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Vitespen

Cancer Vaccine

USAN

HSPPC-96 (former name)
Oncophage®

Cancer vaccine based on autologous, tumor-derived heat shock protein-peptide complexes

Glucose-regulated protein 94 (GRP94)

Endoplasmin (human tumor rejection antigen 1)

CAS: 492448-75-6

EN: 264271

Abstract

Heat shock proteins (HSPs) are highly conserved stress-induced proteins which function as chaperones for trafficking peptides inside different cellular compartments and delivering peptides. Tumor-derived HSP-peptide complexes (HSPPCs) can be used for vaccination against malignancies. An HSP-based vaccine is a personalized vaccine that carries the fingerprint of a given tumor, circumventing the need for identifying tumor antigens for each individual cancer. Vitespen (Oncophage®, formerly HSPPC-96) is a novel vaccine preparation with a promising role in cancer management. This vaccine has essentially no toxicity and autoimmunity has not been observed. Vitespen has been extensively studied in multiple clinical trials in the phase I and II setting, demonstrating activity in several malignancies, including pancreatic cancer, colorectal cancer, chronic myelogenous leukemia (CML) and non-Hodgkin's lymphoma (NHL), as well as phase III trials in kidney cancer and metastatic melanoma. The vaccine just recently received approval from the Russian authorities for use in intermediate-risk kidney cancer.

Background

Heat shock proteins (HSPs) are highly conserved stress-induced proteins which function as chaperones for trafficking peptides inside different cellular compartments and delivering peptides, including immunogenic peptides, to be presented by major histocompatibility complex (MHC) class I molecules. HSPs exist across all species and are classified by their molecular weights in five distinct families: HSP-110, -90, -70, -60 and -28 (1). Within these families, glucose-regulated protein 94 (GRP94, or glycoprotein 96 [gp96]), HSP-90, HSP-70, HSP-110 and GRP170 have been shown to induce cellular immunity against tumors from which they were isolated. HSP itself is not immunogenic, but as a carrier or chaperone of antigenic peptides it forms a complex which induces tumor-specific immunity (2). Thus, tumor derived HSP-peptide complexes (HSPPCs) can be used for vaccination against malignancies.

An HSP-based vaccine is a personalized vaccine that carries the fingerprint of a given tumor, circumventing the need for identifying tumor antigens for each individual cancer. The principle works across all tumor types and bypasses the need for identifying specific tumor-associated antigens. Thus, HSP-based vaccines can be generated from various kinds of malignancies using essentially the same method of preparation. Despite this simple and practical approach, the number of vaccine doses that can be prepared from an individual tumor can be limited and varies depending on tumor size.

It has also been reported that HSP itself, independent of the bound peptide, can induce the production of cytokines, such as IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor α (TNF-α), from antigen-presenting cells (APCs) (3), and IL-1β, IL-6 and TNF-α from monocytes (4). Furthermore, HSP-70 is known to induce the maturation of immature dendritic cells, as evidenced by upregulation of costimulatory molecules such as CD40, CD83 and CD86, although dendritic cell differentiation from monocyte precursors is reduced by HSP-70 (5). HSP-70 and the *C*-terminal domain of HSP-70 also stimulate the proliferation and cytotoxic activity of natural killer (NK) cells (6, 7). This NK cell-stimulating activity does not appear to involve the above-mentioned HSP receptors, but rather the NK receptor CD94 (8). These "natural adjuvant effects" of HSP are considered to be critical for the generation of an efficient immune response.

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The proposed mechanism of immune response induced by vitespen (Oncophage®, formerly HSPPC-96) is illustrated in Figure 1. Once the vaccine is injected intradermally, peptides chaperoned by gp96 or HSP-70 can be taken up by APCs, *i.e.*, macrophages and dendritic cells. Although CD91 is known to play an essential role as a receptor for HSPs (9-11), CD91-independent HSP internalization pathways have also been reported (12), such as the Toll-like receptor 2/4 pathway (13) or the lipopolysaccharide receptor CD14-dependent pathway (4). Internalized vaccine is modulated in the APCs, which finally present the tumor-derived peptide on their MHC class I molecules.

