**SANTAVAC™: A Novel Universal Antigen Composition for Developing Cancer Vaccines**

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**Abstract**: Background: Development of a universal cancer vaccine for the prevention of all cancers has been under development for many years. Antiangiogenic cancer vaccines elicit immune responses with the potential of destroying tumor vasculature endothelial cells without affecting vasculature integrity in normal tissues. The methods used in the development of antigen compositions comprising these vaccines have been recently improved and described in this report in the context of SANTAVAC™ development - the first cancer vaccine based on endothelial cell heterogeneity.

Methods: The present report summarizes data related to SANTAVAC™ development, including technical key points associated with optimal SANTAVAC™ production, a description of the composition required for preparing cancer vaccines with the highest predicted efficacy and safety, and a strategy for SANTAVAC™ large-scale implementation. Patents related to SANTAVAC™ and other universal cancer vaccines are also described.

Results: SANTAVAC™ was shown to be the most promising antigen composition for anti-cancer vaccination, allowing for immune targeting of the tumor vasculature in experimental models with a high predicted efficacy (up to 60), where efficacy represents the fold decrease in the number of endothelial cells with a tumor-induced phenotype and directly related to predicted arrest of tumor growth.

Conclusion: The use of SANTAVAC™ as a universal antigenic composition may spur vaccine development activities resulting in a set of therapeutic or prophylactic vaccines against different types of solid cancers.

**Keywords**: Cancer, vaccine, antigens, universal, endothelial cells, proteomic footprinting, SANTAVAC.

1. **ANTIANGIOGENIC CANCER VACCINES**

Effective treatments for most types of cancers have yet to be developed, and cancer remains the second leading cause of death. Therefore, novel and effective cancer-preventative therapies including cancer vaccines are crucially needed [1]. Destruction of the tumor endothelium as consequence of vaccination has advantages over vaccines that elicit immune responses specific to cancer cells because the tumor endothelium is genetically stable and is less likely to develop escape mutations [2]. Moreover, the endothelial cell (EC) to cancer cell ratio in tumors is approximately 1:100 and because vascular integrity is essential to tumor growth and metastasis, destruction of only a few ECs can lead to vascular obstruction resulting in arrest of tumor growth [3-6]. For these reasons, vaccines targeting the tumor vasculature represent a promising approach for preventing solid tumor growth and metastasis, and can justifiably be referred to as ‘universal cancer vaccines’ (UCV).

The simplest way to target the immune response against the tumor vasculature is by immunizing with antigens derived from ECs resulting in the elicitation of immune responses specific for a comprehensive array of target cell antigens [7-9]. The degree of antigenic composition similarity between a vaccine derived from tumor ECs and non-tumor ECs would directly affect the vaccine efficacy and the chances of eliciting side effects...
resulting in damage to healthy vasculature. Therefore, EC heterogeneity is considered critical to the design of EC-based cancer vaccines because of their ability to induce immune responses targeting tumor vasculature ECs.

EC heterogeneity has been well described [10, 11], including morphology, gene expression profiles, and antigen composition differences between different organs and even tissues within the same organ. Moreover, the gene expression profile of ECs is significantly influenced by growth factors released by tumors that induce angiogenesis required to build the tumor vasculature [12-19]. These data suggest that EC heterogeneity was the cornerstone for EC-based vaccine design capable of eliciting immune responses selectively targeting the tumor vasculature, thereby preventing undesired damage to other vessels that may lead to systemic vasculitis with internal hemorrhage and destruction of internal organs. This is a significant safety concern since it has been shown that experimental autoimmune vasculitis developed following immunization with ECs [20, 21] and several cell-based immunizations have been associated with the elicitation of autoimmunity in animals and humans [22-27].

