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Review

Heat Shock Protein for Use in Cancer Vaccine Therapy: Immune Response and Immunotherapy

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Abstract: Considering what was said about Heat Shock Proteins (HSPs) role in the presentation of tumor antigens, today, before using tumor cell extracts as antigens in vaccine preparation, researchers try to increase the efficiency of antigen presentation by APCs via inducing these proteins; however, since different cell lines express different amounts of HSPs under the influence of different temperatures and different incubation times, discussing the optimal temperature and post-temperature incubation for cancer cell lines that may cause the next stages of the production of cancer cell vaccines, will be very useful. Through systematic search in PubMed/Medline using ("Tumor"[Title/Abstract] OR "Cancer"[Title/Abstract] OR "Neoplasm"[Title/Abstract]) AND ("vaccine"[Title/Abstract] OR "vaccination"[Title/Abstract]) AND ("heat shock protein"[Title/Abstract] OR "HSP"[Title/Abstract]) from 1980 to 2025. Overall, 18 articles were selected from a total of 414. The results of clinical trials confirm the use of HSPs in cancer vaccine therapy. A total of 18 studies, including 1,116 cases with different forms of cancer, were reviewed. These studies spanned different trial phases (I–III) and utilized a range of HSP-based vaccines to evaluate their safety, immunogenicity, and efficacy. HSPPC-96 was the most commonly investigated vaccine. These results indicate that the specific tumor-specific effects of the HSPs' immune response, including cluster of differentiation 4 (CD4+), interferon gamma (IFN-γ), and CD8+, lead to a much stronger vaccination. These outcomes underscore the complexity of the tumor microenvironment and the necessity for tailored approaches to maximize therapeutic success. Advances in vaccine formulation, production, and combination therapies offer promising pathways to address these challenges.

Keywords: Heat Shock Proteins (HSPs); Immune Responses; Immunotherapy; Cancer; Vaccine

1. Introduction

An adjuvant is a helper, which is capable of enhancing or modulating immune responses, whether humoral or cellular, against the antigen of interest [1]. Some of the most effective adjuvants used are derived from com-

ponents of microorganisms, such as lipopolysaccharides. Studies have shown that endogenous adjuvants exist in the cell cytoplasm, which enhances the lymphocyte response to specific antigens. Types of endogenous adjuvants include heat shock proteins (HSPs), bacterial compounds (toxins, lipopolysaccharides, peptidoglycans), acid-based adjuvants, and cytokines [2,3].

HSPs are a natural means of protecting cells against environmental and physiological stresses [4]. Stress refers to any sudden change in a cell's environment that the cell is unprepared to handle. Stressed cells, with the assistance of heat shock proteins (HSPs), either manage to overcome the stress and survive or ultimately fail and die [5]. HSPs were first identified due to their chaperone role during various environmental stresses, pathological factors, or physiological stresses [6]. HSPs are present in the cytoplasm, mitochondria, and nucleus of cells and act as chaperones for proteins. Their task as molecular chaperones is in various procedures such as protein folding, assembly, and antigen processing under both stress and physiological circumstances [7]. HSPs are among the most abundant cellular proteins, but are present at much lower levels under physiological conditions compared to stress conditions. Since stresses imposed on the cell lead to the induction of HSPs, the proteins are also called stress proteins.

Numerous functions have been described for these proteins, for example, Homeostatic functions include preventing protein aggregation, restoring the soluble state of loosely assembled proteins, helping in the folding of freshly produced polypeptide chains or the refolding of misfolded and damaged proteins, targeting severely damaged proteins to the cellular destruction machinery, segregating damaged proteins into larger complexes in cases of extensive cellular damage, processes of protein transport between different compartments of the cell, and post-translational modifications of messenger proteins [8,9]. Also, HSPs impose functions affecting the body's immune system, such as induction of maturation in antigen-presenting cells, influence on antigen-presenting pathways and major histocompatibility complex (MHC), especially class I, induction of cell responses to CD8+ T cells, and activation of Natural killer (NK) cells [10–12]. Finally, HSPs influence the course of the disease, such as diabetes [13], neurodegenerative disorders, such as cancer [14], ischemia [15], wound healing [16], and some other diseases [17]. At the same time, HSPs play key roles in immunity, unlike cancers, by interfering with the function of antigen-presenting cells (APCs), NK cells, and T lymphocytes [18].

Many researchers have pointed out the functional role of HSPs when tumor-specific antigens are present [19]. Both tumor-derived HSPs, imposing a molecular weight of 70 kDa (HSP70) and GP96 (96 kDa), facilitate the insertion of antigenic peptides into MHC class I molecules [20,21]. In addition, it has been shown that the binding of HSP70 and GP96 to their specific receptors affects the phenotypic and functional maturation of APCs [22]. Following such binding leads to an increase in the expression of MHC class II molecules, co-stimulatory molecules CD83 and CD86, along with the secretion of pro-inflammatory cytokines such as IL-12 and TNFα (Tumor necrotizing factor-α) [23]. In addition, other studies have shown that the HSP-peptide complex, either *in vivo* [24] or *in vitro* [25], can cross-present the cognate peptide and activate CTLs (Cytotoxic T lymphocytes) both *in vitro* and *in vivo* through human APCs. Importantly, it has been shown that in the presentation of antigenic peptide with HSP70, compatibility between the cell line from which this HSP is derived and the responding T cells is not necessary [26]. Even in the absence of tumor-derived peptides, HSP70 can stimulate the secretion of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α by APCs through stimulation of TLR2 and TLR4 receptors [27]. This function of extracellular members of the HSP70 family, such as the extracellular form of HSP72 (eHSP72), in nonspecific stimulation of innate immunity, is referred to as chaperone function [28,29].

Another effect of HSP70, which is not related to the presence of associated peptides, is the stimulation of NK cell activity. It has been shown using flow cytometry that the membrane form of HSP70 is present on the surface of tumor cells but not healthy cells [30]. This will result in specific recognition of tumor cells and not healthy cells by NK cells. The sensitivity of tumor cells to lysis mediated by these cells is proportional to the presence of HSP70 molecules on the surface of tumor cells. HSP molecules stimulate both arms of the immune response, namely innate immunity (activation and maturation of dendritic cells and activation of NK cells) and acquired immunity (antigen cross-presentation and activation of CD8+ T cells) [31].

Considering what was said about the role of HSPs in the presentation of tumor antigens, today, before using tumor cell extracts as antigens in vaccine preparation, researchers try to increase the efficiency of antigen presentation by APCs via inducing these proteins; however, since different cell lines express different amounts of HSPs under the influence of different temperatures and different incubation times, discussing the optimal temperature and post-temperature incubation for cancer cell lines that can lead to the next stages of the production of cancer

cell vaccines, will be very useful.

2. Methods

2.1. Cancer Vaccines

Vaccines against cancer are used to either cure present tumors (vaccines with therapeutic objectives) or for cancer prevention (vaccines with preventive targets). Mutual vaccine types may help reduce the size of the cancer. Vaccines with therapeutic objectives are usually prescribed to patients with cancer and are intended to boost the natural defense of the human body against malignancies. These types of vaccines prevent existing cancers from growing further, prevent cancers that have been treated from coming back or kill cancer cells that previous treatments have not killed. On the other hand, both types of prophylactic or preventive vaccines are given to disease-free or healthy people and are intended to target cancer-causing viral agents and prevent virus-associated infections [32]. Currently, the Food and Drug Administration (FDA) has permitted and approved two vaccines to prevent virus-associated infections that may cause malignancies [33]. The first vaccine, the vaccine against hepatitis B, averts contamination with the hepatitis B virus, an infectious agent that is believed to lead to hepatocellular cancer (HCC). The other vaccine, the known Gardasil vaccine, is reported to prevent human papillomavirus (HPV16 and HPV18)-associated infections, which are responsible for around 70% of cervical malignancies globally. Gardasil vaccine also prevents HPV types 6 and 11-associated infections, which it is believed to cause approximately 90% of genital warts [34]. So far, there are currently no approved vaccines with therapeutic effects against cancer; however, several therapeutic vaccines are being tested extensively in humans.

Scientists hope to find a vaccine that includes cancer-specific antigens, helping the immune system target cancer cells while leaving normal cells unharmed [35]. Researchers have introduced various strategies to stimulate the immune system's response to tumors [36]. A strategy is to detect uncommon or exclusive antigens on cancer cells that are rarely found in healthy, normal cells. Other methods include enabling tumor antigens to produce or be more likely to elicit an immune response, such as (a) making minor variations to its amino acid structure, (b) introducing the tumor antigen-related gene in a viral vector, and (c) vector gene-addition for a larger number of genes-associated immune-stimulatory molecules to the tumor-specific antigen. Another approach is to attach a completely foreign (external) substance to the tumor molecule, known as an adjuvant. Sometimes, using an adjuvant as bait, they trick the immune system into attacking the antigen/adjuvant combination (vaccine) and the patient's tumor.