Preparation

To prepare HSP-based vaccines from autologous tumors, a tumor specimen is first surgically removed from a patient with cancer. Ideally, the tumor should be firm, homogeneous-looking and comprised of non-necrotic tissue without friable, fatty or cystic tissue. In general, a minimum of 1-5 g of tumor tissue for low-dose vaccination, and up to 10 g of tumor tissue for high-dose vaccination, is required to yield a sufficient amount of the HSP gp96-peptide complex (HSPPC-96) vaccine. The required size of the tumor, however, varies depending on the tumor type and the intended vaccine dose and schedule. The specimen is homogenized and passed through sequential concanavalin-A and DEAE Sephacel columns, using a liquid chromatography system or the Biocad system. Fractions of HSPPC-96 are stored at –80 ºC. Although the actual laboratory process takes 1 or 2 days, the whole commercial process, from bed to bench and back to bed, takes 6-8 weeks (14, 15).

The ability to purify HSP from a cancer depends on the cancer type. HSP can be purified for vaccine preparation from most colorectal cancers, renal cell carcinomas, lym-

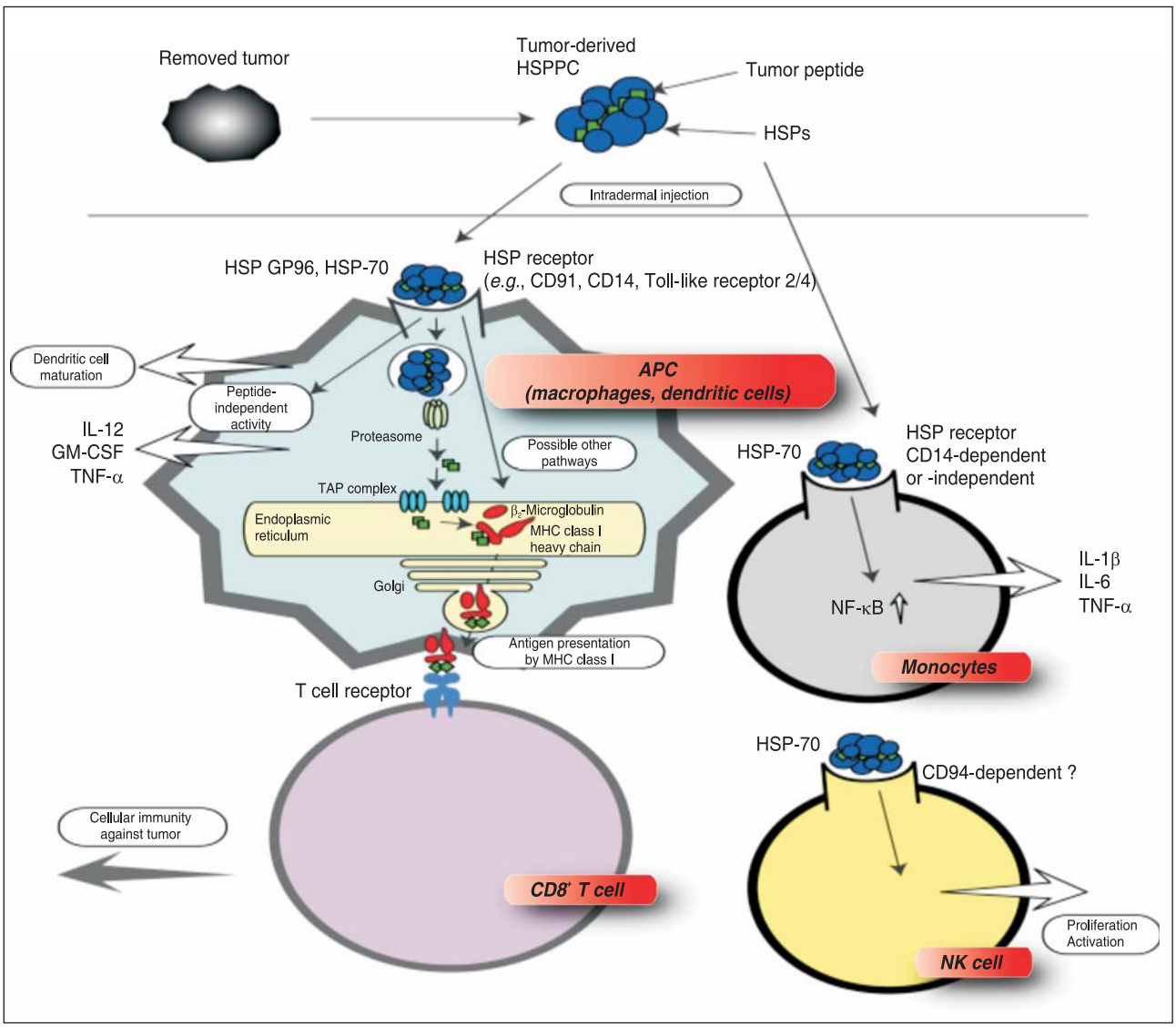

Fig. 1. A proposed mechanism of action for HSPPC-based vaccines.

