

Harnessing the Immune System to Fight Cancer: The Promise of Genetic Cancer Vaccines

Luigi Aurisicchio^{1,2} and Gennaro Ciliberto^{1,3}

¹*Takis, via di Castel Romano, 100, 00128 Rome*

²*Biogem scarl, via Camporeale, 83131 Ariano Irpino (AV),*

³*Dipartimento di Medicina Sperimentale e Clinica, Università degli studi di Catanzaro "Magna Graecia" Campus Germaneto, 88100 Catanzaro Italy*

1. Introduction

In spite of significant progress in recent years towards the development of new targeted therapies Cancer is still a largely unmet medical need and the leading cause of death in industrialized countries (Globocan Project, 2008). Cancer is continuously increasing and is associated with a variety of factors, including genetic predisposition, infectious agents, exposure to mutagens, as well as lifestyle factors (Minamoto et al, 1999). Cancer is linked to the occurrence of genetic and epigenetic changes (Heng et al, 2010) and indeed tumour cells harbor hundreds of these modifications as also witnessed by the recent results of genome wide analyses of cancer genomes (Sastre, 2011). This feature of cancer cells implies that they can be recognized as foreign entities and eliminated by our immune system, and is at the basis of the theory of immunosurveillance (Dunn et al, 2004).

Several studies have shown that it is possible to establish clear correlates between the nature, density and location of immune cells within distinct tumour regions and the risk of disease relapse (reviewed in Mleknic et al, 2011). Compelling data have recently led to propose that an immune classification of patients, based on the density and the immune location within the tumour may have a prognostic value even superior to the standard TNM classification (Bindea et al, 2011; Fridman et al, 2011). In recent years a better knowledge of the immune system has led to an evolution of the initial concept of immunosurveillance into a more articulated theory of immunoediting (Schreiber et al, 2011). Cancer immunoediting acts as an intrinsic tumour suppressor mechanism that engages after cellular transformation has occurred and intrinsic tumour suppressor mechanisms have failed. One can envisage the existence of three sequential steps during clinical tumour evolution: elimination, equilibrium, and escape. In the first step, innate and adaptive immunity are capable of destroying transformed cells before they give rise to tumour masses. If this process is maximally efficient, then the host remains tumour free. If, however, cancer cell variants are not destroyed, they can enter into an equilibrium phase, in which their outgrowth is held in check by immunological mechanisms, which are principally due to the activation of IL-12/IFN γ -dependent adaptive immunity, mainly driven by antigen-specific CD8⁺ and CD4⁺

T cells. Equilibrium may still represent the end stage of the process and may restrain outgrowth of occult cancers for the lifetime of the host. However, as a consequence of constant immune selection pressure placed by the host on genetically/epigenetically unstable tumour cells, cancerous cells that are no longer recognized by adaptive immunity may emerge, become insensitive to immune effectors mechanisms and in addition they can induce the creation of an immunosuppressive environment. When tumour cells enter the escape phase in which their growth is no longer blocked by immunity, equilibrium is lost and disease becomes apparent. Re-establishing this equilibrium is the realistic goal of cancer immunotherapy.

In spite of being the object of intensive efforts over the past decades, Cancer Immunotherapy has seen many more clinical failures than successes. However, very recently major breakthroughs have been achieved, and these have led us to believe that this approach may become an established platform for the therapy of cancer within the next decade. One can envisage three distinct avenues for Cancer immunotherapy: a) Adoptive Cell Therapy (ACT); b) systemic immune-modulators; c) therapeutic cancer vaccines. ACT is based upon the possibility to isolate, *in vitro* expand and re-inject in immunodepleted hosts, tumour-specific T cells. This approach has seen its best demonstration in the treatment of patients with advanced metastatic melanoma. Superb clinical results have been obtained with objective response rates of up to 49-72% and disease control in some cases lasting several years (Rosenberg and Dudley, 2009). Although evolution of this approach such as genetic modification of T cells to redirect their effector cell specificity may open up to broader applications (Morgan et al, 2010), this strategy has several limitations that currently limit its wide applicability: it is patient specific, very expensive, requires hospitalization and can only be executed in highly specialized clinical centers. In contrast, systemic immunomodulators such as monoclonal antibodies against CTLA-4 or PD-1/PD-L1, do not suffer the manufacturing and delivery problems shown by ACT. On March 2011, FDA approved Ipilimumab (Yervoy® - BMS) (Culver et al, 2011), a human monoclonal antibody against CTLA-4 for the treatment of metastatic melanoma, based on the results of a randomized, controlled Phase III, where Ipilimumab showed statistically increased overall survival compared with controls (Hodi et al, 2010). Although the clinical development of anti PD-1 antibodies is at an earlier stage as compared to anti CTLA-4, results are highly promising both for efficacy and tolerability (Kline and Gajewski, 2010). Finally cancer vaccines recently gained increased visibility due to the demonstration that Sipuleucel-T, a immune cell vaccine for the treatment of hormone refractory prostate cancer, is capable of increasing overall survival of cancer patients (Kantoff et al, 2010). These results led to FDA approval as Provenge® (Dendreon) in year 2010 (Cheever and Higano, 2011). This recent approval has acted as a strong injection of enthusiasm in an area that has long suffered major setbacks.

In this review we will focus mainly on recent developments for therapeutic cancer vaccines and will not discuss in detail ACT and systemic immunomodulators (Klebanoff et al, 2011). Major emphasis will be given to aspects that are critical to increase vaccine immunogenicity and probability of success in the clinic. We believe these are mainly: a) efficient vaccine delivery systems, b) development of response biomarkers, c) modified clinical endpoints and d) combinatorial treatments with chemotherapy or other agents. In analyzing vaccine delivery systems a greater attention will be given to genetic vaccines which we believe represent the most promising methods to elicit immune responses against a wide variety of tumour antigens

especially when administered in combined immunization protocols (heterologous prime/boost). We invite the reader to other recent excellent reviews for aspects of tumour immunology and cancer immunotherapy that we may have missed in our work (Steer et al, 2010; Klebanoff et al, 2011; Palucka et al, 2011; Vergati et al, 2010; Aldrich et al, 2010) .

2. Tumour immunology

Our immune system has the intrinsic capability of recognizing tumour cells as foreign entities and to mount responses capable of impacting upon disease evolution. In this section of the chapter we review the main evidences for this spontaneous response, what are the targets of this response, which are the principal components of the immune system involved and what is curtailing this response leading to tumour escape and lack of control of the immune system over cancer.

2.1 Immunosurveillance and Immunoediting

The key studies that unequivocally demonstrated the role of the immune system in the control of cancer development date back to about a decade ago when mouse models of immunodeficiency on pure genetic backgrounds became available. These studies showed that interferon- γ (IFN- γ) is a key factor responsible for the immunological rejection of transplanted tumour cells (Dighe et al, 1994). Furthermore, mice lacking IFN- γ response (either as a consequence of IFN- γ receptor or STAT1 inactivating mutations) or adaptive immunity as a whole (RAG2 $-/-$ deficient mice) are more susceptible to carcinogen induced or spontaneous tumours (Kaplan et al, 1998; Shankaran et al, 2001, Street et al, 2002). These evidences collectively demonstrated that the immune system can function as an extrinsic tumour suppressor. However, as mentioned in the introduction section, a new emerging concept in cancer immunology is that the immune system is not simply a component that protects the host against tumour development, but rather an agent that shapes tumour quality. In other words, tumours that develop in an immunocompetent organism are the resultant of a selection process imposed by the host and by the type of immune response that the host immune system is capable to mount. This concept was originated by pivotal studies that demonstrated that tumours developing in immunocompetent mice have a different molecular profile, are less immunogenic than tumours developing in immunodeficient hosts and progress more rapidly when implanted into naïve wt recipient mice (Dunn et al 2002).

Although both natural and acquired immunity are required to fully exert this control mechanism, the principal contribution comes from adaptive immunity and in particular from the development of tumour-antigen-specific T cells, mainly CD8⁺, but also CD4⁺. Indeed the fundamental principle of cancer immunology is that tumour cells express antigens (TAAs - tumour associated antigens) that differentiate them from normal cells. The existence of tumour antigens has been abundantly demonstrated both in mouse and human studies (Novellino et al, 2005). In the case of human cancers, identification of tumour antigens was made possible *via* the development of methods that used as probes antibodies and CD8⁺ T cells derived from patients and capable of reacting with the autologous tumours (Sahin et al, 1997; Coulie et al, 1997). In the next section we will describe in more detail the types and nature of TAAs under study.

What is happening in the tumour cells that makes them “invisible” or “poorly visible” to the immune system? Certainly the most common mechanism is believed to be loss of tumour

antigen expression, which can occur in at least three possible ways: a) downmodulation of tumour antigen gene expression consequent mainly to epigenetic changes; b) downregulation of MHC class I protein expression and antigen presentation to the cell surface; c) alterations in tumour cells of the machinery responsible for antigen processing and peptide loading onto MHC molecules. In addition to this, it has to be taken into account that tumour cells develop mechanisms of resistance to apoptosis and to the cytotoxic effects of immunity through, for instance, upregulation of anti-apoptotic BCL-2 proteins or activation of transcription factors such as STAT-3. All these processes are strongly favoured by the genetic/epigenetic instability intrinsic of tumour cells, which in the presence of a continuous selection favors the emergence of “immune stealth” clones.

If we analyze in detail the three phases of immunosurveillance/immunoediting, namely Elimination, Equilibrium and Escape, the phase where we have more direct proof of the activity of the immune system is the Equilibrium phase. This phase can represent a type of tumour dormancy where growth of tumour cells is kept at bay for a long period of time, even for the entire life of an organism. Strong evidence for this phenomenon first came when immunocompetent mice treated with low dose carcinogens such as methylcholantrene, were shown to harbor occult cancers for an extended period of time (Koebel et al, 2007). Intriguingly, when these mice were subjected to treatments that selectively affected adaptive immunity, but not innate immunity, tumours rapidly developed, thus demonstrating that equilibrium is established only when a Tumour Antigen Specific CD8⁺ and CD4⁺ response has occurred. This may explain the clinical findings of aggressive tumour arising in organs from a donor apparently cured from cancer, when transplanted into a patient (MacKie et al, 2003).

Although studies of tumour development in mice served as the main driver for the formulation of the cancer immunosurveillance/immunoediting hypothesis, strong demonstration has also been obtained in humans by three different types of evidence. As mentioned before, the first is the demonstration that cancer patients develop detectable levels of antibodies and T cell responses to tumour antigens (Dougan and Dranoff, 2009). The second one is that patients affected by immunodeficiencies are at higher risk of developing cancers (Dunn et al, 2002). The third and strongest one is that intratumoural infiltration by cells of the immune system correlates with disease evolution. In this respect several studies have shown that the quantity, quality, and spatial distribution of tumour infiltrating lymphocytes correlate with patients survival. In fact, tumour infiltration by IFN- γ producing CD8⁺ and CD4⁺ T cells has been associated with an improved prognosis for patients with several different cancer types, including melanoma (Clemente et al, 1996; van Houdt, 1998), colorectal cancer (Chiba et al, 2004) and ovarian cancer (Nelson, 2008). More recent studies in colorectal cancer have extended these findings and have shown, through a global analysis of the tumour microenvironment from both a morphological standpoint and from a system biology approach, that the nature, functional orientation, density and location of cells of the adaptive immune system within distinct tumour regions influence the risk of relapse (Mlecnik et al, 2011). The same authors have come to the conclusion that the density and the immune cell location within the tumour may have a prognostic value superior to the standard TNM classification, and that tumour spread is statistically dependent upon the extent of the host-immune reaction (Bindea et al, 2011).

2.2 Tumour associated antigens (TAAs)

In the past years, several TAAs have been identified having unique expression patterns or being overexpressed by cancer cells. These antigens, under appropriate conditions, can be

recognized by components of the immune system (Campi et al, 2003; Frenoy et al, 1987; Kawashima et al, 1998). Therefore, many current vaccination strategies are designed to induce antibody as well as cell-mediated immune responses against the antigen of interest. A high number of TAAs has been discovered and evaluated in pre-clinical and clinical studies with different results. A list of well-known TAAs subdivided in four main categories is provided in Table 1. Among the most studied and validated TAAs, vaccinations against CEA (Marshall et al, 2003), HER-2/*neu* (Shumway et al, 2009), TERT (Vonderheide, 2008), EpCAM (Chaudry et al., 2007), survivin (Andersen and Thor, 2002), prostate-specific antigens (Doehn et al., 2008) provided good immunologic results. In light of the increasing interest and potential for cancer immunotherapy, the National Cancer Institute recently conducted an interesting pilot project to prioritize cancer antigens and to develop a priority-ranked list of cancer vaccine target antigens based on predefined and pre-weighted objective criteria (Cheever et al., 2009). **Shared TAAs**

Among the shared TAAs, the following three main groups can be identified: (1) cancer-testis (CT) antigens, (2) differentiation antigens, and (3) widely occurring overexpressed antigens. Among shared tumour-specific antigens, *cancer-testis* (CT) antigens are expressed in histologically different human tumours and, among normal tissues, in spermatocytes/spermatogonia of the testis and occasionally in placenta. CT antigens result from the reactivation of genes which are normally silent in adult tissues but are transcriptionally activated in different tumour histotypes (De Smet et al., 1999). Many CT antigens have been identified and used in clinical trials, although little is known about their specific functions, especially with regard to malignant transformation. This group of TAAs includes MAGE-A1 (Chaux et al., 1999) and NY-ESO-1 (Jager et al., 1998). *Differentiation antigens* are shared between tumours and the normal tissue of origin and found mostly in melanomas and normal melanocytes (Gp100, Melan-A/Mart-1, and Tyrosinase), although they are also found in epithelial tissues and tumours such as prostate tumours (prostate-specific antigen [PSA]). To variable extent, normal tissues can be target of the elicited immunity against shared TAAs. An example is the vitiligo developing as a consequence of the immune response in melanoma patients undergoing immunotherapy. Vaccine-induced T cells recognizing gp100 and tyrosinase are present at the *vitiligo* lesions and normal melanocytes are eliminated by the immune system (Jacobs et al., 2009). Importantly, this effect has been associated to a clinical response. Additionally, expression of several oncofetal antigens appears to be increased in many adult cancer tissues, including carcinoembryonic antigen (CEA), which is highly expressed in colon cancer (Tsang et al., 1995).

TAAs from this group, despite representing self-antigens, have been and still are commonly used in current cancer vaccination trials, often together with CT antigens. Widely occurring, overexpressed TAAs have been detected in different types of tumours as well as in many normal tissues, and their overexpression in tumour cells can reach the threshold for T cell recognition, breaking the immunological tolerance and triggering an anticancer response. Among the most interesting TAAs of this group are the antiapoptotic proteins (survivin) (Schmidt et al., 2003), hTERT (Vonderheide et al., 2008), and tumour suppressor proteins (e.g., p53) (Umano et al, 2001).

Unique tumour antigens. Unique TAAs are products of random somatic point mutations induced by physical or chemical carcinogens and therefore expressed uniquely by individual tumours and not by any normal tissue, representing the only true tumour-specific antigens (Ags) (reviewed in Parmiani et al., 2007). Such Ags characterize each single

neoplasm and were shown to be diverse between tumours induced in the same animal or even in different tissue fragments from the same tumour nodule. A relevant feature of unique Ags is their potential resistance to immunoselection if the mutated protein is crucial to the oncogenic process and thus indispensable for maintaining the neoplastic state. As a consequence, unique Ags should elicit an immune response clinically more effective than that of shared Ags. However, identification of unique tumour antigens for solid human tumours requires sequencing of the whole genome of each individual tumour in order to identify mutated genes and select peptides whose motifs are predicted to be presented by the patient's HLA alleles. Moreover, each tumour bears highly heterogeneous sets of defects in different genes which need to be further verified for their substantial contribution to the tumour development and progression and, consequently, for their relevance as vaccine targets (Fox et al., 2009). An interesting class of potential TAAs is associated with fusions between different proteins. Best example is the Bcr-Abl fusion protein, the driving force in chronic myelogenous leukemia (CML) (Daley et al., 1990). By establishing a causal link between a specific chromosomal lesion and a specific malignancy, BCR-ABL also pioneered cancer therapy: the TK inhibitor, imatinib (Gleevec), was introduced as the first widely used targeted therapeutic (Druker et al., 2001). Similar discoveries led to the characterization of causative fusions in other hematological malignancies. A variety of prostate cancer gene fusions have been identified so far (reviewed in Shah and Chinnaiyan, 2009), characterized by 5'-genomic regulatory elements, most notably the androgen-controlled prostate specific gene, transmembrane protease serine 2 (TMPRSS2), fused to members of the erythroblastosis virus E26 transforming sequence family of transcription factors, most notably ERG, leading to the overexpression of oncogenic transcription factors. This class of potential TAAs is matter of extensive studies and holds promise for personalized vaccine applications.