In addition to detecting and combating tumor cells, the immune system also has other ways to prevent them from appearing. It protects the body from viral infections that can lead to the development of tumors. It also fights others with high mutagenic potential, meaning that their infection may alter the genetic material of the cells. It fights by destroying foreign pathogens. When there is an infection, the body reacts by creating inflammation around it. However, this inflammation must be effectively resolved, as this environment can sometimes lead to the emergence of tumors. The immune system recognizes and destroys cancer cells through specific biomarkers. But how does the immune system recognize cancer cells? The answer lies in certain surface proteins found on all cells, called surface antigens. The surface antigens of tumor cells are different from those of normal cells. Therefore, the immune system cells can recognize these antigens and recognize if they match a mutated cell. The immune system's response to cancer begins with the recognition of mutated cells, thanks to the aforementioned antigens. These antigens are examined by T lymphocytes located in the lymph nodes. After this, the T lymphocytes undergo a series of changes and are then activated. This gives them the ability to travel through the blood vessels. Through them, the T lymphocytes reach the tumor guided by a concentration gradient of specific signaling molecules. Here, they recognize tumor cells that have specific proteins on their surface and destroy them. They do this by stimulating another series of immune system cells, for example, macrophages.

Strengthening the immune system is a simple way to protect the body from infectious and viral diseases. Strengthening the immune system can protect you from cold viruses, flu, and serious diseases such as cancer. The truth is that our immune system is very complex and is therefore affected by many factors. Eating healthy foods, being active, and maintaining a healthy weight are some of the most important factors that affect strengthening our immune system. Following a healthy lifestyle along with consuming some specific foods can greatly help improve the functioning of the immune system [37]. Cancer vaccines aim to treat existing cancer or prevent cancer from developing.

Therapeutic cancer vaccines are designed to boost the body's natural defense against cancer that has spread in the body. They may prevent existing tumors from growing, prevent tumors from coming back after treatment, or kill cancer cells that have not been killed by previous treatments [38]. Dendritic cells (DC) are important immune cells that play a key role in shaping the body's immune response. These cells are mainly generated in the bone marrow and circulate in the blood to reach the sites where they are needed. These cells are capable of inducing a primary immune response in dormant T lymphocytes. To do this, dendritic cells take up antigens from an invading cell, process them, and then present them, along with accessory molecules, on their cell surface. Due to this ability to process and present antigens, dendritic cells are known as antigen-presenting cells (APCs). The crucial role of these cells in shaping T-cell responses makes them popular research targets for the development of immunomodulatory therapies for conditions such as autoimmune disease, cancer, and various types of transplants. Knowledge and understanding of dendritic cells have grown significantly over the past three decades. However, the use of these cells as a form of immunotherapy is still in its infancy [39]. Cancer vaccines are considered a promising approach in the field of immunotherapy and cancer treatments. The goal of these vaccines is to strengthen and improve the function of the immune system to identify and destroy cancer cells in the body. Unlike other traditional vaccines that all humans receive from childhood to adulthood as a barrier to prevent infections, cancer vaccines are designed to treat the cancers that patients have or to prevent the recurrence of this deadly disease. Cancer vaccines can also be used in combination with other treatment methods such as surgery, chemotherapy, or radiation therapy [40]. The concept of cancer vaccines stems from the fact that the immune system can identify and destroy cancer cells, but cancer cells often find ways to escape the immune system. The goal of cancer vaccines is to block these escape routes and increase the body's ability not only to prevent cancer but also to fight cancer [41].

2.2. HSPs in Various Cancers

Early diagnosis of this cancer is crucial in avoiding the progression of the disease. HSPs are a huge group of extremely conserved proteins that impose a dominant role in cellular processes. They possess antioxidant and anti-inflammatory properties [42], protect the nucleus and cell membranes from damage, and prevent cell apoptosis. The HSP family is the most temperature-sensitive group of these proteins [43]. Increased expression of HSPs has been shown in many different cancers. For example, increased expression of HSP70 and HSP10 has been reported as a marker for hepatocarcinoma and lung, pancreatic, ovarian, and colorectal cancers [44–46]. As a marker in HSP70 immunohistochemistry, increased expression has also been observed in advanced breast cancer. Jagadish et al. [47] showed that expression of HSP70 was increased in 23% of patients with cancer. They also pointed to a detection of HSP70 from cancer cells that considerably decreases the enlargement of cells and arrests the replication of cells in experimental studies on animals, and suggested that HSP70 may lead to oncogene downregulation and the upregulation of genes responsible for tumor suppression and apoptosis-promoting molecules. Numerous studies have also revealed that higher HSP expression is related to poorer differential diagnosis of cancer, augmented proliferation, metastasis in lymph nodes, enlarged tumor size, the mutation in p53 genes, and advanced cancer stages [48–50]. Gunaldi et al. [51] reported significantly higher HSP levels in cancer patients than controls, but no such difference has existed among diverse forms of cancer. They recommended that HSP could be involved in the process of signaling, multiplying, passing, and offensive ability of cancer. They also advised that the expression of HSP may be induced by cancer-related genes and mutations in genes responsible for tumor suppression, which inhibit cell death and lead to uncontrolled cancer cell proliferation. Hence, Souza et al. [52] studied the expression of HspBP1 in primary breast cancer patients after 7-year follow-up, and showed that though the expression of this HSP was elevated in tumor tissue, it is inversely associated with cancer aggressiveness. Also, de Feritas et al. [53] in an observational study on women with breast cancer assessed the HSPA1A concentration in their serum samples and compared it with controls. They showed that the expression of HSP70 is not only raised in female cases suffering from breast cancer, but also can be considered as a possible biological marker for cancer and its progression. Research has also exposed that approximately a quarter of patients with breast cancer possess anti-p53 antibodies in their circulatory system and a complex of p53-HSP70, indicating that HSP70 could play a role in p53 antigen presentation, and ultimately elicit a specific immune response [54,55]. Since some cancers are immersed in the salivary environment, analysis of salivary proteomes from oral cancer patients is a promising approach to finding potential biomarkers for this disease. Saliva is a fluid that is easily accessible compared to tissue sampling. Therefore, a huge salivary sample could be collected and analyzed, providing a robust study with sufficient statistical

power to reveal true and characteristic signs of the disease. In addition, the identified protein biomarkers can be used to diagnose or monitor the disease in body fluids noninvasively. Squamous cell carcinoma antigen 2, calcycline, cathepsin G, peroxiredoxin II, annexin I, and HSP70 thioredoxin are among the proteins whose salivary levels have been increased in oral cancer [56]. Saliva HSP70 has been measured in patients with breast cancer, and the diverse nature of HSP70 presenting in serum and saliva revealed no correlation between these HSPs [57]. Salivary levels of this protein cannot accurately determine the origin of the cancer. Testing for specific antibodies could be more specific, and further studies of HSP70 are recommended to clarify this. It is also recommended that further studies in different types of breast cancer compare HSP70 levels before, during, and after breast cancer treatment.

2.3. Clinical Trial Studies Using HSPs as Vaccine in Cancer Treatment

Through systematic search in PubMed/Medline using ("Tumor" OR "Cancer" OR "Neoplasm" AND ("vaccine" OR "vaccination") AND ("heat shock protein" OR "HSP") from 1980 to 2025 (**Table 1**). We did not use any language limits. We also checked the reference list of selected articles to find any relevant articles.

Table 1. Search results using selected keywords.

Search	Query	Results
#11	Search: **((((Tumor[Title/Abstract]) OR (Cancer[Title/Abstract])) OR (Neoplasm[Title/Abstract])) AND ((vaccine[Title/Abstract]) OR (vaccination[Title/Abstract]))) AND ((heat shock protein[Title/Abstract]) OR (HSP[Title/Abstract]))** Sort by: **Most Recent** ("Tumor"[Title/Abstract] OR "Cancer"[Title/Abstract] OR "Neoplasm"[Title/Abstract]) AND ("vaccine"[Title/Abstract] OR "vaccination"[Title/Abstract]) AND ("heat shock protein"[Title/Abstract] OR "HSP"[Title/Abstract])	414
#10	Search: **(heat shock protein[Title/Abstract]) OR (HSP[Title/Abstract])** Sort by: **Most Recent**	41,957
#9	Search: **(vaccine[Title/Abstract]) OR (vaccination[Title/Abstract])** Sort by: **Most Recent**	378,753
#8	Search: **vaccination[Title/Abstract]** Sort by: **Most Recent**	210,923
#7	Search: **vaccine[Title/Abstract]** Sort by: **Most Recent**	284,168
#6	Search: **((Tumor[Title/Abstract]) OR (Cancer[Title/Abstract])) OR (Neoplasm[Title/Abstract])** Sort by: **Most Recent**	3,304,607
#5	Search: **Neoplasm[Title/Abstract]** Sort by: **Most Recent**	100,083
#4	Search: **Cancer[Title/Abstract]** Sort by: **Most Recent**	2,391,016
#3	Search: **Tumor[Title/Abstract]** Sort by: **Most Recent**	1,544,551
#2	Search: **HSP[Title/Abstract]** Sort by: **Most Recent**	16,455
#1	Search: **heat shock protein[Title/Abstract]** Sort by: **Most Recent** "heat shock protein"[Title/Abstract]	32,696

2.4. PICO Used in Study Selection

Potential studies were included if they were designed as clinical studies or clinical trials about vaccine therapy in patients diagnosed with various cancer types, assessing efficacy, immune response, survival, and safety, as well as limitations and side-effects (**Table 2**).