2. SANTAVAC™ - THE FIRST ANTIGENIC COMPOSITION FOR VACCINATION BASED ON EC HETEROGENEITY

Recently, EC surface profiling experiments have been performed demonstrating that EC heterogeneity was important to vaccine design approaches. Tumor type-specific changes were observed on the surface of cultured human microvascular endothelial cells (HMECs) in the presence of tumor-conditioned medium collected from different cancer cell types [28-35]. Changes in cell surface antigen profiles were characterized by cell proteomic footprinting (CPF), an advanced proteomics approach used to define cell phenotypes via mass spectrometric analysis of the extracellular surface (Fig. 1A, 1B) [36]. A patent related to CPF was issued in 2007 [37]. Findings from previous studies have motivated the use of primary HMECs (Fig. 1C) for these experiments since these cells are involved in tumor angiogenesis, and microvasculature-derived ECs exhibited functional differences compared to large vessel-derived ECs [38, 39], including differences in responses to growth stimulators [40, 41] and extracellular protein expression patterns [42-44]. Examining the relationships between surface profiles within the HMEC group by principle component analysis (PCA) (Fig. 1D) of corresponding CPF demonstrated that tumors induced reproducible tumor type-specific changes to the HMEC surface profile, which ranged from relatively insignificant to pronounced, and that tumor-induced changes to the cell surface profile directly defined HMEC escape from cytotoxic T lymphocyte (CTL)-mediated cell death in an in vitro model of human antiangiogenic vaccination [28, 30]. Taken together, these findings provided useful information regarding the design of SANTAVAC™ (Set of All Natural Target Antigens for Vaccination Against Cancer) - the compositions of specifically derived HMEC surface antigens divergent from those expressed by ECs in normal tissues that prevents the elicitation of autoimmune reactions. SANTAVAC™ can be prepared with different adjuvants to produce vaccines specific against different types of solid cancers that can be tested in vivo for immunogenicity and safety. Between 2007-2015, patents related to SANTAVAC™ development were issued [32-35].

3. SANTAVAC™ COMPOSITION

SANTAVAC™ design processes have focused on using cells as the source of native EC antigens for the elicitation of immune responses against target cells [9, 32-35, 45, 46]. Whole cells possess a set of cell-surface antigens critical to vaccine efficacy [47, 48], in contrast to many ubiquitous intracellular antigens that could elicit various adverse autoimmune responses (Fig. 1A) [31]. To exclude intracellular content from SANTAVAC™, the original approach for the collection of cell surface antigens is used [32]. That is, cell surface targets are accessible to proteases whose enzymatic byproducts can be isolated after in vitro proteolytic cleavage [29, 32, 49-52]. While trypsinizing the surface of live cancer cells yielded a digest containing less than 1% of the total cell protein content, this trypsin digest was more effective than whole cells at eliciting an antitumor immune response [49]. Thus, the digest’s composition, comprised of proteolytically cleaved cell surface targets, was directly related to the killing rate of target cells in cytotoxicity assays (CTA) [36, 50]. These findings suggested that a set of proteolytically cleaved cell surface targets represented the cell’s ‘antigenic essence’ fit for use in vaccine
formulations. SANTAVAC™ represents the ‘anti- 
genic essence’ of HMEC following tumor-induced 
changes [32-35].

The induction of HMEC needed to produce 
SANTAVAC™ requires cancer cells to influence 
the HMEC surface antigen profile. We previously 
demonstrated that tumor-induced HMEC hetero-
genicity was a result of differences in the strength 
of tumor-derived growth signals (independent of 
the tumor) [28]. Based on these observations, it 
was hypothesized that tumors would affect the 
HMEC surface expression profile in the same 
manner in vivo, and that tumor-induced changes to 
the HMEC antigentic profile would be a conse-
quence of the magnitude of the growth stimulus. 
Since stimuli of different strengths can be deliv-
ered simultaneously by tumors in vivo depending 
on the distance from the tumor cells, it can be ex-
pected that HMECs with different target surface 
profiles would also be present in the tumor-
associated vasculature. Obviously, destruction of 
any type of HMEC at any location in the tumor 
vasculature or in vessels near the tumor would 
lead to vessel obstruction and arrest of tumor 
growth. Therefore, this assumption was used to 
define the optimal SANTAVAC™ composition 
that most effectively targeted the immune re-