phomas and melanomas. In contrast, it has been technically difficult to obtain HSP from gastric and pancreatic cancer, owing to proteolysis of the tumor sample in the vaccine preparation. Recent advances in purification techniques to prevent proteolysis have improved the yield of vaccine preparation from these malignancies (14, 15).

Preclinical Pharmacology

Immunogenicity is a unique property of HSPs, as seen in mice immunized with HSP-peptide complex-primed anti-ovalbumin T cells (16). Other peptide-binding proteins failed to prime immune responses. Mice immunized with tumor-derived HSPs are protected against a subsequent challenge with the same tumor, as shown in a large number of tumor models. In almost all of the antigenic systems tested, T cell responses have been primarily responsible for the rejection of tumors. Furthermore, treatment of mice bearing several different tumor types demonstrated an antitumor effect, resulting in reduction of tumor size and improvement in survival (17). The immune cells involved in this tumor rejection were shown to be T cell-dependent, while also displaying a role for NK cells. Such studies provided the basis for the development of HSP-based vaccines. Table I summarizes selected studies revealing the potential of HSPs to induce immunity in mice against syngeneic cancer cells.

Clinical Studies

Several clinical trials have evaluated the safety and efficacy of vitespen in patients with cancer (Table II). The first human phase I clinical trial was conducted by Janetzki *et al*. in patients with advanced solid tumors (18). In this study, 16 patients were treated with 25 μg of vitespen administered by s.c. injection weekly for 4 consecutive weeks. $CD8^+$-restricted responses against autologous tumors were observed in 6 of 12 evaluated patients. No major clinical responses were reported, but no significant vaccine-related toxicity was observed either. Data from several vitespen trials in various types of cancer are summarized below.

Melanoma

Melanoma is one of the most immunogenic malignancies, for which immunomodulators such as interferon and interleukin have been used as treatment modalities. Eton *et al*. presented data from a phase I trial in 36 patients using 2.5, 25 or 100 μg vitespen weekly for 4 doses (19). No clinical toxicity was observed. One patient at each dose level had disease stabilization or a mixed response after initial progression, all lasting more than 7 months. Eleven of 12 stage IV adjuvant patients remained free of disease for a median of 11+ months.

The results from a phase II vitespen vaccine study in patients with metastatic melanoma were recently reported (20). Among 64 patients who underwent surgical resection of metastatic tumors required for vaccine production, 42 were able to receive the vaccine. Patients were vaccinated starting 5-8 weeks after resection using 5 or 50 μg of vitespen given s.c. or intradermally. Thirty-nine patients were assessable after 1 cycle of 4 weekly intradermal vaccinations. Twenty-one patients received a

Table I: Animal models of immunity to cancer elicited by tumor-derived HSPPC.

Cancer type	Model	Molecule(s)	Ref.
Fibrosarcoma	Meth A	HSP gp96 HSP-70	40-42 43
	CSM5	HSP gp96	41
	CSM13	HSP gp96	44
Hepatoma	Zajdela	HSP gp96	45
Squamous cell carcinoma	UV6138	HSP gp96	46
	UV6139SJ	HSP gp96 HSP-70	17, 46 17
Colon carcinoma	CT26	HSP-110/GRP170 HSP gp96/HSP-70	17, 42, 47 17
Melanoma	B16	HSP gp96 HSP-70	17, 48, 49 49
Lung carcinoma	D122	HSP gp96/HSP-70	17
Leukemia	A20	HSP gp96 HSP-70	50 50, 51
Lymphoma	15/0	HSP gp96/HSP-70	52
Prostate	Dunning G	HSP gp96	53
	TRAMP-C2	HSP-70	49

HSPPC, heat shock protein-peptide complex.

Table II: List of clinical trials with vitespen vaccine.