Table 2. PICO used in study selection.

Criteria	Description
Population (P)	Patient with any type of cancer
Intervention (I)	Using any HSP product for vaccine therapy
Comparison (C)	Comparing to baseline or before vaccination
Outcome (O)	Efficacy, Immune response, survival, and safety

3. Results

3.1. RCTs on HSPs

Overall, of 414 articles, 18 were selected. The selected studies include 1116 cases diagnosed with various cancer types. The results of clinical trials investigating the use of HSPs in cancer vaccine therapy are summarized in **Table 3.** A total of 18 studies, including 1,116 patients with various types of cancer, were reviewed. These studies spanned different trial phases (I–III) and utilized a range of HSP-based vaccines to evaluate their safety, immunogenicity, and efficacy. HSPPC-96 was the most commonly investigated vaccine. Bloch et al. [58,59] conducted Phase II trials on patients with GBM, reporting vaccine-related adverse events (VR-AE) rates of 74% and 68%, respectively. These studies suggested that HSPPC-96 could improve survival outcomes in GBM patients. Ji et al. [60] performed a Phase I trial of HSPPC-96 in GBM patients, with VR-AE observed in 30% of cases. Crane et al. [61] also investigated HSPPC-96 in a Phase I trial with 12 GBM patients, reporting a significant immune response but no documented adverse events. In melanoma, Pilla et al. [62] and Testori et al. [63] assessed the efficacy of HSPPC-96. Pilla's Phase II trial involving 20 patients observed VR-AE in 80% of participants, whereas Testori's Phase III trial with 322 patients reported a lower VR-AE rate of 13.6%. Wood et al. [64] investigated the vaccine in RCC patients during a Phase III trial, documenting a 49.7% VR-AE rate but no significant improvement in survival outcomes. HSP70 was studied in CIN by Trimble et al. [65] in a Phase I trial, with a VR-AE rate of 33%. In CML, Li et al. (2005) found HSP70 to be feasible and safe, with no adverse events reported. HSP105 was explored by Shimizu et al. [66] in colorectal and esophageal cancer patients, showing immunological efficacy without any documented adverse events. Other HSP-based vaccines, such as HspE7 and Hsp65, were evaluated in CIN and HNC, respectively. Van Doorslaer et al. [67] and Einstein et al. [68] studied HspE7 in CIN patients, with VR-AE rates ranging from 0% to 91%, depending on the study. In HNC, Victora et al. [69] reported immunostimulatory effects in a Phase I trial of Hsp65 without adverse events.

Table 3. Clinical trials using HSPs as vaccine in cancer treatment.

Study ID, Country	HSPs	Population	Cancer	Trial Phase	VR-AE	Trial Registration	Finding
Shimizu et al., 2019, Japan [66]	HSP105	30 patients	CRC or EC	Phase I	None	-	Induce immunological effects
Ji et al., 2018, China [60]	HSPPC-96	20 patients	GBM	Phase I	30% (6720 cases)	NCT02122822	A safe and effective strategy for treatment
Bloch et al., 2017, USA [59]	HSPPC-96	46 patients	GBM	Phase II	74% (34746 cases)	NCT00905060	May improve survival for patients
Bloch et al., 2014, USA [58]	HSPPC-96	41 patients	GBM	Phase II	68% (28/41 cases)	NCT00293423	A safe and effective treatment
Crane et al., 2013, USA [61]	HSPPC-96	12 patients	GBM	Phase I	None	-	A significant immune response
Van Doorslaer et al., 2009, USA [67]	HspE7	57 patients	CIN	Phase II	None	NCT00075569	A significant immune response
Victora et al., 2009, Brazil [69]	Hsp65	21 patients	HNC	Phase I	None	-	Induced some degree of immunostimulation
Trimble et al., 2009, USA [65]	HSP70	15 patients	CIN	Phase I	33% (5/15 cases)	NCT00121173	A safe and well tolerated
Wood et al., 2009, USA [64]	HSPPC-96	318 patients	RCC	Phase III	49.7% (158/318 cases)	NCT00033904	No improvement survival for patients
Testori et al., 2008, Italy [63]	HSPPC-96	322 patients	Melanoma	Phase III	13.6% (44/322 cases)	-	A significant immune response
Roman et al., 2007, USA [70]	HspE7	21 patients	CIN	Phase II	None	-	A safe and effective strategy for treatment
Einstein et al., 2007, USA [68]	HspE7	64 patients	CIN	Phase II	91% (58/64 cases)	NCT00075569	Activity but no safety
Maki et al., 2007, USA [71]	HSPPC-96	10 patients	PA	Phase I	80% (8/10 cases)	-	The feasibility of vaccine for PA
Oki et al., 2007, USA [72]	HSPPC-96	20 patients	NHL	Phase II	15% (3/20 cases)	-	Was very well tolerated, but it had limited efficacy
Palefsky et al., 2006, USA [73]	HspE7	15 patients	AIN	phase I/I	60% (9/15 cases)	-	Well tolerated with clinical activity
Pilla et al., 2006, Italy [62]	HSPPC-96	20 patients	Melanoma	Phase II	80% (16/20 cases)	-	Feasible with mild local and systemic toxicity
Li et al., 2005, USA [74]	HSP70	20 patients	CML	Phase I	None	-	Feasible and safe
Belli et al., 2002, Italy [75]	HSPPC-96	64 patients	Melanoma	Phase I	None	-	Feasible with no significant toxicity

Note: Heat shock protein 105 (HSP105); colorectal cancer (CRC) and esophageal cancer (EC); Vaccine related Adverse events (VR -AE); Heat shock protein peptide complex-96 (HSPPC-96); glioblastoma multiform (GBM); Cervical intraepithelial neoplasia (CIN); 65-kDa heat-shock protein of Mycobacterium leprae (Hsp65); head and neck carcinoma (HNC); renal cell carcinoma (RCC); Pancreas adenocarcinomas (PA); Anal intraepithelial neoplasia (AIN); Chronic Myelogenous Leukemia (CML); non-Hodgkin lymphoma (NHL).

3.2. Variability in Adverse Event Rates HSPs

Bloch et al. [59] reported adverse events (AVs) associated with autologous HSPs in patients with glioblastoma. Not only were all types of AVs reported in almost all patients (44 of 46, 96%), but also vaccine-related AVs in approximately 75% of patients, but with no serious types. However, the most common and predominant AV was the reaction in minor injection sites. Ji et al. [60] also reported several slight dose-related AVs, but the most predominant one was fatigue in 20% of patients. They reported only one serious AV in the form of a focal neurological deficit. Wood et al. [64] reported AVs with a high frequency of 95% mostly mild to moderate, with no serious AV. The most frequent AV was the reaction in minor injection sites at a frequency of 50%. Testori et al. [63] reported pyrexia and fatigue as the most common AVs. Trimble et al. [65] reported well-toleration results related to the HSP vaccine with fewer AVs, mostly mild to moderate, and no serious AV. They also reported the reaction in minor injection sites as the most frequent AV. Other clinical trials reported some degrees of immunostimulation, but without pathological autoimmunity. Despite the aforementioned AVs, the HSP vaccine showed promising results in overall survival and

was well-tolerated.

3.3. Using HSPs as Adjuvants Against Cancer

These results indicate that the specific tumor-specific effects of the HSPs' immune response, including CD4+, INF γ, and CD8+, lead to a much stronger vaccination (**Table 4**). In summary, HSPs and tumor cells together can enhance immunogenicity. It can be concluded that the simultaneous use of HSPs and tumor-specific peptide complexes has a synergistic effect in generating a stronger immune response in tumor cells, and the use of HSPs as an active carrier for antigen presentation (CD4+ and CD8+) can be a suitable strategy for tumor immunotherapy. Further studies in identifying the mechanism and other immune cells involved in this type of response are of particular importance.

Table 4. HSPs as adjuvants for cancer when stimulating immune response.

