Heat Shock Proteins (HSPs) Based Anti-Cancer Vaccines

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Abstract: The importance of HSPs themselves in antigen presentation and cross-presentation remains controversial. Most studies agree that as part of their molecular chaperone function, HSPs can bind and present tumor associated antigens to professional antigen presenting cells through MHC class I and class II molecules, leading to the activation of anti-tumor CD8+ and CD4+ T cells. The regulation of the innate and adaptive immune responses by HSPs is still a matter of intense research. HSPs are seen as important anticancer vaccine adjuvants. They are used through different delivery systems: HSPs/antibodies, peptide/protein-HSP complexes, tumor antigen/HSP gene fusion, viral peptides/HSP complexes or gene fusion, viral proteins/bacterial HSP fusion. In preclinical models different administration routes, subcutaneous, intradermal, intramuscular or even peroral (under special conditions) can be used, and the animal toxicities are non-significant. The HSP-based vaccines can induce specific and non-specific cellular immune responses all of which are important to induce tumor rejection. In addition, the antibodies generated after vaccination are emerging as important protagonist in the antitumoral response. This response is significantly enhanced when the suppressive tumor microenvironment and the immune suppressing effector cells are blocked. Several clinical studies have been carried out and are ongoing, immunizing cancer patients with autologous tumor derived HSP-peptide complexes (HSPPCs). The most promising results have been observed in patients with melanoma and renal clear cell cancer without advanced disease. There are clinical trials with HSP-based anticancer vaccines other than with HSPPCs (including patients with non-Hodgkin lymphoma, high-grade transitional cell carcinoma of the bladder, high-grade cervical dysplasia, etc).

Keywords: Cancer, heat shock proteins, immune system, vaccines.

INTRODUCTION

There is a great interest in developing anticancer vaccines due to the accumulating evidence that the immune defensive mechanisms can successfully attack cancer cells after vaccine stimulation, and because in contrast to chemotherapy and radiotherapy, they have relatively low toxicity. In fact the toxicity (high temperature, nausea and diarrhoea) may come from the consequence of tumor killing as has been reported when genetically engineered immune T cells attacked a refractory chronic lymphocytic leukemia [1]. Although the vaccines are designed to specifically attack cancer cells, they might induce immunity against antigens normally present in the patient. This may occur particularly with some of the approaches using heat shock proteins (HSPs) where relatively "crude HSP-antigen complexes" are injected; at least one serious adverse event (autoimmune thyroiditis of grade 2 severity) has been reported [2]. HSPs are very important molecular chaperones for maintaining cellular homeostasis; they form a complex family of proteins [3]. They operate in a complex interplay with synergistic/overlapping multiplicity of functions converging in cell protection. The HSPs can be both constitutively expressed and expressed in response to proteotoxic stresses (stress-responsive proteins). In cancer they are usually over-expressed by different mechanisms and with important consequences in relation to cell survival, tumor aggressiveness and resistance to therapies [4-6].

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ROLES OF HSPs IN THE IMMUNE SYSTEM

Antigen Presentation and Regulation of Immune Cells

It is outside the scope of this review to present the roles of the HSPs in the immune system, we will present some of the principal features and controversies only. HSPs are translocated from intracellular to extracellular environment and the mechanisms of this change location are not elucidated. Extracellular HSPs have been reported to play and essential role in the immune system, in particular in the stimulation of the innate and adaptive immunity [7, 8]. Several experiments performed with tumor cells and tissues have demonstrated that HSPs, as part of their molecular chaperone function, can bind tumor associated antigens (TAAs) to be presented to professional antigen presenting cells (APCs) [including dendritic cells (DCs)] through MHC class I and class II molecules leading to the activation of anti-tumor CD8+ and CD4+ T cells. Therefore, HSPs are involved in antigen presentation and cross-presentation, activation

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and maturation of DCs, activation of macrophages and lymphocytes, and cytokine production. At present HSP-specific cell surface receptors on immune cells have not been identified, the receptors involved in HSP binding [LRP/CD91, Toll-Like Receptors (TLRs), Scavenger receptors, CD40, and c-type Lectins] have affinity to different types of molecules [9]. This is one of the reasons why the importance of HSPs themselves in antigen presentation and cross-presentation remains controversial. The immunological functions of HSPs have been attributed to the chaperoned peptides (TAAs, etc) or contaminants (LPS, etc) present in HSP preparations and not to the HSP themselves [10]. Highly purified HSPs do not have cytokine effects; they do not meet the definition of endogenous ligands of TLRs, danger signals, or alarmins (Fig. **1**). Therefore, the molecular mechanisms by which they exert their immunological functions remain unclear.

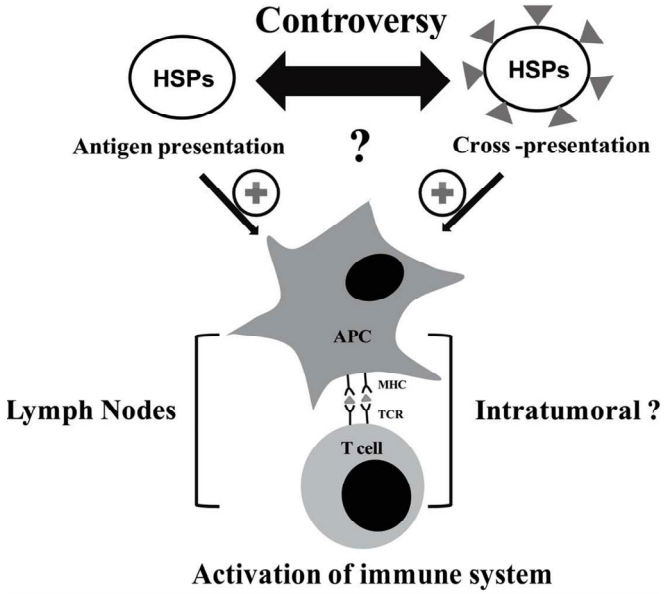

Fig. (1). Controversies on the implications of the HSPs in the immune response: are they immunogenic by themselves? Are they necessary or essential to enhance antigen presentation? After antigen presentation, T cell activation takes place in secondary lymphoid organs but in certain cases this process can occur inside tumor areas.

Hsp70: Hsp70-1 (HSPA1L) and Hsp70-2 (HSPA1B) gene are located within the human leukocyte antigen (HLA) class III region. A recent article by Jolesch *et al.* [11] describes the mechanisms by which Hsp70 cross-talks with the immune system, this HSP seems to enter the DCs *via* endocytosis. Dr. Multhoff´s laboratory [12] has demonstrated an unusual surface localization for Hsp70 in tumors, it was found in the plasma membrane. These tumor cells expressing membrane Hsp70 are frequently resistant to standard treatment but might be recognized and eliminated by pre-activated natural killer (NK) cells (see updated information on this in the article by Multhoff *et al.*. in this issue). Recent studies are showing more evidence that certain HSPs are specifically involved in antigen presentation. Exosomes derived from heat-treated ascitic cells from gastric cancer patients (which show high concentrations of Hsp70 and Hsp60) are able to promote DC maturation and to induce tumor-specific CTL response more efficiently than non heat-treated isolated exosomes [13]. Fang *et al.* [14] have identified a novel Hsp70 family member, termed Hsp70-like protein 1 (Hsp70L1), and demonstrated an interaction between this protein and DCs through TLR4. Hsp70L1 showed a potent T helper cell (Th1) immune response, but a more efficient induction was obtained by Hsp70L1/antigen fusion protein. However, the underlying mechanism involved in Hsp70L1 adjuvant activity remains to be elucidated. On the other hand, HSP gene polymorphisms have been associated with autoimmunity and with various cancers. A recent report presented a possible connection between Hsp70 overexpression and development of autoimmune disease, particularly multiple sclerosis (MS) where Hsp70 could be promoting immune recognition chaperoning myelin [15]. In this study Hsp70 expression was analyzed in peripheral blood mononuclear cells from MS patients, healthy controls, and in patients with rheumatoid arthritis. It was found that heat induced Hsp70 significantly more (up to six fold) in MS patients compared with healthy controls, indicating that immune cells from MS patients are more susceptible to Hsp70 induction under stress conditions.