Cancer type	Clinical stage	Phase	Patients	Dose/schedule	Ref.
Gastric cancer	Advanced	I	10	2.5/25/100 μg QW x 4	30
	Advanced	I	15	2.5/15/25/100 μg QW x 4-9	31
Melanoma	Stage IV	I/II	36	2.5/25/100 μg QW x 4	19
	Stage IV	II	39	5/50 μg QW x 4 then Q2W x 4	20
	Stage IV	III	350+		C-100-21 (NCT00039000)
Renal cell carcinoma	Stage IV	I/II	38	2.5/25/100 μg QW x 3	22
	Stage IV	II	70	25 μg QW x 4 then Q2W	23
	Stage IV	II	61	25 μg QW x 4 then Q2W	24
	Stage I-IV	III	650+	25 μg QW x 4 then Q2W	C-100-12 (NCT00126178; NCT00033904)
Pancreatic cancer	Stage I-III	I	10	5 μg QW x 4	32
			9	6 injections over 6 months	33
Lymphoma	Low-grade	II	10	25 μg QW x 4 then Q2W	26, 27
	Low-grade	II	20	25 μg QW x 4 then Q2W	28
Colorectal cancer	Adjuvant	II	29	2.5/25/100 μg QW x 8	34
			15	6 injections over 6 months	33
Chronic myelogenous leukemia	Chronic phase	I	14	50 μg QW x 8	35
Ovarian cancer	III/IV	I	5	25 μg QW x 8	29

QW, weekly; Q2W, biweekly.

second cycle, which consisted of 4 additional biweekly injections. Of 28 patients with measurable disease, 2 had a complete response and 3 had stable disease at the end of follow-up. An enzyme-linked immunospot (ELISpot) assay using peripheral blood mononuclear cells from 23 patients showed a significantly increased number of post-vaccination melanoma-specific T cell spots in 11 patients, with clinical responders displaying a high frequency of increased T cell activity.

In a phase III study in patients with stage IV melanoma, patients were randomized to receive vitespen or any another treatment which included IL-2 and/or dacarbazine/temozolomide and/or tumor resection (physician's choice) (21). Vitespen was administered s.c. weekly for 4 weeks, then biweekly until disease progression or vaccine depletion. Preliminary data demonstrated that patients who received 10 or more doses of vaccine with M1b-stage disease survived longer than those who were randomized to the physician's choice arm. For those with M1a-stage disease, patients in the vaccine arm survived longer than those in the physician's choice arm.

Renal cell carcinoma

Renal cell carcinoma (RCC) is another malignancy that is chemoresistant and in which immunomodulators have a significant role in treatment. Amato *et al*. studied the vitespen vaccine in a phase I trial in patients with RCC (22). The schedule was 4 weekly intradermal vitespen doses of 2.5, 25 or 100 μg based on the amount of vitespen that could be prepared, followed by monthly re-evaluation. Revaccination was performed depending on the patient's response. Among 42 patients who entered the study, 29 completed the 4 weekly injections and a 4-week follow-up. One had a complete remission, 3 had a partial remission and 18 showed stable disease or slight progression, whereas 17 experienced progression. A phase II trial from the same team followed, involving 70 patients (23). A dose of 25 μg intradermal vitespen was used. Patients who exhibited disease progression continued with the vaccine plus 11 million units of IL-2 for 4 consecutive weeks. No significant adverse effects were observed. The addition of IL-2 did not appear to provide additional benefit to patients.

Assikis *et al*. presented the results from a phase II study of vitespen for metastatic RCC (24). The vaccination schedule consisted of 25 μg of vitespen by intradermal injection at weekly intervals during weeks 1-4, followed by injection every 2 weeks until progression. Sixty-one patients received at least one dose of vaccine. No significant adverse effects were observed. One patient had a complete remission, 2 had a partial remission and 18 had stable disease. Of those who pro-

gressed, 7 of 16 responded clinically to the addition of IL-2. Median progression-free survival (PFS) was 18 weeks and 25 weeks if IL-2 was added; 30% of patients were alive after 2 years.

More recently, an ongoing randomized phase III trial investigating the use of vitespen *versus* observation in patients with RCC postnephrectomy reported high levels of accrual and vaccine production (25). Endpoints are recurrence-free survival and overall survival.