Heat Shock Proteins (HSPs) Reference	Immunogenes	Tumor Models	Immune Response
GP96 [76]	GP96 15-mer peptide	BALB/c mouse model	Specific antibody against CD4+ and CD8+
HSP110/Grp170 [77]	TRP2 peptide complex	C57BL/6 mouse model	CD8+ T cell
HSP70 [78]	TGF-beta	Non-immunogenic rat carcinoma	Suppressing expression of active TGF-beta
HSP70 [79]	DC&HSP70 peptide complex	Cell line	INF γ and T cell
HSP70 [80]	MOSEC/luc and HSP70	C57BL/6 mouse model	CD4+, CD8+ T& NK cell
HSP90 [81]	p485 and p527	MMTV *neu*-transgenic mice	higher rearrangement of TCRβ

4. Discussion

The use of heat shock proteins HSPs as cancer vaccine adjuvants represents a significant development in immunotherapy, primarily due to their dual role in stress responses and immune modulation. Unlike conventional adjuvants derived from microorganisms or synthetic compounds, HSPs naturally enhance antigen presentation by binding and stabilizing tumor-derived peptides, facilitating their recognition by APCs. This intrinsic ability to activate both innate and adaptive immunity positions HSPs as unique and versatile tools in cancer immunotherapy. These molecules—originally discovered for their role in stress responses—have demonstrated the ability to act as molecular chaperones, facilitating tumor antigen presentation and stimulating robust immune responses. The results of clinical trials summarized in **Table 3** illustrate their potential and underscore critical areas for further exploration.

The evaluation of HSPPC-96, the most extensively studied HSP-based vaccine, highlights its application in GBM. This vaccine demonstrates unique effectiveness in GBM due to the tumor's high immunogenicity and the ability of HSPPC-96 to chaperone a wide range of tumor-specific antigens. GBM's immunosuppressive microenvironment, characterized by regulatory T cells and immunosuppressive cytokines, makes antigen-specific therapies particularly challenging, yet HSPPC-96 has shown promise in overcoming these barriers by promoting robust cytotoxic T lymphocyte activation and modulating immune checkpoints. Bloch et al. [58,59] and Ji et al. [60] observed high rates of vaccine-related immune activation. The capacity of HSPPC-96 to chaperone tumor antigens and promote cytotoxic T lymphocyte responses aligns with preclinical evidence supporting its efficacy. However, the variation in adverse event rates between studies suggests a need for optimizing vaccine administration. For example, tailoring patient selection based on immune profiles may enhance outcomes, particularly in immunosuppressive tumor microenvironments such as GBM.

In melanoma, the efficacy of HSPPC-96 was further supported by trials conducted by Pilla et al. [62] and Testori et al. [63]. The observed reduction in vaccine-related adverse events between Phase II and III trials points to improvements in vaccine formulation and dosing. This trend underscores the importance of iterative clinical testing in refining therapeutic approaches. Melanoma, characterized by its high mutational burden, offers an ideal model for antigen-rich vaccines like HSPPC-96. These results affirm the value of HSP-based vaccines in tumors with abundant

antigenic targets.

Conversely, limited efficacy in RCC trials, as reported by Wood et al. [64], highlights challenges in addressing tumors with "cold" immune microenvironments. RCC's resistance to immunotherapy is attributed to several factors, including low baseline T cell infiltration, an abundance of immunosuppressive cytokines such as IL-10 and TGF-β, and the presence of regulatory T cells that dampen immune responses. Additionally, the metabolic characteristics of RCC, such as reliance on aerobic glycolysis, create a hypoxic and acidic tumor microenvironment that further hinders T-cell activity and antigen presentation. Overcoming these barriers may require innovative strategies such as combining HSP-based vaccines with immune checkpoint inhibitors or metabolic modulators, which could reprogram the tumor microenvironment to support a more robust anti-tumor immune response. RCC's resistance to immunotherapy may stem from low baseline T cell infiltration and an immunosuppressive cytokine milieu. Combining HSP-based vaccines with checkpoint inhibitors, which modulate immune checkpoints like PD-1/PD-L1, could help overcome these barriers. Preclinical studies have already shown synergistic effects between these modalities, warranting their inclusion in future RCC vaccine trials.

In CIN, Trimble et al. [65] demonstrated moderate adverse event rates and promising safety profiles for HSP70. Differences in vaccine efficacy across CIN trials—as seen in the higher adverse event rates in studies by Einstein et al. [68] may reflect heterogeneity in patient populations, including variations in HPV genotypes. These findings emphasize the need for personalized vaccine strategies that account for underlying etiological factors.

HSP105, studied in colorectal and esophageal cancer patients [66], demonstrated immunological efficacy without significant adverse events. This molecule engages immunological pathways by promoting dendritic cell maturation and enhancing antigen cross-presentation to T cells. These mechanisms are particularly relevant in gastrointestinal cancers, where immune evasion is often driven by suboptimal antigen presentation. HSP105's ability to activate cytotoxic T lymphocytes and induce robust immune responses highlights its therapeutic potential. Moreover, its favorable safety profile reinforces its applicability in combination with strategies aimed at overcoming tumor-driven immunosuppression. This outcome reinforces HSP105's ability to induce dendritic cell maturation and antigen cross-presentation. Its favorable safety profile suggests the potential for broader application across gastrointestinal malignancies. Future trials should investigate its use in combination with other immunomodulatory agents to further enhance therapeutic efficacy.

Notably, HspE7 and Hsp65 vaccines demonstrated wide variability in safety and efficacy outcomes. Van Doorslaer et al. [67] reported consistent immunostimulatory effects in CIN patients, whereas Einstein et al. [68] observed higher adverse event rates. This discrepancy may arise from differences in vaccine formulations or patient selection. In HNC, Victora et al. [69] documented immunostimulatory effects with Hsp65, emphasizing its potential in cancers with localized antigen expression.

An important fact about HSPs as a group of homologous protein families is that they are encoded by a multigene family that plays important roles in homeostasis, regulation of apoptosis, protein folding, and maintenance of other physiological processes, including spermatogenesis [82]. Therefore, tumor cell-isolated HSPs are hypothetically tumor antigen-rich proteins. Considering HSP-peptide complexes' role in the stimulation and development of APCs, they can activate a polyclonal response of T lymphocytes in response to tumor antigens. In these circumstances, even if the tumor loses some of its antigens under the selective pressure of the immune system, numerous T-cell clones will be available to destroy tumor cells [83].

According to the danger theory, activation of the immune system is dependent on the recognition by the innate immune system of danger molecules released from stressed and damaged cells or danger molecules derived from the pathogen [84]. Both membrane and soluble forms of HSP will raise alarm bells for the immune system [85]. Induction of maturation in dendritic cells, along with antigen cross-presentation, will prevent unresponsiveness of the immune system. Using this approach will require us to identify individual tumor-specific antigens. HSP-peptide complexes from an individual's tumor will ensure the presence of maximum tumor-specific and unique antigens [86]. Heat stress is the most important method used to induce HSP in cells. Typically, non-lethal heat at temperatures above 40°C for one hour is used to induce HSP in cell culture medium [87].

gp96 and HSP70 are among the most important tumor-derived HSPs that facilitate the entry of antigenic peptides into MHC class I molecules [88]. Of course, the distribution of HSP molecules within the cell is different. Normally, gp96 molecules are present in the endoplasmic reticulum, and HSP70 is present in the endoplasmic reticulum. Thus, it seems that in order to obtain the maximum potential of tumor antigens together with HSPs, it is necessary

to evaluate the optimal conditions that cause the maximum induction of both molecules simultaneously [89]. In the past, not many studies have been conducted to determine the best temperature and incubation time for HSP induction. However, in a study conducted on hepatocellular carcinoma, the best conditions for each tumor strain were identified.

The variability in immunological responses across trials underscores the importance of context-specific vaccine design. Factors such as tumor antigenicity, microenvironmental conditions, and expression of HSP molecules were determined to be 42°C for half an hour to one hour, followed by 5 to 18 hours of incubation at 37°C [90,91]. These results indicate that before designing anti-tumor vaccines based on HSP, it is better to find the optimal temperature and incubation times, and patient immune status plays a critical role in determining vaccine efficacy. Standardizing trial methodologies and incorporating biomarker-driven approaches could address these challenges, enabling more accurate assessments of vaccine performance.

Beyond efficacy and safety, the scalability and feasibility of HSP-based vaccine production merit consideration. Recent advances in recombinant protein technology have significantly lowered production costs by streamlining purification processes and improving yield. For example, the use of microbial expression systems, such as *E. coli*, has enabled the cost-effective production of HSP-based vaccines without compromising their immunogenic properties. Additionally, synthetic biology approaches have facilitated the design of highly stable HSP constructs that require less stringent storage conditions, enhancing accessibility in low-resource settings. These innovations have made HSP vaccines more viable for widespread clinical use and expanded their potential application in global cancer immunotherapy efforts. Advances in recombinant protein technology and synthetic biology have streamlined HSP vaccine manufacturing, reducing costs and increasing accessibility. These innovations position HSP-based vaccines as viable options for widespread clinical use, particularly in resource-limited settings.

