliest forms of antitumor vaccines and have been extensively studied in human trials. The concept of using autologous whole cells remains appealing because these vaccines should contain a large repertoire of TAA potentially targeted by the immune system, including TAA unique to the patient tumor. However, the main drawback of autologous tumor cell vaccines is the difficulty of obtaining tumor cells from patients with visceral disease and their scarce availability in the adjuvant setting. Allogeneic preparations, which are made out tumor cell lines grown in vitro, overcome the limitation of cancer cell source. However, it can be objected that such vaccine preparations introduce dominant allogeneic antigens (i.e. HLA molecules) that might overwhelm the induction of an effective immune response to more relevant but weaker antigens (i.e. TAA). Several efforts have been made to increase the immunogenicity of these vaccines. Autologous tumor cells have been aptenized by reacting them with dinitrophenyl (DNP), exploiting the ability of haptens to induce an immune response to the carrier antigen they are coupled with (135). More recently, both autologous and allogeneic tumor cells have been transfected with cytokines (e.g. GM-CSF, IL-2, etc.) in order to recruit and activate APC at the site of vaccine injection, thus favoring the process of TAA uploading and ultimately TAA presentation to T-cells in secondary lymphoid organs (reviewed in (12)).
The efficacy of autologous/allogeneic whole cell tumor vaccines has not been proven by randomized trials so far performed in the adjuvant setting, although there might be a survival advantage for patients with immunological response to vaccination (123,136,137). Other randomized trials are under way in both the therapeutic and adjuvant setting.

4.3.1.2. Tumor lysate vaccines
The ability of tumor lysates to induce a long-lasting antitumor immunity was first demonstrated in animal models employing viral oncolysis (138). Subsequent studies demonstrated that tumor cell lines lysed mechanically or enzymatically can also evoke an effective immune response (139).

In humans, randomized studies carried out in the therapeutic and adjuvant settings have not demonstrated the efficacy of lysate vaccines (140), although a survival benefit has been described for patients who might better respond to vaccination based on their HLA pattern (141).

4.3.1.3. Heat shock proteins
Heat shock proteins (HSP) are intracellular proteins that act as chaperones for peptides, including TAA-derived peptides (142). DC express CD91, a specific receptor for HSP whose engagement leads to maturation of these APC (143). Therefore, HSP released by necrotic cells function both as endogenous danger signal and as a vehicle to cross-present TAA by DC. HSP can be isolated and used as polyvalent autologous cancer vaccine preparation of undefined TAA, thus obviating the need to identify TAA epitopes recognized by CTL.

On the basis of the encouraging results obtained in animal models (144) and pilot studies conducted in humans (145), HSP are being tested in phase III randomized trials enrolling patients affected with resected renal cell cancer or melanoma.

4.3.1.4. Shed antigen vaccines
This partially purified allogeneic polyvalent vaccine is made from surface material shed into a culture medium under proper laboratory conditions by a pool of selected tumor cell lines (146). This vaccine formulation provides, in theory, a broad array of TAA epitopes released from HLA molecules present on malignant cells, without the risk of contamination with irrelevant allogeneic antigens and perhaps immunosuppressive factors present inside malignant cells.

In the adjuvant setting, a significant disease-free (but not overall) survival advantage has been observed in a randomized phase III trial of patients with resected stage III melanoma (147).

4.3.2. Antigen-defined vaccines
4.3.2.1. Carbohydrate vaccines
Carbohydrates represent the epitope of several TAA including glycosphingolipids and glycoproteins. They are either overexpressed by cancer cells (e.g. gangliosides) or unique TAA originating from the altered glycosylation of mucins characteristic of tumor progression (148).

In a randomized trial, the efficacy of a ganglioside-based vaccine was compared to IFN-α for the adjuvant treatment of high-risk melanoma patients (TNM stage II-III) and the results showed that IFN-α guaranteed a survival advantage over vaccination (149). Nevertheless, it was also noted that patients with humoral response to the vaccine did show a better clinical outcome as compared to non-responders. Currently, a phase III study (ganglioside versus control) is under way in patients with stage II cutaneous melanoma. A large randomized trial using the aberrant mucin sialyl-Tn epitope has been recently completed but results have not been published yet (150).

4.3.2.2. Peptide vaccines
A large number of studies demonstrated the existence of tumor-specific antigens on mouse and human

tumors that can be recognized by autologous CTL. Most of these antigens consist of peptides that are presented to T-cells by HLA molecules The majority of TAA-derived peptides identified so far are presented in association with class I HLA molecules and are recognized by tumor-specific CTL, although the number of TAA epitopes presented in association with class II HLA molecules and recognized by helper T-cells is growing. Although tens of T-cell-defined TAA peptides are currently available (19), their clinical use is limited to patients expressing the appropriate HLA molecule and to tumors expressing the TAA from which the vaccine peptide derives.

The majority of TAA epitopes derive from normal proteins, for which immune tolerance may prevent immunogenicity. One of the strategies employed to deal with this phenomenon is to synthetically modify the sequence of natural epitopes at amino acid residues that are crucial for the interaction with the HLA/T-cell receptor molecules (151).
Both objective tumor responses and survival benefit (particularly in immune responders) have been reported with the use of such ASI strategy, alone or in combination with IL-2 (125,152-154). Randomized trials are ongoing in the therapeutic setting.

4.3.2.3. Recombinant protein vaccines
The use of recombinant proteins is attractive because they are molecularly defined and easily produced by means of recombinant technology. Moreover, their administration enables the patient's own immune system to recognize HLA class I and/or II restricted peptides, thus overcoming the requirement of knowing their sequence in advance, which is necessary for peptide-based vaccines. Idiotype-based vaccines for patients with B-cell malignancies belong to this group of ASI (reviewed in (13)). Although the experience is still limited and only one randomized trial has been performed, some survival advantage has been reported in patients developing an immune response to the vaccine (124,155,156).

4.3.2.4. Recombinant virus vaccines
The finding that viral infections lead to the presentation of viral peptides in association with MHC class I and class II HLA molecules on the surface of infected cells has led to the design of strategies in which viruses are used as immunization vehicles. Viruses such as vaccinia, poxvirus and adenovirus are potentially ideal vectors for the delivery of TAA, because of their ability to directly infect and activate APC (157). To circumvent the development of anti-virus neutralizing antibodies that diminished the host response to TAA during subsequent vaccination boosts, investigators have tested viral vectors (i.e. avipox virus) with the ability to infect human cells and express the transgene for 2-3 weeks without replicating. Recombinant viruses have also been utilized to encode and express molecules acting as immunological adjuvants (e.g. B7.1). Although some objective responses have been observed, none of the few studies thus far performed has demonstrated any significant clinical benefit (reviewed in (12)).

4.3.2.5. DNA vaccines
Naked DNA vaccines consist of the specific gene encoding the TAA of interest cloned into a bacterial plasmid engineered for optimal expression in eukaryotic cells (158). Notably, DNA vaccines also contain nucleotide sequences (e.g. oligo-CpG) that can act as immunological adjuvants (159). Plasmid DNA, which can also express immunological adjuvants other than oligo-CpG (e.g. cytokines) and costimulatory molecules, has the advantages that it is readily deliverable, molecularly defined and can be easily constructed and produced in large quantities.

No major objective responses have been yet described in humans, but the implementation of this ASI strategy in the clinical setting is still in its infancy (reviewed in (12)).

4.3.2.6. Anti-idiotype vaccines
Conventional network theory postulates that a mirror-image antibody can be generated to any specific antibody (160). Vaccination with TAA-specific mouse antibodies (Ab1) results in the formation of autologous antibodies (Ab2) against the vaccine. The variable part of these induced antibodies fits to the idiotype of Ab1, and therefore strongly resembles the epitope of the original TAA. Consequently, this anti-idiotype can be used as a surrogate vaccine in place of the natural TAA by stimulating cellular and/or humoral (i.e. production of Ab3 antibodies) immune responses. This vaccination strategy requires relatively low amounts of vaccine preparation and allows for the vaccination towards non-protein antigens (e.g. carbohydrates) that are difficult to be cloned. Furthermore, in preclinical models anti-idiotype antibodies are particularly effective in breaking immune tolerance to TAA (161). Blocking antibodies against the murine immunoglobulin can be produced by the host. This phenomenon can be avoided by using the technique of humanized antibodies (162).

In the small number of patients so far enrolled in non-randomized studies, some clinical benefit has been reported, particularly in immune responders (126,163). Randomized phase III trials are being carried out.

4.3.3. Dendritic cell based vaccines
The fate of TAA largely depends on their ability to be internalized and processed by DC, the most effective APC. DC expressing high levels of HLA class I and class II as well as costimulatory molecules have been demonstrated to be effective in presenting TAA peptides to enhance cellular immunity both in vitro and in vivo (164). The use of TAA/peptide-loaded DC requires prior knowledge of patient HLA types and the sequences of the relevant antigens/epitopes. To overcome this limitation, tumor cells or their mRNA content (165) have been used as immunogens. As a further development of the DC-based vaccination strategy, some investigators have proposed the fusion of cancer cells with DC to generate cell hybrids with the characteristics of an antigen-presenting cell able to process endogenously provided TAA (166).

Despite the strong preclinical evidence supporting the use of DC in humans for antitumor vaccination, the results of clinical trials so far reported are conflicting (167-172). The labor-intensive and time-consuming methods required for the preparation of such vaccines has hampered the conduction of large prospective studies in humans. Nevertheless, a comparative phase III trial of DC-based vaccination for the treatment of advanced prostate carcinoma is underway.