Like other Hsps, Hsp72 (HSPA1A) is present at elevated levels in various human tumors, and its expression often correlates with increased tumor cell proliferation, poor response to chemotherapy, and poor survival [4-6]. In addition to its classical intracellular localization, Hsp72 is also expressed on the plasma membrane of malignantly transformed cells or tumor derived exosomes (TDEs). Recently, it was shown that TDE-associated Hsp72 was responsible of the suppressive activity of myeloid-derived suppressor cells (MDSCs) by activating Stat3. Indeed, TDE-associated Hsp72 triggered Stat3 activation in MDSCs in a TLR2/MyD88-dependent manner through autocrine production of IL-6 [16].

Hsp90: Endogenous Hsp90 (HSPC) seems to have an important role on DCs in cross-presentation pathways and that the mechanism is at least different from its role in endogenous MHC I processing pathway [17]. A recent article has presented evidence on the involvement of Hsp90 with the Treg [18]. When histone/protein deacetylase HDAC-6 was blocked (impairing the acetylation of Hsp90) the activity of Foxp3+ Tregs was altered, then Hsp90 can control the function of these Treg cells which have implications in autoimmunity and transplant rejection. Kasperkiewicz *et al.* [19] have studied anti-Hsp90 treatment in autoimmunity to type VII collagen. The *in vivo* studies showed that type VII collagen-specific plasma cells, and germinal center B cells were unaffected by anti-Hsp90 treatment. However, T-cell proliferation was potently inhibited with Hsp90 inhibitors identifying this kind of cells as targets of the treatment in autoimmunity to type VII collagen.

Hsp60: It has been reported that the production of Treg depends on the HSP type. Bacterial (but not

mouse) Hsp60 caused naïve T cells to differentiate into CD4+, CD25+, Foxp3+ T cells (Treg) [20]. This bacterial Hsp60 exists in the appendix and large intestine. Hsp60 from endogenous or exogenous microorganisms might produce a local pro-inflammatory reaction with cross-reacting antibodies and cell-mediated response with immuno-pathogenic outcomes in malignant transformation and autoimmune diseases. It has also been shown that high metastatic B16-F10 murine melanoma cells produced more Hsp60 than low metastatic B16-F1 cells and that Hsp60 released by tumor cells caused a persistent activation of TLR2, critically activating the transcription factor Stat3 and leading to the release of immunosuppressive cytokines and chemokines [21].

Hsp27: This protein is essential in the expression and release of LPS-induced pro-inflammatory factors. Phosphorylation of Hsp27 by MAPKAPK2/3 is also very important in controlling the excessive production of pro inflammatory factors by LPS stimulation. Hsp27 enhances NF-KB but not MAPKs signaling pathway through its reaction with tumor necrosis factor receptor-associated factor 6 (TRAF6) and increasing the association of TRAF6-IKKc (TNFα-mediated IKB kinase) [22]. In addition, a novel immuno-regulatory activity of extracellular Hsp27 has been associated to human breast cancer progression [23]. Soluble Hsp27 caused the differentiation of monocytes to tolerogenic macrophages inducing severe unresponsiveness/ anergy in T cells. These Hsp27-differentiated macrophages lose tumoricidal activity and become highly pro-angiogenic. Finally, Hsp27 (among other proteins) has been associated to streptococcal-induced autoimmune response in psoriasis; Hsp27 was immunogenic for T cells from psoriasis patients acting as an autoantigen [24].

Fig. (**2**) shows a summary of some the roles of these HSPs in the immune system.

In conclusion, there are some controversies on the specific roles of the HSPs on the immune system. Most researches agree that in the context of MHC class I or II cross-presentation the HSPs can present antigens to APCs. But can the HSPs-TAAs be incorporated *via* specific receptors or by endocytosis? Can HSPs regulate the activity and maturation of DCs also? Can the HSPs by themselves regulate the innate and adaptive immune responses? Which are the roles of the HSPs in autoimmunity? Can they act as natural anti-inflammatory agents? These topics are still a matter of intense research.

STRATEGIES FOR USING THE HSPS AS ANTICANCER VACCINES

Immune Attack to Cancer Cells

Along the evolution our immune system has turned into a sophisticated complex and powerful machinery whose main objective is to protect the body against potentially dangerous agents (microbes, pre- and cancerous cells). There is considerable evidence that both the innate immune system components (immediate and unspecific response) and the adaptive immune system components (delayed and specific response) can recognize and eliminate tumor cells. T cells (NKT cells and CTLs) are believed to carry out an immune surveillance function; they have the ability to detect newly transformed cells (tumor antigen recognition). Conversely, tumor cells have elicited strategies to evade both types of immune responses. At present, there is a discussion on the dual role of the immune system on tumor biology: host-protection and tumor-promotion. This has led to coin the concept “immune-edition of cancer” which is expertly revised elsewhere [25].

Tumor cells can induce malfunction or evade the immune system through different mechanisms:

a) Down-regulation of MHC class I genes.

b) Loss or masking of tumor antigens.

c) Production of inhibitory molecules that affect DCs leading to T cell anergy (tumors can increase IL-10 levels which reduce expression of CD80 and CD86 on DCs, both of which are required for T cell activation). In the absence of CD80 and CD86, T cells become tolerant to the tumor and do not mount an antitumor immune response.

d) Production of immunosuppressive molecules [nitric oxide, hydrogen peroxide, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), galectin, and indoleamine 2,3-dioxygenase (IDO)];

e) Recruitment of immunosuppressive cells [Treg cells and myeloid-derived suppressor cells (MDSCs)];

f) Expression of molecules which produce blockage of T cell-induced apoptosis (soluble Fas receptor, Decoy receptors, among others).

Moreover, some tumors do not induce a strong immune response; during early embryonic development all the lymphocytes that recognize normal cell antigens ("self antigens") are destroyed to prevent an immune response against one's own tissue. This process is called self tolerance. Since tumor cells are not extremely different from normal cells, the first also can be immunologically tolerated.

Since the immune system plays a crucial role in preventing and eradicating cancer, it is possible to boost the immune system to enable it to combat cancer more effectively through the design of cancer vaccines. For a long time, immunotherapies have been an attractive promise for the effective treatment against cancer and several are now into clinical phases. Immunotherapy is considered the fourth modality of cancer treatment after chemotherapy, radiation therapy and surgery. Included in immunotherapy, there are many approaches with anticancer vaccines within which are the HSP-based vaccines (Fig. **3**).