Lastly, combination therapies integrating HSP vaccines with emerging immunotherapeutic modalities represent a promising avenue for future research. The synergistic potential of HSPs with checkpoint inhibitors, oncolytic viruses, and adoptive T-cell therapies could enhance tumor-specific immune responses while mitigating resistance mechanisms. Investigating these combinations in preclinical and clinical settings will be essential to unlocking the full therapeutic potential of HSP-based cancer vaccines. Though combining HSP-based vaccines with other immunotherapies could be a promising treatment modality, exploring this issue in depth through large-scale research will be beneficial. Identifying specific combination strategies that show the most promise and understanding the potential risks or challenges could help in better decision-making about the best vaccine-based intervention for people suffering from various malignancies.

Efficacy and safety of vaccines, unlike the effectiveness of treatment, depends on various factors, including adherence to the vaccine schedule, type of disease, vaccine strain (some vaccine strains are more effective than others), non-responsiveness (some people do not respond at all to certain vaccines, the reason may be in the person's race, ethnicity, or genetics) [92]. Currently, a large number of potential vaccines are being studied for cancer. Knowing the genetic predisposition to certain diseases, such as cancer, is very important because, as studies and research progress, it will become necessary to conduct genetic tests on the population to determine who should be vaccinated against certain diseases [93]. Vaccine effectiveness is best measured by double-blind clinical trials, especially phase III [94]. These examine the best-case scenarios of vaccine protection under controlled conditions and are typically required before a new vaccine can be licensed by the Food and Drug Administration (FDA) and other global regulatory authorities. Given that, we showed that most of the conducted trials on using the HSP vaccine against cancer were in phase 1 or II. So, conducting more advanced phase III trials could better help to overcome such limitations. Moreover, the current trials, including patients with various diseases, could be another source.

4.1. Patient-Specific Factors Influence the Efficacy of HSP-Based Vaccines

In evaluating the effectiveness of vaccines, several factors are influential, including the age of the individuals, the health status of the participants, the type of strain circulating in the country under study, the type of vaccine under study and the availability of vaccines, compliance with the demographic characteristics of the vaccinated individuals such as genetic and ethnicity, as well as the disease-protocols in the population under study and the time of the study [95]. However, the vaccines used in the countries were of different types, affecting the effectiveness of vaccines, such as changes in the cold chain during transportation and storage, the way vaccines are administered, the age of individuals, the type of strain circulating in a country, and the presence of medical conditions and

underlying and previous diseases such as heart disease, are among the factors that can affect the effectiveness of vaccines.

4.2. The Challenges of Scaling Up HSP-Based Vaccine Production and Possible Barriers

The human immune system is delicately balanced, and this is one of the challenges associated with HSP vaccines. On the one hand, it needs to recognize carcinogens and other organisms. On the other hand, the immune response must not overreact to healthy tissues in the body. Another feature of the immune system is that it can fight and protect against many types of cancers [96]. For example, the lymphocytes that help in immune responses, called helper T cells, come in different types. The first (Th1) is expressed in response to some cancer cells, while the second (Th2) controls other types [97,98]. Activating the wrong pathway can increase the level of inflammation and worsen the disease. As part of normal immune responses, lymphocytes and other cells produce immune signaling chemicals called cytokines [99]. These coordinate and stimulate immune responses, but if these responses are excessive, they can cause inflammation, which in severe cases can lead to the shutdown of vital organs, including the lungs, heart, and kidneys [100]. Another challenge with these vaccines is the lack of suitable animal models for testing. Mice make antibodies in response to disease or infection, and their immune systems are well-known [101]. However, mice do not naturally develop various symptoms alongside disease or infection, so it is impossible to test whether a vaccine protects against the disease. This issue can be overcome to some extent by using older or genetically modified mice or other animals.

Commercialization and the length of the processes are organizational factors making barriers to large-scale production, along with issues such as assessing the safety, efficacy, and safety of a new drug throughout extensive and long preclinical and clinical studies [102], and emphasizing compliance with intellectual property rights, pricing, and contract negotiation, among other issues [103]. Commercialization involves a variety of activities, including important technical and commercial processes, that transform a new technology into a useful product or service. This process includes market assessment, product design, manufacturing engineering, intellectual property rights management, market development and management, capital raising, and personnel training. These challenges can be addressed by explaining scientific advances and redefining safety markers, reducing clinical trial time, facilitating licensing, and reducing costs.

5. Recommendations

Identifying barriers to vaccine design and production and analyzing the relationships between them provides the basis for sound decision-making and policy-making by military health system managers. Causal factors in vaccine production have been identified, and efforts are being made to eliminate them in military health systems. Considering the production of two vaccines in the armed forces, it is necessary to pay attention to vaccine production infrastructure as a key strategy. Despite the obstacles, vaccine production strengthened the self-confidence of policymakers and, with their support, a courageous decision was made with great risk.

6. Future Perspective

One of the latest advances in cancer research is the development of personalized vaccines for cancer patients. Thousands of people with cancer will soon be able to take part in a trial of a new cancer vaccine [104]. The treatment is designed to prime the body's immune system to target cancer cells and reduce the risk of the disease coming back. Experts also hope that the vaccines will have fewer side effects than standard chemotherapy. Precision oncology is the best weapon to defeat cancer, which involves studying the genetic makeup and molecular characteristics of cancer tumors in each patient [105]. Precision oncology identifies changes in cells that may cause cancer to develop and spread. After applying this method, personalized treatments can be developed. Unlike general treatments such as chemotherapy, precision oncology-based treatments are targeted and target specific tissues and cells. This means they damage fewer healthy cells and therefore have fewer side effects. Comparative analysis of HSP-based vaccines versus other immunotherapies (e.g., checkpoint inhibitors, CAR-T cells), in terms of the efficacy, safety, and limitations of HSP-based vaccines with other immunotherapies, will be worthwhile in near-future studies.

7. Conclusions

HSP-based cancer vaccines represent a transformative step in immunotherapy, leveraging their unique capacity to chaperone tumor-specific antigens and activate both innate and adaptive immune responses. The clinical trials reviewed demonstrate significant potential across various cancers, with promising efficacy in GBM, melanoma, and gastrointestinal malignancies, albeit with variability in outcomes for RCC, CIN, and HNC. These findings underscore the complexity of tumor microenvironments and the need for tailored approaches to maximize therapeutic success. Advances in vaccine formulation, production, and combination therapies offer promising pathways to address these challenges. Identifying predictive biomarkers can help identify patients who are most likely to benefit from cancer vaccines; biomarkers can also be useful in monitoring treatment response and making the best treatment decisions for the patient [106]. In a new study, researchers have presented a model that could help improve cancer treatment using a type of biomarker [107]. The scientists also found new features that indicate which cancer markers are most likely to trigger an immune response [108]. While the safety profiles of HSP-based vaccines are largely favorable, optimizing administration protocols and integrating biomarkers for patient stratification remains critical. Recent breakthroughs in recombinant protein technology and synthetic biology have enhanced the scalability and accessibility of these vaccines, laying the groundwork for their broader application, particularly in resource-limited settings. Future research must prioritize the integration of HSP-based vaccines with emerging immunotherapeutic strategies, including immune checkpoint inhibitors and oncolytic therapies, to overcome immune resistance and improve clinical outcomes. By addressing current limitations and leveraging ongoing innovations, HSP-based vaccines hold the potential to redefine cancer immunotherapy, offering hope for more effective and accessible treatments for diverse patient populations.

Author Contributions

Conceptualization, D.L., S.H.K., H.L.R.A., O.F.B., M.S.F., and T.H.K.; validation, H.L.R.A. and O.F.B.; formal analysis, D.L., S.H.K., M.S.F., and T.H.K.; data curation, D.L., S.H.K., M.S.F., and T.H.K.; writing—original draft preparation, D.L. and S.H.K.; writing—review and editing, H.L.R.A. and O.F.B. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Conflicts of Interest

The authors declared no conflict of interest.