4.4. Future perspectives in cancer vaccine development
4.4.1. Novel targets

In the search for the ideal TAA suitable for anticancer vaccination, investigators are looking for antigens with three main characteristics: *a)* maximal immunogenicity, *b)* wide expression by different tumor types, and *c)* maximal tumor specificity (173). In animal models, immunization with xenogeneic homologs of TAA appears to be a promising approach to break immune tolerance to weakly immunogenic self-antigens such as most TAA (174-176). In humans, similar immunological responses have been reported (177), although no data are yet available on tumor response.

Most TAA so far utilized in the clinical setting play a non-vital role in the metabolism of malignant cells (e.g. gp100, MAGE-3, CEA). Accordingly, selection of tumor cells not expressing these TAA can be the only effect of anticancer vaccination. A new class of TAA is represented by antigens involved in cancer cell survival. This is the case of the anti-apoptotic protein survivin (178) and the cell survival-related ribonucleoprotein telomerase (179), which are expressed by most tumor types. Preclinical and clinical preliminary results are encouraging (180-185). A further development of this strategy is to indirectly target tumor cells by immunizing the host against tumor angiogenesis-related antigens (e.g. VEGF receptor-2, FGF receptor-1) in order to induce tumor necrosis by shortening its blood supply (174-176).

4.4.2. Peripheral tolerance

Low-affinity autoreactive T-cells can avoid negative selection in the thymus. Indeed a low level of autoreactivity is required for positive thymic selection. In normal circumstances, after maturation is complete, these autoreactive T-cells are likely to be either ignorant (that is, they simply do not "see" their target epitope) or anergic (defined as a state of induced unresponsiveness). In the first case, they do not have any contact with the antigen. Naturally occurring and vaccine-induced TAA specific T-cells can be negatively regulated by host factors, such as 1) immunosuppressive cytokines (e.g. prostaglandin-E2, TGF-β) (186-188) or soluble factors (e.g. ROS, nitric oxide) produced by tumor cells or tumor infiltrating macrophages (189-194); 2) suppressor cells such as CD4$^+$/CD25$^+$ T-cells (T regulatory cells) (104,195), IL-13 secreting NKT cells (104,196), CD11b$^+$/Gr-1$^+$ suppressor cells (197,198), and tolerogenic DC (42,199). T-cell inhibition by suppressor cells is mediated by soluble factors (e.g. TGFβ, IL-13) or cell-to-cell contact involving inhibitory receptors (e.g. CTLA-4) (200) and ligands (e.g. B7-H1) (201). These and other recent insights on the molecular pathways involved in

T-cell unresponsiveness to TAA (202-204) are spurring the development of novel strategies aimed at counteracting this phenomenon. For instance, the blockade of CTLA-4 (205,206) or B7-H1 (201), neutralization of TGF-β (207,208) or IL-13 (209), and inhibition of tolerogenesis related enzymes (e.g. indoleamine 2,3-dioxygenase, IDO) (210,211) improve the antitumor immune reaction and increase tumor rejection rates in preclinical models.

4.4.3. Immunological adjuvants

Immunological adjuvants (IA) are agents of very different nature (e.g. microbial extracts, cytokines aluminum hydroxide, and so on) that mixed with an antigen enhance the immune response against that antigen after immunization (212,213). Even though at present only two IA are approved for clinical use worldwide (i.e. aluminum-based salts and MF59, a squalene-oil/water emulsion), many other substances (e.g. incomple Freund's adjuvant, IFA; saponin QS-21; several cytokines, and so forth) that increase the immunogenicity of vaccines have been tested and proven to be effective in animal models and humans.

Generally, IA are believed to activate innate immunity mediators such as DC and NK cells that ultimately stimulate T-cell function by secreting cytokines and freeing TAA and "danger" signals (e.g. heat shock proteins, HSP; double stranded DNA, dsDNA) from tumor cells (36,214). As it has been demonstrated that NK cells and DC efficiently cooperate to start an efficient T-cell response (105,215), more and more attention is being paid to the appropriate stimulation of the innate immunity arm while eliciting an adaptive immune reaction to a given TAA. Recently, bacterial DNA has been found to have strong immunostimulatory activity due to the presence of unmethylated CpG dinucleotides (216). These bacterial products can be bound by several receptors of the toll like receptor (TLR) family, which are expressed by DC and NK cells (217). Their engagement induces the maturation/activation of innate immunity cells, ultimately favoring the stimulation of the T-cell activity (218) Although this novel category of IA has been already implemented in the clinical setting (218), the efficacy of cancer vaccines based on these IA is not yet defined.

Among IA, cytokines are molecules with known effects on innate/adaptive immunity cells, and a growing number of them (e.g. IL-2, IL-7, IL-12, GM-CSF, IFN-α, IFN-γ) is being tested as IA for antitumor vaccination in clinical trials. These molecules act as "danger" signals in alerting the immune system: by promoting the differentiation/activation of DC and stimulating NK cell cytotoxic activity, they are considered powerful natural adjuvants for the development of cancer vaccines (219-221). The efficacy of some cytokines more recently identified (e.g. IL-15, IL-18, IL-21) has been proven in vaccination preclinical models (38,222,223), but is still to be tested in clinical trials.

5. CONCLUDING REMARKS

Despite the enormous theoretical potential of this type of biotherapy, the clinical results of cancer vaccines

are still limited and no vaccination regimen should be currently proposed to patients outside experimental clinical trials. The initial enthusiasm for anticancer ASI is being tempered by clinical results that, so far, have not met the expectations, as pointed out in an extensive review of the literature (>3,000 patients) (12,13), in which the mean overall (tumor disappearance or shrinkage ≥50%) response rate is 10% (range 0-42%) (224). Although it should be remembered that most patients are heavily treated with conventional chemotherapy regimens before undergoing ASI, suggesting that they are likely affected with particularly aggressive/resistant tumors as compared to those submitted to first-line chemotherapy, these figures are too low for an approach with therapeutic ambitions.

This disappointing clinical evidence can be at least in part justified by the difficulty of appropriately exploit the anticancer potential of a highly complex cellular/molecular network such as the immune system: as an example, different types of immune cells (e.g. DC) can show different if not opposite functions and interact with a number of other immune cells belonging to both the innate and the adaptive arm of the immune system. Therefore, the net effect of the immune system activity depends upon several variables that likely are not controlled by current vaccine formulations. Obviously, only by dissecting the physiology of the immune system in a greater detail will allow investigators to fully exploit the anticancer potential of ASI (225,226).

The myriad of vaccine preparation protocols is making the assessment of their therapeutic efficacy particularly challenging: in this respect, the urgent need for the standardization of procedures that has been repeatedly advocated (227,228) would greatly benefit from the definition of the best procedures for clinical grade anticancer vaccines, particularly in the case of cellular vaccines (e.g. DC-based ASI).

Clinical results so far obtained in humans allow us to state that the immune system can be actively polarized against malignant cells by means of a variety of ASI strategies, and that in same cases this is associated with tumor regression. This implies that under some unique circumstances, the naturally "dormant" immune effectors can actually be put at work and used as endogenous weapons against malignant cells. Consequently, the main challenge of tumor immunologists appears to lie on the ability of reproducing those conditions in a larger set of patients. Several ASI trials have shown that vaccine effectiveness correlates with a particular immunological settings of patients or immune response to the vaccination (141,229), strengthening the idea that the combination of certain tumor/host immune-related features (antigenicity, antigen presentation capabilities and so on) can be successfully turned into a therapeutic immune response by vaccination. Opposite findings (immune response without tumor regression) should not be regarded as the proof confuting the above statement, but rather suggest that some immunological parameters commonly utilized for monitoring vaccinated patients are not suitable for the detection of an effective immune response (116). In this

regard, the identification of novel surrogates of effective T-cell activation (e.g. LAMP-1) might usefully serve the cause in the near future (230). Moreover, the multilevel comparison (by means of high-throughput technologies, such as DNA microarray and proteomics platforms) of the multitude of dynamic immunological variables found within the tumor microenvironment in patients responding versus non-responding to anticancer vaccination might be particularly useful to describe the cascade of cellular/molecular events leading to immune-mediated tumor eradication (89,91,231-233).

Finally, as in the case of some standard chemotherapeutic regimens (234), the potential of ASI might be better exploited in the adjuvant rather than in the therapeutic setting (235). Results from recent randomized studies (147,236,237) support the hypothesis that ASI might be more effective in the presence of minimal residual disease, owing to a favorable effector/target ratio as compared to the scenario of metastatic disease.

The complexity of the immune network and the still enigmatic host-tumor interactions make these tasks at the same time challenging and fascinating. Recent tumor immunology findings are giving new impetus to the development of more effective ASI strategies and might revolutionize the way of designing the next generation of cancer vaccines (2,32). The improved knowledge of the mechanisms underlying immunological tolerance and the greater importance attributed to the interactions between innate and adaptive immune responses are leading to a more comprehensive immunotherapeutic approach that takes into consideration the multiple variables determining an effective immune attack on cancer.

In the near future, the implementation of these insights in the clinical setting and the completion/conduction of comparative randomized phase III trials will allow oncologists to define the actual role of ASI in the fight against malignancy.