Non-Hodgkin's lymphoma

A phase II study of vitespen vaccine therapy in patients with indolent non-Hodgkin's lymphoma (NHL) was recently performed (26, 27). Patients were enrolled if they had newly diagnosed or relapsed low-grade lymphoma, including Waldenström's macroglobulinemia. Patients received 4 doses of 25 μg of autologous tumor-derived vitespen every 2 weeks and were then evaluated for tumor response status at week 14. If the tumors were improving or stable, patients continued treatment with 25 μg vitespen at 2-week intervals until disease progression or the available vaccine was finished, not to exceed 1 year of therapy. A full tumor assessment was performed every 3 months. The preliminary results from the first 14 patients showed that vaccine could be prepared from 10 patients. One patient with relapsed marginal-zone lymphoma had a partial remission and 2 patients had a minor response; 3 had stable disease. Vaccination was well tolerated, without adverse effects.

Twenty patients with indolent NHL were enrolled in a phase II trial and treated with vitespen vaccine (28). They were treated with 25 μg by intradermal injection weekly for 4 weeks and then every 2 weeks thereafter. This continued until either disease progression, grade 3 or 4 toxicity, or the vaccination supply was finished. One patient with marginal-zone lymphoma showed a response to therapy but did not meet criteria for complete or partial remission. Many of the patient's skin nodules exhibited signs of response at 3 months and after biopsy showed significant reactive lymphocyte infiltration and the absence of lymphoma cells. Eight patients had stable disease lasting more than 6 months and 4 had stable disease for more than a year. The median FFS (failure-free survival) was 5.2 months.

Ovarian cancer

The vitespen vaccine is currently being evaluated in patients with ovarian cancer in a consolidative setting (29). Patients with stage III/IV ovarian cancer and no evidence of progressive disease after standard carboplatin/taxane therapy will receive 8 weekly doses of 25 μg vitespen intradermally (n=12) or vitespen plus GM-CSF (n=12). Recent reports indicate that 5 patients completed the vaccination with vitespen. All demonstrated increased NK cell activity against K-562 cells. No grade 2 or higher toxicities were observed. At the time of the first report, none of the patients had been treated with vitespen + GM-CSF.

Gastric cancer

Tumor vaccination with vitespen was first used in patients who underwent curative surgery for gastric cancer (30). At 4-8 weeks after surgery, patients were vaccinated 4 times in weekly intervals with 2.5, 25 or 100 μg of vitespen. Vaccine preparation was successful in 10 of 13 patients. After a median follow-up of 9 months from surgery, 5 of 10 patients who received the vaccine remained disease-free.

Another phase I study was conducted in patients with gastric carcinoma undergoing surgery with curative intent (31). Patients were vaccinated 4-9 times at weekly intervals with 2.5, 15, 25 or 100 μg intradermal vitespen. Vaccine was prepared in 18 of 21 patients; 3 were not eligible because of tumor recurrence and 15 patients were vaccinated. No toxicity or autoimmunity was observed. After a median follow-up of 32 months from surgery, 3 patients remained disease-free; 12 patients had tumor recurrence. The median disease-free and overall survival of the 15 vaccinated patients was 7 and 16+ months, respectively, with 9 of 15 patients still alive at the time of reporting. Immune monitoring showed an expansion of $CD8^+CD45RO^+$ memory T cells in 8 of 11 and an expansion of $CD8^+$ T cells with specific TCR-V-β subtypes in 2 of 9 evaluated patients.

Pancreatic cancer

Pancreatic cancer is often found in an advanced stage and has an extremely poor prognosis even after complete resection of the tumor. New treatment modalities therefore need to be investigated to improve the prognosis. In this type of malignancy, there were some technical difficulties in the preparation of vitespen from resected tumors due to the small size of these tumors and proteolytic enzymes in the pancreatic tissue. With improved techniques, 10 of 20 patients with resected pancreatic cancer were vaccinated with 4 weekly doses of 5 μg of tumor-derived vitespen (32). Three patients were alive and disease-free at 5.0, 2.6 and 2.7 years, with a median overall survival of 2.2 years. ELISpot assay of autologous T cells against APCs loaded with autologous HSPPC-96 showed a substantial increase in ELISpot number in 1 patient of the first 5 examined. Two of 5 patients had increases in ELISpot number of borderline significance.

A pilot study was performed in order to investigate whether antitumor immunity was induced by HSP-70 by purifying HSP-70-peptide complexes from patients with pancreatic cancer (33). Each patient was injected with autologous HSP six times over half a year. This trial also included patients with colorectal cancer. All patients showed expansion of $CD8^+$ T lymphocytes and NK cells. Seventeen of 24 patients who received vaccination with HSP-70 elicited a specific response of potent $CD8^+$ T lymphocytes cytotoxic to the carcinoma cell line *in vitro*. These results suggest that HSP immunization leads to immune responses.