References

1. Guerrero Manriquez, G.G.; Tuero, I. Adjuvants: Friends in Vaccine Formulations Against Infectious Diseases. *Hum. Vaccin. Immunother.* **2021**, *17*, 3539–3550. [CrossRef]
2. Quintana, F.J.; Cohen, I.R. Heat Shock Proteins as Endogenous Adjuvants in Sterile and Septic Inflammation. *J. Immunol.* **2005**, *175*, 2777–2782. [CrossRef]
3. Kensil, C.; Mo, A.; Truneh, A. Current Vaccine Adjuvants: An Overview of a Diverse Class. *Front. Biosci.* **2004**, *9*, 2972–2988. [CrossRef]
4. Mengal, K.; Kor, G.; Kozák, P.; et al. Heat Shock Proteins Adaptive Responses to Environmental Stressors and Implications in Health Management of Decapods. *Aquac. Rep.* **2023**, *30*, 101564. [CrossRef]
5. Fulda, S.; Gorman, A.M.; Hori, O.; et al. Cellular Stress Responses: Cell Survival and Cell Death. *Int. J. Cell Biol.* **2010**, *2010*, 214074. [CrossRef]
6. Asea, A. Initiation of the Immune Response by Extracellular Hsp72: Chaperokine Activity of Hsp72. *Curr. Immunol. Rev.* **2006**, *2*, 209–215. [CrossRef]
7. Park, C.J.; Seo, Y.S. Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. *Plant Pathol. J.* **2015**, *31*, 323–333. [CrossRef]
8. Bakthisaran, R.; Tangirala, R.; Rao, C.M. Small Heat Shock Proteins: Role in Cellular Functions and Pathology. *Biochim. Biophys. Acta Proteins Proteom.* **2015**, *1854*, 291–319. [CrossRef]
9. Ranek, M.J.; Stachowski, M.J.; Kirk, J.A.; et al. The Role of Heat Shock Proteins and Co-Chaperones in Heart Failure. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2018**, *373*, 20160530. [CrossRef]
10. Binder, R.J. Functions of Heat Shock Proteins in Pathways of the Innate and Adaptive Immune System. *J. Immunol.* **2014**, *193*, 5765–5771. [CrossRef]
11. Murshid, A.; Gong, J.; Calderwood, S.K. The Role of Heat Shock Proteins in Antigen Cross Presentation. *Front. Immunol.* **2012**, *3*, 63. [CrossRef]
12. Gaston, J.S. Heat Shock Proteins and Innate Immunity. *Clin. Exp. Immunol.* **2002**, *127*, 1–3. [CrossRef]
13. Rowles, J.E.; Keane, K.N.; Heck, T.G.; et al. Are Heat Shock Proteins an Important Link Between Type 2 Diabetes and Alzheimer Disease? *Int. J. Mol. Sci.* **2020**, *21*, 1–20. [CrossRef]
14. Leak, R.K. Heat Shock Proteins in Neurodegenerative Disorders and Aging. *J. Cell Commun. Signal.* **2014**, *8*, 293–310. [CrossRef]
15. Szyller, J.; Bil-Lula, I. Heat Shock Proteins in Oxidative Stress and Ischemia/Reperfusion Injury and Benefits From Physical Exercises: A Review to the Current Knowledge. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 6678457. [CrossRef]
16. Guo, J.; Chang, C.; Li, W. The Role of Secreted Heat Shock Protein-90 (Hsp90) in Wound Healing – How Could It Shape Future Therapeutics? *Expert Rev. Proteomics* **2017**, *14*, 665–675. [CrossRef]
17. Zügel, U.; Kaufmann, S.H. Role of Heat Shock Proteins in Protection From and Pathogenesis of Infectious Diseases. *Clin. Microbiol. Rev.* **1999**, *12*, 19–39. [CrossRef]
18. Mukherjee, A.G.; Wanjari, U.R.; Namachivayam, A.; et al. Role of Immune Cells and Receptors in Cancer Treatment: An Immunotherapeutic Approach. *Vaccines* **2022**, *10*, 1–24. [CrossRef]
19. Zhou, Q.; Guan, X.Y.; Li, Y. Roles of Heat Shock Proteins in Tumor Immune Microenvironment. *Vaccines Cell. Mol. Med.* **2024**, *5*, 3. [CrossRef]
20. Sastry, S.; Linderoth, N. Molecular Mechanisms of Peptide Loading by the Tumor Rejection Antigen/Heat Shock Chaperone gp96 (GRP94). *J. Biol. Chem.* **1999**, *274*, 12023–12035. [CrossRef]
21. Calderwood, S.K.; Theriault, J.R.; Gong, J. Message in a Bottle: Role of the 70-kDa Heat Shock Protein Family in Anti-Tumor Immunity. *Cell Stress Chaperones* **2005**, *35*, 2518–2527. [CrossRef]
22. Thériault, J.R.; Mambula, S.S.; Sawamura, T.; et al. Extracellular HSP70 Binding to Surface Receptors Present on Antigen Presenting Cells and Endothelial/Epithelial Cells. *FEBS Lett.* **2005**, *579*, 1951–1960. [CrossRef]
23. Stocki, P.; Wang, X.N.; Dickinson, A.M. Inducible Heat Shock Protein 70 Reduces T Cell Responses and Stimulatory Capacity of Monocyte-Derived Dendritic Cells. *J. Biol. Chem.* **2012**, *287*, 12387–12394. [CrossRef]
24. Liu, B.; DeFilippo, A.M.; Li, Z. Overcoming Immune Tolerance to Cancer by Heat Shock Protein Vaccines. *Mol. Cancer Ther.* **2002**, *1*, 1147–1151. [CrossRef]
25. Li, Y.; Subjeck, J.; Yang, G.; et al. Generation of Anti-Tumor Immunity Using Mammalian Heat Shock Protein 70 DNA Vaccines for Cancer Immunotherapy. *Vaccine* **2006**, *24*, 5360–5370. [CrossRef]
26. Callahan, M.K.; Chaillot, D.; Jacquin, C.; et al. Differential Acquisition of Antigenic Peptides by Hsp70 and Hsc70 under Oxidative Conditions. *J. Biol. Chem.* **2002**, *277*, 33604–33609. [CrossRef]
27. Borges, T.J.; Wieten, L.; van Herwijnen, M.J.; et al. The Anti-Inflammatory Mechanisms of Hsp70. *Front. Immunol.* **2012**, *3*, 95. [CrossRef]

28. Campisi, J.; Leem, T.H.; Fleshner, M. Stress-Induced Extracellular Hsp72 Is a Functionally Significant Danger Signal to the Immune System. *Cell Stress Chaperones* **2003**, *8*, 272–286. [CrossRef]
29. Radons, J. The Human HSP70 Family of Chaperones: Where Do We Stand? *Cell Stress Chaperones* **2016**, *21*, 379–404. [CrossRef]
30. Yazdi, M.; Kafshgari, M.H.; Moghadam, F.K.; et al. Crosstalk Between NK Cell Receptors and Tumor Membrane Hsp70-Derived Peptide: A Combined Computational and Experimental Study. *Adv. Sci.* **2024**, *11*, 2305998. [CrossRef]
31. Guzhova, I.V.; Margulis, B.A. HSP70-Based Anti-Cancer Immunotherapy. *Hum. Vaccin. Immunother.* **2016**, *12*, 2529–2535. [CrossRef]
32. Verma, C.; Pawar, V.A.; Srivastava, S.; et al. Cancer Vaccines in the Immunotherapy Era: Promise and Potential. *Vaccines* **2023**, *11*, 1–19. [CrossRef]
33. Enokida, T.; Moreira, A.; Bhardwaj, N. Vaccines for Immunoprevention of Cancer. *J. Clin. Investig.* **2021**, *131*, e147147. [CrossRef]
34. Mehrizi, T.Z.; Ataei-Pirkooh, A.; Eshrati, B.; et al. Investigating Factors Affecting the Effectiveness of Gardasil 4, Cervarix, and Gardasil 9 Vaccines Considering the WHO Regions in Females: A Systematic Review. *Cancer Epidemiol.* **2025**, *95*, 102759. [CrossRef]
35. Zhao, Y.; Baldin, A.V.; Isayev, O.; et al. Cancer Vaccines: Antigen Selection Strategy. *Vaccines* **2021**, *9*, 1–12. [CrossRef]
36. Wu, Z.; Li, S.; Zhu, X. The Mechanism of Stimulating and Mobilizing the Immune System Enhancing the Anti-Tumor Immunity. *Front. Immunol.* **2021**, *12*, 682435. [CrossRef]
37. Ruzzi, F.; Riccardo, F.; Conti, L.; et al. Cancer Vaccines: Target Antigens, Vaccine Platforms and Preclinical Models. *Mol. Aspects Med.* **2025**, *101*, 101324. [CrossRef]
38. de Gruijl, T.D.; van den Eertwegh, A.J.; Pinedo, H.M.; et al. Whole-Cell Cancer Vaccination: From Autologous to Allogeneic Tumor- and Dendritic Cell-Based Vaccines. *Cancer Immunol. Immunother.* **2008**, *57*, 1569–1577. [CrossRef]
39. Lee, K.W.; Yam, J.W.P.; Mao, X. Dendritic Cell Vaccines: A Shift From Conventional Approach to New Generations. *Cells* **2023**, *12*, 1–18. [CrossRef]
40. Larocca, C.; Schlom, J. Viral Vector-Based Therapeutic Cancer Vaccines. *Cancer J.* **2011**, *17*, 359–371. [CrossRef]
41. Sellers, R.S.; Nelson, K. Chapter 9 - Vaccines. In *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, 4th ed.; Haschek, W.M., Rousseaux, C.G., Wallig, M.A., et al., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 335–396. [CrossRef]
42. Kumar, V.; Roy, S.; Behera, B.K.; et al. Heat Shock Proteins (Hsps) in Cellular Homeostasis: A Promising Tool for Health Management in Crustacean Aquaculture. *Life* **2022**, *12*, 1748. [CrossRef]
43. DeNicola, D.M. 6 - Periphyton Responses to Temperature at Different Ecological Levels. In *Algal Ecology*; Stevenson, R.J., Bothwell, M.L., Lowe, R.L., Eds.; Academic Press: San Diego, CA, USA, 1996; pp. 149–181. [CrossRef]
44. Gao, G.; Liu, S.; Yao, Z.; et al. The Prognostic Significance of Hsp70 in Patients With Colorectal Cancer: A PRISMA-Compliant Meta-Analysis. *Biomed. Res. Int.* **2021**, *2021*, 5526327. [CrossRef]
45. Liu, C.C.; Jan, Y.J.; Ko, B.S.; et al. 14-3-3σ Induces Heat Shock Protein 70 Expression in Hepatocellular Carcinoma. *BMC Cancer* **2014**, *14*, 425. [CrossRef]
46. Feng, J.; Zhan, Y.; Zhang, Y.; et al. Increased Expression of Heat Shock Protein (HSP) 10 and HSP70 Correlates With Poor Prognosis of Nasopharyngeal Carcinoma. *Cancer Manag. Res.* **2019**, *11*, 8219–8227. [CrossRef]
47. Jagadish, N.; Agarwal, S.; Gupta, N.; et al. Heat Shock Protein 70-2 (HSP70-2) Overexpression in Breast Cancer. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 150. [CrossRef]
48. Rothammer, A.; Sage, E.K.; Werner, C.; et al. Increased Heat Shock Protein 70 (Hsp70) Serum Levels and Low NK Cell Counts After Radiotherapy: Potential Markers for Predicting Breast Cancer Recurrence? *Radiat. Oncol.* **2019**, *14*, 78. [CrossRef]
49. Ciocca, D.R.; Clark, G.M.; Tandon, A.K.; et al. Heat Shock Protein Hsp70 in Patients With Axillary Lymph Node-Negative Breast Cancer: Prognostic Implications. *J. Natl. Cancer Inst.* **1993**, *85*, 570–574. [CrossRef]
50. Mahjabeen, I.; Sheshe, S.; Shakoor, T.; et al. Role of Genetic Variations of DNA Damage Response Pathway Genes and Heat-Shock Proteins in Increased Head and Neck Cancer Risk. *Future Oncol.* **2022**, *18*, 3519–3535. [CrossRef]
51. Gunaldi, M.; Afsar, C.U.; Okuturlar, Y.; et al. Elevated Serum Levels of Heat Shock Protein 70 Are Associated With Breast Cancer. *Tohoku J. Exp. Med.* **2015**, *236*, 97–102. [CrossRef]