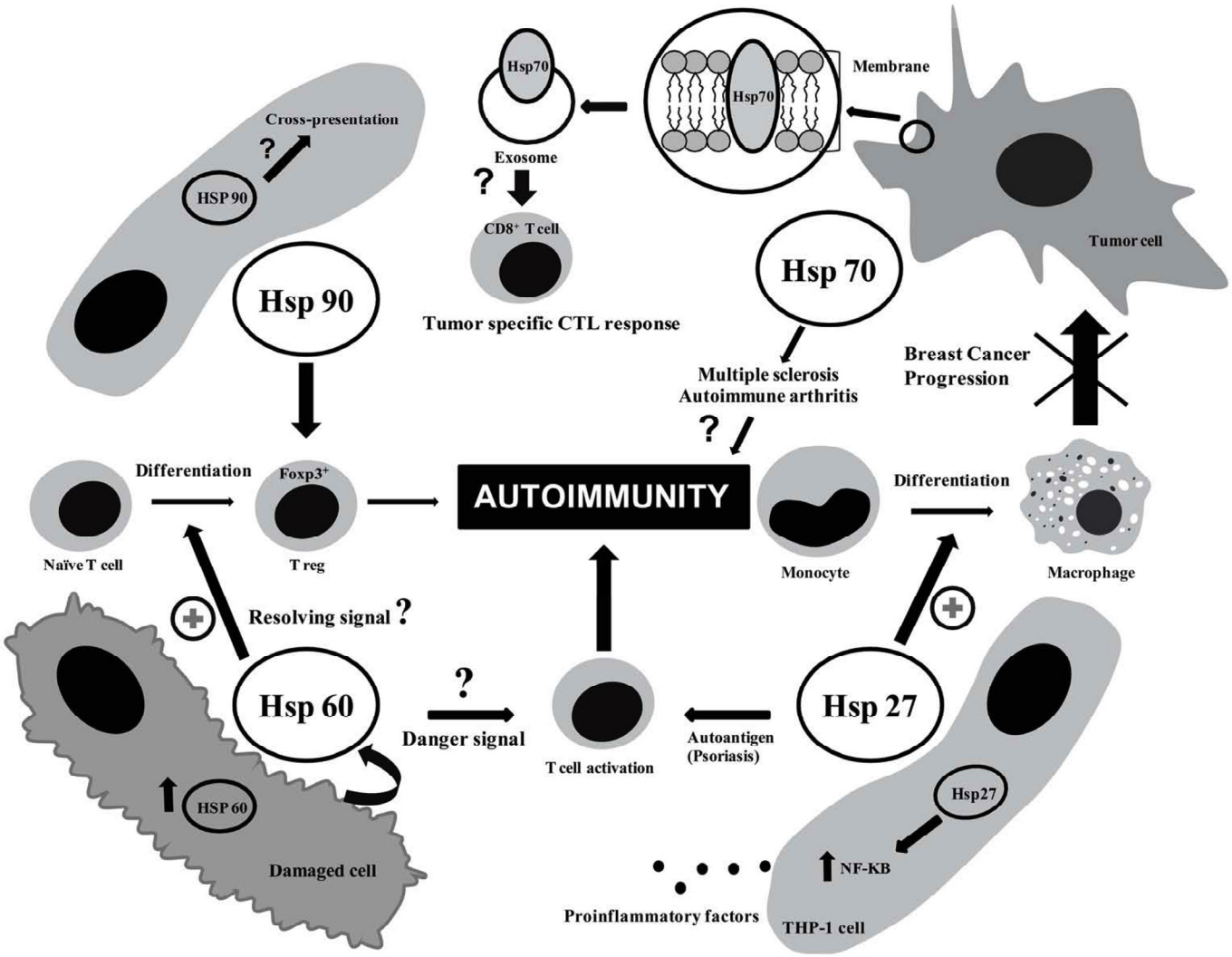

Fig. (2). HSPs in autoimmunity, in immune cell differentiation, and on the tumor behavior. **Hsp70** in the plasma membrane of some tumor cells can be released by exosomes which are able to promote tumor specific responses (innate and adaptive). Exosomes derived from heat-treated ascitic cells from gastric cancer patients (which show high concentrations of Hsp70 and Hsp60) are able to promote DC maturation and induce tumor-specific CTL response more efficiently than non heat-treated isolated exosomes. Hsp70 has been found up-regulated in some autoimmune diseases, the underlying mechanisms are uncertain. Endogenous **Hsp90** seems to have an important role in cross-presentation pathways. Extracellular Hsp90 has been related to the functional control of Treg cells, which have implications in autoimmunity and transplant rejection. **Hsp60** has been associated to autoimmunity causing naïve T cells differentiation into Treg cells. It is up-regulated in stressed cells, and released acting as a danger signal. Intracellular **Hsp27** is essential in the expression and release of LPS-induced pro-inflammatory factors in human macrophages (cell line THP-1). It has been associated to streptococcal-induced autoimmune response in psoriasis, acting as an auto-antigen. In breast cancer, Hsp27 promoted the differentiation of monocytes to tolerogenic macrophages (losing its tumoricidal activity).

HSPs as Adjuvants to Cancer Immunotherapy

The study conducted by Dr. Pramod Srivastava in 1986 [26], was the pivot to the development of vaccines based on HSPs. Immunization of mice with gp96 purified from tumors might trigger an antitumoral immune response causing tumor rejection and inhibition of metastatic tumor progression. The immune response against cancer was attributed to HSP cross-presentation of peptide chaperoned antigens in APCs, which induced a strong and sustained cytotoxic activity from CTLs. Complementary studies have elucidated that the antitumor immunity of HSP-peptide complexes is the consequence of the association between peptides and HSPs and not due to HSPs or to the presence of other contaminating proteins. Besides the immunogenicity due to the peptide-HSP complex, is the outcome of the immuno-modulatory ability peptide-independent of HSPs, to evoke an innate immune response *via* stimulating the elaboration of cytokines such as IL-1, IL-2, TNF-α, GM-CSF, and the release of NO and C-C chemokines by macrophages and DCs. For this reason certain HSPs (e.g., Hsp72) have "chaperokine" effects which describe the dual role of an extracellular heat shock protein as both chaperone and cytokine [27]. Dr. Asea´s group [28] has recently shown

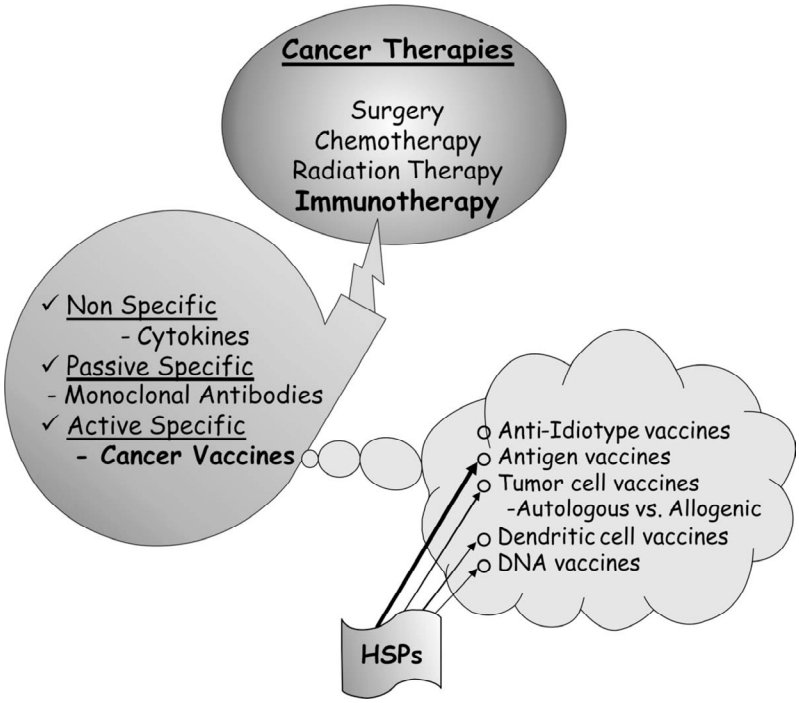

Fig. (3). Schematic representation of the main strategies used to treat cancer, showing the place of the HSPs to generate anticancer vaccines.

that Hsp72 free of endotoxin contamination can retain its chaperokine function. HSPs also may encourage the killer activity of NK cells thereby contributing to tumor immunity. Therefore, the wide variety of immune mechanisms developed by the HSPs allows them to operate as a powerful polyvalent vaccine. Added to this, HSPs raise immunity to a wide range of the cancer epitopes thus preventing the difficulties in identifying epitopes of individual cancers to efficiently abrogate primary and metastatic disease. Studies in murine models have demonstrated that HSPs extracted from several types of tumors, specially gp96 and Hsp70, are potent inducers of adaptive immunity by stimulating tumor-specific CTL responses.