6. ACKNOWLEDGEMENTS:

7. REFERENCES

1. Blagosklonny, M. V. From the war on cancer to translational oncology. *Cancer Biol Ther*, 1: 711-714 (2002)

2. Blattman, J. N. and Greenberg, P. D. Cancer immunotherapy: a treatment for the masses. *Science*, 305: 200-205 (2004)

3. Burnet, F. M. The concept of immunological surveillance. *Prog Exp Tumor Res*, 13: 1-27 (1970)

4. Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., and Schreiber, R. D. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol*, 3: 991-998 (2002)

5. Houbiers, J. G., van der Burg, S. H., van de Watering, L. M., Tollenaar, R. A., Brand, A., van de Velde, C. J., and

Melief, C. J. Antibodies against p53 are associated with poor prognosis of colorectal cancer. *Br J Cancer*, 72: 637-641 (1995)

6. Rosenberg, S. A. Development of cancer immunotherapies based on identification of the genes encoding cancer regression antigens. *J Natl Cancer Inst*, 88: 1635-1644 (1996)

7. Boon, T. and van der Bruggen, P. Human tumor antigens recognized by T lymphocytes. *J Exp Med*, 183: 725-729 (1996)

8. Anichini, A., Molla, A., Mortarini, R., Tragni, G., Bersani, I., Di Nicola, M., Gianni, A. M., Pilotti, S., Dunbar, R., Cerundolo, V., and Parmiani, G. An expanded peripheral T cell population to a cytotoxic T lymphocyte (CTL)-defined, melanocyte-specific antigen in metastatic melanoma patients impacts on generation of peptide-specific CTLs but does not overcome tumor escape from immune surveillance in metastatic lesions. *J Exp Med*, 190: 651-667 (1999)

9. Mukherji, B., Chakraborty, N. G., and Sivanandham, M. T-cell clones that react against autologous human tumors. *Immunol Rev*, 116: 33-62 (1990)

10. Weiss, R. A. Viruses, cancer and AIDS. *FEMS Immunol Med Microbiol*, 26: 227-232 (1999)

11. Penn, I. Post-transplant malignancy: the role of immunosuppression. *Drug Saf*, 23: 101-113 (2000)

12. Mocellin, S., Mandruzzato, S., Bronte, V., Lise, M., and Nitti, D. Part I: Vaccines for solid tumours. *Lancet Oncol*, 5: 681-689 (2004)

13. Mocellin, S., Semenzato, G., Mandruzzato, S., and Riccardo Rossi, C. Part II: Vaccines for haematological malignant disorders. *Lancet Oncol*, 5: 727-737 (2004)

14. Prehn, R. and Main, J. Immunity to methycholanthrene-induced sarcomas. *J Natl Cancer Inst*, 18: 769-778 (1957)

15. Radvanyi, L. Discovery and immunologic validation of new antigens for therapeutic cancer vaccines. *Int Arch Allergy Immunol*, 133: 179-197 (2004)

16. Rosenberg, S. A. A new era for cancer immunotherapy based on the genes that encode cancer antigens. *Immunity*, 10: 281-287 (1999)

17. Rosenberg, S. A. Progress in human tumour immunology and immunotherapy. *Nature*, 411: 380-384 (2001)

18. Mandruzzato, S., Brasseur, F., Andry, G., Boon, T., and van der Bruggen, P. A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. *J Exp Med*, 186: 785-793 (1997)

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

20. Guermonprez, P., Valladeau, J., Zitvogel, L., Thery, C., and Amigorena, S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol*, 20: 621-667 (2002)

21. van der Merwe, P. A. and Davis, S. J. Molecular interactions mediating T cell antigen recognition. *Annu Rev Immunol*, 21: 659-684 (2003)

22. Novellino, L., Castelli, C., and Parmiani, G. A listing of human tumor antigens recognized by T cells: March 2004 update. *Cancer Immunol Immunother* (2004)

23. Spiotto, M. T., Fu, Y. X., and Schreiber, H. Tumor immunity meets autoimmunity: antigen levels and dendritic cell maturation. *Curr Opin Immunol*, 15: 725-730 (2003)

24. Bretscher, P. The two-signal model of lymphocyte activation twenty-one years later. *Immunol Today*, 13: 74-76 (1992)

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

26. Perales, M. A., Blachere, N. E., Engelhorn, M. E., Ferrone, C. R., Gold, J. S., Gregor, P. D., Noffz, G., Wolchok, J. D., and Houghton, A. N. Strategies to overcome immune ignorance and tolerance. *Semin Cancer Biol*, 12: 63-71 (2002)

27. Acres, B., Paul, S., Haegel-Kronenberger, H., Calmels, B., and Squiban, P. Therapeutic cancer vaccines. *Curr Opin Mol Ther*, 6: 40-47 (2004)

28. Schwartz, R. T cell anergy. *Annu Rev Immunol*, 21: 305-334 (2003)

29. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. Immunobiology of dendritic cells. *Annu Rev Immunol*, 18: 767-811 (2000)

30. Raulet, D. H. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol*, 5: 996-1002 (2004)

31. Skoberne, M., Beignon, A. S., and Bhardwaj, N. Danger signals: a time and space continuum. *Trends Mol Med*, 10: 251-257 (2004)

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

33. Finn, O. J. Cancer vaccines: between the idea and the reality. *Nat Rev Immunol*, 3: 630-641 (2003)

34. Moser, M. and Murphy, K. M. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol*, 1: 199-205 (2000)

35. Ikeda, H., Chamoto, K., Tsuji, T., Suzuki, Y., Wakita, D., Takeshima, T., and Nishimura, T. The critical role of type-1 innate and acquired immunity in tumor immunotherapy. *Cancer Sci*, 95: 697-703 (2004)

36. Dredge, K., Marriott, J. B., Todryk, S. M., and Dalgleish, A. G. Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. *Cancer Immunol Immunother*, 51: 521-531 (2002)

37. Tatsumi, T., Kierstead, L. S., Ranieri, E., Gesualdo, L., Schena, F. P., Finke, J. H., Bukowski, R. M., Mueller-Berghaus, J., Kirkwood, J. M., Kwok, W. W., and Storkus, W. J. Disease-associated bias in T helper type 1 (Th1)/Th2 CD4(+) T cell responses against MAGE-6 in HLA-DRB10401(+) patients with renal cell carcinoma or melanoma. *J Exp Med*, 196: 619-628 (2002)

38. Tatsumi, T., Gambotto, A., Robbins, P. D., and Storkus, W. J. Interleukin 18 gene transfer expands the repertoire of antitumor Th1-type immunity elicited by dendritic cell-based vaccines in association with enhanced therapeutic efficacy. *Cancer Res*, 62: 5853-5858 (2002)

39. Trinchieri, G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol*, 3: 133-146 (2003)

40. Portielje, J. E., Gratama, J. W., van Ojik, H. H., Stoter, G., and Kruit, W. H. IL-12: a promising adjuvant for cancer vaccination. *Cancer Immunol Immunother*, 52: 133-144 (2003)

41. Liu, Y. J. IPC: Professional Type 1 Interferon-Producing Cells and Plasmacytoid Dendritic Cell Precursors. *Annu Rev Immunol* (2004)

42. Steinman, R. M., Hawiger, D., and Nussenzweig, M. C. Tolerogenic dendritic cells. *Annu Rev Immunol*, 21: 685-711 (2003)

43. De Vries, I. J., Lesterhuis, W. J., Scharenborg, N. M., Engelen, L. P., Ruiter, D. J., Gerritsen, M. J., Croockewit, S., Britten, C. M., Torensma, R., Adema, G. J., Figdor, C. G., and Punt, C. J. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. *Clin Cancer Res*, 9: 5091-5100 (2003)

44. Morse, M. A., Mosca, P. J., Clay, T. M., and Lyerly, H. K. Dendritic cell maturation in active immunotherapy strategies. *Expert Opin Biol Ther*, 2: 35-43 (2002)

45. Yang, L. and Carbone, D. P. Tumor-host immune interactions and dendritic cell dysfunction. *Adv Cancer Res*, 92: 13-27 (2004)

46. McIlroy, D. and Gregoire, M. Optimizing dendritic cell-based anticancer immunotherapy: maturation state does have clinical impact. *Cancer Immunol Immunother*, 52: 583-591 (2003)

47. Hornung, V., Guenthner-Biller, M., Bourquin, C., Ablasser, A., Schlee, M., Uematsu, S., Noronha, A., Manoharan, M., Akira, S., de Fougerolles, A., Endres, S., and Hartmann, G. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nat Med*, 11: 263-270 (2005)

48. Kusmartsev, S., Cheng, F., Yu, B., Nefedova, Y., Sotomayor, E., Lush, R., and Gabrilovich, D. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. *Cancer Res*, 63: 4441-4449 (2003)

49. Liu, B., DeFilippo, A. M., and Li, Z. Overcoming immune tolerance to cancer by heat shock protein vaccines. *Mol Cancer Ther*, 1: 1147-1151 (2002)

50. Daniels, G. A., Sanchez-Perez, L., Diaz, R. M., Kottke, T., Thompson, J., Lai, M., Gough, M., Karim, M., Bushell, A., Chong, H., Melcher, A., Harrington, K., and Vile, R. G. A simple method to cure established tumors by inflammatory killing of normal cells. *Nat Biotechnol*, 22: 1125-1132 (2004)