Colorectal cancer

Vitespen vaccination was also used in patients with colorectal cancer and liver metastasis (34). Twenty-nine consecutive patients received autologous tumor-derived vitespen, following the schedule of 4 weekly injections followed by 4 biweekly injections after 8 weeks. A class I HLA-restricted T cell-mediated anti-colon cancer response was observed in 15 patients. No relevant toxicity was seen. Patients with an immune response had a statistically significant clinical advantage over nonresponding subjects (2-year overall survival = 100% *vs*. 50%; disease-free survival = 51% *vs*. 8%). The observed effect of postvaccine immune response on patients' outcome was independent of the clinical prognostic categories of favorable *versus* unfavorable, according to the Memorial Sloan-Kettering Cancer Center (MSKCC) score (scored based on the size and number of tumor nodules, the clearance of surgical margins, the status of liver hilum lymph nodes, and the CEA level).

As discussed above for pancreatic carcinoma, HSP-70 vaccination in patients with colorectal cancer showed a specific immune response against cell lines *in vitro* (33). No significant toxicity was reported using the vaccine and expansion of the $CD8^+$ T lymphocyte and NK cell populations was observed.

Chronic myelogenous leukemia

Li *et al*. presented an updated phase I study of the vitespen vaccine combined with imatinib mesilate in patients with chronic-phase chronic myelogenous leukemia (CML) (35). Patients were immunized weekly with 50 μg intradermally for 8 weeks. Of the 18 eligible patients, 14 had completed the study. The vaccines were successfully prepared for all patients and were well tolerated. Ten of 14 patients had either a reduction in the number of Philadelphia chromosome-positive (Ph^+) cells or a decreased level of BCR/ABL transcript in a polymerase chain reaction (PCR)-based assay. Moreover, all patients remained in chronic phase at the end of vaccinations.

Marin *et al*. (36) reported complete cytogenetic responses (CCyR) in patients with Ph^+ CML in first chronic phase who were cytogenetically positive following prior treatment with imatinib monotherapy. AG-858, another autologous polyvalent HSP-peptide complex vaccine, was administered at 50 μg intradermally once weekly for up to 8 weeks. No serious adverse events related to the vaccine were reported. The data suggest that AG-858 vaccination may induce CCyR in those with imatinib-resistant CML with low cytogenetic disease burden. Future studies are planned to evaluate whether booster vaccinations affect the duration of response.

Vitespen is also being evaluated in a phase I/II trial in patients with recurrent or progressive glioma (37) and in a phase II trial in patients with non-small cell lung cancer (NSCLC) (38). The vaccine holds fast track and orphan drug designations from the U.S. FDA for both kidney cancer and metastatic melanoma, as well as European orphan drug status for kidney cancer. The Russian Ministry of Public Health has approved its use for the treatment of kidney cancer patients with an intermediate risk for disease recurrence (39).

Conclusions

Vitespen is a novel vaccine preparation with a promising role in cancer management. This vaccine has essentially no toxicity and autoimmunity has not been observed. HSP-70 and vitespen have been extensively studied in multiple clinical trials in the phase I and II setting, demonstrating activity in several malignancies. Phase III studies have been completed for RCC and malignant melanoma.

New strategies will need to be evaluated to explore enhancing vaccine efficacy. For example, the potential benefit of using a combination of multiple different chaperone proteins to vaccinate patients, such as HSP-70 and vitespen, may increase the peptide repertoire, which may improve vaccine efficacy. Furthermore, because metastatic lesions could potentially have developed different subclones that express different tumor-associated peptides from that of the primary tumor, it is possible to improve the immune response by vaccination with vitespen derived from primary and metastatic sites. These hypotheses will need to be addressed by carefully designed studies.

The obvious limitation of an HSP-based vaccine is the limited amount of vaccines that can be purified from a particular tumor. It is not known whether the response elicited by tumor-derived vitespen will be long lasting and will not require repeated vaccination. Since the treatment will have to be stopped when the vaccine preparation is exhausted, the dose schedule for the use of vitespen is critical and needs to be clarified in clinical trials. Moreover, a powerful method to purify vaccine preparation with higher yield needs to be investigated.

Source

Antigenics, Inc. (US).

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