One of the major goals in the design of peptide vaccines is based on obtaining a sustained and powerful immune response. In this sense the use of adjuvants has taken a special interest. Adjuvants are important tools to enhance vaccine performance through different ways: a) by targeting the antigen to APC, b) stimulating cytokines that direct T helper cell or CTLs responses, and c) promoting cell-mediated immunity. The advantages in the use of these compounds include: increase magnitude, availability, quality and persistence of the immune response to antigens, with minimal toxicity. The addition of adjuvants to some vaccines can substantially reduce the amount of antigen or the number of immunizations required to achieve the desired response. The application of peptides in vaccines requires the assistance from adjuvants mainly to operate as a primer catalyzing the specific immune response to tumoral antigens. The nature of the adjuvant is varied: mineral salts, emulsions, viral particles, bacterial toxins and cytokines, among others, and according to its main effect can be classified into vehicles or immunostimulants. Dubensky and Reed have presented a review on this [29]. In cancer vaccines, HSPs are used as adjuvants through different delivery systems: HSPs/antibodies, peptide/protein-HSP complexes, tumor antigen/HSP gene fusion, viral peptides/HSP complexes or gene fusion, viral proteins/bacterial HSP fusion, etc. Table **1** summarizes the strategies involving the utilization of HSPs as adjuvants in cancer vaccines.

In 2007, we (DRC, FDC-C) published a pilot study using HSP-based vaccines [54]. This approach combined two types of adjuvants: the mineral salt hydroxyapatite (HA) plus HSPs, and this therapeutic vaccine based on HA ceramic particles and self-antigens could be safely administered and showed some encouraging clinical results in cancer patients. This vaccine had Hsp27 which we now know that can produce a down regulation of the immune response. Then, we are carrying studies to know whether this particular HSP should be avoided in the vaccine formulation. The challenge for the future is to find the suitable combination of adjuvants that help to develop the strongest immune responses to attack cancer effectively.

PRECLINICAL AND CLINICAL STUDIES

Limitations

The HSP-based anticancer vaccines have been first tested using animal models in order to find: a) the

Table 1. Examples where Different HSPs have been Used as Adjuvants to Generate Vaccines

Hsp	Immunogen	Model Studied/Tumor	Immune Response	Authors
Gp96	15-mer gp96 peptide mimotope	BALB/c mice/ Copenhagen rats/ MAT-LyLu cells	Specific antibodies, $CD4^+$ and $CD8^+$ T cells	[30]
Hsp110 Grp170	TRP2 peptide complexes	C57BL/6 and BALB/c mice/challenged with B16-gp100 tumor cells	$CD8^+$ T cells	[31]
Hsp70/*Others	Hsp70/peptide complexes of DC/ tumor cells fused	OVCA cells/human breast cancer cells	T cells with higher levels of IFN-γ	[32]
Hsp70	Hsp70.PC-F	C57BL/6 wild-type mice/ B16/MUC1 tumor cells	CTL cells	[33]
Hsp70	SigmE7/HuHSP70 DNA vaccine	Female C57BL/6 mice challenged with TC-1 tumor cells	$CD8^+$ T cells and anti-tumor effects	[34]
Hsp65	DNA vaccine boosting with Hsp65-Grp6 murine chimeric protein	Male C57BL/6 mice/RM-1 prostate carcinoma cells	Grp-Specific Antibodies	[35]
Hsp70	MOSEC/luc cells expressing Hsp70	Female C57BL/6 mice/ MOSEC/luc and TC-1/ luc cells	$CD8^+$ T, $CD4^+$ T and NK cells	[36]
Gp96	GP96-sr(scFv) antibodies	C57BL/6 and BALB.B mice	Cytotoxic T cells	[37]
Microbial Agents				
Gp96	Recombinant Gp96/ HBV peptide complexes	Female BALB/c (H-2d) mice	$CD8^+$ T cells and peptide specific CTLs	[38]
Hsp70	HSV-2 peptides-Hsp70 plus QS-21 saponin	Naïve BALB/c mice and Female Hartley guinea pig challenged with live HSV-2 virus	$CD4^+$ and $CD8^+$ T cells	[39]
Hsp110	mHsp110-E7complex	C57BL/6 mice challenged with TC-1 tumor cells	Cytotoxic T cells/ IFN-γ production	[40]
Hsp70	E7/Hsp70 protein fusion packaged in AAV1 or 2	C57BL/6 mice challenged with TC-1 tumor cells	CTL response and IFN-γ production	[41]
HspA	Trivalent fusion vaccine rHHU	SPF female BALB/c mice/ *H. pylori* infection	Specific antibodies production and Th1/ Th2-type responses	[42]
Hsp70	MtHsp70–LMP2A peptide complexes	C57BL/6-Tg (HLA-A2.1) 1Enge/J transgenic homozygous mice/B16/ LMP2A tumor cells	Cytotoxic T cells	[43]
Hsp70	E7/Hsp70 DNA vaccine combined with apigenin	Female C57BL/6 mice (H-2Kb and I-Ab) challenged with TC-1 tumor cells	Primary and memory E7-specific $CD8^+$ T cells Reduced tumor size	[44]
Hsp70	PC61plus E7/Hsp70 DNA vaccine	Female C57BL/6 mice challenged with TC-1/ E7 tumor cells	$CD8^+$ T cells and $CD4^+$ $CD25^+$ Tregs depletion	[45]
Hsp65/Hsp70	pCR3.1-VS-Hsp65-TP-GRP6-M2 plus mHsp70 plasmid	male C57BL6 mice/murine RM-1 prostate carcinoma cell line	Specific antibodies and Th cells	[46]
Hsp65	*M. bovis* BCG Hsp65/ E7 fusion protein	Women with CIN III	Clinical responses	[47]
Hsp60	Hsp60/E6/E7 chimeric DNA vaccine	C57BL/6J mice challenged with TC-1 cells /lung hematogenous spread model	E6- or E7-specific $CD8^+$ T cells	[48]
Hsp65	*M. bovis* BCG HSP65/E7 fusion protein	Female C57BL/6 ($H\text{-}2^b$) mice challenged with TC-1 tumor cells	$CD8^+$ T cells (with cytolytic and cytokine secretion activities) and memory $CD8^+$ T cells	[49]
Hsp70	HPV16 mE6delta/mE7/ TBHsp70delta fusion protein vaccine	Hu-PBL- SCID mouse/E7/ESCC cell line EC9706	$CD8^+$ T cells, IFN-γ, TNF-α, granzyme B and perforin	[50]
Hsp70	E7/Hsp70 DNA vaccine	C57BL/6 mice challenged with TC-1/E7 tumor cells	$CD8^+$ T cells	[51]
Hsp70	E7/Hsp70 DNA vaccine	Female C57BL/6 mice challenged with TC-1 tumor cells	$CD8^+$ T cells and specific B cells	[52]
Gp96	BHV-1 peptides/Gp96 complexes	Female BALBc (ByJ) mice ($H\text{-}2^d$)	CTLs and antibodies	[53]