51. Whitmore, M. M., DeVeer, M. J., Edling, A., Oates, R. K., Simons, B., Lindner, D., and Williams, B. R. Synergistic activation of innate immunity by double-stranded RNA and CpG DNA promotes enhanced antitumor activity. *Cancer Res*, 64: 5850-5860 (2004)

52. Mazzoni, A. and Segal, D. M. Controlling the Toll road to dendritic cell polarization. *J Leukoc Biol*, 75: 721-730 (2004)

53. Engleman, E. G., Brody, J., and Soares, L. Using signaling pathways to overcome immune tolerance to tumors. *Sci STKE*, 2004: pe28 (2004)

54. Ishii, K. J., Gursel, I., Gursel, M., and Klinman, D. M. Immunotherapeutic utility of stimulatory and suppressive oligodeoxynucleotides. *Curr Opin Mol Ther*, 6: 166-174 (2004)

55. Wooldridge, J. E. and Weiner, G. J. CpG DNA and cancer immunotherapy: orchestrating the antitumor immune response. *Curr Opin Oncol*, 15: 440-445 (2003)

56. Colombo, M. P. and Trinchieri, G. Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev*, 13: 155-168 (2002)

57. Roncarolo, M., Bacchetta, R., Bordignon, C., Narula, S., and Levings, M. Type 1 T regulatory cells. *Immunol Rev*, 182: 68-79 (2001)

58. Levings, M., Bacchetta, R., Schulz, U., and Roncarolo, M. The role of IL-10 and TGF-b in the differentiation and effector function of T regulatory cells. *Int Arch Allergy Immunol*, 129: 263-276 (2002)

59. Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J. R., Zhang, L., Burow, M., Zhu, Y., Wei, S., Kryczek, I., Daniel, B., Gordon, A., Myers, L., Lackner, A., Disis, M. L., Knutson, K. L., Chen, L., and Zou, W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*, 10: 942-949 (2004)

60. Viguier, M., Lemaitre, F., Verola, O., Cho, M. S., Gorochov, G., Dubertret, L., Bachelez, H., Kourilsky, P., and Ferradini, L. Foxp3 expressing CD4+CD25(high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J Immunol*, 173: 1444-1453 (2004)

61. Wang, H. Y., Lee, D. A., Peng, G., Guo, Z., Li, Y., Kiniwa, Y., Shevach, E. M., and Wang, R. F. Tumor-specific human CD4+ regulatory T cells and their ligands: implications for immunotherapy. *Immunity*, 20: 107-118 (2004)

62. Kuwana, M. Induction of anergic and regulatory T cells by plasmacytoid dendritic cells and other dendritic cell subsets. *Hum Immunol*, 63: 1156-1163 (2002)

63. Roncarolo, M. G., Levings, M. K., and Traversari, C. Differentiation of T regulatory cells by immature dendritic cells. *J Exp Med*, 193: F5-9 (2001)

64. Nielsen, M. B. and Marincola, F. M. Melanoma vaccines: the paradox of T cell activation without clinical response. *Cancer Chemother Pharmacol*, 46: S62-66 (2000)

65. Chakraborty, N. G., Chattopadhyay, S., Mehrotra, S., Chhabra, A., and Mukherji, B. Regulatory T-cell response and tumor vaccine-induced cytotoxic T lymphocytes in human melanoma. *Hum Immunol*, 65: 794-802 (2004)

66. Yang, S. C., Hillinger, S., Riedl, K., Zhang, L., Zhu, L., Huang, M., Atianzar, K., Kuo, B. Y., Gardner, B., Batra, R. K., Strieter, R. M., Dubinett, S. M., and Sharma, S. Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. *Clin Cancer Res*, 10: 2891-2901 (2004)

67. Prasad, S. J., Farrand, K. J., Matthews, S. A., Chang, J. H., McHugh, R. S., and Ronchese, F. Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4+CD25+ regulatory T cells. *J Immunol*, 174: 90-98 (2005)

68. Yang, Y., Huang, C. T., Huang, X., and Pardoll, D. M. Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. *Nat Immunol*, 5: 508-515 (2004)

69. Belardelli, F., Ferrantini, M., Parmiani, G., Schlom, J., and Garaci, E. International meeting on cancer vaccines: how can we enhance efficacy of therapeutic vaccines? *Cancer Res*, 64: 6827-6830 (2004)

70. Malmberg, K. J. Effective immunotherapy against cancer: a question of overcoming immune suppression and immune escape? *Cancer Immunol Immunother*, 53: 879-892 (2004)

71. Mapara, M. Y. and Sykes, M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. J Clin Oncol, 22: 1136-1151 (2004)

72. Seliger, B., Wollscheid, U., Momburg, F., Blankenstein, T., and Huber, C. Characterization of the major histocompatibility complex class I deficiencies in B16 melanoma cells. *Cancer Res*, 61: 1095-1099 (2001)

73. Yang, T., McNally, B. A., Ferrone, S., Liu, Y., and Zheng, P. A single-nucleotide deletion leads to rapid degradation of TAP-1 mRNA in a melanoma cell line. *J Biol Chem*, 278: 15291-15296 (2003)

74. Mocellin, S., Wang, E., and Marincola, F. M. Cytokines and Immune Response in the Tumor Microenvironment. *J Immunother*, 24: 392-407 (2001)

75. Walker, P. R., Saas, P., and Dietrich, P. Y. Role of Fas ligand (CD95L) in immune escape: the tumor cell strikes back. *J Immunol*, 158: 4521-4524 (1997)

76. Townsend, S. E. and Allison, J. P. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. *Science*, 259: 368-370 (1993)

77. Schultze, J., Nadler, L. M., and Gribben, J. G. B7-mediated costimulation and the immune response. *Blood Rev*, 10: 111-127 (1996)

78. Lenschow, D., Walunas, T., and Bluestone, J. CD28/B7 system of T cell costimulation. *Annu Rev Immunol*, 14: 233-258 (1996)

79. Salomon, B. and Bluestone, J. A. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu Rev Immunol*, 19: 225-252 (2001)

80. La Motte, R. N., Sharpe, A. H., Bluestone, J. A., and Mokyr, M. B. Host B7-1 and B7-2 costimulatory molecules contribute to the eradication of B7-1-transfected P815 tumor cells via a CD8+ T cell-dependent mechanism. *J Immunol*, 162: 4817-4823 (1999)

81. Ohnmacht, G. A., Wang, E., Mocellin, S., Abati, A., Filie, A., Fetsch, P., Riker, A. I., Kammula, U. S., Rosenberg, S. A., and Marincola, F. M. Short-term kinetics of tumor antigen expression in response to vaccination. *J Immunol*, 167: 1809-1820 (2001)

82. Chappell, D. B., Zaks, T. Z., Rosenberg, S. A., and Restifo, N. P. Human melanoma cells do not express Fas (Apo-1/CD95) ligand. *Cancer Res*, 59: 59-62 (1999)

83. Mocellin, S., Marincola, F., Riccardo Rossi, C., Nitti, D., and Lise, M. The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle. *Cytokine Growth Factor Rev*, 15: 61-76 (2004)

84. Mocellin, S., Panelli, M. C., Wang, E., Nagorsen, D., and Marincola, F. M. The dual role of IL-10. *Trends Immunol*, 24: 36-43 (2003)

85. Chen, L., McGowan, P., Ashe, S., Johnston, J., Li, Y., Hellstrom, I., and Hellstrom, K. E. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med*, 179: 523-532 (1994)

86. Wang, E. and Marincola, F. M. A natural history of melanoma: serial gene expression analysis. *Immunol Today*, 21: 619-623 (2000)

87. Mocellin, S., Ohnmacht, G. A., Wang, E., and Marincola, F. M. Kinetics of cytokine expression in melanoma metastases classifies immune responsiveness. *Int J Cancer*, 93: 236-242 (2001)

88. Wang, E., Miller, L. D., Ohnmacht, G. A., Mocellin, S., Perez-Diez, A., Petersen, D., Zhao, Y., Simon, R., Powell, J. I., Asaki, E., Alexander, H. R., Duray, P. H., Herlyn, M., Restifo, N. P., Liu, E. T., Rosenberg, S. A., and Marincola, F. M. Prospective molecular profiling of melanoma metastases suggests classifiers of immune responsiveness. *Cancer Res*, 62: 3581-3586 (2002)

89. Mocellin, S., Rossi, C., Pilati, P., Nitti, D., and Marincola, F. Quantitative real time PCR: a powerful ally in cancer research. *Trends Mol Med*, 9: 189-195 (2003)

90. Mocellin, S., Provenzano, M., Rossi, C. R., Pilati, P., Nitti, D., and Lise, M. DNA Array-Based Gene Profiling: From Surgical Specimen to the Molecular Portrait of Cancer. *Ann Surg*, 241: 16-26 (2005)

91. Mocellin, S., Wang, E., Panelli, M., Pilati, P., and Marincola, F. M. DNA array-based gene profiling in tumor immunology. *Clin Cancer Res*, 10: 4597-4606 (2004)

92. Tian, Q., Streuli, M., Saito, H., Schlossman, S. F., and Anderson, P. A polyadenylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells. *Cell*, 67: 629-639 (1991)

93. Anderson, P. TIA-1: structural and functional studies on a new class of cytolytic effector molecule. *Curr Top Microbiol Immunol*, 198: 131-143 (1995)