Fig. (1). Profiling of critical cell surface vaccine targets identified by cell proteomic footprinting. (A) Depiction of 
the relative ratios of surface targets accessible to the immune system and the remaining undesired cellular content. 
Adapted from [31]. (B) Cell proteomic footprinting. Adherent cell cultures were washed to remove traces of culture 
medium and subsequently treated with a protease. Released cell surface protein fragments were collected and subjected 
to mass spectrometry analysis. The set of peptides obtained represents the proteomic footprint. (C) Examples of cell 
proteomic footprints for non-ECs (HepG2) and HMECs induced to grow in the presence of non-tumor stimuli provided 
by EC growth supplement (HMEC_ECGS) or HepG2 cancer cells (HMEC_HepG2). Adapted from [28, 36]. (D) Principle 
component analysis (PCA) of cell footprints obtained from HMECs and control non-ECs (HepG2 and MCF-7) that 
were projected in the space of the first two principal components. PCA shows the degree of difference between cell 
surface profiles. Cell surface profiles are shown for HMECs stimulated to grow in the presence of EC growth supple-
ment (HMEC_ECGS and HMEC_HepG2), human breast adenocarcinoma MCF-7 cell-conditioned medium (HMEC_MCF-7 
and HMEC_CondMCF-7), LNCap human prostate adenocarcinoma cell-conditioned medium (HMEC_LNCap and HMEC_CondLNCap), 
or HepG2 human hepatocellular carcinoma cell-conditioned medium (HMEC_HepG2 and HMEC_CondHepG2). Superscript 
numbers correspond to different HMEC primary cultures. Adapted from [28].
response. This was measured using CTAs of tumor-induced HMECs and the predicted efficacy in vivo was measured for respective compositions.

4. PREDICTED EFFICACY OF ALLOGENEIC AND AUTOLOGOUS SANTAVAC™

A critical key to finding the optimal SANTAVAC™ composition with maximum efficacy was based on the observation that HMEC targets that had a surface profile induced by human prostate adenocarcinoma (LNCap) cells were effectively killed by a SANTAVAC™ formulation generated from HMECs induced by human hepatocellular carcinoma (HepG2) cells [28]. These findings supported the in vitro design of autologous SANTAVAC™ with a targeting efficacy of 1.7 (i.e., 1.7 tumor ECs were destroyed before one EC in normal tissue was destroyed) [28, 53]. This efficacy provides a therapeutic window in which tumor HMEC cells could be killed by SANTAVAC™-induced immune responses before normal tissue HMECs (including ECs involved in angiogenesis) are adversely affected.

Because the obtained experimental data were limited by the availability of specific antigen compositions, gaps in the experimental data were filled by approximation of experimental data. Analysis of the data suggested that the efficacy of the autologous SANTAVAC™ may exceed 18 (Fig. 2C). To reach such an efficacy level, the SANTAVAC™ composition and the profile of target HMECs should be quite similar (correlation coefficient for their CPF should exceed 0.82) [30]. Additionally, it was concluded that specific experimental points were required to directly observe the maximum efficacy for SANTAVAC™ in experiments designed to establish the maximum allogeneic SANTAVAC™ efficacy.

Although alloanitgens elicit lower target cell killing rates than autoantigens, the use of alloanitgens allows for the use of the patient’s own biomaterial to be excluded from vaccine preparation, thereby simplifying research, development, and facilitating vaccine implementation in clinical practice. Moreover, correctly prepared alloanitgen compositions that induce low target cell killing rates also exhibited low killing rates of HMECs stimulated to proliferate by normal cells, thereby providing a therapeutic window similar to one elicited by autoantigens. For example, high killing rates were observed for HepG2-stimulated target HMECs, where allogeneic SANTAVAC™ was prepared from MCF-7-stimulated HMEC cells and the observed targeting efficacy equaled 4 (Fig. 2A, 2B) [28, 30]. This promising observation led to subsequent experiments that filled experimental gaps that better estimated the maximum efficacy of alloanitgens. In this study, two types of efficacy were described in relation to the allogeneic SANTAVAC™ vaccine: i) Efficacy I allowed for an in vitro estimation of the number of tumor vasculature endothelial cells that would be destroyed before one normal tissue endothelial cell (even if a cell is actively proliferating and involved in angiogenesis) was destroyed, and ii) Efficacy II allowed for an in vitro estimation of vaccine efficacy in the context of suppression of HMEC proliferation of the tumor vasculature and is a reflection of the potential for the vaccine to arrest tumor growth, i.e. describes the vaccine’s therapeutic efficacy. It was found that moderate tumor-induced changes to the HMEC surface antigen profile would be preferable in the production of allogeneic SANTAVAC™ resulting in an efficacy I equal to 17.3 (predicted safety) and efficacy II equal to 60 (predicted capacity to arrest tumor growth) (Fig. 2D, 2E) [54]. This optimal cell surface profile of HMEC was induced by a 15% HepG2 cell conditioned medium preparation. This allogeneic SANTAVAC™ composition (final composition) currently is considered the most efficient and efficacious for further vaccine development activities and clinical trials [54].