Viral Antigens. Some viruses, such as human papillomavirus (HPV) and hepatitis B virus (HBV) can induce cancer. As a matter of fact, HBV vaccination in newborns has eradicated hepatocellular carcinoma (HCC) in populations at high risk (McMahon et al, 2011; Blumberg et al., 2010). The high-risk HPV types (e.g., HPV16) are causally related to the development of anogenital lesions, including vulvar intraepithelial neoplasia (VIN), and their subsequent progression to invasive squamous cell carcinoma. The expression of viral antigens (hence non-self proteins) such as HPV E6 and E7 proteins by cancer cells can represent the mechanism through which the tumour becomes visible to the immune system. Recently, promising results have been obtained by vaccination of patients with HPV16 E6/E7 synthetic long-peptide vaccine (Van der Burg and Melief, 2011), providing an important proof of concept for the development of therapeutic cancer vaccines against cervical and anal cancers.

Stromal Antigens. During transformation, reciprocal interactions occur between neoplastic and adjacent normal cells, i.e. fibroblasts, endothelial, and immunocompetent cells. In general, stroma cells contribute 20–50% to the tumour mass, but the stromal compartment may account for up to 90% in several carcinomas. In contrast to cancer cells, tumour stroma cells are genetically more stable so that at least some immune evasion mechanisms of tumours do not apply to these cells. Nevertheless, stroma cells differ from their normal counterparts by upregulation or induction of various antigens (reviewed in Hofmeister et al., 2006). Some of the tumour stroma-associated antigens (TSAAs) are highly selective for the tumour microenvironment. TSAAs are also expressed by a broad spectrum of solid tumours, thus

therapies designed to target tumour stroma are not restricted to a selected tumour type. Cancer-associated fibroblasts (CAFs) are reactive fibroblasts with a phenotype differing from that of quiescent fibroblasts in normal adult tissue. CAFs contribute to the development of cancer by secreting growth promoting factors such as TGF- β , matrix degrading enzymes, and angiogenic factors, e.g. MMPs or vascular endothelial growth factor (VEGF). Endothelial cells have a major part in tumour progression since they are necessary for angiogenesis. Tumour endothelial cells (TECs) express surface receptors and secrete factors that sustain their own growth by an autocrine pathway. Another target cell population for immune intervention present in the tumour microenvironment are tumour-associated macrophages (TAMs, see also paragraph 2.3). Among the proteins explored as promising stromal immunotherapy targets it is worth mentioning Fibroblast Activation Protein a (FAP α , seprase), a surface glycoprotein selectively expressed in reactive stromal fibroblasts of solid tumours, Carbonic Anhydrase IX (CAIX) an important pH regulator, Matrix Metalloproteases (MMP) such as MMP11 (Peruzzi et al., 2009), extracellular angiogenic factors, such as Vascular Endothelial Growth factor (VEGF) and its receptor VEGFR2 and basic Fibroblast Growth Factor (bFGF). Tumour endothelial markers (TEMs), among them TEM1 and TEM8, are overexpressed during tumour angiogenesis and prostate-specific membrane antigen (PSMA) is another endothelial cell surface molecule of particular interest for vascular targeting. In conclusion, ideal target stromal proteins are those selectively induced or upregulated in the tumour *micromilieu*, and confer a growth or survival advantage to the tumour.

Shared Antigens	Features	Type of Tumour	Examples
Cancer Testis (CT) Ags	Expressed only by tumours and testis	Melanoma, lymphoma, bladder, breast, colon, lung	BAGE, GAGE, MAGE, NY-ESO-1
Differentiation Ags	Expressed also by normal cells	Melanoma, prostate, colon, breast	Gp100, MART-1, tyrosinase, CEA, PSA
Overexpressed Ags	Expressed by tumor cells prevalently	Liver, colon, breast, ovary, bladder, prostate, esophagus, lymphoma	p53, Her2/ <i>neu</i> , survivin, hTERT
Unique Antigens	Features	Type of Tumour	Examples
Unique	Expressed by a single tumor	Melanoma, NSCLC, RCC	CD α -actin-m, K-4/m, β -actin-m, Myosin-m
Viral Antigens	Features	Type of Tumour	Examples
Viral	Encoded by genome of oncogenic viruses	HCC, anogenital lesions	HBV, HPV E6 and E7
Stromal Antigens	Features	Type of Tumour	Examples
Fibroblast TSAA	Expressed by CAFs	Ubiquitous	FAP α , CAIX, MMP11
Endothelial TSAA	Expressed by TECs	Ubiquitous	VEGF, VEGFR2, TEMs, PSMA

Table 1. Classification and examples of TAAs and TSAAs.

2.3 Immune suppression mechanisms

A strong and persistent immune response against cancer is necessary but not sufficient to controlling tumour growth in the escape phase. For example, while robust T cell responses generated by vaccinations against HPV are capable of successfully controlling pre-malignant intraepithelial neoplasias (Welters et al, 2010), in clinical trials of tumour vaccines against large, invasive malignancies the effective generation of tumour antigen-specific T cells is not predictive of clinical efficacy (Radoja et al, 2001). Although this discrepancy may be due in part to differences in the affinity/avidity of effector T cells developing against self *vs* exogenous antigens, the principal cause is believed to be the establishment of an immunosuppressive state within the tumour microenvironment (reviewed by Gajewski et al, 2006). This immunosuppression is not due to a single mechanism but to the concerted action of several processes. In first instance the presence of regulatory T cells (Tregs) and Myeloid-derived suppressor cells (MDSCs), which play a direct inhibitory role on host-protective antitumour responses.

Tregs are CD4⁺ T cells which constitutively express CD25 and the transcription factor FoxP3 (Nishikawa and Sakaguchi, 2010). It is unclear what proportion of intratumoural Tregs react with specific tumour antigens (Wang et al, 2005), or instead are recruited through the recognition of shared self-antigens co-expressed by tumour cells (Darrasse-jeze et al, 2009). At any rate, their inhibitory function is exerted *via* the production of immunosuppressive cytokines such as IL-10 and TGF β , the expression of negative co-stimulatory receptors such as CTLA-4 and PD-1, and the expression of IDO. IDO (indoleamine 2,3-dioxygenase) is an enzyme responsible for a rate-limiting step in tryptophan catabolism and is strongly induced in the tumour environment by IFN- γ . The immunosuppressive effect of IDO expression is due both to reduction of local levels of tryptophan and to the generation of cytotoxic catabolites kynurenins, which affect T cell activity and dendritic cell survival (Soliman et al, 2010). Several studies have shown that in several cancer types the presence of regulatory CD4⁺CD25⁺ T cells in tumours inversely correlates with disease outcome (Woo et al, 2001; Curiel et al, 2004)

MDSCs or Tumour Associated Macrophages (TAMs) are a heterogenous group of myeloid progenitor cells and immature myeloid cells that inhibit lymphocyte function by inducing Tregs, producing TGF β , depleting essential aminoacids as tryptophan, arginine and cysteine, and inducing down-regulation of L selectin on T cells (Ostrand-Rosenberg, 2010; Lindenberg et al, 2011). T cells must have an L-selectin phenotype to home to lymphnodes and inflammatory sites where they encounter antigens and are activated. TAMs therefore, perturb T cell trafficking and inhibit T cell activation. Furthermore, immunosuppression appears to be enhanced by active angiogenesis and angiogenic cytokines like VEGF (Johnson et al, 2007), also through a possible direct effect on dendritic cells.

Recent studies have shown that a symbiotic relationship exists between tumour cells and TAMs, in which tumour cells attract TAMs and sustain their survival, with TAMs responding to microenvironmental factors in tumours such as hypoxia by producing important mitogens, growth factors and enzymes that stimulate tumour growth angiogenesis (Bingle et al, 2002). Actually it seems that in response to different stimuli, TAMs differentiate into subsets capable of stimulating different pro-tumourigenic functions. For example in areas of invasion TAMs promote cancer cell motility, in perivascular areas they promote metastasis, and in avascular and perinecrotic areas hypoxic TAMs stimulate angiogenesis (Lewis and Pollard, 2006). Finally in a very recent study it has been shown that

macrophage infiltration in tumours is able to affect chemotherapy (De Nardo et al, 2011). Indeed these authors have shown that TAM depletion in highly infiltrated tumours increased the antitumour efficacy of paclitaxel, and this was at least in part due to their suppression of the antitumour functions of cytotoxic T cells. This study therefore confirms the high complexity of the immune cell interactions in tumours (DeNardo et al, 2010) and shows that cross-talk between TAMs and cytotoxic T cells impairs effective tumour eradication by immune mechanisms.

Unraveling the mechanisms at the basis of immunosuppression in the tumour microenvironment has led to the definition of novel targets for therapeutic intervention. Agents directed against these new targets, such as for example IDO or PD-1, may act in concert with cancer vaccines to enhance their efficacy, in particular in conditions of advanced tumour development. We believe that the clinical efficacy of anti CTLA-4 antibody Ipilimumab is in part linked to the inhibition of immunosuppressive processes. Indeed recent studies have demonstrated that maximal anti-tumour effects of CTLA-4 blockade are due to the concomitant blockade not only of effector T cells, but also of Tregs (Peggs et al, 2009). Also, it cannot be excluded that at least in part the clinical efficacy of the anti-VEGF antibody Bevacizumab is due to inhibition of the immunosuppressive function of VEGF (Chouaib et al, 2010).

3. Types of cancer vaccines

Different technologies have been employed to develop cancer immunotherapies. These include passive immunotherapy, based on the adoptive transfer of *ex-vivo* activated immune cells, immunomodulators (including cytokines) or tumour specific antibodies; and active immunotherapy, aimed at activating the patient's own immune system via the administration of a therapeutic vaccine. To date, active cancer immunotherapy trials have included therapeutic vaccination with recombinant viral vectors encoding TAAs, recombinant proteins with appropriate adjuvants, antigen-loaded Dendritic Cells (DCs), DNA encoding tumour-associated antigens, heat shock proteins and synthetic peptides (see next paragraphs). However, apart from melanoma, in which impressive clinical responses have been observed in a small proportion of patients, the recent success of Sipuleucel-T (see paragraph 3.1.2.1) and the promise of PROSTVAC-VF (see paragraph 3.2.1), most results have been disappointing. Therefore, the continuous development of novel vaccine strategies and technologies is needed to improve recognition, immune response, effector functions, and trafficking of T cells induced by vaccination. These goals may be achieved by the concurrent administration of novel immunotherapeutics with an immunopotentiating profile (see section 4.3).

3.1 Cell-based vaccines

A first category of cancer vaccines under evaluation is based on delivery of cells. As pointed out before, we will not discuss in this chapter ACT but only Whole Tumour Cell Vaccines and Dendritic Cells vaccines.

3.1.1 Whole cell vaccines

Autologous tumour cells are an obvious source of TAAs for vaccination purposes, since, by definition, all relevant candidate TAAs should be contained within them. In early clinical

trials, individualized tailor-made vaccines prepared from whole tumour cells were associated with limited activity, presumably due to the already biased nature of the host immune response to specific TAAs (Vermorcken et al. 1999; Jocham et al., 2004). However, due to the mechanism of immunologic tolerance, this approach has resulted in poor immunogenicity and different categories of adjuvants have been evaluated in the past years (de Gruijl et al., 2008).

Perhaps the most explored approach in the clinic is GVAX (Cell Genesys). Autologous tumour cells, transduced with GM-CSF were shown to induce tumour-specific immunity and durable anti-tumour responses in a number of trials. The efficacy of GVAX depends on the cross-presentation of vaccine-derived TAAs to specific cytotoxic T lymphocytes (CTLs) *in vivo* (Hege et al., 2006). This process of cross-priming is facilitated by the activation of Dendritic Cells (DC), by GM-CSF. This finding led to the realization that allogeneic cells would also present a viable source of TAAs, which would be taken up by DCs and then presented in the context of appropriate MHC alleles to autologous CTLs. Advantages of the use of allogeneic cells are obvious: (1) through the use of antigenically well-defined cell lines one has access to a sustained and virtually limitless source of material, (2) the use of cell lines allows for a highly standardized and large-scale production of vaccine, (3) the use of a single batch of allo-vaccines for all vaccinees, independent of HLA haplotype, eliminates variability in the quality and composition of the vaccines and facilitates reliable comparative analysis of clinical outcome. Eliminating the need for the continuous production of tailor-made individual vaccines simplifies the logistics, reduces the laboriousness of vaccine production and distribution, and increases its cost-effectiveness.

3.1.2 Dendritic cell vaccines

Dendritic Cells (DCs) collect antigens from various tissues and carry them to secondary lymphoid organs to ultimately activate antigen-specific T cells. Myeloid DCs and plasmacytoid DCs are the 2 main subsets of DCs (Palucka et al, 2011). Through toll-like receptors (TLRs) 7 and 9, plasmacytoid DCs recognize viral nucleic acids and secrete type I interferon (IFN). Three myeloid DC subsets localize to the skin. Langerhans cells (LCs) are found in the epidermis, while CD1 α +DCs and CD14+DCs are found in the dermis. CD14+DCs produce interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-12, GM-CSF, membrane cofactor protein-1, and tumour growth factor- β . LCs produce IL-15, which is a growth and maintenance factor for CD8 $^+$ T cells and natural killer cells. LCs are more efficient in cross presentation and prime higher avidity T cells with reported greater capacity for cell kill. Although DC biology is complicated, it is clear that these cells are the critical regulators of adaptive T-cell and B-cell responses. These findings have provided the rationale for *ex vivo* antigen loading of DCs for the preparation of vaccines. DCs have been loaded with tumour antigens in the form of peptides, proteins, tumour lysates, and mRNAs. Alternatively, they have been fused with tumour cells or infected with viral vectors encoding tumour-associated antigens (reviewed in Le et al., 2010).

Clinical development of DC-based cancer vaccines has several aspects that make this technology not ideal for application on a large scale. The first aspect is the difficulty to set up standardized procedures for the reliable production of functioning DCs. Currently, it is difficult to demonstrate that each preparation has the same levels of processed and presented antigen, and can induce an equivalent degree of immune response after administration. Quality control in the processing of cellular products is critical to the

integrity of the product. Large amounts of autologous peripheral blood mononuclear cells must be cultured in the presence of several cytokines making their off-the-shelf marketability challenging. There are critical issues not only in ensuring the proper maturation status of the DCs but also in the precise selection of appropriate subsets of DCs required to elicit the desired response. Other aspects include the significant costs of manufacturing the product and the huge amount of labor required to produce a viable product within a short time frame.

3.1.2.1 The Sipuleucel-T (Provenge) experience

Despite the above described technical hurdles, a immune cell-based vaccine, Sipuleucel-T, recently received Food and Drug Administration approval based on a successful phase III trial showing improvement in overall survival (OS) in men with asymptomatic or minimally symptomatic metastatic advanced castrate resistant prostate cancer (CRPC). The key to manufacturing feasibility of Sipuleucel-T is the absence of DC purification in the preparation. The preparation of a Sipuleucel-T product involves a leukapheresis to obtain the peripheral blood of the patient. The leukapheresed specimen is then transferred to the company manufacturing facility. The cell pellet containing DCs (CD54⁺), T lymphocytes (CD3⁺), B lymphocytes (CD19⁺), monocytes (CD14⁺), and natural killer cells (CD56⁺) is exposed to PA2024, an engineered antigen-cytokine fusion protein consisting of Prostate Acidic Protein (PAP) and GM-CSF. GM-CSF facilitates uptake of the fusion protein by DCs and promotes DC stimulation. PAP is the tumour antigen used in this vaccine. The final product is transported to the patient at 4°C and infused intravenously within 8 hours of formulation. Because the product is a mixture of cell types, the precise mechanism of action has not been established. It is not clear if induction of anti-prostate cancer responses involves *in vivo* activation of T cells by the loaded DCs in the preparation. It is also possible that T cells in the preparation are activated by *ex vivo* manipulations and that this therapy actually represents an alternative form of adoptive T-cell therapy. The paucity of available immunologic data to date precludes mechanistic dissection of this drug.