94. Akdis, C. A. and Blaser, K. Mechanisms of interleukin-10-mediated immune suppression. *Immunology*, 103: 131-136 (2001)

95. Moore, K. W., de Waal Malefyt, R., Coffman, R. L., and O'Garra, A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol*, 19: 683-765 (2001)

96. Berman, R. M., Suzuki, T., Tahara, H., Robbins, P. D., Narula, S. K., and Lotze, M. T. Systemic administration of cellular IL-10 induces an effective, specific, and long-lived immune response against established tumors in mice. *J Immunol*, 157: 231-238 (1996)

97. Salazar-Onfray, F., Petersson, M., Franksson, L., Matsuda, M., Blankenstein, T., Karre, K., and Kiessling, R. IL-10 converts mouse lymphoma cells to a CTL-resistant, NK-sensitive phenotype with low but peptide-inducible MHC class I expression. *J Immunol*, 154: 6291-6298 (1995)

98. Giovarelli, M., Musiani, P., Modesti, A., Dellabona, P., Casorati, G., Allione, A., Consalvo, M., Cavallo, F., di Pierro, F., De Giovanni, C., and et al. Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory. *J Immunol*, 155: 3112-3123 (1995)

99. Kundu, N., Beaty, T. L., Jackson, M. J., and Fulton, A. M. Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. *J Natl Cancer Inst*, 88: 536-541 (1996)

100. Huang, S., Xie, K., Bucana, C. D., Ullrich, S. E., and Bar-Eli, M. Interleukin 10 suppresses tumor growth and

metastasis of human melanoma cells: potential inhibition of angiogenesis. *Clin Cancer Res*, 2: 1969-1979 (1996)

101. Adris, S., Klein, S., Jasnis, M., Chuluyan, E., Ledda, M., Bravo, A., Carbone, C., Chernajovsky, Y., and Podhajcer, O. IL-10 expression by CT26 colon carcinoma cells inhibits their malignant phenotype and induces a T cell-mediated tumor rejection in the context of a systemic Th2 response. *Gene Ther*, 6: 1705-1712 (1999)

102. Mocellin, S., Panelli, M., Wang, E., Rossi, C. R., Pilati, P., Nitti, D., Lise, M., and Marincola, F. M. IL-10 stimulatory effects on human NK cells explored by gene profile analysis. *Genes Immun*, 5: 621-630 (2004)

103. Matzinger, P. Tolerance, danger, and the extended family. *Annu Rev Immunol*, 12: 991-1045 (1994)

104. Smyth, M. J., Godfrey, D. I., and Trapani, J. A. A fresh look at tumor immunosurveillance and immunotherapy. *Nat Immunol*, 2: 293-299 (2001)

105. Kelly, J. M., Darcy, P. K., Markby, J. L., Godfrey, D. I., Takeda, K., Yagita, H., and Smyth, M. J. Induction of tumor-specific T cell memory by NK cell-mediated tumor rejection. *Nat Immunol*, 3: 83-90 (2002)

106. Agarwala, S. S. and Sabbagh, M. H. Histamine dihydrochloride: inhibiting oxidants and synergising IL-2-mediated immune activation in the tumour microenvironment. *Expert Opin Biol Ther*, 1: 869-879 (2001)

107. Dokka, S., Shi, X., Leonard, S., Wang, L., Castranova, V., and Rojanasakul, Y. Interleukin-10-mediated inhibition of free radical generation in macrophages. Am J Physiol Lung Cell Mol Physiol, 280: L1196-1202 (2001)

108. Bogdan, C., Vodovotz, Y., and Nathan, C. Macrophage deactivation by interleukin 10. *J Exp Med*, 174: 1549-1555 (1991)

109. Hellstrand, K., Brune, M., Naredi, P., Mellqvist, U. H., Hansson, M., Gehlsen, K. R., and Hermodsson, S. Histamine: a novel approach to cancer immunotherapy. *Cancer Invest*, 18: 347-355 (2000)

110. Agarwala, S. S., Glaspy, J., O'Day, S. J., Mitchell, M., Gutheil, J., Whitman, E., Gonzalez, R., Hersh, E., Feun, L., Belt, R., Meyskens, F., Hellstrand, K., Wood, D., Kirkwood, J. M., Gehlsen, K. R., and Naredi, P. Results from a randomized phase III study comparing combined treatment with histamine dihydrochloride plus interleukin-2 versus interleukin-2 alone in patients with metastatic melanoma. *J Clin Oncol*, 20: 125-133 (2002)

111. Mocellin, S., Rossi, C. R., Pilati, P., and Nitti, D. Tumor necrosis factor, cancer and anticancer therapy. *Cytokine Growth Factor Rev*, 16: 35-53 (2005)

112. Yu, P., Lee, Y., Liu, W., Chin, R. K., Wang, J., Wang, Y., Schietinger, A., Philip, M., Schreiber, H., and Fu, Y. X. Priming of naive T cells inside tumors leads to eradication of established tumors. *Nat Immunol*, 5: 141-149 (2004)

113. Simon, R. M., Steinberg, S. M., Hamilton, M., Hildesheim, A., Khleif, S., Kwak, L. W., Mackall, C. L., Schlom, J., Topalian, S. L., and Berzofsky, J. A. Clinical trial designs for the early clinical development of therapeutic cancer vaccines. *J Clin Oncol*, 19: 1848-1854 (2001)

114. Ochsenbein, A. F., Sierro, S., Odermatt, B., Pericin, M., Karrer, U., Hermans, J., Hemmi, S., Hengartner, H.,

and Zinkernagel, R. M. Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. *Nature*, 411: 1058-1064 (2001)

115. Ganss, R., Arnold, B., and Hammerling, G. J. Mini-review: overcoming tumor-intrinsic resistance to immune effector function. *Eur J Immunol*, 34: 2635-2641 (2004)

116. Monsurro, V. V., Wang, E., Yamano, Y., Migueles, S. A., Panelli, M. C., Smith, K., Nagorsen, D., Connors, M., Jacobson, S., and Marincola, F. M. Quiescent phenotype of tumor-specific CD8+ T cells following immunization. *Blood*, 104: 1970-1978 (2004)

117. Anichini, A., Vegetti, C., and Mortarini, R. The paradox of T-cell-mediated antitumor immunity in spite of poor clinical outcome in human melanoma. *Cancer Immunol Immunother*, 53: 855-864 (2004)

118. Shankar, G. and Salgaller, M. L. Immune monitoring of cancer patients undergoing experimental immunotherapy. *Curr Opin Mol Ther*, 2: 66-73 (2000)

119. Keilholz, U., Weber, J., Finke, J. H., Gabrilovich, D. I., Kast, W. M., Disis, M. L., Kirkwood, J. M., Scheibenbogen, C., Schlom, J., Maino, V. C., Lyerly, H. K., Lee, P. P., Storkus, W., Marincola, F., Worobec, A., and Atkins, M. B. Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. *J Immunother*, 25: 97-138 (2002)

120. Lyerly, H. K. Quantitating cellular immune responses to cancer vaccines. Semin Oncol, 30: 9-16 (2003)

121. Whiteside, T. L. Methods to monitor immune response and quality control. *Dev Biol* (Basel), 116: 219-228; discussion 229-236 (2004)

122. Clay, T. M., Hobeika, A. C., Mosca, P. J., Lyerly, H. K., and Morse, M. A. Assays for monitoring cellular immune responses to active immunotherapy of cancer. *Clin Cancer Res*, 7: 1127-1135 (2001)

123. Habal, N., Gupta, R. K., Bilchik, A. J., Yee, R., Leopoldo, Z., Ye, W., Elashoff, R. M., and Morton, D. L. CancerVax, an allogeneic tumor cell vaccine, induces specific humoral and cellular immune responses in advanced colon cancer. *Ann Surg Oncol*, 8: 389-401 (2001)

124. Moulton, H. M., Yoshihara, P. H., Mason, D. H., Iversen, P. L., and Triozzi, P. L. Active specific immunotherapy with a beta-human chorionic gonadotropin peptide vaccine in patients with metastatic colorectal cancer: antibody response is associated with improved survival. *Clin Cancer Res*, 8: 2044-2051 (2002)

125. Mine, T., Sato, Y., Noguchi, M., Sasatomi, T., Gouhara, R., Tsuda, N., Tanaka, S., Shomura, H., Katagiri, K., Rikimaru, T., Shichijo, S., Kamura, T., Hashimoto, T., Shirouzu, K., Yamada, A., Todo, S., Itoh, K., and Yamana, H. Humoral responses to peptides correlate with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing, peptide-specific cellular responses. *Clin Cancer Res*, 10: 929-937 (2004)

126. Samonigg, H., Wilders-Truschnig, M., Kuss, I., Plot, R., Stoger, H., Schmid, M., Bauernhofer, T., Tiran, A., Pieber, T., Havelec, L., and Loibner, H. A double-blind randomized-phase II trial comparing immunization with antiidiotype goat antibody vaccine SCV 106 versus unspecific goat antibodies in patients with metastatic colorectal cancer. *J Immunother*, 22: 481-488 (1999)

127. Gonzalez, G., Crombet, T., Torres, F., Catala, M., Alfonso, L., Osorio, M., Neninger, E., Garcia, B., Mulet,

A., Perez, R., and Lage, R. Epidermal growth factor-based cancer vaccine for non-small-cell lung cancer therapy. *Ann Oncol*, 14: 461-466 (2003)