Abbreviations and comments. **MtHSP70**: *Mycobacterium Tuberculosis* Hsp70; **HBV**: Hepatitis B Virus; **TRP2**: tyrosinase-related protein 2; **OVCA**: ovarian carcinoma, Hsp70 and ovalbumin (257–264 peptide); **pHis**: polyhistidine; **OVA**: ovalbumin; **Mage-3**: a cancer testis antigen; **GRP**: gastrin-releasing peptide; **PC61**: anti-CD25 monoclonal antibody; **EBV LMPs**: Epstein-Barr virus latent membrane proteins; **AAV**: adeno-associated virus**; HSV**: herpes simplex virus; **MOSEC/luc cells**: murine ovarian cancer cells that express luciferase; **CIN**: cervical intraepithelial neoplasia; **EC9706**: HPV E7-expressing human ESCC cell line; **HS-Exo**: exosomes derived from heat-shocked mouse B lymphoma cells; **BHV-1**: bovine herpesvirus-1; **sr(scFv)**: specific recombinant single-chain Fv. **HSP70.PC-F**: Hsp70 from fusion cells derived from dendritic cells (DC) and murine cancer cells; **rHHU**: recombinant heat shock protein A (HspA), H. pylori adhesin A (Hpa A), and UreB414; ***Others**: Hsp90 and Hsp110. **ESCC**: esophageal squamous cell carcinoma.

administration route, b) the optimal vaccine schedule, c) the possible toxicity, d) the generated immune response(s), and e) the efficacy against a given tumor. However, the animal models (usually mice) do not mimic the situation found in cancer patients that suffer from heterogeneous non-immunogenic tumors that have been growing for a long period of time (large tumor burden). In addition, due to both the disease and to the fact that cancer is more frequent in older patients, these patients usually have a deteriorated immune system, and moreover they have been heavily treated with different anticancer regimens before reaching an experimental therapeutic anticancer vaccine. In contrasts, animal anticancer vaccine models are usually based on the prevention of tumor challenge or the treatment of relatively tiny tumors [55] (Table **2**).

Table 2. Anticancer Vaccines: Salient Features of Animal Studies *Versus* Clinical Studies

Animal Studies
Treatment of immunogenic, small tumors
Animals challenged with tumor cells (more homogeneous tumor population)
The animals usually have a competent immune system
The response is ease to interpret
Clinical Studies
Tumors are initially "nonimmunogenic" (silently generated in the own body)
Large and heterogeneous tumors (mixed bag of cells ⇒ different TAAs[1], secrete cytokines and have immunosuppressive cells ⇒ ⇓ the immune system) [56]
Usually immunodepressed (older patients, advanced disease, chemotherapies)
The clinical and laboratory responses might be more difficult to interpret [57]

[1]TAAs: tumor associated antigens.

We need more animal studies designed with tumors genetically engineered to express a tumor associated antigen (TAA) (e.g., Her-2/neu which is immune-tolerated probably due to its oncofetal origin) to demonstrate the ability for the vaccine to overcome immune tolerance mechanisms.

On the other hand, in cancer patients the anticancer vaccine protocols that the regulatory authorities usually approve for clinical trials are for patients with large tumors, usually with metastatic disease, who has failed standard therapeutic regimens. Although some vaccines may attack large tumor burdens [1], this is not the best scenario to use the body's own adaptive immune system to recognize and eliminate cancer cells. Nowadays there are no doubts that TAA-primed lymphocytes can effectively recognize and destroy cancer cells, but we also know the limitations of the immune system to attack cancer cells. This is exemplified with Sipuleucel-T, a therapeutic cancer vaccine that is generated from a patient's own immune cells and that targets prostatic acid phosphatase (PAP) [58]. Adjuvant immunotherapies should be used after tumor reduction (e.g., after surgery) since the tumor burden will be reduced as well as the levels of immunosuppressive cells (e.g., Treg) and factors (e.g., TGF-beta, prostaglanding E2, IL-10). Then, therapeutic vaccines are moving to be used in the adjuvant setting in patients with poor prognosis (minimal residual disease/micrometastases), and to achieve this there is a need of large randomized immunotherapy trials [59]. Vaccine trials in clinically disease-free patients have shown encouraging initial results and, if they are validated, they may be incorporated in standard treatment algorithms [60]. But here it will be difficult to define the most appropriate parameters to measure the vaccine benefit on the clinical outcome.

Another up to date problem is how to test the clinical efficacy of an anticancer vaccine, tetramer or ELISPOT analysis and many other methods have been tested to evaluate the immunosuppressive cells activated by the vaccine, but there are no agreements on this issue since cellular (and humoral) immune response assays generate highly variable results [61, 62]. For these reasons in a pilot clinical study using a HSP-based therapeutic vaccine we decided to evaluate as endpoints the pathologic and clinical response [53]. Even so, immune responses after an increase in total tumor burden and in the presence of new lesions can occur, making difficult to evaluate the efficacy of anticancer immunotherapies [57] (Table **2**).

Preclinical Studies (Animal Models)

Several animal models and vaccine protocols have been used to tests HSP-based anticancer vaccines (Table **3**). For example, tumor cells have been heated, lysated, and the resulting product exposed to dendritic cells (CD8α+) which were injected to mice challenged with fibrosarcoma cells [71]. In this case only Hsp70 was tested as produced by the heat shock but we can assume that other HSPs were also induced. Various strategies are being investigated to optimize vaccines using recombinant DNA. In one of these strategies, a recombinant replicative adenovirus encoding Hsp70 gene infected primary melanomas, the virus replication killed the tumor cells and the Hsp70-antigenic peptides (released from the tumor cells, perhaps together with other HSPs) activated tumor-specific immune responses supposedly against a large array of TAAs [63]. The results of Pan *et al.* [68] support the concept that *M. tuberculosis* Hsp70 helps in the generation of T cells and, in addition, the authors found that there is a humoral immune response to EBV-induced tumors. In the case of Yamaoka *et al.* [75], the authors used polyhistidine (imidazole-containing polymers) together with Hsp70-antigen peptide to minimize degradation from the endosome/lysosome facilitating cytosolic delivery of Hsp70-associated antigen into the class I presentation pathway. These authors also found antigen-specific CTL responses. In the case of Yong