Phase I and II trials of Sipuleucel-T demonstrated T-cell and antibody responses to the antigen (Burch et al., 2004). Immune responses correlated with improved time to progression (TTP). Two sequential phase III placebo-controlled studies were subsequently conducted in patients with metastatic CRPC, with a primary end point of TTP. Integrated data again suggested a survival benefit but failed to show significance for the predetermined clinical end point. In this combined data set, a total of 225 patients were randomized to Sipuleucel-T (n = 147) or placebo (n = 78). There was a 33% reduction in the risk of death (HR 1.50; 95% CI 1.10– 2.05; P = 0.011). There was only a 4.8% PSA response in the combined analysis. Median survival was 23.2 versus 18.9 months and the percentage alive at 36 months was 33% versus 15% in favor of the treatment groups. Cumulative CD54 up-regulation, a measure of product potency, correlated with Overall Survival (OS). As a result of these studies, Dendreon pursued a new study, known as the Immunotherapy for Prostate Adeno Carcinoma Treatment (IMPACT) trial. OS was the primary end point. Five hundred twelve patients were enrolled in this study. Despite absence of clinical response to Sipuleucel-T or effect on TTP, the study met its primary end point of survival benefit. Subjects in the treatment group experienced a statistically significant increase in median survival vs controls (25.8 vs. 21.7 months respectively) and greater OS at 36 months (31.7% vs. 23%). The final analysis after 349 events demonstrated a median OS benefit of 4.1 months (HR 0.759; 95%CI 0.606–0.951; P = 0.017) (www.dendreon.com).

3.1.3 Heat shock proteins-based vaccines

Another interesting approach in cancer vaccine development is the use of heat shock protein (HSP)-peptide complexes, as natural host vector for vaccination (reviewed in di Pietro et al., 2009). Heat shock proteins are intracellular molecules of a family characterized by members of similar molecular mass (such as hsp70 and hsp90) that act as chaperones for a repertoire of peptides, including normal self-peptides and antigenic peptides. During both protein synthesis and breakdown, heat shock protein complexes are released from cells still associated non-covalently with peptides. Release by necrotic cells function as endogenous danger signals as well as a method to cross-present antigens to DCs. In fact, DCs have a specific receptor for heat shock proteins (CD91) and its engagement leads to their maturation. HSPs complexed with antigenic peptides have been shown to efficiently deliver peptides into the MHC class I processing pathway thus generating cellular immune responses. This phenomenon has been demonstrated in mouse and human tumours. In the latter, hsp70-peptide complexes extracted from melanoma cells have been found to contain well-known peptides on the basis of their ability to stimulate antigen-specific CD8⁺ T cells from melanoma patients' peripheral blood mononuclear cells (PBMCs). This observation has led to the purification of HSP-complexes from the tumours of patients and their administration as vaccines. The immunogenicity of tumour-derived HSP-peptide complexes, like the immunogenicity of experimentally induced tumours of mice and rats, has been shown to be individually tumour specific and not tumour type specific. These observations have led to the conclusion that the relevant tumour-antigenic, immunoprotective peptides are derived from unique rather than from shared tumour antigens.

Heat shock proteins explored for clinical immunotherapy may contain a defined antigen (E7 antigen derived from the human papilloma virus, MAGE tumour antigen, etc) or nondefined tumour antigens, which require the individualized production of heat shock proteins from fresh tumour samples. This could be a limitation, since several grams of tumour tissue must be available for the patient to be eligible for the trial. Following a number of trials (<http://www.agenusbio.com/prophage/past-trials.html>) in a range of tumour types (pancreatic cancer, Kidney cancer, Non-Hodgkin's lymphoma, CRC, gastric cancer) the tumour specific HSP-complexes vaccine named HSP peptide complex-96 (HSPPC-96 or Oncophage® or Prophage; Agenus, Lexington, MA, USA) has been approved in Russia as Oncophage® for the adjuvant treatment of kidney cancer patients at intermediate risk for disease recurrence. Currently Agenus is planning a registration Phase III trial for recurrent and newly diagnosed glioblastoma (<http://www.agenusbio.com/prophage/ongoing-trials.html>) in order to obtain drug approval by EMA.

3.1.4 Peptide vaccines

Peptide-based cancer vaccines represent the most popular approach to direct the immune system against malignant cells, since they are usually made of single epitopes, the minimal immunogenic region of an antigen. Peptides can be synthesized in a standardized manner and their cost of production is relatively low. Thus peptide vaccines have been the technology of choice by several group. Despite the strong rationale, the promising preclinical results and the frequent induction of antigen-specific immune responses in patients, peptide-based cancer vaccines have yielded relatively poor results in the clinical

setting and so far none of advanced clinical trials with peptide vaccines has resulted in statistically significant increase in survival. A particular mention deserve the results of the phase III clinical trial in 676 metastatic melanoma patients, which compared the efficacy of a gp100 peptide vaccine, with that of the fully human anti CTLA-4 antibody ipilimumab, or with the combined agents (Hodi et al, 2010). Ipilimumab when compared to gp100 alone improved median overall survival from 6.4 to 10.1 months (hazard ratio for death, 0.68; $P < 0.001$). More importantly no difference in survival was detected between the Ipilimumab alone vs Ipilimumab plus vaccine groups (median overall survival 10.1 vs 10.0 months, hazard ration with ipilimumab plus gp100, 1.04; $P = 0.76$). Based on these results ipilimumab was recently approved by FDA for the treatment of unresectable stage III and IV melanoma, with the name of Yervoy® (BMS). A possible interpretation for the lack of efficacy of a peptide vaccine in a patient population otherwise responsive to immunotherapy is the necessity to generate a polyclonal immune response directed simultaneously against several MHC class I epitopes. This could not be achieved in the Ipilimumab trial cited above because of the use of a single peptide. In order to overcome this limitation alternative approaches are being undertaken which make use of a combination of immunogenic peptides.

Advances in the engineering of peptides and in our understanding of the molecular mechanisms underlying an effective immune response against tumours have renewed the enthusiasm for peptide-based vaccination regimens in the setting of cancer and a variety of clinical trials are being conducted based on the use of peptides (Aurisicchio and Ciliberto, 2010). In this respect promising results in phase II studies have been obtained by Immatix (www.immatix.com). This technology consists in the vaccination of patients with multiple tumour-associated peptides (TUMAPs) that can be isolated from tumour specimens and identified by mass spectrometry (Dengjel et al, 2006). The most advanced product, IMA901, a combination of several TUMAPs for the treatment of renal cell carcinoma, completed a Europe-wide multi-center Phase II clinical trial and has recently commenced a Phase III trial. Another advanced peptide vaccine is L-BLP25 (Stimuvax) currently under development by MerckSerono. L-BLP25 is a peptide vaccine that targets the exposed core peptide of MUC1, a mucinous glycoprotein which is overexpressed and aberrantly glycosylated in many human malignancies. MUC1 is associated with cellular transformation and can confer resistance to genotoxic agents. In preclinical studies, L-BLP25 induced a cellular immune response characterized by T-cell proliferation in response to MUC1 and production of IFN- γ (reviewed in Gridelli et al., 2009). Phase I and II trials have established the dose and schedule of the vaccine as well as its excellent safety profile. A randomized phase II trial of maintenance L-BLP25 versus best supportive care in patients with stage IIIB/IV non-small cell lung cancer showed a strong survival trend in favor of L-BLP25 (median survival, 30.6 versus 13.3 months) in a subgroup of patients with locoregional stage IIIB disease (Butts et al., 2011). These promising results are being tested in three phase III trials (START, INSPIRE and STRIDE). The START and the INSPIRE studies are Phase III, multi-center, randomized, double-blind, placebo-controlled clinical trial designed to evaluate the efficacy, safety and tolerability of Stimuvax in subjects suffering from unresectable, stage IIIA or IIIB non-small cell lung cancer (NSCLC) who have had a response or stable disease after at least two cycles of platinum-based chemo-radiotherapy. The primary endpoint of the START study is overall survival (OS). STRIDE is a randomized, double-blind, controlled, multi-center Phase III study designed to determine if Stimuvax can extend progression free survival in patients

treated with hormonal therapy who have inoperable, locally advanced, recurrent or metastatic breast cancer. Overall survival, quality of life, tumour response and safety will also be assessed in this study.

3.1.5 Protein vaccines

Isolated recombinant proteins have been successfully employed for antiviral vaccines. However, soluble proteins are poorly immunogenic and require appropriate adjuvants and delivery systems to induce the desirable level and type of immune responses. For optimal performance, antigen delivery vehicles should closely mimic the composition and immunological processing of actual pathogens; they should actively or passively target APCs such as DCs; protect the antigenic protein from spontaneous degradation; direct the nature of the resulting immune response (i.e., cellular versus humoral responses) and, lastly, induce APC maturation by interacting with elements of the innate immune system such as Toll-like receptors (TLRs). Several strategies have been reported including directly conjugating TLR ligands to protein antigens or co-encapsulating immunostimulatory agents and proteins in liposomes or hydrophobic polymeric particles (Beaudette et al., 2009).

The most advanced approach is the one being pursued for the development of MAGE-A3 antigen specific immunotherapy (ASCI). MAGE-A3 ASCI is a therapeutic cancer vaccine directed against tumour antigen MAGE-A3, which is overexpressed in subset of patients affected by various cancers, and is being developed by GSK (Tyagi and Mirakhur, 2009). The vaccine is delivered as highly purified recombinant protein in conjunction with GSK's own proprietary adjuvant System. The most advanced development for the MAGE-A3 vaccine is a Phase III trial called MAGRIT (MAGE-A3 as Adjuvant Non-Small Cell Lung Cancer Immunotherapy), which began in October 2007 aimed at recruiting 2270 patients randomized to ASCI or placebo. The objective of the MAGRIT trial is to investigate the efficacy of MAGE-A3 ASCI in preventing cancer relapse, when administered after tumour resection, in patients with MAGE-A3 positive stages IB, II and IIIA NSCLC and is going to be the largest-ever trial in the adjuvant treatment for NSCLC. Results of the MAGRIT trial are expected in late 2011 and may lead to registration of this product in the coming years.

3.2 Genetic vaccines

Genetic vaccines represent promising methods to elicit immune responses against a wide variety of antigens, including TAAs. A variety of vectors have been utilized in the past, each of them presenting advantages and drawbacks with respect to "classic" protein-based vaccines. The main advantage of genetic vaccines is that they allow a) endogenous expression of the antigen of interest by muscle and/or antigen-presenting cells, which maximize antigen processing through the endogenous pathway and epitope display on MHC class I molecules, and b) easy molecular engineering of the targeted tumour antigen which help to boost significantly self-antigen immunogenicity.

3.2.1 Viral vaccines

Viral infection results in the presentation of virus-specific peptides in association with both MHC class I and MHC class II on the surface of infected cells. Based on this observation, several strategies have been designed to use viruses as immunization vehicles to elicit antigen-specific immune responses. In this approach, the cDNA encoding one or more antigens, is inserted into a viral vector. The resulting recombinant viruses are used as

vaccine, obtaining the *in vivo* expression of the selected antigen(s) and its presentation to the immune system. A variety of gene therapy viral vectors have been adapted to cancer immunotherapy. For vaccination purposes, the ideal viral vector should be safe with respect to disease-causing potential, transmissibility and long-term persistence in the host. It should enable efficient presentation of expressed antigens to the immune system while preferably exhibiting low intrinsic immunogenicity so that it can be administered repeatedly to boost relevant specific immune responses, often necessary to break immune tolerance to self antigens.

Tumour antigen DNA sequences have been inserted into attenuated pox viruses that are unable to replicate in mammalian hosts (such as modified vaccinia Ankara, fowlpox, or canarypox). Vaccinia poxvirus (VV) was demonstrated to be safe and very effective in the induction of potent cellular and humoral immune response in several tumour model systems (Gómez et al. 2011). For Carcinoembryonic Antigen (CEA), VV as well as ALVAC, a variant of the canary poxvirus, have been successfully used in colorectal cancer patients. As an avian virus, ALVAC has an advantage over vaccinia in that it is unable to replicate in human cells and thus has a very favorable safety profile. Combination of vaccinia followed by multiple injection of ALVAC revealed to be efficient in terms of elicited anti-CEA immune response and overall patient survival (Marshall et al., 2005). Another successful story of a vaccine based on this technology is PROSTVAC-VF (Bavarian Nordic, Kvistgård, Denmark). PROSTVAC-VF is a vaccine against Prostate Specific Antigen (PSA) that includes a number of costimulatory molecules. Three well-characterized costimulatory molecules were found to be synergistic when added to the poxvirus system. This triad, which includes B7.1 (CD80), ICAM-1 (CD54), and LFA-3 (CD58), is designated as TRICOM and has been added to both the vaccinia priming vector and the fowlpox boosting vector. With PSA as the encoded antigen, this configuration constitutes PROSTVAC-VF, vaccinia-PSA-TRICOM, and fowlpox-PSA-TRICOM. Interestingly, a randomized, controlled, and double-blinded phase II study was designed and powered for the short-term end point of PFS, and it failed to find an association between treatment arm and progression. However, a strong association between treatment arm and OS was observed (Kantoff et al., 2010). The estimated hazard ratio is 0.56 (95% CI, 0.37 to 0.85), and the observed difference in median survival of 8.5 months suggests a significant therapeutic benefit. PROSTVAC-VF immunotherapy is, therefore, a promising approach, and a larger pivotal phase III trial is being planned. Another Poxvector based vaccine is Trovax, a vector directed against a tumour enriched surface marker named 5T4 (Kim et al, 2010). Clinical trials with Trovax showed good safety profile, immunologic responses to the target antigen and efficacy in relation to a defined biomarker strategy (see section 4.2). TG4010 is an MVA vector developed by Transgene (Strasbourg, France) that incorporates the MUC1 antigen, which is overexpressed in the majority of cancers. A second gene, interleukin-2 is also incorporated as an immune stimulus. The vaccine has been tested in breast, kidney, prostate and lung cancers with encouraging results in phase II. For RCC, thirty-seven patients with progressive, MUC1-positive tumours received TG4010 10^8 pfu/inj weekly for 6 weeks, then every 3 weeks until progression, when TG4010 was continued in combination with interferon- α 2a and interleukin-2. Assessments included clinical response (primary endpoint), safety, time to treatment failure (TTF), OS, and immune response. No objective clinical responses occurred, but median OS was 19.3 months for all patients and 22.4 months for combination therapy

recipients. MUC1-specific CD8⁺ T cell responses were associated with longer survival (Oudard et al., 2011).

Another emerging viral system for vaccination is Adenovirus (Ad). Ads are very efficient vehicles for gene delivery and have been extensively characterized for gene therapy purposes (reviewed in Dharmapuri et al., 2009). Ad gene therapy products have recently been demonstrated to be safe, well-tolerated and capable of successful gene transfer to target cells. The high immunogenicity of E1-deleted first generation Ad recombinants has largely excluded their use for somatic gene therapy but re-directed their development as vaccine carriers. Ad vaccines have been shown to induce the highest degree of B- and CD8⁺ T-cell responses in experimental animals, including rodents, canines, and primates against a variety of immunogens derived from a variety of infectious agents (e.g., viruses, parasites, or bacterial pathogens) and tumour cells, including tumour-associated antigens (TAAs). Most clinical trials with Ad vectors have been conducted in oncology. Among the others, intratumour (IT) injections of Ad containing the wild-type p53 tumour suppressor gene showed clinical efficacy when combined with chemotherapy and led to the clinical development of Advexin® and Gendicine®. Advexin and Gendicine® are recombinant Ad5 vectors with an E1 region that is replaced by a human wild type p53 expression cassette containing a Cytomegalovirus (CMV) or Rous sarcoma virus (RSV) promoter, respectively. In October 2003, the State Food and Drug Administration (SFDA) of China approved Gendicine as the first commercialized gene therapy product in the world. Another example is Onyx-015, the first engineered replication-selective virus to be used in humans. It is an Ad2/Ad5 hybrid with deletions in E1B and E3B region and replicates exclusively in cells with inactive p53, activated p14ARF and late viral mRNA transport. Onyx-015 has been tested in more than 15 clinical trials by direct IT injection (up to 5×10^9 vp) and resulted in transient antitumoural effects (objective response rate 14%).