128. Brett, B. T., Smith, S. C., Bouvier, C. V., Michaeli, D., Hochhauser, D., Davidson, B. R., Kurzawinski, T. R., Watkinson, A. F., Van Someren, N., Pounder, R. E., and Caplin, M. E. Phase II study of anti-gastrin-17 antibodies, raised to G17DT, in advanced pancreatic cancer. *J Clin Oncol*, 20: 4225-4231 (2002)

129. Lee, P. P., Yee, C., Savage, P. A., Fong, L., Brockstedt, D., Weber, J. S., Johnson, D., Swetter, S., Thompson, J., Greenberg, P. D., Roederer, M., and Davis, M. M. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat Med*, 5: 677-685 (1999)

130. Monsurro, V., Nagorsen, D., Wang, E., Provenzano, M., Dudley, M. E., Rosenberg, S. A., and Marincola, F. M. Functional heterogeneity of vaccine-induced CD8(+) T cells. *J Immunol*, 168: 5933-5942 (2002)

131. Karanikas, V., Lurquin, C., Colau, D., van Baren, N., De Smet, C., Lethe, B., Connerotte, T., Corbiere, V., Demoitie, M. A., Lienard, D., Dreno, B., Velu, T., Boon, T., and Coulie, P. G. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant canarypox virus. *J Immunol*, 171: 4898-4904 (2003)

132. Fong, L., Hou, Y., Rivas, A., Benike, C., Yuen, A., Fisher, G. A., Davis, M. M., and Engleman, E. G. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc Natl Acad Sci U S A*, 98: 8809-8814 (2001)

133. Nielsen, M. B., Monsurro, V., Migueles, S. A., Wang, E., Perez-Diez, A., Lee, K. H., Kammula, U., Rosenberg, S. A., and Marincola, F. M. Status of activation of circulating vaccine-elicited CD8+ T cells. *J Immunol*, 165: 2287-2296 (2000)

134. Panelli, M. C., Wang, E., Monsurro, V., and Marincola, F. M. The role of quantitative PCR for the immune monitoring of cancer patients. *Expert Opin Biol Ther*, 2: 537-544 (2002)

135. Berd, D. Autologous, hapten-modified vaccine as a treatment for human cancers. *Vaccine*, 19: 2565-2570 (2001)

136. Mocellin, S., Rossi, C. R., Lise, M., and Nitti, D. Colorectal cancer vaccines: principles, results, and perspectives. *Gastroenterology*, 127: 1821-1837 (2004)

137. Hsueh, E. C. and Morton, D. L. Antigen-based immunotherapy of melanoma: Canvaxin therapeutic polyvalent cancer vaccine. *Semin Cancer Biol*, 13: 401-407 (2003)

138. Austin, F. C. and Boone, C. W. Virus augmentation of the antigenicity of tumor cell extracts. *Adv Cancer Res*, 30: 301-345 (1979)

139. Hsueh, E. C. Tumour cell-based vaccines for the treatment of melanoma. *BioDrugs*, 15: 713-720 (2001)

140. Sondak, V. K. and Sosman, J. A. Results of clinical trials with an allogenic melanoma tumor cell lysate vaccine: Melacine. *Semin Cancer Biol*, 13: 409-415 (2003)

141. Sosman, J. A., Unger, J. M., Liu, P. Y., Flaherty, L. E., Park, M. S., Kempf, R. A., Thompson, J. A., Terasaki, P. I., and Sondak, V. K. Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: impact of HLA class I antigen expression on outcome. *J Clin Oncol*, 20: 2067-2075 (2002)

142. Srivastava, P. K., Menoret, A., Basu, S., Binder, R. J., and McQuade, K. L. Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world. *Immunity*, 8: 657-665 (1998)

143. Binder, R. J., Han, D. K., and Srivastava, P. K. CD91: a receptor for heat shock protein gp96. *Nat Immunol*, 1: 151-155 (2000)

144. Tamura, Y., Peng, P., Liu, K., Daou, M., and Srivastava, P. K. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science*, 278: 117-120 (1997)

145. Janetzki, S., Palla, D., Rosenhauer, V., Lochs, H., Lewis, J. J., and Srivastava, P. K. Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: a pilot study. *Int J Cancer*, 88: 232-238 (2000)

146. Bystryn, J. C., Henn, M., Li, J., and Shroba, S. Identification of immunogenic human melanoma antigens in a polyvalent melanoma vaccine. *Cancer Res*, 52: 5948-5953 (1992)

147. Bystryn, J. C., Zeleniuch-Jacquotte, A., Oratz, R., Shapiro, R. L., Harris, M. N., and Roses, D. F. Double-blind trial of a polyvalent, shed-antigen, melanoma vaccine. *Clin Cancer Res*, 7: 1882-1887 (2001)

148. Dennis, J. W., Granovsky, M., and Warren, C. E. Glycoprotein glycosylation and cancer progression. *Biochim Biophys Acta*, 1473: 21-34 (1999)

149. Kirkwood, J. M., Ibrahim, J. G., Sosman, J. A., Sondak, V. K., Agarwala, S. S., Ernstoff, M. S., and Rao, U. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. *J Clin Oncol*, 19: 2370-2380 (2001)

150. Ibrahim, N. K. and Murray, J. L. Clinical Development of the STn-KLH Vaccine (Theratope(R)). *Clin Breast Cancer*, 3 Suppl 4: S139-143 (2003)

151. Parmiani, G., Castelli, C., Dalerba, P., Mortarini, R., Rivoltini, L., Marincola, F. M., and Anichini, A. Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? *J Natl Cancer Inst*, 94: 805-818 (2002)

152. Rosenberg, S. A., Yang, J. C., Schwartzentruber, D. J., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Dudley, M. E., Schwarz, S. L., Spiess, P. J., Wunderlich, J. R., Parkhurst, M. R., Kawakami, Y., Seipp, C. A., Einhorn, J. H., and White, D. E. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med*, 4: 321-327 (1998)

153. Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dreno, B., Tessier, M. H., Rankin, E., Parmiani, G., Arienti, F., Humblet, Y., Bourlond, A., Vanwijck, R., Lienard, D., Beauduin, M., Dietrich, P. Y., Russo, V., Kerger, J., Masucci, G., Jager, E., De Greve, J., Atzpodien, J., Brasseur, F., Coulie, P. G., van der Bruggen, P., and Boon, T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide

encoded by gene MAGE-3 and presented by HLA- A1. *Int J Cancer*, 80: 219-230 (1999)

154. Jager, E., Gnjatic, S., Nagata, Y., Stockert, E., Jager, D., Karbach, J., Neumann, A., Rieckenberg, J., Chen, Y. T., Ritter, G., Hoffman, E., Arand, M., Old, L. J., and Knuth, A. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc Natl Acad Sci U S A*, 97: 12198-12203 (2000)

155. Neidhart, J., Allen, K. O., Barlow, D. L., Carpenter, M., Shaw, D. R., Triozzi, P. L., and Conry, R. M. Immunization of colorectal cancer patients with recombinant baculovirus-derived KSA (Ep-CAM) formulated with monophosphoryl lipid A in liposomal emulsion, with and without granulocyte-macrophage colony-stimulating factor. *Vaccine*, 22: 773-780 (2004)

156. Marchand, M., Punt, C. J., Aamdal, S., Escudier, B., Kruit, W. H., Keilholz, U., Hakansson, L., van Baren, N., Humblet, Y., Mulders, P., Avril, M. F., Eggermont, A. M., Scheibenbogen, C., Uiters, J., Wanders, J., Delire, M., Boon, T., and Stoter, G. Immunisation of metastatic cancer patients with MAGE-3 protein combined with adjuvant SBAS-2: a clinical report. *Eur J Cancer*, 39: 70-77 (2003)

157. Bonnet, M. C., Tartaglia, J., Verdier, F., Kourilsky, P., Lindberg, A., Klein, M., and Moingeon, P. Recombinant viruses as a tool for therapeutic vaccination against human cancers. Immunol Lett, 74: 11-25 (2000)

158. Kumar, V. and Sercarz, E. Genetic vaccination: the advantages of going naked. *Nat Med*, 2: 857-859 (1996)

159. Krieg, A. M. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol*, 20: 709-760 (2002)

160. Perelson, A. S. Immune network theory. *Immunol Rev*, 110: 5-36 (1989)

161. Bhattacharya-Chatterjee, M., Chatterjee, S. K., and Foon, K. A. Anti-idiotype vaccine against cancer. *Immunol Lett*, 74: 51-58 (2000)

162. Losman, M. J., Qu, Z., Krishnan, I. S., Wang, J., Hansen, H. J., Goldenberg, D. M., and Leung, S. O. Generation and monitoring of cell lines producing humanized antibodies. *Clin Cancer Res*, 5: 3101s-3105s (1999)

163. Wagner, U., Kohler, S., Reinartz, S., Giffels, P., Huober, J., Renke, K., Schlebusch, H., Biersack, H. J., Mobus, V., Kreienberg, R., Bauknecht, T., Krebs, D., and Wallwiener, D. Immunological consolidation of ovarian carcinoma recurrences with monoclonal anti-idiotype antibody ACA125: immune responses and survival in palliative treatment. *Clin Cancer Res*, 7: 1154-1162 (2001)

164. Figdor, C. G., De Vries, I. J., Lesterhuis, W. J., and Melief, C. J. Dendritic cell immunotherapy: mapping the way. *Nat Med*, 10: 475-480 (2004)