Table 3. Preclinical Animal Models

Hsp Used	Immunogen	Tumors	Immune Response	Authors
Hsp70	Recombinant replicative Ad[1] overexpressing Hsp	Mice with established melanomas	Systemic antitumor T cell activity	[63]
Hsp70	Engineered tumor cells that secrete antigens with Hsp70	Mice injected with Hsp70 secreting tumors	Tumor rejection (70%), NK and T cell mediation	[64]
Hsp70	DNA vaccine PSCA-Hsp70	Mice with prostate PSCA-expressing tumors	CD8+ T-cell response⇓ tumor growth	[65]
Hsp70, others	Chaperone-rich cell lysate embedded with BCR-ABL peptide	Pre-established murine leukemia	T cell activation	[66]
Hsp70	A20-derived Hsp70(leukemia)	Mice received the vaccine only (no tumors)	Specific antibodies induced complement-dependent cytotoxicity, activation of type-2 helper T-cells	[67]
Hsp70 (M. tuberculosis)	Chimeric gene containing the EBV LMP1/LMP2 epitope and Hsp70	Mice challenged with nasopharyngeal carcinoma	CTL activity *in vitro* and humoral immune response.	[68]
Hsp70	Hsp70 with peptides, combined to psBTLA	Mice challenged with TC-1 cervical cancer	Improved antitumor immunity	[69]
Hsp70	Nanoemulsion with MAGE1-Hsp70 and SEA complex protein	Mice challenged with melanoma B16 cells	Antitumor response *via* s.c. and p.o. administration	[70]
Hsp70, others?	CD8α+ dendritic cells pulsed with heat-treated fibrosarcoma lysate	Mice challenged with WEHI-164 cell line	Increased lymphocyte immune response with increased IFN-γ	[71]
Hsp70, gp96, others?	Heat shocked tumor cell lysate-pulsed DCs (followed by radio-frequency ablation)	Mice challenged with B16 melanoma cells (local cancer recurrence)	Prime-boost vaccination efficiently protected local recurrence (mediated mainly by CD8+ T cells)	[72]
Hsp70	DNA vaccine CMV- OVAhsp70-CD11c-IL-15	Mice challenged with B16OVA melanoma cells	DC engineered by the DNA vaccine migrated to lymph nodes for potent T cell priming	[73]
Hsp70	Hsp70-enriched exosomes (heat shocked cancer cells)	Regression of established tumors	MHC independent therapy	[74]
Hsp70	Plasmid DNA vector (His-Hsp70-pep)	Mice challenged with OVA expressing tumor cell line (EL4)	Antigen-specific CTL	[75]
Hsp70	Mage3-expressing DNA construct	Mice challenged with TC-1/Mage3 cells	CD4+/CD8+ T cell and antibody response	[76]
Hsp70	TAAs, attenuated recombinant S. typhimurium	Mice challenged with B16F10 melanoma	Specific CTL response and tumor protection	[77]
Hsp70	DNA vaccine, Hsp70 fused to Her2/neu	Mice with TUBO cells (mammary cancer)	Tumor progression	[78]
Hsp65	DNA vaccine boosting with Hsp65-GRP6	Mice challenged with prostate cancer cells (RM-1)	Antibody-mediated mechanism of tumor destruction	[79]
Hsp65	Fusion protein with β-hCG and Hsp65	Mice challenged with hepatocarcinoma H22 cells	Cellular and humoral immune response	[80]
Hsp65	Recombinant Hsp65-GnRH copies	Mice challenged with mammary tumor cells (EMT-6)	Specific anti-GnRH antibodies	[81]
Hsp65 (mycobacterial)	Tumor cell lysates lung cancer cells	Mice challenged with Lewis lung cancer cells	activation of CD8+ T cells and others	[82]
Gp96	Pooled gp96 vaccines from myeloma cell lines	Mice challenged with myeloma cells, and mice with established tumors	CD4+ and CD8+ T-cell response, IFN-γ–producing	[83]
Gp96	DNA vaccine gp96+Her-2/neu	Her-2/neu+ mammary tumors	⇓ CD4+ CD25+ Foxp3+ (Treg), ⇑ IFN-γ/IL-4 bi-phasic growth pattern of the tumors	[84]
Gp96	G22+gp96 DNA vaccine	Mice challenged with CMT-93-G22 (colon cancer cells, transfected)	Gp96 did not enhanced G22-specific humoral response, but enhanced cellular CD8+ response	[85]
Hsp110	Hsp110–heparanase complex	Mice challenged with B16 melanoma cells	IFN-γ production, cytotoxic T cells	[86]

(Table 3) contd…..

Hsp Used	Immunogen	Tumors	Immune Response	Authors
Grp170	Prostate cancer cells transfected with grp170 (secretable)	Mice challenged and with established tumors (TRAMP-C2 prostate cancer cells)	CD8+ T-cell dependent tumor protection	[87]
Grp170	grp170-PSCA complex (recombinant)	Mice challenged with RM-1 prostate cancer Cells	⇑ IFN-γ secretion, CD8+ T-cells	[88]
Grp170	Colon cancer cells secreting grp170	Mice challenged with colon cancer cells	Tumor rejection, CD8-dependent response, full-length TAAs	[89]

[1]Abbreviations and comments used. **Ad**: adenovirus; **EBV**: Epstein Barr Virus; **LMP**: latent membrane proteins (of EBV); **His-Hsp70-pep**: vector encoding polyhistidine, Hsp70 and ovalbumin (257–264 peptide); **GRP**: gastrin-releasing peptide; **GnRH**: gonadotrophin-releasing hormone; **Mage-3**: is a cancer testis antigen; **G22**: a pcDNA3.1-G22 plasmid encoding a human anti-idiotype single chain antibody; **psBTLA**: plasmid soluble B and T lymphocyte attenuator (inhibitory receptor, CD28 superfamily); **SEA**: Staphylococcal enterotoxins A; **MAGE-1**: melanoma antigen; **TUBO** cells: cloned cell line (derived from a lobular carcinoma, transgenic for the rat HER2/neu oncogene); **PSCA**: prostate stem cell antigen; **OVA**: ovalbumin.

et al. [79] the tested vaccine produced anti-carrier protein immunity (antibodies against Hsp65) in addition to antibodies against the antigen (GRP: gastrin releasing peptide). The efficacy of this vaccine was due to the GRP-specific antibodies that neutralized the self-peptide GRP thus blocking an important signal pathway of the tumor cells. Xiangbing *et al.* [80] found that the Hsp65 and β-hCG tandem repeat immunogen generated specific anti-β-hCG antibody (IgM and IgG) and stimulated the proliferation activity of splenocytes, interestingly the authors also found reduced angio-genesis. Using a similar approach, vaccination with the fusion protein Hsp65 (C-terminus)–GnRH (6 copies) evoked specific and strong humoral response inhibiting the growth of mammary cancer and interestingly causing atrophy of the ovary and uterus [81].

The Her-2/neu cell surface oncoprotein is an interesting target for immunotherapy, Her-2/neu positive breast cancer patients have poor prognosis [90], they can be treated with humanized antibodies, and there are many vaccine protocols under evaluation [91]. In an experimental mammary cancer model, human Hsp70 was fused to the extracellular C-terminal domain of rat Her-2/neu, this DNA vaccine could significantly increased survival and reduced metastasis [92]. In contrast, when human Hsp70 was fused to the N-terminal domain of Her-2/neu there was tumor progression indicating that the N-terminal domain must be left free [78]. On the other hand, the C-terminal domain of Hsp70 seems to be the crucial part in eliciting antigen-independent responses (including NK cell stimulation) against tumor challenges, but a better antitumor response was obtained by immunization with multiple Hsp70 fusion proteins [93].

One problem with peptide vaccines is that they are HLA-restricted, which limits the number of patients that can receive any one vaccine. HSPs may contribute to solve this because vaccination with high molecular weight whole protein chaperone complexes (e.g., Hsp110, grp170) bind large full-length antigen proteins at approximately an equivalent molar ratio [88]. This could enhance epitope exposure to immune cells (theoretically exposing multiple MHC class I/II epitopes to circumvent HLA restriction).

Significant loss of antitumor response was noted when NK cells were depleted suggesting that the vaccine can more effectively inhibit tumor growth by inducing both the innate immune responses and TAA-specific adaptive immune responses *via* the Hsp70-associated adjuvant function (Table **3**). This is consistent with the work of Qian *et al.* [83], they have shown that NK cells play a role in a Gp96-based vaccine against myeloma, but that IFN-γ and both CD4+ and CD8+ T cells are the major effectors in this vaccine model. In addition, the authors examined whether depleting Tregs, blocking B7H1-negative T-cell signaling, and neutralizing IL-10, further improved the efficacy of the pooled gp96 vaccine, and as expected this happened. In a similar way, Han *et al.* [69] reported that the increased antitumor immunity of the Hsp70 vaccine model that they used (Table **3**), was associated with higher expression of Th1 cytokines (IL-2 and IFN-γ) and decreased transcription of IL-10, TGF-β, and Foxp3 in the tumor microenvironment. Another strategy to modulate the immune response has been presented by Wang *et al.* [94]. These authors used a Hsp110-based vaccine model with recombinant TAAs to treat mice with established melanoma and renal cancer finding that temsirolimus-treated CD8+ cells had greater IF-γ and cytotoxic T-cell responses.

Fig. (**4**) shows a summary of the strategies used to generate HSP-based vaccines in animal models to attack cancer cells.