For development of cancer vaccines, several groups are currently assessing the immunologic and clinical activity of Ad vectors expressing TAAs, such as prostate serum antigen (PSA), HER-2/*neu*, carcinoembryonic antigen (CEA) and telomerase (hTERT) (see www.clinicaltrials.gov). It will be of great interest to verify how local and systemic suppression exerted by the tumour itself as well as pre-existing immunity to Ad will impact the outcome of these studies.

In conclusion, viral vectors appear promising tools for cancer vaccines. From a practical standpoint, viral vectors also meet criteria that enable their large scale industrialization. These include; efficient growth on a cell substrate acceptable to regulatory authorities; total genetic stability with respect to attenuation and presence of the foreign gene(s), scalability to large doses; easy purification of the vector virus away from cellular debris, and stability in the final formulation.

3.2.2 DNA plasmid vaccines

The inoculation of plasmid DNA coding for a protein antigen by means of a simple intramuscular or intradermal injection currently represents an easily performed vaccine approach that is safe for host and relatively inexpensive. DNA delivery vehicles contain a gene expression cassette bearing the coding region of an antigen gene regulated by a promoter usually with constitutive activity (like the cytomegalovirus early enhancer-promoter). Simple injection of naked DNA sequences results in gene expression and the generation of immune responses. A possible mechanism of how DNA immunization works

is the following: the protein antigen is produced by the target cells (usually skeletal myocytes or dermal fibroblasts, depending upon the injection route) that usually lack the co-stimulatory molecules needed as part of the CTL activation process. Subsequently, the antigen is taken up by host APCs, processed, and cross-presented to the immune system in the draining lymph nodes, although direct transfection of rare APCs residing at the injection site has also been demonstrated (Liu, 2011).

In mouse models, DNA vaccines have been successfully used to generate strong cellular immune responses against a wide variety of tumour antigens and to exert a preventive or therapeutic effect on tumour growth (Liu, 2011). However, clinical trials for DNA vaccines have shown that, albeit immune responses can be generated in humans, there is a need for increased potency if this vaccine technology is to be effective. The reasons for the failure of DNA vaccines to induce potent immune responses when scaled up from mice to man have not been fully elucidated. However, it is reasonable to assume that low levels of antigen production, inefficient cellular delivery of DNA plasmids and insufficient stimulation of the innate immune system have roles in low potency of DNA vaccines (Ulmer et al., 2006). In the design of more potent DNA vaccines, clearly regimens, plasmid dose, timing, adjuvants, alternative delivery systems and/or routes of vaccination are being considered. Methods for enhancing DNA plasmid delivery include tattooing, Gene gun, Ultrasound, Laser and DNA electroporation (reviewed in Bolhassani et al., 2011). In particular, here we will focus our attention on DNA electroporation.

3.2.3 DNA electroporation

In vivo electroporation of plasmid DNA (DNA-EP) has emerged as a safe method resulting in greater DNA uptake leading to enhanced protein expression in the treated muscle, and in a concomitant increase in immune responses to the target antigen in a variety of species (Auricchio et al., 2007; Peruzzi et al., 2010a). For its properties, DNA-EP is a desirable vaccine technology for cancer vaccines since it is repeatable several times, as required for the maintenance of anti-tumour immunity (Peruzzi et al., 2010b). This approach uses brief electrical pulses that create transient “pores” in the cell membrane, thus allowing large molecules such as DNA or RNA to enter the cell cytoplasm. Immediately following cessation of the electrical field, these pores close and the molecules are trapped in the cytoplasm (Andre et al., 2010). Typically, milli- and microsecond pulses have been used for EP. In addition to the increased permeability of target cells, EP may also enhance immune responses through increased protein expression, secretion of inflammatory chemokines and cytokines, and recruitment of APCs at the EP site (Liu, 2011). As a result, both antigen-specific humoral and cellular immune responses are increased by EP mediated delivery of plasmid DNA in comparison with levels achieved by intramuscular injection of DNA alone. Indeed, the addition of *in vivo* EP has been associated with a consistent enhancement of cell-mediated and humoral immune responses in small and large animals, supporting its use in humans.

Several devices have been developed for DNA-EP. Cytopulse has developed two clinical vaccine delivery systems: DermaVax™ and Easy Vax™. Easy Vax™ primarily targets the epidermis layer of skin and has been used in mass-scale prophylactic virus vaccination. In contrast, Derma Vax™ primarily targets the dermis layer of skin. Clinical trials in progress and planned using DermaVax™ include Prostate cancer (Phase I/II) and Colorectal cancer (Phase I/II). In this study, DNA vaccine was delivered by intradermal electroporation to

treat colorectal cancer (El-porCEA; ID: NCT01064375). The purpose of this study was to evaluate the safety and immunogenicity of a CEA DNA immunization approach in patients with colorectal cancer. Altogether, the electroporation with DNA vaccines has been investigated in several clinical trials for cancer therapy. They include: (1) Intratumoural IL-12 DNA plasmid (pDNA) [ID: NCT00323206, phase I clinical trials in patients with malignant melanoma]; (2) Intratumoural VCL-IM01 (encoding IL-2) [ID: NCT00223899; phase I clinical trials in patients with metastatic melanoma]; (3) Xenogeneic tyrosinase DNA vaccine [ID: NCT00471133, phase I clinical trials in patients with melanoma]; (4) VGX-3100 [ID: NCT00685412, phase I clinical trials for HPV infections], and 5) IM injection prostate-specific membrane antigen (PSMA)/pDOM fusion gene [ID: UK-112, phase I/II clinical trials for prostate cancer] (Bodles-Bakhop et al., 2009). Inovio (Oslo, Norway) has developed electroporators suitable for muscle DNA-EP, such as MedPulser®. Plasmid V930 is DNA vaccine candidate being developed by Merck. This vaccine is designed to target cancers expressing the antigens HER-2/*neu* and/or CEA, which include breast, colorectal, ovarian, and non-small cell lung cancer (ID: NCT00250419). V934 is a DNA plasmid that encodes human Telomerase (hTERT). The biologic is a Merck proprietary, therapeutic DNA vaccine candidate designed to target cancers expressing the antigen hTERT (ID: NCT00753415), including non-small cell lung carcinoma; breast cancer; melanoma; upper GI tract (e.g. esophagus, stomach, gallbladder) in collaboration with Geron Corp. (Menlo Park, CA, USA). Both vaccines are undergoing Phase I studies using MedPulser® DNA Delivery System in combination with Ad vectors.

Attempts to further enhance immune responses elicited by DNA vaccines are focusing on the use of codon optimization in order to enhance expression in eukaryotic cells. In fact, the potency of current gene delivery methods that include plasmid DNA and viral vectors can also be improved through increasing the expression efficiency of the encoded antigens. Elevated percentages of AU in human mRNA have been shown to result in instability, increased turnover, and low expression levels of the encoded proteins. These findings have prompted modification of the target gene coding sequence through reduction of the AT content with the assumption that these modifications could result in improved mRNA stability and increased expression. These changes have been justified by the observation that for highly expressed genes, G or C is generally preferred over A or T. In fact, optimization of the codon usage of the target gene has been shown in a variety of experimental systems to lead to enhanced expression and increased immunogenicity (Auriscchio et al., 2007; Peruzzi et al., 2010a, b).

Another strategy to enhance the efficacy of DNA vaccine is the development of gene fusions in which antigens are linked to various immunoenhancing elements (reviewed in Stevenson et al., 2004). The enhancement of immune responses is particularly relevant for cancer vaccines because of the limited immunogenicity of tumour antigens and the need to overcome tolerance. Enhancement of immune response to target antigens has been demonstrated in animal models by vectors encoding antigens fused to heat shock protein 70 (HSP70), the Fc portion of IgG1, lysosome-associated membrane protein (LAMP), the universal helper T (Th) epitope from tetanus toxin (DOM) (Facciabene et al., 2006) and Heat labile enterotoxin B from *E. Coli* (Facciabene et al., 2007). A DOM-PSMA fusion DNA vaccine delivered *via* DNA-EP resulted in highest antibody response to DOM in prostate cancer patients (Low et al., 2009), while LTB fusions are currently being evaluated in the clinical trials for CEA and hTERT cited above.

In conclusion, DNA vaccines have several promising features. They are simpler and cheaper to produce. DNA immunization is not associated with anamnestic immune responses against the vector. On the other hand, it appears that efficacy must be improved, especially for cancer vaccines targeting 'self' antigens.

3.3 Heterologous prime/boost

There are emerging evidences that vaccination schedules comprising more than one delivery method against the same antigen(s) (i.e., genetic vectors, genetic vector/protein, genetic vector/peptides, etc.) may be beneficial to overcome the 'therapeutic immunity' threshold and adequately harness the immune system to fight cancer. In particular, genetic vectors, if appropriately combined with each other and with other agents (immunomodulators and/or chemotherapy) hold promise for clinical development (reviewed in Lu et al., 2009).

The sequential administration of DNA and a viral vector in different combinations may result in synergistic immune activation. Preclinical murine and primate models have shown that this heterologous prime-boost regimen induces 10- to 100-fold higher frequencies of T cells than do naked DNA or recombinant viral vectors alone (Ribas et al., 2003). In addition to the enhanced immune response, the therapeutic proof of concept of DNA/viral vector combination has recently been achieved in dogs affected by B cell lymphoma (Peruzzi et al., 2010b). In this study, the best performing vaccination schedule consisted of Ad followed by DNA-EP boosters, concurrently with chemotherapy (see also section 4.1). Another strategy is the sequential administration of two different viral vectors carrying the same tumour antigen gene, which bypasses the limitation of the development of neutralizing antibodies to the viral backbone by boosting with a different vector without shared viral epitopes (see paragraph 3.2.1).

4. Cancer vaccines clinical development

Due to the complexity of tumour immunology, the success of cancer vaccines points to important considerations for clinical development: (1) evaluation of vaccines in predictive preclinical animal models. (2) Biomarker strategies for patient selection. This turns to be a crucial aspect as revealed by the experience of different cancer vaccine products under evaluation (see next paragraph); (3) value of vaccinating at early stages of cancer progression. Advances in early cancer detection methods and the development of efficacious cancer vaccines will allow vaccination of patients with various types of cancer at early stages of disease, when they still have an intact immune system. This may become the most promising strategy for preventing tumour progression. (4) use of heterologous prime-boost technologies (see previous paragraph). (5) combination with other therapies. Some classes of chemotherapy drugs could act as immunomodulators by affecting antigen cross-presentation, inducing a cytokine storm, reducing the number of regulatory T cells and activating homeostatic lymphoid proliferation. (6) autoimmunity as a possible side effect of cancer immunotherapy. In those instances when intense immunotherapy is necessary the incidence of autoimmunity in early or especially in advanced cancer should be evaluated in the short or long term following immunotherapy.

4.1 Pre-clinical models

Cancer immune therapy and its translation to the clinic are strictly dependent on efficient vaccine technology, delivery systems and evaluation in appropriate pre-clinical models. For

cancer vaccines the most widely used models for immunologic and anti-tumoural studies are transgenic rodents expressing the human TAA which show central and/or peripheral tolerance to the antigen of interest (see Fig. 1). The mouse is an excellent and reproducible system: mice from a given strain are inbred, with same MHC-I, allow experimentation on a large number of subjects. The mouse immune system is well-known and all reagents are available. Nevertheless, the translational relevance of cancer vaccines additionally needs a suitable, outbred therapeutic large animal model. In fact, scaling up experimental protocols from rodents to humans is often not a straightforward procedure, and this particularly applies to cancer vaccines, where vaccination technology must be especially effective to overcome a variety of immune suppressive mechanisms.

Nonhuman primates such as macaques are valid models to determine the safety and immunogenicity of candidate vaccines that are being developed for implementation in humans. In fact, the immune response is similar to that expected in humans. In the last two decades numerous immunogenicity studies have been performed in nonhuman primates utilizing pre-clinical candidate vaccines, most of them utilizing recombinant proteins of bacterial or viral origin as immunogen or genetic vectors coding for viral antigens (Shiver et al., 2002; Montgomery et al., 1993; Jeong et al., 2004). This is a reasonable approach when dealing with bacterial or viral diseases, where the organism recognizes the antigen as an exogenous protein and consequently the elicited immune-response is generally strong and effective against the target pathogen. Similarly, other studies involving the use of TAAs have been conducted with human proteins or vectors encoding for human TAAs: in these reports, the elicited immune response is not expected to be fully predictive of the possible outcome in human patients, since the antigen is recognized as a non-self protein. Therefore, the evaluation of the immune response against the 'self' antigen requires cloning of the nonhuman primate ortholog gene and evaluation of a technology able to break immune tolerance (Aurisicchio et al., 2007; Fattori et al., 2009). Still, evaluation of antitumour efficacy cannot be performed in nonhuman primates.

Another emerging model in oncology is the dog. The dog is extensively used in drug discovery and development because of its similarities to human anatomy and physiology. Compared with other animal models, dogs naturally develop cancers that share many characteristics with human malignancies (Paoloni et al., 2008). Cancers in pet dogs are characterized by tumour growth over long periods of time in the setting of an intact immune system, interindividual and intra-tumoural heterogeneity, the development of recurrent or resistant disease, and metastasis to relevant distant sites. Thanks to their large population size, cancer rate in pets is sufficient to power clinical trials, including assessment of new drugs. Examples include non-Hodgkin lymphoma, osteosarcoma, melanoma, prostate carcinoma, lung carcinoma, head and neck carcinoma, mammary carcinoma and soft tissue sarcoma, which share similar histological appearance, tumour genetics, biological behavior and response to conventional therapies with human cancers. With the recent release of the canine genome sequence, the dog is now also amenable to comparative genomic and expression analysis, including tumour samples (Uva et al., 2009). However, to date the application of cancer vaccines in dogs has been poorly explored. A xenogeneic DNA-based vaccine strategy for melanoma is the only example in the setting of minimal residual disease in dogs (Bergman et al., 2006) that led to the successful approval and the commercial launch of a veterinary biological (Merial US). Furthermore, recently we have been able to successfully evaluate a heterologous prime-boost Ad-vector /DNA-EP based

vaccine against telomerase in canine subjects affected by B cell lymphoma (Peruzzi et al., 2010b). Unfortunately, the canine immune system is not deeply characterized and there are not many tools available to further evaluate the impact of a vaccination strategies. However, we expect that pet dog nonclinical studies will increasingly gain interest to help a better definition of the safety and activity of new anticancer agents and the identification of relevant biomarkers associated with response or exposure to these drugs.

Animal Models for Cancer Vaccines

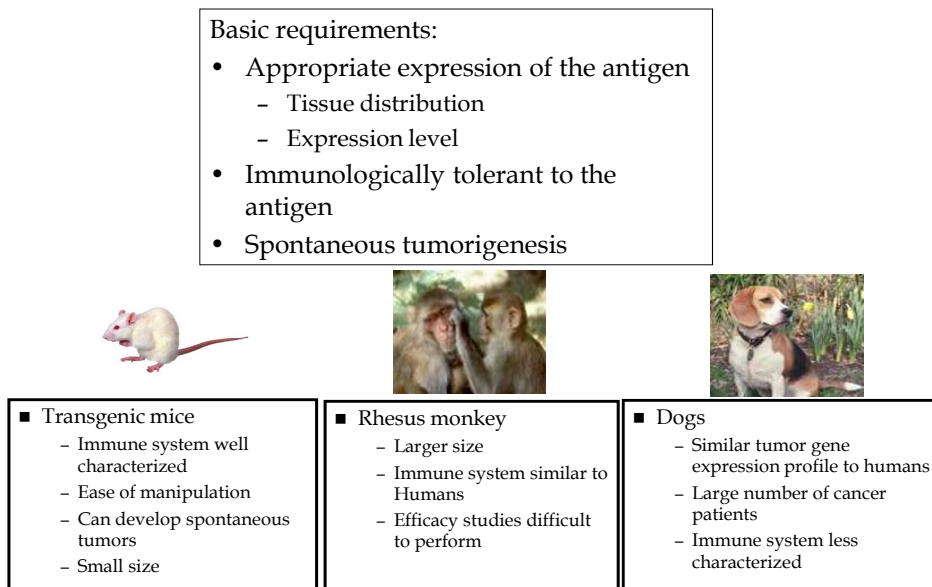


Fig. 1. Animal models for Cancer Vaccines. Advantages and drawbacks of each model are indicated.