165. Nair, S. K., Morse, M., Boczkowski, D., Cumming, R. I., Vasovic, L., Gilboa, E., and Lyerly, H. K. Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. *Ann Surg*, 235: 540-549 (2002)

166. Phan, V., Errington, F., Cheong, S. C., Kottke, T., Gough, M., Altmann, S., Brandenburger, A., Emery, S., Strome, S., Bateman, A., Bonnotte, B., Melcher, A., and Vile, R. A new genetic method to generate and isolate small, short-lived but highly potent dendritic cell-tumor cell hybrid vaccines. *Nat Med*, 9: 1215-1219 (2003)

167. Hirschowitz, E. A., Foody, T., Kryscio, R., Dickson, L., Sturgill, J., and Yannelli, J. Autologous dendritic cell vaccines for non-small-cell lung cancer. *J Clin Oncol*, 22: 2808-2815 (2004)

168. Thurner, B., Haendle, I., Roder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., Bender, A., Maczek, C., Schreiner, D., von den Driesch, P., Brocker, E. B., Steinman, R. M., Enk, A., Kampgen, E., and Schuler, G. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med*, 190: 1669-1678 (1999)

169. Panelli, M. C., Wunderlich, J., Jeffries, J., Wang, E., Mixon, A., Rosenberg, S. A., and Marincola, F. M. Phase 1 study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma- associated antigens MART-1 and gp100. *J Immunother*, 23: 487-498 (2000)

170. Banchereau, J., Palucka, A. K., Dhodapkar, M., Burkeholder, S., Taquet, N., Rolland, A., Taquet, S., Coquery, S., Wittkowski, K. M., Bhardwaj, N., Pineiro, L., Steinman, R., and Fay, J. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res*, 61: 6451-6458 (2001)

171. Schuler-Thurner, B., Schultz, E. S., Berger, T. G., Weinlich, G., Ebner, S., Woerl, P., Bender, A., Feuerstein, B., Fritsch, P. O., Romani, N., and Schuler, G. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide- loaded monocyte-derived dendritic cells. *J Exp Med*, 195: 1279-1288 (2002)

172. Whiteside, T. L., Zhao, Y., Tsukishiro, T., Elder, E. M., Gooding, W., and Baar, J. Enzyme-linked immunospot, cytokine flow cytometry, and tetramers in the detection of T-cell responses to a dendritic cell-based multipeptide vaccine in patients with melanoma. *Clin Cancer Res*, 9: 641-649 (2003)

173. Mocellin, S., Rossi, C. R., and Nitti, D. Cancer vaccine development: on the way to break immune tolerance to malignant cells. *Exp Cell Res*, 299: 267-278 (2004)

174. Niethammer, A. G., Xiang, R., Becker, J. C., Wodrich, H., Pertl, U., Karsten, G., Eliceiri, B. P., and Reisfeld, R. A. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med*, 8: 1369-1375 (2002)

175. He, Q. M., Wei, Y. Q., Tian, L., Zhao, X., Su, J. M., Yang, L., Lu, Y., Kan, B., Lou, Y. Y., Huang, M. J., Xiao, F., Liu, J. Y., Hu, B., Luo, F., Jiang, Y., Wen, Y. J., Deng, H. X., Li, J., Niu, T., and Yang, J. L. Inhibition of tumor growth with a vaccine based on xenogeneic homologous fibroblast growth factor receptor-1 in mice. *J Biol Chem*, 278: 21831-21836 (2003)

176. Wei, Y. Q., Huang, M. J., Yang, L., Zhao, X., Tian, L., Lu, Y., Shu, J. M., Lu, C. J., Niu, T., Kang, B., Mao, Y. Q., Liu, F., Wen, Y. J., Lei, S., Luo, F., Zhou, L. Q., Peng, F., Jiang, Y., Liu, J. Y., Zhou, H., Wang, Q. R., He, Q. M., Xiao, F., Lou, Y. Y., Xie, X. J., Li, Q., Wu, Y., Ding, Z. Y., Hu, B., Hu, M., and Zhang, W. Immunogene therapy of tumors with vaccine based on Xenopus homologous

vascular endothelial growth factor as a model antigen. *Proc Natl Acad Sci U S A*, 98: 11545-11550 (2001)

177. Fong, L., Brockstedt, D., Benike, C., Breen, J. K., Strang, G., Ruegg, C. L., and Engleman, E. G. Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. *J Immunol*, 167: 7150-7156 (2001)

178. Ambrosini, G., Adida, C., and Altieri, D. C. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*, 3: 917-921 (1997)

179. Buys, C. H. Telomeres, telomerase, and cancer. *N Engl J Med*, 342: 1282-1283., 2000.

180. Pisarev, V., Yu, B., Salup, R., Sherman, S., Altieri, D. C., and Gabrilovich, D. I. Full-length dominant-negative survivin for cancer immunotherapy. *Clin Cancer Res*, 9: 6523-6533 (2003)

181. Zeis, M., Siegel, S., Wagner, A., Schmitz, M., Marget, M., Kuhl-Burmeister, R., Adamzik, I., Kabelitz, D., Dreger, P., Schmitz, N., and Heiser, A. Generation of cytotoxic responses in mice and human individuals against hematological malignancies using survivin-RNA-transfected dendritic cells. *J Immunol*, 170: 5391-5397 (2003)

182. Siegel, S., Wagner, A., Schmitz, N., and Zeis, M. Induction of antitumour immunity using survivin peptide-pulsed dendritic cells in a murine lymphoma model. *Br J Haematol*, 122: 911-914 (2003)

183. Frolkis, M., Fischer, M. B., Wang, Z., Lebkowski, J. S., Chiu, C. P., and Majumdar, A. S. Dendritic cells reconstituted with human telomerase gene induce potent cytotoxic T-cell response against different types of tumors. *Cancer Gene Ther*, 10: 239-249 (2003)

184. Hernandez, J., Garcia-Pons, F., Lone, Y. C., Firat, H., Schmidt, J. D., Langlade-Demoyen, P., and Zanetti, M. Identification of a human telomerase reverse transcriptase peptide of low affinity for HLA A2.1 that induces cytotoxic T lymphocytes and mediates lysis of tumor cells. *Proc Natl Acad Sci U S A*, 99: 12275-12280 (2002)

185. Nair, S. K., Heiser, A., Boczkowski, D., Majumdar, A., Naoe, M., Lebkowski, J. S., Vieweg, J., and Gilboa, E. Induction of cytotoxic T cell responses and tumor immunity against unrelated tumors using telomerase reverse transcriptase RNA transfected dendritic cells. *Nat Med*, 6: 1011-1017 (2000)

186. Wynn, T. A. IL-13 effector functions. *Annu Rev Immunol*, 21: 425-456 (2003)

187. Gorelik, L. and Flavell, R. A. Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nat Med*, 7: 1118-1122 (2001)

188. Terabe, M., Matsui, S., Park, J. M., Mamura, M., Noben-Trauth, N., Donaldson, D. D., Chen, W., Wahl, S. M., Ledbetter, S., Pratt, B., Letterio, J. J., Paul, W. E., and Berzofsky, J. A. Transforming growth factor-beta production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. *J Exp Med*, 198: 1741-1752 (2003)

189. Saio, M., Radoja, S., Marino, M., and Frey, A. Tumor-infiltrating macrophages induce apoptosis in activated CD8+ T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide. *J Immunol*, 167: 5583-5593 (2001)

190. Moochhala, S. and Rajnakova, A. Role of nitric oxide in cancer biology. *Free Radic Res*, 31: 671-679 (1999)

191. Bogdan, C. Nitric oxide and the immune response. *Nat Immunol*, 2: 907-916 (2001)

192. Mellqvist, U. H., Hansson, M., Brune, M., Dahlgren, C., Hermodsson, S., and Hellstrand, K. Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. *Blood*, 96: 1961-1968 (2000)

193. Herberman, R. B. Cancer immunotherapy with natural killer cells. *Semin Oncol*, 29: 27-30 (2002)

194. Betten, A., Dahlgren, C., Mellqvist, U. H., Hermodsson, S., and Hellstrand, K. Oxygen radical-induced natural killer cell dysfunction: role of myeloperoxidase and regulation by serotonin. *J Leukoc Biol* (2004)

195. 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)

196. Ahlers, J. D., Belyakov, I. M., Terabe, M., Koka, R., Donaldson, D. D., Thomas, E. K., and Berzofsky, J. A. A push-pull approach to maximize vaccine efficacy: abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L. *Proc Natl Acad Sci U S A*, 99: 13020-13025 (2002)

197. Apolloni, E., Bronte, V., Mazzoni, A., Serafini, P., Cabrelle, A., Segal, D. M., Young, H. A., and Zanovello, P. Immortalized myeloid suppressor cells trigger apoptosis in antigen-activated T lymphocytes. *J Immunol*, 165: 6723-6730 (2000)

198. Bronte, V., Apolloni, E., Cabrelle, A., Ronca, R., Serafini, P., Zamboni, P., Restifo, N. P., and Zanovello, P. Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood*, 96: 3838-3846 (2000)

199. Mehrotra, S., Stevens, R., Zengou, R., Chakraborty, N. G., Butterfield, L. H., Economou, J. S., Dorsky, D. I., and Mukherji, B. Regulation of melanoma epitope-specific cytolytic T lymphocyte response by immature and activated dendritic cells, in vitro. *Cancer Res*, 63: 5607-5614 (2003)