5. KEY POINTS PERTAINING TO SANTA-VAC™ PREPARATION

Several important technical points pertaining to vaccine preparation include the use of proteolytically cleaved cell surface antigens. Although the protease-based isolation of cell surface molecules [55-58] and the use of cancer cells for vaccination have long been described, this approach has some important limitations in terms of protease purity and the isolation of cell surface components using protease-mediated mechanisms versus the use of fluid shearing approaches.

Although earlier work demonstrated a possible loss of cell mass after trypsinization without any apparent change in cell viability [59], subsequent studies demonstrated that protease treatment affected cellular integrity [60-64] at levels sufficient...
Prediction of the optimal SANTAVAC™ composition by cell surface profiling and cytotoxicity assays. (A) Results of cytotoxicity assays (CTAs) and cell surface profiling. Data represent the cytotoxicity of effector CTLs against target HMECs versus the similarity between surface profiles of target HMECs and HMECs used to produce SANTAVAC™ used in the CTAs. Data represent the mean value of three independent measurements. The similarity between cell surface profiles is presented as the correlation coefficient \( r \) between corresponding proteomic footprints. 1 - 2 - First letter corresponds to HMECs used as a source of SANTAVAC™ and the second letter corresponds to the target HMECs used in the same CTA. ‘Autologous’ and ‘allogeneic’ correspond to areas of the autologous and allogeneic antigens, respectively. Different letters are used to identify HMECs stimulated to grow by EC growth supplement (‘G’), by MCF-7 cells (‘M’), by LNCap cells (‘L’), or HepG2 cells (‘H’). Data were scaled to bring all controls to equal values (25,000 cells, see CONTROL line). Dashed lines show examples of linear dependence between CTA data. Adapted from [28] (B) Predicted efficacy I for allogeneic and autologous SANTAVAC™ measured on the data depicted in plot ‘A’. (C) Maximal efficacy I for autologous SANTAVAC™ calculated by data approximation from the plot ‘A’. Adapted from [30]. (D) Efficacy I of target cell killing by allogeneic SANTAVAC™ in CTA. Efficacy I was calculated as a ratio of the number of non-stimulated cells in control wells (i.e., HMEC0%) to the number of tumor-stimulated cells in experimental wells. Efficacy I allows \textit{in vitro} estimation of SANTAVAC™ efficacy by demonstrating how many endothelial cells were destroyed in the tumor vasculature before 1 endothelial cell was destroyed in healthy tissue (used to establish vaccine safety). Adapted from [54]. (E) Efficacy II of target cell killing by allogeneic SANTAVAC™ in CTA. Efficacy II was calculated as a ratio of the number of tumor-stimulated cells in control wells (i.e., HMEC5%, HMEC15%, or HMEC25%) to the number of tumor-stimulated cells in experimental wells, i.e., the percentage of tumor-conditioned medium in control wells was same as in the experimental wells. Efficacy II allows \textit{in vitro} estimation of the SANTAVAC™ efficacy by demonstrating the degree of HMEC proliferation suppression in the tumor vasculature and used to establish the degree by which the vaccine can arrest tumor growth (vaccine therapeutic effect). ‘Control’ indicates the data related to the control (■) in CTA where fibroblast-associated antigens were used to simulate CTL. Percent values indicated by superscripts correspond to the percentage of tumor-conditioned medium used to stimulate target HMEC or HMEC used to generate SANTAVAC™. The most effective final SANTAVAC™ composition intended for clinical trials is indicated (‘√’). Adapted from [54].
to affect cell viability [59, 62, 65, 66]. More recent studies have utilized a more highly purified trypsin preparation with an activity of 15,000 U/mg resulting in cell lysis rates of less than 0.1% [49]. These data demonstrated that mammalian cells could be treated with trypsin without inducing lysis if a sufficiently pure trypsin preparation was used, thereby providing the foundation for preparing SANTAVAC™ formulations consisting of pure cell surface targets (i.e., without contamination by intracellular content released by damaged cells). Moreover, the use of highly pure trypsin prevents contamination of the SANTAVAC™ preparations by trypsin admixtures and by trypsin autolysis products [32, 36].