4.2 Biomarker strategies

Drug failures in Oncology often originate from a lack of understanding of the biology of the drug, its mechanism of action (MOA), the complexity of patient physiology, and inadequate characterization of patient tumours. Poor understanding of the criteria required for patient selection for the drug may lead to misapprehensions of the drug’s potential for safety and efficacy. It is these misapprehensions that can persist through to late development until the clinical program crashes in a late and costly failure. Clearly, there is an urgent need for detailed information on new anticancer drugs to help make critical development decisions at the earliest possible point, speeding up the development process and enabling valuable time and resources to be placed where they can do the most good.

Molecular biomarkers are widely recognized as being integral to this solution. They provide a set of tools which can provide invaluable information to support two major development concerns:

1. Does the drug perform according to the expected mechanism of action?
2. Which patients will experience benefit in disease management utilizing the drug?

Thus, appropriately selected biomarkers can be used to confirm the MOA, while patient selection biomarkers can be used to guide the selection of the most appropriate patients for therapies. Correct use of biomarkers for patient selection can enrich the treatment population by identifying those most likely to benefit from the treatment. This reduces the risk to the non-responder population and, by allowing earlier assessment of therapeutic efficacy, substantially shrinks the costs of development.

Over the past two decades molecular biomarkers have become established components of clinical research in a way few could have foreseen. Today > 50% of new molecular entities are estimated to have a biomarker element also in development (Carden et al, 2010).

Nowadays the most successful examples of biomarkers for patient selection are HER2 positivity for treating breast cancer patients with Herceptin® and lack of KRAS mutations for treatment of colorectal cancer patients with Erbitux® or Vectibix®. They have completely different histories. The development of Herceptin was guided from its earliest stages by the use of the selection biomarker - HER2 - as an integral part of the original development plan for the product (Pietras et al, 1998). However, in the case of Erbitux® (cetuximab) and Vectibix® (panitumumab) in colorectal cancer, the original hypothesis that the level of epidermal growth factor receptor (EGFR) expression was critical for success of the antibody turned out to be wrong. Only recently the crucial role of KRAS wild type (or non-KRAS mutant carrying) tumour cells has been found to be a necessary element for Erbitux functioning and thus has been introduced into the drug's label (<http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm172905.htm>).

All the considerations above need to be applied also to the development of cancer vaccines and cancer immunotherapeutics in general. It is inconceivable nowadays to imagine that a new immunotherapy will be efficacious when administered to all patients affected by a given cancer pathology. Therefore a rigorous biomarker strategy is absolutely essential to avoid failures in cancer vaccines development (Gajewski et al, 2010; Disis, 2011). As a corollary, we believe that one of the main reasons for the several failures of previous cancer vaccines in phase III is the lack of biomarkers for patients selection, and this in spite of radiological evidences of direct anti-tumour efficacy observed in subsets of patients during precedent Phase II trials. Therefore, several attempts are currently being made to rescue vaccines "failed" in Phase III trials through a rigorous retrospective analysis of data collected in Phase III in order to identify biomarkers that can allow to predict patients who will most benefit from treatment, and then to restart Phase II and Phase III development.

This is the case for example of Trovax (see paragraph 3.2.1). Trovax was developed through a series of Phase I and II trials, until it was brought into a Phase III trial called TRIST (TroVax Renal Immunotherapy Trial) in patients with advanced or metastatic renal cell carcinoma. The study enrolled 733 patients divided in two arms: 1) Trovax + IL2 or IFN α or sunitinib; 2) Placebo + IL2 or IFN α or sunitinib (Amato et al, 2010). The primary predefined endpoint, namely survival (80% power, HR= 0.725; α = 0.05), was not met. However, subsequent analysis showed survival advantage in certain subsets of patients, and this opened up to studies aiming at identifying factors which could maximize benefit. In particular immunological monitoring suggested that 5T4 antibody responses were associated with increased survival (Harrop et al, 2011). However, it was necessary to show that 5T4 antibody responses was not simply linked to the general health status of the patients. This

was possible through the identification of an “immune response surrogate”, capable of predicting antibody responses with a reasonable level of accuracy. This was indeed identified in baseline platelet levels. In fact elevated platelet levels inversely correlate with anti 5T4 antibody responses and therapeutic efficacy. This new biomarker is currently being analyzed in additional ongoing trials and will likely be used to inform future strategies for renewed Phase II/III development of TroVax.

As mentioned in the introduction section, ipilimumab, the fully human monoclonal antibody directed against CTLA-4 has had a luckier developmental fate and was recently approved by FDA for the treatment of metastatic melanoma. A 3.7-month survival benefit was observed in the registration Phase III trial in the ipilimumab arm vs control gp-100 peptide vaccine arm was achieved (hazard ratio 0.68; $P = 0.003$), which met the predefined primary endpoint (Hodi et al, 2010). However if we look only at tumour responses only a minority of patients treated with ipilimumab or with the other anti CTLA-4 antibody under development, tremelimumab, achieve radiographic responses (Sarnaik and Weber, 2009). In the search of biomarkers capable of predicting early efficacy of these two antibodies immunological monitoring has been an integral part of their clinical development. Approaches to immunological monitoring have included: 1) monitoring the frequency of specific populations of cells in peripheral blood or tumour; 2) monitoring changes in expression levels of specific markers on immune cells; 3) quantifying antigen specific immune responses including antibody and CD8⁺ or CD4⁺ T cell responses; 4) monitoring changes in peripheral cytokine levels of cytokines produced by specific immune cell populations. This has led to the identification of several endpoints that may correlate with a variety of clinical parameters (reviewed in Callahan et al, 2010). The most robust correlation to date is with the rate of absolute lymphocyte counts (ALC), which was shown to correlate with clinical benefit (Berman et al, 2009). Also, inducible costimulator (ICOS) a member of the immunoglobulin gene family, seems to be involved. In some studies a correlation between increased frequency of circulating CD4⁺ICOS^{high} T cells and Overall Survival has been shown (Chen et al, 2009; Vonderheide et al, 2010; Charton et al, 2010). Promising biomarkers also appear to be increases in CD54RO and HLA-DR on circulating CD4⁺ and CD8⁺ T cells (Comin-Anduix et al, 2008), poly-epitope antigen specific immune responses (Yuan et al, 2008) and degree of intratumoural Treg infiltration (Ribas et al, 2009). Finally perhaps the most recent but probably most promising biomarker appears to be the change in circulating levels of Th17 as shown in a recent study on 75 patients (Sarnaik et al, 2011), where higher changes in Th-17 inducible frequency was a surrogate marker of freedom from relapse ($p=0.047$). These biomarkers have been so far identified in small retrospective trials, but their validation awaits larger prospective studies. Also, another present limitation of these biomarkers is that they belong more to the category of efficacy biomarkers than to that of stratification biomarkers and therefore, do not look as promising tools to stratify patients that are expected to better respond to therapy.

A clever biomarker strategy has been applied for MAGE-A3 ASCI by GSK (see paragraph 3.1.5). The MAGRIT study applies stringent patient stratification criteria based upon the level of expression of MAGE-A3 in patients' tumours (approximately 40% of NSCLC patients), which are believed to have greater chances of responding to therapy, mirroring the same strategy adopted during Herceptin® development. Furthermore, during the early phases of MAGE-A3 ASCI clinical development a multiple gene signature predictive of response to therapy was derived with an unbiased approach from microarray analysis of RNA extracted from peripheral lymphocytes of treated patients, in the attempt to establish a

correlation between gene expression and disease relapse. The MAGRIT trial will have as an additional objective the validation of this predictive signature in a prospective manner.

4.3 Combination therapies

The greatest potential for cancer vaccines will derive from the possibility to combine these treatments with existing and forthcoming therapeutics in order to create synergies while mitigating side effects (Andersen et al, 2010). Understanding the molecular basis for synergies poses significant scientific challenges, together with the definition of the best protocols for combinations. The establishment and optimization of dosing and scheduling of multiple treatments will require intensive pre-clinical studies as well as the conduct of well designed clinical study protocols with the consequence of significantly increasing development costs. Furthermore, for the combination of experimental drugs the ability to conduct combination studies will require that Companies that are commercially pursuing different drugs and vaccine candidates must come to specific agreements.

4.3.1 Combining vaccines with chemotherapy

It is now clear that chemotherapy, instead of having a general immunodepressant effect, when given in particular combination schedules, can have a potent immunostimulatory effect and may enhance cancer vaccine efficacy. Several pre-clinical studies have been at the basis of what is now called chemoimmunotherapy, a strategy which is structured upon the possibility to enhance cancer vaccine efficacy through well studied combinations with available chemotherapeutic agents. Promising clinical results have been obtained, which are waiting for confirmation in larger randomized trials (Zitvogel et al, 2008).

A leading example is that of cyclophosphamide (CTX), an alkylating agent that has been used for a long time at high dosages as a potent cytotoxic and lymphoablative drug. In recent years careful studies have shown that low doses CTX (also called metronomic CTX) have instead immunostimulatory and antiangiogenic effects, opening up new applications for cancer immunotherapy. By promoting IFN α secretion, CTX influences dendritic cells homeostasis, leading to preferential expansion of CD8 α^+ DCs, i.e. the main subset involved in cross-presentation of cell-derived antigens (Schiavoni et al, 2011; Moschella et al, 2011). Furthermore CTX induces tumour cell death with consequent DCs uptake of tumour apoptotic material, and CD8+ T-cell cross priming. Finally CTX induces a T-helper 17 (Th17) status, capable of shifting the Treg/Teffector equilibrium in favor of tumour regression (Sistigu et al, 2011). Another drug that is being combined with cancer vaccines with promising results is dacarbazine, due to its known effect in stimulating cytokine production, modulating Treg numbers and favouring homeostatic proliferation of effector T cells (Nisticò et al, 2009).

Chemotherapy-induced cell death can also be qualitatively immunogenic through upregulation of surface calreticulin. This process, called immunogenic apoptosis has been observed with chemotherapeutic agents such as oxaliplatin and anthracyclines and is activated by pre-apoptotic ER stress. Calreticulin, a protein usually residing in the endoplasmic reticulum is translocated onto the plasma membrane surface and triggers cell engulfment by dendritic cells and tumour associated antigens presentation (Zitvogel et al, 2010). Finally, other chemotherapeutic agents such as gemcitabine have been shown to favor depletion of TAMs and may enhance vaccine efficacy through removal of their negative regulation on effector T cells (Suzuki et al, 2007).

4.3.2 Combining vaccines with immunomodulators

From the mechanistic standpoint these are the combinations that should work best. Depending upon their mechanism of action immunomodulators are expected either to increase vaccine immunogenicity by potentiating antigen-specific CD8⁺ and/or CD4⁺ T cell responses, or to increase vaccine effectiveness by impairing one or more of those immunosuppressive mechanisms that operate at the level of tumour microenvironment. It was unfortunate that these expectations were not met in the Phase III trial that led to registration of Ipilimumab. In that 3 arms trial, Ipilimumab alone was as effective at increasing OS as the combination of Ipilimumab plus a peptide gp100 vaccine (Hodi et al, 2010). In other words, adding a therapeutic melanoma vaccine on top of Ipilimumab did not provide additional advantage. Several are the possible explanations of this failure, but we believe, as discussed above (see section 3.1.4) that peptide vaccines, especially those monospecific, i.e. directed against a single epitope, are not potent enough to show efficacy, in particular in large trials like this one, where no patients stratification criteria are being applied.

Nevertheless, we believe that vaccines plus immunomodulators combinations hold a great potential; however, they need to be studied in detail starting from rigorous preclinical studies. Furthermore, success of the same immunomodulators in one combination cannot be automatically extrapolated to another combination, because this may be affected by the combined vaccine/immunomodulators mechanism of action, and by the disease under study. For example we have observed that the same immunomodulators, namely an IMO TLR9 agonist exerts different effects when co-delivered with two genetic vaccines targeting different tumour antigens in two distinct pre-C models. In the BALB/*NeuT* model repeated vaccinations against HER2 with DNA electroporation plus systemic IMO administration proved to be the most effective treatment in the eradication of advanced mammary tumours (Aurisicchio et al, 2009). In contrast this was not the case when the same systemic IMO was co-delivered together with a genetic telomerase vaccine in an immunocompetent mouse model of melanoma (Conforti et al, 2010). We believe this discrepancy is due to the fact that in the first case the anti-HER2 vaccine acts primarily through the induction of antitumoural antibody responses that are strongly enhanced by systemic IMO in mice (Aurisicchio et al, 2009). In contrast, the telomerase vaccine mechanism of action is exerted via the induction of antigen-specific cytotoxic CD8⁺ responses (Mennuni et al, 2008) which are not increased by systemic IMO delivery.

Among the most promising approaches to combinations is the one with agents capable to target Tregs (Golovina and Vonderheide, 2010). For example, in a transgenic CEA preclinical model we have observed that administration of an anti CEA vaccine plus an antibody against CD25 strongly enhanced CEA specific CD4⁺ and CD8⁺ antigen-specific immunity and exerted a strong tumour protection (Elia et al, 2007). Indeed a single infusion of daclizumab (Zenapax), a monoclonal antibody against CD25, in patients with metastatic breast cancer is associated with a strong and prolonged decrease of circulating CD25⁺ FoxP3⁺ Tregs. When a peptide vaccine was administered after Zenapax infusion, at the nadir of circulating Tregs, a strong generation of antigen-specific immunity was observed.

In a very recent study, the administration of an agonistic CD40 antibody was shown, when combined with gemcitabine in a small cohort of patients with pancreatic ductal adenocarcinoma to induce tumour regressions (Beatty et al, 2011). Although in theory antibodies against CD40 are believed to act through reversion of immune suppression and induction of antitumour T cell responses, this was shown not to be the case in this trial

and in a relevant mouse model. Surprisingly the antibody seemed to act via a new and unsuspected mechanism of action, which consisted in the stimulation of macrophages which infiltrated the tumours, became tumouricidal and facilitated depletion of tumour stroma.

In conclusion, we believe that combinations of immunomodulators like Zenapax, TLR agonists, anti-CTLA4 antibodies, anti-PD1 antibodies, IDO inhibitors, etc. together with cancer vaccines, may have great potential to increase vaccine effectiveness and to prolong survival, but careful mechanistic studies have to be conducted to identify the best combination and the most appropriate delivery schedule for the two agents.

4.3.3 Combining vaccines with other targeted therapies

The availability of an expanding repertoire of targeted therapies against cancer opens up tremendous possibility for combinations with therapeutic cancer vaccines. This is still a largely unexplored area. However we believe that, in parallel with the clinical progress and the increasing number of FDA and EMA approved vaccines, this area will be the object of extensive investigations. Based on the mechanism of action for example anti-angiogenic agents are expected to act synergically with cancer vaccines. The same concept can be applied to combinations with anti-apoptotic agents targeting Bcl-2 members. Finally we have to be aware that some cancer targeted therapies may exert a negative effect on immune responses. This is for example the case of sorafenib, which has been shown in a preclinical model to significantly affect the immunostimulatory capacity of DCs (Hipp et al, 2008), or of HDAC inhibitors which are able to increase the suppressive functions of Tregs (Akimova et al, 2010)

4.3.4 Adverse effects of cancer Immunotherapy

Cancer Immunotherapy has been initially advocated as being very specific for cancer cells and to have fewer side effects than conventional therapies. This concept is confirmed by reports from cancer vaccines clinical trials of cases of patients experiencing complete responses in the absence of any serious adverse event (Suso et al, 2011). An even more significant example is the very benign toxicity profile of Sipuleucel-T (Plosker, 2011). It has to be pointed out however, when examining large trials, that vaccine-related adverse events, albeit rare and usually mild, are being observed. For example in a recent meta-analysis of 500 cases of advanced cancer patients treated with therapeutic peptide vaccines, 6 severe adverse events (SAEs) were related to the vaccine itself (Yoshida et al, 2011). They consisted mainly in local skin reactions or cellulitis around the injection sites. In some cases, more systemic effects such as edemas of the head and neck regions, colitis, rectal bleeding and bladder-vaginal fistulae were reported.