200. Alegre, M. L., Frauwirth, K. A., and Thompson, C. B. T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol*, 1: 220-228 (2001)

201. Curiel, T. J., Wei, S., Dong, H., Alvarez, X., Cheng, P., Mottram, P., Krzysiek, R., Knutson, K. L., Daniel, B., Zimmermann, M. C., David, O., Burow, M., Gordon, A., Dhurandhar, N., Myers, L., Berggren, R., Hemminki, A., Alvarez, R. D., Emilie, D., Curiel, D. T., Chen, L., and Zou, W. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med*, 9: 562-567 (2003)

202. Tzachanis, D., Freeman, G. J., Hirano, N., van Puijenbroek, A. A., Delfs, M. W., Berezovskaya, A., Nadler, L. M., and Boussiotis, V. A. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. *Nat Immunol*, 2: 1174-1182 (2001)

203. Soares, L., Seroogy, C., Skrenta, H., Anandasabapathy, N., Lovelace, P., Chung, C. D., Engleman, E., and Fathman, C. G. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. *Nat Immunol*, 5: 45-54 (2004)

204. Overwijk, W. W., Theoret, M. R., Finkelstein, S. E., Surman, D. R., de Jong, L. A., Vyth-Dreese, F. A., Dellemijn, T. A., Antony, P. A., Spiess, P. J., Palmer, D. C., Heimann, D. M., Klebanoff, C. A., Yu, Z., Hwang, L.

N., Feigenbaum, L., Kruisbeek, A. M., Rosenberg, S. A., and Restifo, N. P. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J Exp Med*, 198: 569-580 (2003)

205. Hodi, F. S., Mihm, M. C., Soiffer, R. J., Haluska, F. G., Butler, M., Seiden, M. V., Davis, T., Henry-Spires, R., MacRae, S., Willman, A., Padera, R., Jaklitsch, M. T., Shankar, S., Chen, T. C., Korman, A., Allison, J. P., and Dranoff, G. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A*, 100: 4712-4717 (2003)

206. Davila, E., Kennedy, R., and Celis, E. Generation of antitumor immunity by cytotoxic T lymphocyte epitope peptide vaccination, CpG-oligodeoxynucleotide adjuvant, and CTLA-4 blockade. *Cancer Res*, 63: 3281-3288 (2003)

207. Kobie, J. J., Wu, R. S., Kurt, R. A., Lou, S., Adelman, M. K., Whitesell, L. J., Ramanathapuram, L. V., Arteaga, C. L., and Akporiaye, E. T. Transforming growth factor beta inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines. *Cancer Res*, 63: 1860-1864 (2003)

208. Wang, T., Niu, G., Kortylewski, M., Burdelya, L., Shain, K., Zhang, S., Bhattacharya, R., Gabrilovich, D., Heller, R., Coppola, D., Dalton, W., Jove, R., Pardoll, D., and Yu, H. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat Med*, 10: 48-54 (2004)

209. Terabe, M., Matsui, S., Noben-Trauth, N., Chen, H., Watson, C., Donaldson, D. D., Carbone, D. P., Paul, W. E., and Berzofsky, J. A. NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol*, 1: 515-520 (2000)

210. Munn, D. H. and Mellor, A. L. IDO and tolerance to tumors. *Trends Mol Med*, 10: 15-18 (2004)

211. Friberg, M., Jennings, R., Alsarraj, M., Dessureault, S., Cantor, A., Extermann, M., Mellor, A. L., Munn, D. H., and Antonia, S. J. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. *Int J Cancer*, 101: 151-155 (2002)

212. Berzofsky, J. A., Ahlers, J. D., and Belyakov, I. M. Strategies for designing and optimizing new generation vaccines. *Nat Rev Immunol*, 1: 209-219 (2001)

213. Schijns, V. E. Immunological concepts of vaccine adjuvant activity. *Curr Opin Immunol*, 12: 456-463 (2000)

214. Gallucci, S., Lolkema, M., and Matzinger, P. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med*, 5: 1249-1255 (1999)

215. Moretta, A. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol*, 2: 957-964 (2002)

216. Medzhitov, R. CpG DNA: security code for host defense. *Nat Immunol*, 2: 15-16 (2001)

217. Schnare, M., Barton, G. M., Holt, A. C., Takeda, K., Akira, S., and Medzhitov, R. Toll-like receptors control activation of adaptive immune responses. *Nat Immunol*, 2: 947-950 (2001)

218. Wittig, B., Marten, A., Dorbic, T., Weineck, S., Min, H., Niemitz, S., Trojaneck, B., Flieger, D., Kruopis, S., Albers, A., Loffel, J., Neubauer, A., Albers, P., Muller, S., Sauerbruch, T., Bieber, T., Huhn, D., and Schmidt-Wolf, I. G. Therapeutic vaccination against metastatic carcinoma by expression-modulated and immunomodified autologous tumor cells: a first clinical phase I/II trial. *Hum Gene Ther*, 12: 267-278 (2001)

219. Forni, G. and Boggio, K. Cytokine gene-engineered vaccines. *Curr Opin Mol Ther*, 1: 34-38 (1999)

220. Belardelli, F. and Ferrantini, M. Cytokines as a link between innate and adaptive antitumor immunity. *Trends Immunol*, 23: 201-208 (2002)

221. Parmiani, G., Rivoltini, L., Andreola, G., and Carrabba, M. Cytokines in cancer therapy. *Immunol Lett*, 74: 41-44 (2000)

222. Ma, H. L., Whitters, M. J., Konz, R. F., Senices, M., Young, D. A., Grusby, M. J., Collins, M., and Dunussi-Joannopoulos, K. IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-gamma. *J Immunol*, 171: 608-615 (2003)

223. Meazza, R., Lollini, P. L., Nanni, P., De Giovanni, C., Gaggero, A., Comes, A., Cilli, M., Di Carlo, E., Ferrini, S., and Musiani, P. Gene transfer of a secretable form of IL-15 in murine adenocarcinoma cells: effects on tumorigenicity, metastatic potential and immune response. *Int J Cancer*, 87: 574-581 (2000)

224. Mocellin, S., Mandruzzato, S., Bronte, V., and Marincola, F. M. Correspondence 1: Cancer vaccines: pessimism in check. *Nat Med*, 10: 1278-1279 (2004)

225. Gilboa, E. Opinion: The promise of cancer vaccines. *Nat Rev Cancer*, 4: 401-411 (2004)

226. Goldman, B. Cancer vaccines: finding the best way to train the immune system. *J Natl Cancer Inst*, 94: 1523-1526 (2002)

227. Weiner, D. B. and Kim, J. J. Cancer vaccines: is the future now? *Expert Rev Vaccines*, 1: 257-260 (2002)

228. Buchler, T., Kovarova, L., Musilova, R., Bourkova, L., Ocadlikova, D., Bulikova, A., Hanak, L., Michalek, J., and Hajek, R. Generation of dendritic cells using cell culture bags--description of a method and review of literature. *Hematology*, 9: 199-205 (2004)

229. Harris, J. E., Ryan, L., Hoover, H. C., Jr., Stuart, R. K., Oken, M. M., Benson, A. B., 3rd, Mansour, E., Haller, D. G., Manola, J., and Hanna, M. G., Jr. Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. *J Clin Oncol*, 18: 148-157 (2000)

230. Rubio, V., Stuge, T. B., Singh, N., Betts, M. R., Weber, J. S., Roederer, M., and Lee, P. P. Ex vivo identification, isolation and analysis of tumor-cytolytic T cells. *Nat Med*, 9: 1377-1382 (2003)

231. Mosca, P. J., Lyerly, H. K., Ching, C. D., Hobeika, A. C., Clay, T. M., and Morse, M. A. Proteomics for monitoring immune responses to cancer vaccines. *Curr Opin Mol Ther*, 5: 39-43 (2003)

232. Mocellin, S., Riccardo Rossi, C., Traldi, P., Nitti, D., and Lise, M. Molecular oncology in the post-genomic era: the challenge of proteomics. *Trends Mol Med*, 10: 24-32 (2004)

233. Mocellin, S., Rossi, C., Nitti, D., Lise, M., and Marincola, F. Dissecting tumor responsiveness to immunotherapy: the experience of peptide-based melanoma vaccines. *Biochim Biophys Acta Rev Cancer*, 1653: 61-71 (2003)

234. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project. *J Clin Oncol*, 10: 896-903 (1992)

235. Mocellin, S., Rossi, C. R., Lise, M., and Marincola, F. M. Adjuvant immunotherapy for solid tumors: from promise to clinical application. *Cancer Immunol Immunother*, 51: 583-595 (2002)

236. Jocham, D., Richter, A., Hoffmann, L., Iwig, K., Fahlenkamp, D., Zakrzewski, G., Schmitt, E., Dannenberg, T., Lehmacher, W., von Wietersheim, J., and Doehn, C. Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. *Lancet*, 363: 594-599 (2004)

237. Kuang, M., Peng, B. G., Lu, M. D., Liang, L. J., Huang, J. F., He, Q., Hua, Y. P., Totsuka, S., Liu, S. Q., Leong, K. W., and Ohno, T. Phase II randomized trial of autologous formalin-fixed tumor vaccine for postsurgical recurrence of hepatocellular carcinoma. *Clin Cancer Res*, 10: 1574-1579 (2004)

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