The use of HSPs has not always resulted in an improved immune response. For example DNA-based vaccines encoding chimeric proteins containing prostate-specific antigen (PSA) fused to Hsp65 and Hsp70 did not increase the levels of PSA-specific CTLs and the protection against tumor challenge compared with the mice that received the conventional PSA-expressing DNA plasmid alone [95]. In addition, McLaughlin *et al.* [96] used recombinant bovine Hsp110 to chaperone the envelope E2 glycoprotein of bovine viral diarrhea virus. In contrast to expected, cellular and humoral responses to E2 were reduced when Hsp110 was added in the vaccine formulation. Finally, using an *in vitro* melanoma model, Bleifuss *et al.* [97] have reported that despite the presence of active chaperones, melanoma cell-derived chaperone-rich cell lysates failed to transfer endogenously expressed melanoma-associated antigens to DC for cross-presentation and cytotoxic T cell recognition. The chaperones tested in this study were Hsp/Hsc70,

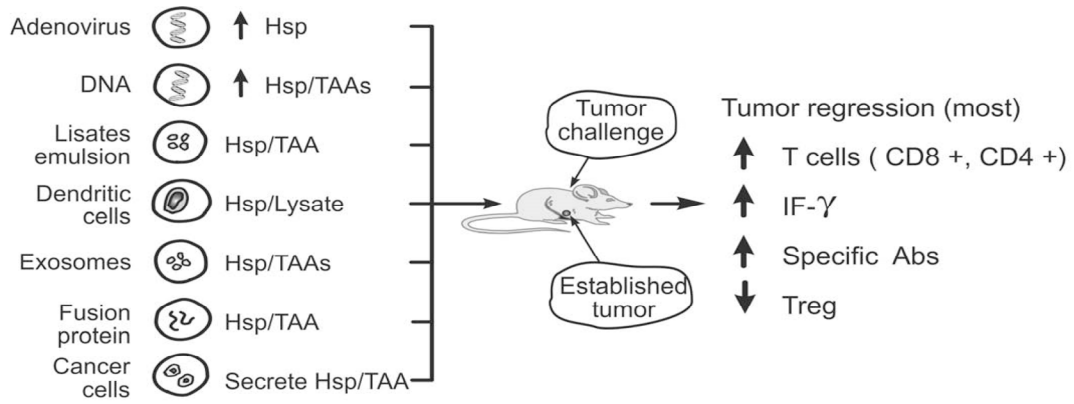

Fig. (4). Schematic representation of the main strategies used to induce the immune response to cancer cells in mice using HSPs. The animals can be immunized directly with lysates, emulsions or exosomes containing the HSPs with the associated TAAs. Dendritic cells can be first stimulated with the HSP/TAA and then injected into mice. Different DNA constructs producing HSPs alone or HSPs with TAAs, or fusion proteins, are inoculated into mice to induce a specific antitumoral response. Another strategy is to inoculate cancer cells transfected with vectors that secrete HSPs to make the cells more immunogenic.

Hsp90, Grp94/gp96 and calreticulin enriched by a free-solution isoelectric focusing technique. The authors conclude that the chaperone-rich cell lysates have limitations regarding cross-presentation of endogenously expressed melanoma-associated antigens, but that they may be utilized as *vehicles to enhance the delivery of exogenous antigens* for DC-mediated cross-presentation and T cell stimulation.

In conclusion, the animal studies show that:

a) Certain HSPs (e.g., gp96) alone can induce an antitumor response, but they are more effective and produce long lasting antitumor responses when used as adjuvants to TAAs.

b) Several high molecular weight HSPs (Hsp65, Hsp70, gp96, Hsp110, grp170) act as multifunctional antigen delivery system to improve the immunogenicity of anticancer vaccines (with some exceptions as shown in the Table **3**).

c) The higher molecular weight HSPs seem to present the TAAs more efficiently (because they bind large full-length proteins).

d) The vaccines can induce specific and non-specific cellular and humoral immune responses all of which are important to induce tumor rejection. However, there are reports where the HSP-based vaccines have not been effective.

e) The antitumor response is significantly enhanced when the suppressive tumor microenvironment and the immune suppressing effector cells are blocked.

In addition, different administration routes, subcutaneous, intradermal, intramuscular or even peroral (under special conditions) can be used, and the animal toxicities are non-significant.

Clinical Studies

In a previous review Parmiani *et al.*. (2002) [98] presented a classification of the immunogenic human TAAs (peptides) recognized by T cells, the approaches to increase their immunogenicity, and the peptide-based vaccination trials in cancer patients (including those based on HSPs). They also reviewed the advantages and disadvantages of *ex vivo* assays to assess the antivaccine T-cell responses (e.g., 51Cr-release or cytokine-release assay, ELISPOT, etc.). One of their judicious conclusions was “it may be premature to declare that cancer vaccines are an effective antitumor approach”. Almost one decade has past, scientists have improved considerably the anticancer vaccine strategies [1, 99, 100], we have seen an explosion of preclinical and clinical vaccine studies, and nowadays the vaccines are under way to be incorporated in the adjuvant setting (like Sipuleucel-T) [58], with many anticancer vaccines in phase II and III clinical trials [91, 101]. So where are the HSP-based vaccines?

Following the pioneer work of Pramod Srivastava on immunotherapy with HSP preparations [102], several clinical studies have been carried out immunizing cancer patients with autologous tumor derived HSP-peptide complexes (HSPPCs). Therefore, vitespen® (formerly Oncophage®) was the first autologous cancer-derived vaccine based on gp96. Binder (2008) [103], di Pietro *et al.* (2009) [104], and Wood and Mulders (2009) [105] have made excellent reviews on its activity in melanoma and other tumors. In melanomas, the vaccine produced activation of both innate and adaptive immunity and showed higher activity in M1a + M1b patient sub-groups, whereas a significant lower benefit was observed in disseminated melanoma patients. In a phase III trial, only 2 patients (out of 322 patients) showed serious adverse events possibly related to the vaccine administration.

Unfortunately there were not clear correlations between the observed immunological effects and the antitumor activity of the vaccine, which exclude individual patient selection of a biologically active dose. The authors have also summarized the clinical trials using this vaccine in several other malignancies including gastric cancer, recurrent high grade gliomas, renal cancer, pancreatic cancer, ovarian cancer, colorectal cancer, chronic myelogenous leukaemia, and non-Hodgkin's lymphoma [103-105]. The most promising results have been observed in patients with melanoma and renal clear cell cancer. Eton *et al.* (2010) [106] enrolled 36 melanoma patients *with advanced disease* (most with metastases), they were able to obtain the HSPPC-gp96 vaccine from tumor weighing as little as 2 g, with no serious toxicities. They noted no DTH responses to DNCB, while the IFNγ-producing cell count rose modestly and showed no correlations with the clinical outcome. Finally, among 16 patients with indicator lesions, there was no major objective response in the 16 patients (81% had clear progression), and among patients with stage IV disease treated in the adjuvant setting, a median time to progression of 30.4 months (with 82% alive at 10 years) was reported as encouraging, but it was impossible to determine whether the HSPPC-96 treatment contributed directly to this outcome, concluding that the antitumor activity was modest [106]. Chi and Dudek (2011) [107] have made a systematic review and meta-analysis of phase II and III clinical trials of different vaccines against *metastatic* melanoma (data on 4,375 patients), concluding that no evidence was found that vaccine therapy provides better overall disease control or overall survival compared with other treatments. This again reinforces the concept that the immune system is more effective to attack cancer cells in patients with minimal residual disease.

In a multicentre phase III trial, patients were randomly assigned to receive either vitespen (n=409) or observation alone (n=409) after nephrectomy (patients at high risk of recurrence, locally advanced disease) [2]. No difference in recurrence-free survival (primary endpoint, median follow-up of 1.9 years) was seen between patients given vitespen and those who received no treatment, the recurrence events were reported in 19 (15.2%) patients in the vitespen group and in 31 (27.0%) patients in the observation group (hazard ratio 0.576, 95% CI 0.324-1.023; p=0.056), concluding that a possible improvement in recurrence-free survival in patients with early stage disease who received vitespen will require further validation. Russia issued a registration certificate in 2008 for the use of vitespen® for the adjuvant treatment of kidney cancer patients at intermediate-risk for disease recurrence.