The occurrence of autimmunity is particularly evident in the case of therapies with systemic immunomodulators more than with cancer vaccines. Indeed, Immune-related adverse events (IRAEs) are being commonly observed in patients after CTLA-4 blockade and most likely reflect the drug mechanism of action and corresponding effects on the immune system (Weber, 2007). Immunotoxicities resulting from Ipilimumab treatment can range from relatively minor conditions, such as skin depigmentation, to severe toxicities against crucial organ systems, such as liver, heart and lung. In the Ipilimumab registration trial Grade 3 or 4 IRAEs occurred in 10 to 15% of patients treated and seven deaths were associated with IRAEs (Hodi et al, 2010). Treatment-related toxicity correlates with better

responses in some cases, and it is likely that serious adverse events from immune-mediated reactions will increase in frequency and severity as immunotherapeutic approaches become more effective (Amost et al, 2011). Hence, scientists and physicians should be on guard for SAEs associated with augmented immune responses and strategies will have to be developed to avoid or circumvent these side effects.

The use of viral vectors in past gene-therapy trials has been shown to cause the occurrence of leukemogenesis (Dunbar, 2007). This phenomenon has been linked to the use of retroviral vectors and is due to their integration into the host genome and the activation of adjacent proto-oncogenes. It is, therefore, important to carefully analyze whether genetic vaccines that make use of either naked DNA or viral vectors may raise similar issues. It has to be pointed out, however, that genetic vaccines bear two significant differences when compared to gene therapy with retroviral vectors. In first instance DNA, also following electroporation (Wang et al, 2004), as well as Adenoviral (Jager and Ehrhardt, 2007) or Pox vectors used for cancer vaccines have a very low or null chromosomal integration respectively in the host genome. The second aspect is that vaccines are inoculated at peripheral sites in the body such as dermal tissue or skeletal muscles which are mainly composed of terminally differentiated and mitotic quiescent cells. At any rate, Regulatory Agencies require the inclusion of genome integration and genotox studies for any new genetic vaccine as part of the documentation to be included in IND filings.

5. Clinical endpoints

Cancer Vaccines and Cancer Immunotherapy in general act via a gradual build up of immune responses in the body that eventually are expected to affect cancer growth and propagation. The realization that the kinetics of this process are relatively slow as compared to the more immediate effects of chemotherapy have led to the conclusion that the conventional clinical trial endpoints cannot be applied as such also to Cancer Immunotherapy trials, and that there was a need for the establishment of new and specific criteria. Several initiatives in this direction were started over the past years and were coordinated by the Cancer Immunotherapy Consortium of the Cancer Research Institute (CIC-CRI) in collaboration with the International Society for Biological Therapy of Cancer in USA and with the Association for Cancer Immunotherapy (C-IMT) in Europe. They led to the issuance in year 2009 of a guidance document by FDA (see next paragraph) whose principles are summarized below (for a detailed description, please refer to Hoos et al, 2010).

Essentially three novel endpoint considerations, which require extensive validation by prospective assessment in clinical studies, were formulated: 1) Harmonize assays directed to assess cellular immune response to tumour antigens in order to minimize assay variability among clinical sites. The goal is to obtain a reproducible biomarker that eventually will allow to establish more precise correlations between immune response and clinical efficacy; 2) Adopt new criteria for antitumour response which are adapted from the standard Response Evaluation Criteria in Solid Tumours (RECIST) criteria; 3) Use different statistical methods for trial design and assessment of survival outcomes.

The Cancer Vaccine Clinical Trial Working group first proposed that immunoassays should be performed at least at three different time points, one baseline and two follow up. At least two assays should be used in parallel to provide relevant data to inform go/nogo decision for further development. Furthermore, the cutoff values for an immune response should be established prospectively both to define a positive vs negative response and to define the

proportion of patients needed to conclude for a positive outcome. With respect to assay harmonization it was soon realized that several assays are being used (ELISPOT, IFN- γ intracellular staining, HLA-peptide multimer-staining, etc) with principles and procedures different in different laboratories. This hampers data reproducibility and comparisons among studies. Immunomonitoring proficiency panels were launched to address these issues for individual assays. These panels have worked by accrual of patients' samples and preparation of peripheral blood mononuclear cells, which were then shipped and tested across multiple laboratories, using their respective protocols. Results were then centrally analyzed. The ELISPOT panel has been the longest running panel, and its results have led to initial ELISPOT harmonization guidelines (Janetzki et al, 2007), which are directed to address key variables across different laboratories that influence assay outcome, but do not impose assay standardizations (Hoos et al, 2010).

Investigators rely on RECIST criteria to assess clinical activity of anticancer agents (Eisenhauer et al, 2009). These criteria nicely capture the effects of chemotherapeutic agents and measure tumour shrinkage. These criteria are used to distinguish Progressive Disease (PD) vs Stable Disease (SD), Partial Response (PR) or Complete Response (CR) and inform about trial continuation or discontinuation of experimental new therapies. However, it has become evident with time that RECIST criteria do not offer a complete description of the response to immunotherapeutic agents and need to be adjusted. This is due to the fact that the dynamics of antitumour effects of immunotherapeutic agents are in general much slower than chemotherapies and that in some cases patients with a stable disease, or a progressive disease at early time points, experience tumour regression at a later time. The Cancer Vaccine Clinical Trial Working Group addressed this issue, concluded that the appearance of measurable clinical activity for immunotherapies may take longer than for cytotoxic therapies (also after conventional progressive disease has been declared) and that application of standard RECIST criteria, may lead to inappropriate trial discontinuation (Hoos et al, 2007). By analyzing data from several different immunotherapy trials on a large number of patients a set of four distinct patterns were detected: immediate response, durable stable disease, response after tumour increase, and response in the presence of new lesions. While the first two patterns are included in conventional RECIST criteria, the other two are not. Therefore, in order to capture all patterns observed, the so called immune related Response Criteria (irRC) were formulated (Wolchok et al, 2009); irPD (immune-related Progressive Disease), irSD (immune-related Stable Disease), irPR (immune-related Partial Response), irCR (immune-related Complete Response) using the same thresholds to distinguish between categories as in the standard RECIST criteria. However, there are two substantial differences: a) Progressive Disease is declared not simply upon the appearance of new tumour lesions but upon the measure of total tumour burden according to a precise formula (Hoos et al, 2010); b) that measure should be confirmed at least at two consecutive time points. Using irRC the appearance of new tumour lesions alone does not constitute therefore irPD if they do not add to the total tumour burden measured at the initiation of the treatment by at least 25%, and if they are not confirmed at the subsequent time point. These new criteria are meaningful because they have received extensive validation in clinical trials with ipilimumab and have shown to correlate with favorable patients survival. However, their prospective evaluation in new trials is required to confirm their clinical utility.

Finally, at variance with chemotherapy, where early clinical effects are possible, immunotherapies often show delayed clinical effects. This is evident when analyzing

Kaplan-Meier survival curves of Provenge trials (Kantoff et al, 2010), where delayed separation of survival curves between active treatment vs control is observed. If this delayed separation is a general phenomenon for immunotherapeutic agents, then the statistical power to differentiate the curves is reduced. Therefore new statistical paradigms need to be established which take this into account in order to avoid miscalculations in the number of patients to be accrued in Phase III registration trials, and in the number of events required to calculate Hazard Ratio and Confidence Interval. It is highly recommended in this case that the quantification to compute the required events comes from previous randomized well designed Phase II trials.

In conclusion to this section we anticipate that the application of these new clinical endpoints is going to positively enhance the probability of success of cancer vaccines, allow faster and more informed GO/NOGO decisions in early clinical development and to prioritize agents that have the best profile to show statistically meaningful survival benefit.

6. Regulatory perspectives

Sipuleucel-T (Provenge®, Dendreon) is the only therapeutic cancer vaccine approved by FDA. However several promising vaccines such as M-Vax, (AVAX Technologies, Inc.) OncoVax (Vaccinogen), TroVax (Oxford Biomedica), ASCI MAGE-A3 (GSK), Oncophage® (Agenus) are in late stage development and are preparing for regulatory review in the United States, Europe, Canada, or other international regions (www.MarketResearch.com). Many of the products with potential approval status over the coming years are already in Phase III development, have orphan drug status, SPA status, or Fast Track status. As a signal of a new open attitude towards cancer vaccines, the FDA has recently issued new draft clinical trial guidelines for makers of therapeutic cancer vaccines intended to treat patients with existing disease (<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm182443.htm>). Most notably, the draft guidance, in line with the new criteria described in section 5, advises that time-to-tumor-progression may not be an appropriate endpoint for cancer vaccines and that immune response launched against the tumour may take longer than the time it takes for it to progress. As the mechanism of action for most cancer vaccines is thought to be mediated through amplifying a native T-cell response, especially cytotoxic T cells, regulators explained that development of a cancer vaccine can present different considerations for clinical trial design than development of a traditional cytotoxic drug or biological product for the treatment of cancer. Consequently, developers of cancer vaccines are now encouraged to move forward with new products, although they need to weigh the advantages and disadvantages of testing their agents in patients with metastatic diseases vs. patients with no evidence of residual disease or minimal burden of disease.

7. Future directions

We believe that therapeutic cancer vaccines have a bright future and that within the next ten years they will become an established therapeutic modality for cancer, in a manner similar to what have now become monoclonal antibodies. This success will strictly depend upon the respect of the four major principles listed below (see also Fig 2);

The “Key” to successful cancer vaccine development

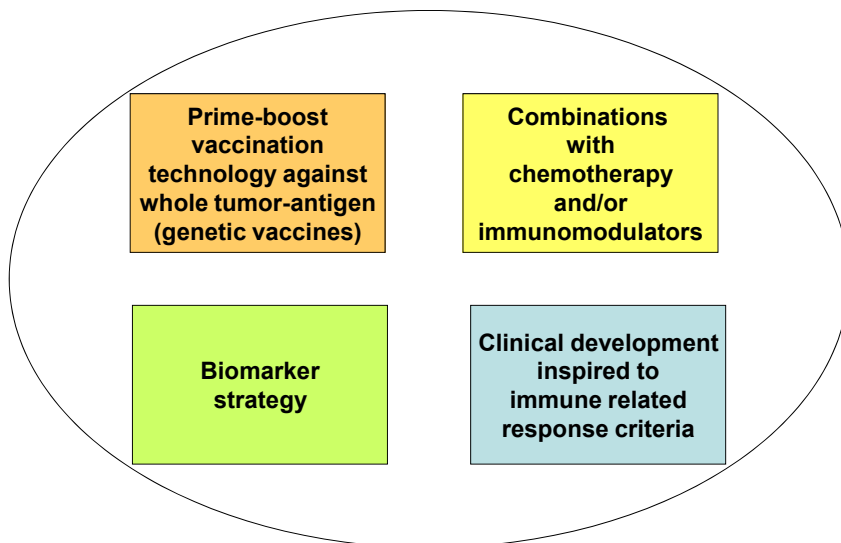


Fig. 2. Components and requirements for successful cancer vaccines development.

1. Use of a well established vaccination technology capable of inducing strong multi-epitope antigen-specific T and B cell responses, while using reproducible and easily scalable technologies. In this respect we believe that the most promising platforms for vaccination are those based on the use of genetic vectors, primarily when used in heterologous prime-boost combinations;
2. Appropriate combinations of vaccines with chemotherapy and/or with immunomodulators;
3. Development of an articulated biomarker strategy, which allows in parallel with clinical development to reproducibly quantify antigen-specific T cell responses as a pharmacodynamic measure of vaccine immunogenicity, and to pre-select the best responders to treatment;
4. A development paradigm that takes into account the evolving scenario and that is constantly inspired to the improved endpoints for cancer immunotherapy trials

8. Acknowledgements

G.C. work was supported in part by a grant AIRC IG 10334. L.A. work was supported in part by a grant AIRC IG 10507.

9. References

Akimova T, Ge G, Golovina T et al (2010) Histone/protein deacetylase inhibitors increase suppressive functions of human FOXP3 + Tregs , *Clin Immunol Sep*; 136 (3): 348-63

- Aldrich JF, Lowe DB, Shearer MH et al (2010) Vaccines and Immunotherapeutics for the Treatment of Malignant Disease, *Clin. Dev. Immunol.* 697158 Epub 2010 Sep 26
- Amato RJ, Hawkins RE, Kaufman HL et al (2010) Vaccination of metastatic renal cancer patients with MVA-5T4: a randomized, double-blind, placebo-controlled phase III study, *Clin Cancer Res* Nov 15; 16(22): 5539-47
- Amos SM, Duong CP, Westwood JA, Ritchie DS, Junghans RP, Darcy PK, Kershaw MH (2011) Autoimmunity associated with immunotherapy of cancer. *Blood.* 2011 Apr 29.
- Andersen MH, Thor SP (2002). Survivin--a universal tumour antigen. *Histol Histopathol*;17(2):669-75.
- Andersen MH, Junker N, Ellebaek E et al (2010) Therapeutic cancer vaccines in combination with conventional therapy , *Journal of Biomedicine an Biotechnology* 237623. Epub 2010 Jun 29
- Andre FM, Mir LM (2010). Nucleic acids electrotransfer in vivo: mechanisms and practical aspects. *Curr Gene Ther.* Aug;10(4):267-80.
- Ascierto PA, Simeone E, Sznol M et al (2010) Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies, *Semin Oncol* Oct; 37(5): 508-16
- Auricchio L, Mennuni C, Giannetti P, Calvaruso F, Nuzzo M, Cipriani B, Palombo F, Monaci P, Ciliberto G, La Monica N. (2007). Immunogenicity and safety of a DNA prime/adenovirus boost vaccine against rhesus CEA in nonhuman primates. *Int J Cancer.* Jun 1;120(11):2290-300.
- Auricchio L, Peruzzi D, Conforti A et al (2009) Treatment of mammary carcinomas in HER-2 transgenic mice through combination of genetic vaccine and an agonist of Toll-like receptor 9, *Clin Cancer Res* Mar 1; 15(5): 1575-84
- Auricchio L, Ciliberto G. (2010). Patented cancer vaccines: the promising leads. *Expert Opin Ther Pat.* May;20(5):647-60
- Beatty GL, Chiorean EG, Fishman MP et al (2011) CD40 agonists alter tumour stroma and show efficacy against pancreatic carcinoma in mice and humans, *Science* Mar 25; 331(6024): 1612-6
- Bei R, Scardino A (2010) TAA polyepitope DNA-based vaccines: a potential tool for cancer therapy, *J Biomed Biotechnol*;102758. Epub 2010 Jun 17
- Beaudette TT, Bachelder EM, Cohen JA, Obermeyer AC, Broaders KE, Fréchet JM, Kang ES, Mende I, Tseng WW, Davidson MG, Engleman EG. (2009). *In vivo* studies on the effect of co-encapsulation of CpG DNA and antigen in acid-degradable microparticle vaccines. *Mol Pharm.*6(4):1160-1169.
- Bergman PJ, Camps-Palau MA, McKnight JA, Leibman NF, Craft DM, Leung C, et al. (2006). Development of a xenogeneic DNA vaccine program for canine malignant melanoma at the Animal Medical Center. *Vaccine*;24(21):4582-5
- Bindea G , Mlecnik B, Fridman WH et al (2011) The prognostic impact of anti-cancer immune response: a novel classification of cancer patients, *Semin Immunopathol* 33(4):335-40.
- Bingle L, Brown NJ, Lewis CE (2002) The role of tumour-associated macrophages in tumour progression : implications for new anticancer therapies, *J Pathol* Mar; 196(3): 254-65
- Blumberg BS. (2010). Primary and secondary prevention of liver cancer caused by HBV. *Front Biosci (Schol Ed).* Jan 1;2:756-63.
- Bodles-Bakhop AM, Heller R, Draghia-Akli R (2009) Electroporation for the Delivery of DNA-based Vaccines and Immunotherapeutics: Current Clinical Developments, *Mol Ther* Apr; vol 17 n.4 : 585-592
- Bolhassani A, Safaiyan S, Rafati S (2011) Improvement of different vaccine delivery systems for cancer therapy, *Mol Cancer* Jan 7; 10:3