52. Souza, A.P.; Albuquerque, C.; Torronteguy, C.; et al. HspBP1 Levels Are Elevated in Breast Tumor Tissue and Inversely Related to Tumor Aggressiveness. *Cell Stress Chaperones* **2009**, *14*, 301–310. [CrossRef]
53. de Freitas, G.B.; Penteado, L.; Miranda, M.M.; et al. The Circulating 70 kDa Heat Shock Protein (HSPA1A) Level Is a Potential Biomarker for Breast Carcinoma and Its Progression. *Sci. Rep.* **2022**, *12*, 13012. [CrossRef]
54. Davidoff, A.M.; Iglehart, J.D.; Marks, J.R. Immune Response to p53 Is Dependent Upon p53/HSP70 Complexes in Breast Cancers. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3439–3442. [CrossRef]
55. Sobhani, N.; D'Angelo, A.; Wang, X.; et al. Mutant p53 as an Antigen in Cancer Immunotherapy. *Cancers* **2020**, *21*, 4087. [CrossRef]
56. Hu, S.; Arellano, M.; Boontheung, P.; et al. Salivary Proteomics for Oral Cancer Biomarker Discovery. *Clin. Cancer Res.* **2008**, *14*, 6246–6252. [CrossRef]
57. Motahari, P.; Zamiri, R.E.; Fatemi, M. Evaluation of Salivary Level of Heat Shock Protein 70 in Patients With Breast Cancer. *Iran. J. Breast Dis.* **2022**, *14*, 21–30. [CrossRef]
58. Bloch, O.; Crane, C.A.; Fuks, Y.; et al. Heat-Shock Protein Peptide Complex-96 Vaccination for Recurrent Glioblastoma: A Phase II, Single-Arm Trial. *Neuro-Oncol.* **2014**, *16*, 274–279. [CrossRef]
59. Bloch, O.; Lim, M.; Sughrue, M.E.; et al. Autologous Heat Shock Protein Peptide Vaccination for Newly Diagnosed Glioblastoma: Impact of Peripheral PD-L1 Expression on Response to Therapy. *Clin. Cancer Res.* **2017**, *23*, 3575–3584. [CrossRef]
60. Ji, N.; Zhang, Y.; Liu, Y.; et al. Heat Shock Protein Peptide Complex-96 Vaccination for Newly Diagnosed Glioblastoma: A Phase I, Single-Arm Trial. *JCI Insight* **2018**, *3*, e122229. [CrossRef]
61. Crane, C.A.; Han, S.J.; Ahn, B.; et al. Individual Patient-Specific Immunity Against High-Grade Glioma After Vaccination With Autologous Tumor Derived Peptides Bound to the 96 KD Chaperone Protein. *Clin. Cancer Res.* **2013**, *19*, 205–214. [CrossRef]
62. Pilla, L.; Patuzzo, R.; Rivoltini, L.; et al. A Phase II Trial of Vaccination With Autologous, Tumor-Derived Heat-Shock Protein Peptide Complexes Gp96, in Combination With GM-CSF and Interferon-Alpha in Metastatic Melanoma Patients. *Cancer Immunol. Immunother.* **2006**, *55*, 958–968. [CrossRef]
63. Testori, A.; Richards, J.; Whitman, E.; et al. Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein Gp96 Peptide Complex Vaccine, With Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group. *J. Clin. Oncol.* **2008**, *26*, 955–962. [CrossRef]
64. Wood, C.; Srivastava, P.; Bukowski, R.; et al. An Adjuvant Autologous Therapeutic Vaccine (HSPPC-96; Vitespen) Versus Observation Alone for Patients at High Risk of Recurrence After Nephrectomy for Renal Cell Carcinoma: A Multicentre, Open-Label, Randomised Phase III Trial. *Lancet* **2008**, *372*, 145–154. [CrossRef]
65. Trimble, C.L.; Peng, S.; Kos, F.; et al. A Phase I Trial of a Human Papillomavirus DNA Vaccine for HPV16+ Cervical Intraepithelial Neoplasia 2/3. *Clin. Cancer Res.* **2009**, *15*, 361–367. [CrossRef]
66. Shimizu, Y.; Yoshikawa, T.; Kojima, T.; et al. Heat Shock Protein 105 Peptide Vaccine Could Induce Antitumor Immune Reactions in a Phase I Clinical Trial. *Cancer Sci.* **2019**, *110*, 3049–3060. [CrossRef]
67. Van Doorslaer, K.; Reimers, L.L.; Studentsov, Y.Y.; et al. Serological Response to an HPV16 E7 Based Therapeutic Vaccine in Women With High-Grade Cervical Dysplasia. *Gynecol. Oncol.* **2010**, *116*, 208–212. [CrossRef]
68. Einstein, M.H.; Kadish, A.S.; Burk, R.D.; et al. Heat Shock Fusion Protein-Based Immunotherapy for Treatment of Cervical Intraepithelial Neoplasia III. *Gynecol. Oncol.* **2007**, *106*, 453–460. [CrossRef]
69. Victora, G.D.; Socorro-Silva, A.; Volsi, E.C.; et al. Immune Response to Vaccination With DNA-Hsp65 in a Phase I Clinical Trial With Head and Neck Cancer Patients. *Cancer Gene Ther.* **2009**, *16*, 598–608. [CrossRef]
70. Roman, L.D.; Wilczynski, S.; Muderspach, L.I.; et al. A Phase II Study of Hsp-7 (SGN-00101) in Women With High-Grade Cervical Intraepithelial Neoplasia. *Gynecol. Oncol.* **2007**, *106*, 558–566. [CrossRef]
71. Maki, R.G.; Livingston, P.O.; Lewis, J.J.; et al. A Phase I Pilot Study of Autologous Heat Shock Protein Vaccine HSPPC-96 in Patients With Resected Pancreatic Adenocarcinoma. *Dig. Dis. Sci.* **2007**, *52*, 1964–1972. [CrossRef]
72. Oki, Y.; McLaughlin, P.; Fayad, L.E.; et al. Experience With Heat Shock Protein-Peptide Complex 96 Vaccine Therapy in Patients With Indolent Non-Hodgkin Lymphoma. *Cancer* **2007**, *109*, 77–83. [CrossRef]
73. Palefsky, J.M.; Berry, J.M.; Jay, N.; et al. A Trial of SGN-00101 (HspE7) to Treat High-Grade Anal Intraepithelial Neoplasia in HIV-Positive Individuals. *AIDS* **2006**, *20*, 1151–1155. [CrossRef]
74. Li, Z.; Qiao, Y.; Liu, B.; et al. Combination of Imatinib Mesylate With Autologous Leukocyte-Derived Heat Shock Protein and Chronic Myelogenous Leukemia. *Clin. Cancer Res.* **2005**, *11*, 4460–4468. [CrossRef]
75. Belli, F.; Testori, A.; Rivoltini, L.; et al. Vaccination of Metastatic Melanoma Patients With Autologous Tumor-Derived Heat Shock Protein Gp96-Peptide Complexes: Clinical and Immunologic Findings. *J. Clin. Oncol.* **2002**, *20*, 4169–4180. [CrossRef]