Since in this vaccine preparation the peptides are derived specifically from each patient (unknown TAA peptides chaperoned by the HSP, customized vaccine), the vaccine received a positive recommendation for orphan drug designation in the treatment of gliomas (granted by the Committee for Orphan Medical Products from the European Medicines Agency and FDA) [104].

High grade gliomas are target for diverse immunotherapies. Immunotherapy for these patients is highly attractive because high grade gliomas are very aggressive and the traditional treatments are not very effective. Moreover, immune cells can traverse the blood-brain barrier and migrate into brain tumors to exert their therapeutic function [108]. These treatments have recently been reviewed by Hickey *et al.*. (2010) [101], including two phase I/II trials with HSPPC (gp96) at University of California, San Francisco for recurrent (NCT00293423) or newly diagnosed (NCT00905060) patients. The enrolled patients are receiving the vaccine with or without concurrent temozolamide chemotherapy.

Table **4** presents the clinical trials with HSP-based anticancer vaccines other than with vitespen. Zappasodi *et al.* [115], identified Hsp105 as the target for antibodies generated after post-vaccination in the responder patients with non-Hodgkin lymphoma [113]. Hsp105 was associated with a significant local increase of granzyme-B(+) killer cells which might contribute to the tumor-restricted necrosis [115]. Consistent with this, another HSP (Hsp65) has been identified as the target for antibodies in patients successfully treated with BCG immunotherapy for high-grade transitional cell carcinoma of the bladder [116]. In addition, a modest but maintained increase in HPV16 E7 specific IgG levels has been reported in women with high-grade cervical dysplasia that received a vaccine consisting of full length HPV16 E7 linked to a HSP from M. bovis [117]. Therefore, the antibodies generated after vaccination are emerging as important protagonist in the antitumoral response, both as effectors mediating the tumor cell killing and as predictive of individual patient outcome. However, further studies on this subject are needed since vaccination with a DNA-Hsp65 did not increase levels of circulating antibodies against Hsp65 or Hsp60 [111]. Perhaps in this case the vaccine was not strong enough due to the lack of TAAs in the vaccine design. Fig. (**5**) shows a summary of the strategies used to generate HSP-based anticancer vaccines in patients.

In conclusion, in cancer patients the HSP-based vaccines and other anticancer vaccines in general are very promising but are not producing a durable/optimal clinical benefit yet. Some of the reasons have been analyzed in recent reviews showing the strategies to improve the immunological response using more effective adjuvants, and interfering suppressive checkpoint blockage pathways and suppressive cellular populations (e.g., using inhibitors of CTLA-4 and oncogenic BRAF) [118-120]. Finally, there are molecular inhibitors of Hsp90 (reviewed elsewhere by Neckers and Workman [121]) which are being explored as new anticancer therapies, but in this case we do not know yet if the oncogenic clients once free of Hsp90 are destroyed and then could escape the immuno-logical response.

Table 4. Hsp-Based Vaccines in Cancer Patients (Excluding Vitespen)

Immunogen	Cancer Type(s)	Trial Phase	Main Results	Authors
Hsp70-peptide complexes combined with imatinib mesylate	Chronic myeloid leukaemia (in chronic phase)	I/II (20 patients)	Minimal toxicity, clinical responses (13 patients), immunologic responses	[109]
Hsp65 (M. bovis fused with HPV E7)	CIN II/III (persistent colposcopic lesions)	II (20 patients)	35% had complete regression of their intraepithelial lesions, correlated with immune response	[110]
gp96, Hsp27, TAAs hydroxiapatite	Various, advanced disease	I (20 patients)	Low toxicity, tumor reduction in some cases, T cells in tumor	[54]
DNA vaccine encoding Hsp65 (M. leprae) (intratumoral injection)	Advanced head and neck carcinoma	I (21 patients)	Modest immunostimulation no autoimmunity	[111]
DNA vaccine (Hsp70 fused with HPV E7)	CIN II/III (HPV16+)	I (15 patients)	Mild adverse events, modest specific T cell response, complete histologic regression in 3 patients	[112]
DCs loaded with killed cells[1]	Relapsed B-cell non-Hodgkin's lymphoma	I/II	Responder showed ⇑ calreticulin and Hsp90 in treated tumor cells (6/18 patients)	[113]
Hsp70-peptide plus IL-2 (ex vivo stimulation of autologous PBL)	Colorectal and lung cancer	I (12 patients)	⇑ cytolytic activity of NK cells and CD94 cell surface density	[114]

Notes and abbreviations. [1]They used autologous tumors, with a cell death protocol based on heat shock, γ-ray, and UVC rays. CIN: cervical intraepithelial neoplasia; PBL: peripheral blood lymphocytes.

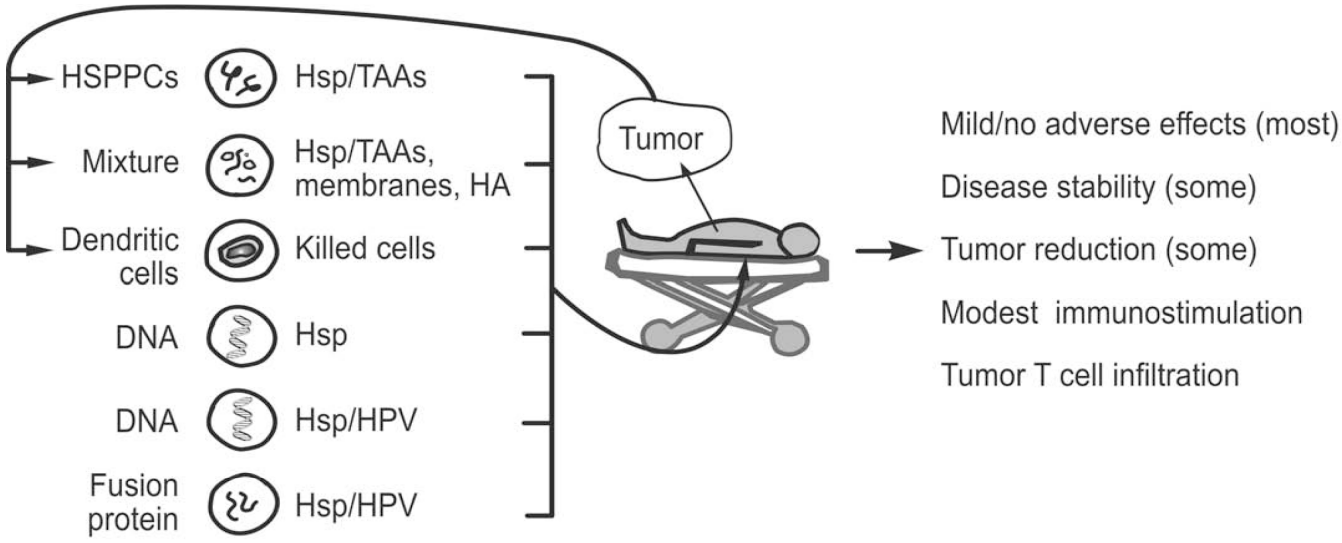

Fig. (5). Schematic representation of the main HSP-based vaccines used in cancer patients. Tumor cells obtained from the patient are first processed to obtain the HSP/TAAs and then directly injected into the patient, or used to stimulate dendritic cells which will then be used for injection. In these cases autologous cancer vaccines are generated which have the advantage of inoculating the patients own TAAs in a form that can be recognized by the immune system. Other strategies are DNA constructs or fusion proteins.

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DECLARATION OF INTERESTS

The authors report no conflicts of interest, except DRC who has presented a patent (with Urodelia, France) on the use of HA to generate anticancer vaccines.

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