- Bozic I, Antal T, Ohtsuki H et al (2010) Accumulation of driver and passenger mutations during tumour progression, *Proc Natl Acad Sci U S A* Oct 26; 107(43): 18545-50
- Burch PA, Croghan GA, Gastineau DA, et al. (2004) Immunotherapy (APC8015, provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: A phase 2 trial. *Prostate.*; 60:197-204
- Butts C, Maksymiuk A, Goss G, Soulières D, Marshall E, Cormier Y, Ellis PM, Price A, Sawhney R, Beier F, Falk M, Murray N. (2011). Updated survival analysis in patients with stage IIIB or IV non-small-cell lung cancer receiving BLP25 liposome vaccine (L-BLP25): phase IIB randomized, multicenter, open-label trial. *J Cancer Res Clin Oncol.* Jul 9.
- Callahan MK, Wolchok JD, Allison JP (2010) Anti-CTLA-4 Antibody Therapy: Immune Monitoring During Clinical Development of a Novel Immunotherapy, *Semin Oncol.* October; 37(5): 473-484
- Campi G CM, Consogno G, et al. (2003) CD4+ T cells from healthy subjects and colon cancer patients recognize a carcinoembryonic antigen-specific immunodominant epitope. *Cancer Res*; 63:8481-6.
- Carden CP, Sarker D, Postel-Vinay S et al (2010) Can molecular biomarker-based patient selection in Phase I trials accelerate anticancer drug development?, *Drug Discov Today* Feb; 15(3-4): 88-97
- Cecco S, Muraro E, Giacomin E et al (2011) Cancer vaccines in phase II/III clinical trials: state of the art and future perspectives, *Curr Cancer Drug Targets* Jan; 11(1): 85-102
- Chaudry MA, Sales K, Ruf P, et al. (2007). EpCAM an immunotherapeutic target for gastrointestinal malignancy: current experience and future challenges. *Br J Cancer*;96(7):1013-9.
- Chaux, P., R. Luiten, N. Demotte, V. Vantomme, V. Stroobant, C. Traversari, V. Russo, E. Schultz, G. R. Cornelis, T. Boon, and B. P. van der. (1999). Identification of five MAGE-A1 epitopes recognized by cytolytic T lymphocytes obtained by in vitro stimulation with dendritic cells transduced with MAGE-A1. *J. Immunol.* 163:2928-2936.
- Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM. (2009). The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res.* Sep 1;15(17):5323-37
- Cheever MA, Higano C (2011) PROVENGE (Sipuleucel-T) in Prostate Cancer: The First FDA Approved Ther Cancer Vaccine, *Clin Cancer Res* Jun; 17(11):3520-6
- Chen H, Liakou CI, Kamat A et al (2009) Anti-CTLA-4 therapy results in higher CD4+ICOShi T cell frequency and IFN-gamma levels in both nonmalignant and malignant prostate tissue, *Proc Natl Acad Sci U S A* Feb 24; 106(8):2729-34
- Chiba T, Ohtani H, Mizoi T et al (2004), Intraepithelial CD8+ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis, *Br J Cancer.* Nov 1;91(9):1711-7
- Chouaib S, Kieda C, Benlalam H (2010), Endothelial cells as key determinants of the tumour microenvironment: interaction with tumour cells, extracellular matrix and immune killer cells, *Crit Rev Immunol.*;30(6):529-45
- Clemente CG, Mihm MC Jr, Bufalino R et al (1996), Prognostic value of tumour infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma, *Cancer.* Apr 1;77(7):1303-10

- Comin-Anduix B, Lee Y, Jalil J et al (2008) Detailed analysis of immunologic effects of the cytotoxic T lymphocyte-associated antigen 4-blocking monoclonal antibody tremelimumab in peripheral blood of patients with melanoma, *J Transl Med* May 1; 6:22
- Conforti A, Cipriani B, Peruzzi D et al (2010) A TLR9 agonist enhances therapeutic effects of telomerase genetic vaccine, *Vaccine* Apr 30; 28(20):3522-30
- Coulie PG, Van den Eynde BJ, van der Bruggen P et al (1997), Antigens recognized by T-lymphocytes on human tumours, *Biochem Soc Trans.* May;25(2):544-8
- Couzin-Frankel J (2010) Immune Therapy Steps Up the Attack, *Science* Oct; vol 330 n. 6003: 440-443
- Culver ME, Gatesman ML, Mancl EE et al (2011) Ipilimumab : a novel treatment for metastatic melanoma, *Ann Pharmacother* Apr; 45 (4) : 510-9
- Curiel TJ, Coukos G, Zou L et al (2004) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival, *Nat Med* Sep; 10(9) :942-9
- Daley GQ, Van Etten RA, Baltimore D. (1990). Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science*;247(4944):824-30.
- Darrasse-Jèze G, Bergot AS, Durgeau A et al (2009), Tumour emergence is sensed by self-specific CD44hi memory Tregs that create a dominant tolerogenic environment for tumours in mice, *J Clin Invest.* Sep; 119(9):2648-62.
- de Gruijl TD, van den Eertwegh AJ, Pinedo HM, et al. (2008). Whole-cell cancer vaccination: from autologous to allogeneic tumour- and dendritic cell-based vaccines. *Cancer Immunol Immunother* ;57(10):1569-77
- DeNardo DG, Andreu P, Coussens LM (2010) Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumour immunity, *Cancer Metastasis Rev* Jun; 29(2):309-16
- Dengjel J, Nastke MD, Gouttefangeas C, Gitsioudis G, Schoor O, Altenberend F, Müller M, Krämer B, Missiou A, Sauter M, Hennenlotter J, Wernet D, Stenzl A, Rammensee HG, Klingel K, Stevanović S (2006). Unexpected abundance of HLA class II presented peptides in primary renal cell carcinomas. *Clin Cancer Res.* Jul 15;12(14 Pt 1):4163-70.
- De Palma M, Lewis CE (2011) Cancer: Macrophages limit chemotherapy, *Nature* Apr 21; 472(7343): 303-4
- De Smet, C., C. Lurquin, B. Lethe, V. Martelange, and T. Boon. (1999). DNA methylation is the primary silencing mechanism for a set of germ line- and tumour-specific genes with a CpG-rich promoter. *Mol. Cell. Biol.* 19:7327-7335
- di Pietro A, Tosti G, Ferrucci PF, Testori A (2009). Heat shock protein peptide complex 96-based vaccines in melanoma: How far we are, how far we can get. *Hum Vaccin.* 2009 Nov 23;5(11).
- Dharmapuri S, Peruzzi D, Aurisicchio L. Engineered adenovirus serotypes for overcoming anti-vector immunity. *Expert Opin Biol Ther.* 2009 Oct;9(10):1279-87.
- Disis ML (2011) Immunologic biomarkers as correlates of clinical response to cancer immunotherapy, *Cancer Immunol Immunother* Mar; 60(3): 433-42
- Doehn C, Bohmer T, Kausch I, et al. (2008). Prostate cancer vaccines: current status and future potential. *BioDrugs*;22(2):71-84
- Dougan M, Dranoff G.(2009) Immune therapy for cancer, *Ann Rev Immunol.* ;27:83-117
- Druker BJ, Talpaz M, Resta DJ, et al. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*;344(14):1031-7.

- Dunbar CE. (2007). The yin and yang of stem cell gene therapy: insights into hematopoiesis, leukemogenesis, and gene therapy safety. *Hematology Am Soc Hematol Educ Program.*: 460-5
- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumour escape, *Nat Immunol.* Nov;3(11):991-8
- Elia L, Aurisicchio L., Facciabene A et al (2007) CD4+CD25+regulatory T-cell-inactivation in combination with adenovirus vaccines enhances T-cell responses and protects mice from tumour challenge, *Cancer Gene Ther* Feb; 14(2): 201-10
- Facciabene A, Aurisicchio L, Elia L, Palombo F, Mennuni C, Ciliberto G, La Monica N. (2006) DNA and adenoviral vectors encoding carcinoembryonic antigen fused to immunoenhancing sequences augment antigen-specific immune response and confer tumour protection. *Hum Gene Ther.* 2006 Jan;17(1):81-92.
- Facciabene A, Aurisicchio L, Elia L, Palombo F, Mennuni C, Ciliberto G, La Monica N. (2007). Vectors encoding carcinoembryonic antigen fused to the B subunit of heat-labile enterotoxin elicit antigen-specific immune responses and antitumour effects. *Vaccine.* Dec 21;26(1):47-58
- Fattori E, Aurisicchio L, Zampaglione I, Arcuri M, Cappelletti M, Cipriani B, Mennuni C, Calvaruso F, Nuzzo M, Ciliberto G, Monaci P, La Monica N (2009). ErbB2 genetic cancer vaccine in nonhuman primates: relevance of single nucleotide polymorphisms. *Hum Gene Ther.* Mar;20(3):253-65.
- Fox, E. J., J. J. Salk, and L. A. Loeb. (2009). Cancer genome sequencing – an interim analysis. *Cancer Res.* 69:4948-4950
- Fridman WH, Mlecnik B, Bindea G et al (2011) Immunosurveillance in human non-viral cancers, *Curr Opin Immunol* Apr; 23(2): 272-8
- Frenoy N SJ, Cahour A, Burtin P (1987). Natural antibodies against the carcinoembryonic antigen (CEA) and a related antigen, NCA, in human sera. *Anticancer Res*;7:1229-33.
- Gajewski TF, Louahed J, Brichard VG (2010) Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy, *Cancer J* Jul-Aug; 16(4): 399-403
- Gajewski TF, Meng Y, Blank C et al (2006), Immune resistance orchestrated by the tumour microenvironment, *Immunol Rev.* Oct; 213:131-45
- Globocan Project by International Agency for Research on Cancer (2008), Cancer Incidence and Mortality Worldwide in 2008, Available from <http://globocan.iarc.fr>
- Golovina TN, Vonderheide RH (2010) Regulatory T cells: overcoming suppression of T-cell immunity, *Cancer J* Jul-Aug; 16(4): 342-7
- Gómez CE, Nájera JL, Krupa M, Perdiguero B, Esteban M. (2011). MVA and NYVAC as vaccines against emergent infectious diseases and cancer. *Curr Gene Ther.* Jun 1;11(3):189-217.
- Gonzalez G, Crombet T, Lage A (2011) Chronic vaccination with a therapeutic EGF-based cancer vaccine: a review of patients receiving long lasting treatment, *Curr Cancer Drug Targets* Jan; 11 (1): 103-10
- Gridelli C, Rossi A, Maione P, Ferrara ML, Castaldo V, Sacco PC. (2009). Vaccines for the treatment of non-small cell lung cancer: a renewed anticancer strategy. *Oncologist.* Sep;14(9):909-20
- Harrop R, Shingler WH, McDonald M et al (2011) MVA-5T4-induced immune responses are an early marker of efficacy in renal patients , *Cancer Immunol Immunother* Jun; 60(6):829-37

- Hege KM, Jooss K, Pardoll D. (2006). GM-CSF gene-modified cancer cell immunotherapies: of mice and men. *Int Rev Immunol* 25(5-6):321-52.
- Heng HH, Liu G, Stevens JB et al (2010) Genetic and epigenetic heterogeneity in cancer: the ultimate challenge for drug therapy, *Curr Drug Targets* Oct; 11(10): 1304-16
- Hipp MM, Hilf N, Walter S et al (2008) Sorafenib, but not sunitinib, affects function of dendritic cells and induction of primary immune responses, *Blood* Jun 15; 111(12): 5610-20
- Hodi FS, O'Day SJ, McDermott DF et al (2010) Improved Survival with Ipilimumab in Patients with Metastatic Melanoma, *N Engl J Med* Aug; 363(8): 711-23
- Hofmeister V, Vetter C, Schrama D, Bröcker EB, Becker JC. (2006). Tumour stroma-associated antigens for anti-cancer immunotherapy. *Cancer Immunol Immunother.* May;55(5):481-94.
- Hood A, Eggermont AMM, Janetzki S et al (2010) Improved Endpoints for Cancer Immunotherapy Trials, *JNCI* vol 102 Issue 18, Sept,2010 : 1388-97
- Hoos A, Parmiani G., Hege K et al (2007) A clinical development paradigm for cancer vaccines and related biologics, *J. Immunother* Jan; 30(1) : 1-15
- Huang RR, Jalil J, Economou JS et al (2011) CTLA4 blockade induces frequent tumour infiltration by activated lymphocytes regardless of clinical responses in humans, *Clin Cancer Res* Jun; 17 (2):4101-9
- Jacobs JF, Aarntzen EH, Sibelt LA, et al. (2009). Vaccine-specific local T cell reactivity in immunotherapy-associated vitiligo in melanoma patients. *Cancer Immunol Immunother*;58(1):145-51
- Jager, E., Y. T. Chen, J. W. Drijfhout, J. Karbach, M. Ringhoffer, D. Jager, M. Arand, H. Wada, Y. Noguchi, E. Stockert, L. J. Old, and A. Knuth. (1998). Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J. Exp. Med.* 187:265-270
- Jager L, Ehrhardt A (2007). Emerging adenoviral vectors for stable correction of genetic disorders. *Curr Gene Ther.* Aug;7(4):272-83
- Jeong SH, Qiao M, Nascimbeni M, Hu Z, Rehermann B, Murthy K, Liang TJ. (2004). Immunization with hepatitis C virus-like particles induces humoral and cellular immune responses in nonhuman primates. *J Virol*;78(13):6995-7003
- Jocham D, Richter A, Hoffmann L, et al. (2004). 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(9409):594-9
- Johnson BF, Clay TM, Hobeika AC et al (2007) Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy, *Expert Opin Biol Ther.* Apr;7(4):449-60
- Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, Manson K, Panicali DL, Laus R, Schlom J, Dahut WL, Arlen PM, Gulley JL, Godfrey WR. (2010). Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol.* Mar 1;28(7):1099-105.
- Kantoff PW, Higano CS, Shore ND et al (2010) Sipuleucel-T immunotherapy for castration-resistant prostate cancer, *N.Engl J Med* Jul 29; 363 (5): 411-22
- Kaplan DH, Shankaran V, Dighe AS et al (1998), Demonstration of an interferon gamma-dependent tumour surveillance system in immunocompetent mice, *Proc Natl Acad Sci U S A.* Jun 23;95(13):7556-61

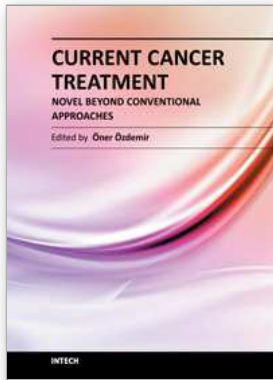
- Kawashima I HS, Tsai V et al. (1998). The multiepitope approach for immunotherapy for cancer: identification of several CTL epitopes from tumour-associated antigens expressed on solid epithelial tumours. *Hum Immunol* ;59:1-14.
- Kim DW, Krishnamurthy V, Bines SD et al (2010) TroVax, a recombinant modified vaccinia Ankara virus encoding 5T4: lessons learned and future development, *Hum Vaccine* Oct; 6 (10): 784-91
- Klebanoff CA, Acquavella N, Yu Z et al (2011) Therapeutic cancer vaccines : are we there yet?, *Immunol Rev* Jan; 239 (1) 27-44
- Kline J, Gajewski TF (2010) Clinical development of mAbs to block the PD1 pathway as an immunotherapy for cancer, *Curr Opin Investig Drugs* Dec; 11(12): 1354-9
- Koebel CM, Vermi W, Swann JB et al (2007), Adaptive immunity maintains occult cancer in an equilibrium state, *Nature*. Dec 6;450(7171):903-7
- Le DT, Pardoll DM, Jaffee EM. (2010) Cellular vaccine approaches. *Cancer J*. Jul-Aug;16(4):304-10
- Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumour microenvironments, *Cancer Res* Jan 15; 66(2): 605-12
- Lindenberg JJ, Fehres CM, van Crujisen H et al (2011), Cross-talk between tumour and myeloid cells: how to tip the balance in favor of antitumour immunity, *Immunotherapy*. Jan;3(1):77-96
- Liu MA. (2011) .DNA vaccines: an historical perspective and view to the future. *Immunol Rev*.Jan;239(1):62-84.
- Low L, Mander A, McCann K, Dearnaley D, Tjelle T, Mathiesen I, Stevenson F, Ottensmeier CH. (2009). DNA vaccination with electroporation induces increased antibody responses in patients with prostate cancer. *Hum Gene Ther*. Nov;20(11):1269-78.
- Lu S. (2009). Heterologous prime-boost vaccination. *Curr Opin Immunol*. Jun;21(3):346-51
- MacKie RM, Reid R, Junor B, (2003) Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery, *N Engl J Med*. 2003 Feb 6;348(6):567-8
- McMahon BJ, Bulkow LR, Singleton RJ, Williams J, Snowball M, Homan C, Parkinson AJ (2011) Elimination of hepatocellular carcinoma and acute hepatitis B in children 25 years after a hepatitis B newborn and catch-up immunization program. *Hepatology*. 2011 May 26. doi: 10.1002/hep.24442.
- Marshall JL (2003). Carcinoembryonic antigen-based vaccines. *Semin Oncol*;30(3 Suppl 8):30-6.
- Marshall JL, Gulley JL, Arlen PM, Beetham PK, Tsang KY, Slack R, Hodge JW, Doren S, Grosenbach DW, Hwang J, Fox E, Odogwu L, Park S, Panicali D, Schlom J. (2005) Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol*. Feb 1;23(4):720-31.
- Mennuni C, Ugel S, Mori F et al (2008) Preventive vaccination with telomerase controls tumour growth in genetically engineered and carcinogen-induced mouse models of cancer. *Cancer Res* Dec 1; 68(23): 9865-74
- Minamoto T, Mai M, Ronai Z. (1999) Environmental factors as regulators and effectors of multistep carcinogenesis. *Carcinogenesis* Apr;20(4):519-27.
- Mlecnik B, Bindea G, Pagès F et al (2011) Tumour immunosurveillance in human cancers , *Cancer Metastasis Rev* 30:5-12
- Mlecnick B, Sanchez-Cabo F., Charoentong P et al (2010) Data integration and exploration for the identification of molecular mechanisms in tumour-immune cells interaction, *BMC Genomics* Feb 10; 11 Suppl 1:S7