76. Shanmugam, A.; Suriano, R.; Goswami, N.; et al. Identification of Peptide Mimotopes of Gp96 Using Single-Chain Antibody Library. *Cell Stress Chaperones* **2011**, *16*, 225–234. [CrossRef]
77. Wang, X.Y.; Sun, X.; Chen, X.; et al. Superior Antitumor Response Induced by Large Stress Protein Chaperoned Protein Antigen Compared With Peptide Antigen. *J. Immunol.* **2010**, *184*, 6309–6319. [CrossRef]
78. Massé, D.; Ebstein, F.; Bougras, G.; et al. Increased Expression of Inducible HSP70 in Apoptotic Cells Is Correlated With Their Efficacy for Antitumor Vaccine Therapy. *Int. J. Cancer* **2004**, *111*, 575–583. [CrossRef]
79. Gong, J.; Zhang, Y.; Durfee, J.; et al. A Heat Shock Protein 70-Based Vaccine With Enhanced Immunogenicity for Clinical Use. *J. Immunol.* **2010**, *184*, 488–496. [CrossRef]
80. Enomoto, Y.; Bharti, A.; Khaleque, A.A.; et al. Enhanced Immunogenicity of Heat Shock Protein 70 Peptide Complexes From Dendritic Cell–Tumor Fusion Cells. *J. Immunol.* **2006**, *177*, 5946–5955. [CrossRef]
81. Kang, J.; Lee, H.J.; Lee, J.; et al. Novel Peptide-Based Vaccine Targeting Heat Shock Protein 90 Induces Effective Antitumor Immunity in a HER2+ Breast Cancer Murine Model. *J. Immunother. Cancer* **2022**, *10*, e004830. [CrossRef]
82. Stetler, R.A.; Gan, Y.; Zhang, W.; et al. Heat Shock Proteins: Cellular and Molecular Mechanisms in the Central Nervous System. *Prog. Neurobiol.* **2010**, *92*, 184–211. [CrossRef]
83. Nayak, D.A.; Binder, R.J. Agents of Cancer Immunosurveillance: HSPs and dsDNA. *Trends Immunol.* **2022**, *43*, 404–413. [CrossRef]
84. Pradeu, T.; Cooper, E.L. The Danger Theory: 20 Years Later. *Front. Immunol.* **2012**, *3*, 287. [CrossRef]
85. Santos, T.G.; Martins, V.R.; Hajj, G.N.M. Unconventional Secretion of Heat Shock Proteins in Cancer. *Int. J. Mol. Sci.* **2017**, *18*, 945. [CrossRef]
86. Milani, V.; Noessner, E.; Ghose, S.; et al. Heat Shock Protein 70: Role in Antigen Presentation and Immune Stimulation. *Int. J. Hyperthermia* **2002**, *18*, 563–575. [CrossRef]
87. Bastaki, N.K.; Albarjas, T.A.; Almoosa, F.A.; et al. Chronic Heat Stress Induces the Expression of HSP Genes in the Retina of Chickens (Gallus gallus). *Front. Genet.* **2023**, *14*, 1085590. [CrossRef]
88. Bendz, H.; Ruhland, S.C.; Pandya, M.J.; et al. Human Heat Shock Protein 70 Enhances Tumor Antigen Presentation Through Complex Formation and Intracellular Antigen Delivery Without Innate Immune Signaling. *J. Biol. Chem.* **2007**, *282*, 31688–31702. [CrossRef]
89. Horváth, I.; Multhoff, G.; Sonnleitner, A.; et al. Membrane-Associated Stress Proteins: More Than Simply Chaperones. *Biochim. Biophys. Acta Biomembr.* **2008**, *1778*, 1653–1664. [CrossRef]
90. Linderoth, N.A.; Popowicz, A.; Sastry, S. Identification of the Peptide-Binding Site in the Heat Shock Chaperone/Tumor Rejection Antigen gp96 (Grp94). *J. Biol. Chem.* **2000**, *275*, 5472–5477. [CrossRef]
91. Schueller, G.; Stift, A.; Friedl, J.; et al. Hyperthermia Improves Cellular Immune Response to Human Hepatocellular Carcinoma Subsequent to Co-Culture With Tumor Lysate Pulsed Dendritic Cells. *Int. J. Oncol.* **2003**, *22*, 1397–1402. [CrossRef]
92. Falahi, S.; Kenarkoohi, A. Host Factors and Vaccine Efficacy: Implications for COVID-19 Vaccines. *J. Med. Virol.* **2022**, *94*, 1330–1335. [CrossRef]
93. Singh, D.N.; Daripelli, S.; Bushara, M.O.E.; et al. Genetic Testing for Successful Cancer Treatment. *Cureus* **2023**, *15*, e49889. [CrossRef]
94. Coutinho, F.A.B.; Amaku, M.; Boulos, F.C.; et al. Analysing Vaccine Efficacy Evaluated in Phase 3 Clinical Trials Carried Out During Outbreaks. *Infect. Dis. Model.* **2024**, *9*, 1027–1044. [CrossRef]
95. Pollard, A.J.; Bijker, E.M. A Guide to Vaccinology: From Basic Principles to New Developments. *Nat. Rev. Immunol.* **2021**, *21*, 83–100. [CrossRef]
96. Corthay, A. Does the Immune System Naturally Protect Against Cancer? *Front. Immunol.* **2014**, *5*, 197. [CrossRef]
97. Yamaguchi, H.; Hiroi, M.; Mori, K.; et al. Simultaneous Expression of Th1- and Treg-Associated Chemokine Genes and CD4(+), CD8(+), and Foxp3(+) Cells in the Premalignant Lesions of 4NQO-Induced Mouse Tongue Tumorigenesis. *Cancers* **2021**, *13*, 1988. [CrossRef]
98. Kogame, M.; Nagai, H.; Shinohara, M.; et al. Th2 Dominance Might Induce Carcinogenesis in Patients With HCV-Related Liver Cirrhosis. *Anticancer Res.* **2016**, *36*, 4529–4536. [CrossRef]
99. Kany, S.; Vollrath, J.T.; Relja, B. Cytokines in Inflammatory Disease. *Int. J. Mol. Sci.* **2019**, *20*, 6008. [CrossRef]
100. Chen, L.; Deng, H.; Cui, H.; et al. Inflammatory Responses and Inflammation-Associated Diseases in Organs. *Oncotarget* **2018**, *9*, 7204–7218. [CrossRef]
101. Kiros, T.G.; Levast, B.; Auray, G.; et al. The Importance of Animal Models in the Development of Vaccines: Innovation in Vaccinology. In *The Science and Practice of Vaccinology*; Gerdts, V., Ed.; Springer: Dordrecht, Netherlands, 2012; pp. 251–264. [CrossRef]

102. Beheshtizadeh, N.; Gharibshahian, M.; Pazhouhnia, Z.; et al. Commercialization and Regulation of Regenerative Medicine Products: Promises, Advances and Challenges. *Biomed. Pharmacother.* **2022**, *153*, 113431. [CrossRef]
103. Sampathkumar, K.; Kerwin, B.A. Roadmap for Drug Product Development and Manufacturing of Biologics. *J. Pharm. Sci.* **2024**, *113*, 314–331. [CrossRef]
104. Srivastava, S.; Singh, D.; Verma, S.K.; et al. Recent Advancements in Cancer Vaccines: A Systematic Review. *Vacunas* **2024**, *25*, 97–108. [CrossRef]
105. Rulten, S.L.; Grose, R.P.; Gatz, S.A.; et al. The Future of Precision Oncology. *Int. J. Mol. Sci.* **2023**, *24*, 9110. [CrossRef]
106. Harrop, R. Cancer Vaccines: Identification of Biomarkers Predictive of Clinical Efficacy. *Hum. Vaccin. Immunother.* **2013**, *9*, 800–804. [CrossRef]
107. Alum, E.U. AI-Driven Biomarker Discovery: Enhancing Precision in Cancer Diagnosis and Prognosis. *Discov. Oncol.* **2025**, *16*, 313. [CrossRef]
108. Passaro, A.; Al Bakir, M.; Hamilton, E.G.; et al. Cancer Biomarkers: Emerging Trends and Clinical Implications for Personalized Treatment. *Cell* **2024**, *187*, 1617–1635. [CrossRef]