- Montgomery DL, Shiver JW, Leander KR, Perry HC, Friedman A, Martinez D, Ulmer JB, Donnelly JJ and Liu MA (1993). Heterologous and homologous protection against influenza A by DNA vaccination: optimization of DNA vectors. *DNA Cell Biol.*;129:777-83.
- Morgan RA, Dudley ME, Rosenberg SA (2010) Adoptive cell therapy: genetic modification to redirect effector cell specificity, *Cancer J.* Jul-Aug; 16(4): 336-41
- Moschella F, Valentini M, Aricò E et al (2011) Unraveling cancer chemoimmunotherapy mechanisms by gene and protein expression profiling of responses to cyclophosphamide, *Cancer Res* May 15; 71(10): 3528-39
- Moschella F, Proietti E, Capone I et al (2010) Combination strategies for enhancing the efficacy of immunotherapy in cancer patients, *Ann N Y Acad Sci* Apr; 1194: 169-78
- Nelson BH (2008) , Impact of T-cell immunity on ovarian cancer outcomes, *Immunol Rev.* Apr;222:101-16
- Nishikawa H, Sakaguchi S.(2010), Regulatory T cells in tumour immunity, *Int J Cancer.* Aug 15;127(4):759-67
- Nisticò P, Capone I, Palermo B et al (2009) Chemotherapy enhances vaccine-induced antitumour immunity in melanoma patients, *Int J Cancer* Jan 1; 124(1): 130-9
- Novellino L, Castelli C, Parmiani G (2005) A listing of human tumour antigens recognized by T cells: March 2004 update, *Cancer Immunol Immunother.* Mar;54(3):187-207
- Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumour immunity, *Cancer Immunol Immunother.* Oct;59(10):1593-600
- Oudard S, Rixe O, Beuselink B, Linassier C, Banu E, Machiels JP, Baudard M, Ringeisen F, Velu T, Lefrere-Belda MA, Limacher JM, Fridman WH, Azizi M, Acres B, Tartour E. (2011). A phase II study of the cancer vaccine TG4010 alone and in combination with cytokines in patients with metastatic renal clear-cell carcinoma: clinical and immunological findings. *Cancer Immunol Immunother.* Feb;60(2):261-71
- Palucka K, Ueno H, Banchereu J (2011) Recent developments in cancer vaccines, *J. Immunol* Feb 1; 186(3): 1325-31
- Paoloni M, Khanna C. (2008). Translation of new cancer treatments from pet dogs to humans. *Nat Rev Cancer*;8(2):147-56.
- Parmiani G, De Filippo A, Novellino L, Castelli C (2007). Unique human tumour antigens: immunobiology and use in clinical trials. *J Immunol.* Feb 15;178(4):1975-9.
- Pedicord VA, Montalvo W, Leiner IM et al (2011) Single dose of anti-CTLA-4 enhances CD8+ T-cell memory formation, function , and maintenance, *PNAS* Jan 4; vol 108 n.1: 266-271
- Peggs KS, Quezada SA, Chambers CA et al (2009), Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumour activity of anti-CTLA-4 antibodies, *J Exp Med.* Aug 3;206(8):1717-25
- Peled N, Oton AB, Hirsch FR et al (2009) MAGE A3 antigen-specific cancer immunotherapeutic, *Immunotherapy* Jan; 1(1):19-25
- Peruzzi D, Mesiti G, Ciliberto G, La Monica N, Aurisicchio L (2010). Telomerase and HER-2/neu as targets of genetic cancer vaccines in dogs. *Vaccine.* Feb 3;28(5):1201-8.
- Peruzzi D, Mori F, Conforti A, Lazzaro D, De Rinaldis E, Ciliberto G, La Monica N, Aurisicchio L. MMP11: a novel target antigen for cancer immunotherapy. *Clin Cancer Res.* Jun 15;15(12):4104-13.
- Peruzzi D, Gavazza A, Mesiti G, Lubas G, Scarselli E, Conforti A, Bendtsen C, Ciliberto G, La Monica N, Aurisicchio L (2010). A vaccine targeting telomerase enhances survival of dogs affected by B-cell lymphoma. *Mol Ther.* Aug;18(8):1559-67.

- Pietras RJ, Pegram MD, Finn RS et al (1998) Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs, *Oncogene* Oct 29 ; 17(17): 2235-49
- Plosker GL (2011), Sipuleucel-T: in metastatic castration-resistant prostate cancer, *Drugs* Jan 1; 71(1): 101-8
- Potebnya GP, Symchych TV, Lisovenko GS (2010) Xenogenic cancer vaccines, *Exp Oncol* Jul; 32(2): 61-5
- Quezada SA, Peggs KS, Simpson TR et al (2011) Shifting the equilibrium in cancer immunoediting: from tumour tolerance to eradication, *Immunol Rev* May; 241(1); 104-18
- Radoja S, Saio M, Schaer D et al (2001), CD8(+) tumour-infiltrating T cells are deficient in perforin-mediated cytolytic activity due to defective microtubule-organizing center mobilization and lytic granule exocytosis, *J Immunol.* Nov 1;167(9):5042-51
- Rech AJ, Vonderheide RH (2009) Clinical use of anti-CD25 antibody daclizumab to enhance immune responses to tumour antigen vaccination by targeting regulatory T cells , *Ann N Y Acad Sci* Sep; 1174: 99-106
- Ribas A, Butterfield L, Glaspy JA, Economou JS (2003). Current Developments in Cancer Vaccines and Cellular Immunotherapy *Journal of Clinical Oncology*, Vol 21, 12: 2415-2432
- Ribas A, Comin-Anduix B, Economou JS et al (2009) Intratumoural immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase patients with melanoma undergoing CTLA4 blockade, *Clin Cancer Res* Jan 1; 15(1): 390-9
- Rodriguez-Paredes M, Esteller M (2011) Cancer epigenetics reaches mainstream oncology, *Nat Med* Mar; 17(3): 330-9
- Rosenberg SA, Dudley ME (2009) Adoptive cell therapy for the treatment of patients with metastatic melanoma , *Curr Opin Immunol* Apr; 21(2):233-40
- Rosenblatt J, Glotzbecker B, Mills H et al (2011) PD-1 Blockade by CT-011, Anti-PD-1 Antibody, Enhances Ex Vivo T-cell Responses to Autologous Dendritic Cell/Myeloma Fusion Vaccine, *J.Immunother* Jun: 34(5); 409-18
- Sahin U, Türeci O, Pfreundschuh M. (1997) , Serological identification of human tumour antigens, *Curr Opin Immunol.* Oct;9(5):709-16
- Sarnaik AA, Yu B, Morelli D et al (2011) Extended dose ipilimumab with a peptide vaccine: immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma, *Clin Cancer Res* Feb 15;17(4): 896-906
- Sarnaik AA, Weber JS (2009) Recent advances using anti-CTLA-4 for the treatment of melanoma, *Cancer J* May-Hun; 15(3) :169-73
- Sastre L (2011) New DNA sequencing technologies open a promising era for cancer research and treatment , *Clin Transl Oncol* May; 13(5):301-6
- Shah RB, Chinnaiyan AM. (2009). The discovery of common recurrent transmembrane protease serine 2 (TMPRSS2)-erythroblastosis virus E26 transforming sequence (ETS) gene fusions in prostate cancer: significance and clinical implications. *Adv Anat Pathol*;16(3):145-53.
- Schiavoni G, Sistigu A, Valentini M et al (2011) Cyclophosphamide synergizes with type I interferons through systemic dendritic cell reactivation and induction of immunogenic tumour apoptosis, *Cancer Res* Feb 1; 71(3): 768-78
- Shiver JW, Fu TM, Chen L, Casimiro D, Davies ME, Evans RK, Zhang ZQ, Simon AJ, Triglia WL, Dubey SA, Huang L., Harris VA, et al. (2002). Replication-incompetent

- adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature*;415:331-35.
- Schmidt, S. M., K. Schag, M. R. Muller, M. M. Weck, S. Appel, L. Kanz, F. Grunebach, and P. Brossart. (2003). Survivin is a shared tumour-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. *Blood* 102:571-576
- Schreiber RD, Old LJ, Smyth MJ (2011) Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion, *Science* March 25, vol 331 : 1565-70
- Shumway NM, Ibrahim N, Ponniah S, et al. (2009) Therapeutic breast cancer vaccines: a new strategy for early-stage disease. *BioDrugs*;23(5):277-87.
- Sistigu A, Viaud S, Chaput N et al (2011) Immunomodulatory effects of cyclophosphamide and implementations for vaccine design, *Semin Immunopathol* May 25,
- Soliman H, Mediavilla-Varela M, Antonia S (2010) Indoleamine 2,3-dioxygenase: is it an immune suppressor? *Cancer J*. Jul-Aug;16(4):354-9
- Steer HJ, Lake RA, Nowak AK et al (2010) Harnessing the immune response to treat cancer , *Oncogene*, 29, 6301-6313
- Stevenson FK, Rice J, Ottensmeier CH, Thirdborough SM, Zhu D. (2004). DNA fusion gene vaccines against cancer: from the laboratory to the clinic. *Immunol Rev*. Jun;199:156-80.
- Street SE, Trapani JA, MacGregor D et al (2002), Suppression of lymphoma and epithelial malignancies effected by interferon gamma, *J Exp Med*. Jul 1;196(1):129-34
- Suso EM, Dueland S, Rasmussen AM, Vetrhus T, Aamdal S, Kvalheim G, Gaudernack G. (2011). hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes. *Cancer Immunol Immunother*. Jun;60(6):809-18.
- Suzuki E, Sun J, Kapoor V et al (2007) Gemcitabine has significant immunomodulatory activity in murine tumour models independent of its cytotoxic effects, *Cancer Biol Ther* Jun; 6 (6) : 880-5
- Tsang, K. Y., S. Zaremba, C. A. Nieroda, M. Z. Zhu, J. M. Hamilton, and J. Schlom. (1995). Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J. Natl. Cancer Inst.* 87:982-990
- Tyagi P, Mirakhur B (2009) MAGRIT: The largest-Ever Phase III Lung Cancer Trial Aims to Establish a Novel Tumour-Specific Approach to Therapy, *Clinical Lung Cancer* Vol 10,n.5, 371-374;
- Ulmer JB, Wahren B, Liu MA: Gene-based vaccines: recent technical and clinical advances.(2006) *Trends in Molecular Medicine*, 12:216-222.
- Umamo, Y., T. Tsunoda, H. Tanaka, K. Matsuda, H. Yamaue, and H. Tanimura. (2001). Generation of cytotoxic T cell responses to an HLA-A24 restricted epitope peptide derived from wild-type p53. *Br. J. Cancer* 84:1052-1057
- Uva P, Aurisicchio L, Watters J, Loboda A, Kulkarni A, Castle J, et al. (2009). Comparative expression pathway analysis of human and canine mammary tumours. *BMC Genomics* ;10(1):135
- Van der Burg SH, Melief CJ (2011). Therapeutic vaccination against human papilloma virus induced malignancies. *Curr Opin Immunol*. Apr;23(2):252-7. Epub 2011 Jan 13.
- Van Houdt IS, Sluijter BJ, Moesbergen LM et al (2008), Favorable outcome in clinically stage II melanoma patients is associated with the presence of activated tumour infiltrating T-lymphocytes and preserved MHC class I antigen expression, *Int J Cancer*. Aug 1;123(3):609-15

- Vergati M, Intrivici C, Huen NY et al (2010) Strategies for Cancer Vaccine Development, *J Biomed Biotechnol* 596432.Epub 2010 Jul 11
- Vermorken JB, Claessen AM, van Tinteren H, et al. (1999). Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. *Lancet*;353(9150):345-50.
- Vonderheide RH (2008). Prospects and challenges of building a cancer vaccine targeting telomerase. *Biochimie*;90(1):173-80
- Vonderheide RH, LoRusso PM, Khalil M et al (2010) Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells, *Clin Cancer Res*, Jul 1; 16(13): 3485-94
- Wang Z, Troilo PJ, Wang X, Griffiths TG, Pacchione SJ, Barnum AB, Harper LB, Pauley CJ, Niu Z, Denisova L, Follmer TT, Rizzuto G, Ciliberto G, Fattori E, Monica NL, Manam S, Ledwith BJ. (2004). Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. *Gene Ther*. Apr;11(8):711-21.
- Wang HY, Peng G, Guo Z et al (2005), Recognition of a new ARTC1 peptide ligand uniquely expressed in tumour cells by antigen-specific CD4+ regulatory T cells, *J Immunol*. Mar 1;174(5): 2661-70
- Weber J. (2007). Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. *Oncologist*. Jul;12(7):864-72.
- Welters MJ, Kenter GG, de Vos van Steenwijk PJ et al (2010), Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses, *Proc Natl Acad Sci U S A*, Jun 29;107(26):11895-9
- Wolchok JD, Hoos A, O'Day S et al (2009), Guidelines for the evaluation of immune therapy activity in solid tumours: immunerelated response criteria, *Clin Cancer Res*, Dec 1; 15(23):7412-20
- Wolchok JD, Weber JS, Hamid O et al (2010) Ipilimumab efficacy and safety in patients with advanced melanoma: a retrospective analysis of HLA subtype from four trials, *Cancer Immun*. Oct 10; 10:9
- Woo EY, Chu CS, Goletz TJ et al (2001), Regulatory CD4+CD25+T Cells in Tumours from Patients with Early-Stage Non-Small Cell Lung Cancer and Late-Stage Ovarian Cancer, *Cancer Research*, June 15; 61 : 4766-4772
- Yoshida K, Noguchi M, Mine T, Komatsu N, Yutani S, Ueno T, Yanagimoto H, Kawano K, Itoh K, Yamada A. (2011) Characteristics of severe adverse events after peptide vaccination for advanced cancer patients: Analysis of 500 cases. *Oncol Rep*. Jan;25(1):57-62.
- Yuan J, Gnjatic S, Li H et al (2008) CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit, *Proc Natl Acad Sci USA* Dec 23; 105(51): 20410-5
- Zitvogel L, Apetoh L, Ghiringhelli F et al (2008) Immunological aspects of cancer chemotherapy, *Nat Rev Immunol* Jan; 8(1): 59-73
- Zitvogel L, Kepp O, Senovilla L et al (2010) Immunogenetic tumour cell death for optimal anticancer therapy: the calreticulin exposure pathway, *Clin Cancer Res* Jun 15; 16(12): 3100-4



Current Cancer Treatment - Novel Beyond Conventional Approaches

Edited by Prof. Oner Ozdemir

ISBN 978-953-307-397-2

Hard cover, 810 pages

Publisher InTech

Published online 09, December, 2011

Published in print edition December, 2011

Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book "Current Cancer Treatment - Novel Beyond Conventional Approaches", consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Luigi Aurisicchio and Gennaro Ciliberto (2011). Harnessing the Immune System to Fight Cancer: The Promise of Genetic Cancer Vaccines, Current Cancer Treatment - Novel Beyond Conventional Approaches, Prof. Oner Ozdemir (Ed.), ISBN: 978-953-307-397-2, InTech, Available from: <http://www.intechopen.com/books/current-cancer-treatment-novel-beyond-conventional-approaches/harnessing-the-immune-system-to-fight-cancer-the-promise-of-genetic-cancer-vaccines>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.