Animal cells are sensitive to fluid shearing in serum-free medium [67-71]. A study by Lau and Tchao (2007) demonstrated that susceptibility of mammalian cells to damage caused by fluid shearing depended on the growth conditions (up to 56% cell death) [68]. To obtain pure cell surface antigen preparations, cells were treated with protease preparations in serum-free medium, however, damage to the cell membrane resulted in the release of the intracellular contents. As shown in Fig. 1A, the amount of cell surface antigens obtained from 100 cancer cells is comparable to the amount of intracellular molecules contained within a single cell. Therefore, a critical aspect to consider in the preparation of cell surface antigen preparations is minimizing cell destruction as a consequence of fluid shearing. When protocol conditions were optimized and careful manipulation of the HMEC cells was maintained, an observed cell death rate of less than 0.1% was achieved after HMEC trypsinization [49], confirming the capacity of SANTAVAC™ production by careful treatment of live cells using a highly pure protease.

6. ANIMAL MODELS FOR SANTAVAC™ VACCINES

Although animal models can be developed to confirm the efficacy of SANTAVAC™ final composition to arrest tumor growth, these models would provide little supporting evidence regarding the efficacy of SANTAVAC™ preparations in humans. For example, is has already been established that mice vaccinated with ECs had delayed tumor growth [32, 72-75]. However, using SANTAVAC™ in mice would not be very efficacious because results obtained as a consequence of xenovaccination [76] would be a poor reflection of SANTAVAC™ efficacy following administration to an allogeneic recipient. To achieve results in mice that paralleled the effect of SANTAVAC™ in humans, vaccines using mouse cells would have to recreated and a ‘mouse-specific’ SANTAVAC™ would have to be generated. However, developing such a formulation for use in a mouse model would not support results for a ‘human-specific’ SANTAVAC™. Therefore, testing of SANTAVAC™ in animals would not further support the efficacy of this vaccination approach for use in humans since SANTAVAC™ is a result of the ‘sharp tuning’ of human allogeneic HMEC phenotypes to produce an efficient antigenic composition efficacious only in humans.

Regarding potential preclinical trials, SANTAVAC™ is a natural product that does not include any non-human substances and only traces of whole proteins derived from the intracellular compartment. Therefore, it is reasonable to test SANTAVAC™ safety in preclinical trials in the context of different adjuvant formulations.

7. OTHER RECENT UCV DEVELOPMENT APPROACHES

There have been several attempts to prepare UCVs, including the better-known vaccines that target telomerase and mucin. Telomerase activity is indispensable for tumor immortalization and growth; therefore, the catalytic and rate-limiting subunit of telomerase (hTERT) is an attractive UVC candidate [77]. Recently, the TeloVac trial in the U.K. assessed whether adding GV1001 (a peptide vaccine representing a 16-aa hTERT sequence [78]) to gemcitabine and capcitabine chemotherapy regimens was beneficial. The Kael-Gemvax Company filled a world patent application in 2013 [79] and now has pending patents in several countries, including the U.S. and E.U.

ImMucin is another immunotherapeutic approach often referred to as a UCV. The Vaxil Biotherapeutics company holds patents [80-82] related to ImMucin, a 21-mer synthetic vaccine composed of the entire signal peptide domain of cell surface-associated mucin 1 [83]. Overexpression of mucin 1 is associated with many cancers and for this reason is considered a cancer therapy target and a candidate for developing a UCV [84]. However, according to ‘The Human Protein Atlas’
(http://www.proteinatlas.org), melanoma, glioma, and skin cancers (among others) have no or low mucin 1 expression levels. Furthermore, it is of concern that there are high mucin 1 expression levels in normal tissues including lung, gastrointestinal tract, liver, female tissues, and kidneys. These high and varied mucin 1 expression levels between cancer cells and normal cells significantly limits ImMucin-based immunotherapy approaches.

Even though the TeloVac and ImMucin vaccines were designed to target different types of cancers and can be considered UCVs, their efficacy in treating primary cancers or in the treatment of relapses would be limited. One study found that specific cancer cell surface antigens were substantially modified under the selective pressures of drug treatment [50] that may be sufficient to allow cancer cells to escape the immune response. This fact, together with poor induction of specific anti-tumor immune responses may be a reason for the negative or poor results observed during the TeloVac [85] and ImMucin [86] clinical trials.

CURRENT & FUTURE DEVELOPMENTS

Cancer is one of the leading causes of death worldwide. In 2012, 14 million new cases were diagnosed and 8.2 million cancer-related deaths were reported worldwide, accounting for 13% of all deaths according to the WHO. It is expected that the number of cancer-related deaths will continue to grow and that by 2030 about 13.1 million new cases will be diagnosed. We predict that introduction of a UCV into clinical practice today in all countries would save millions of human lives.

Due to the large number of different tumor types (at least 30 are common) a clinical trial testing the efficacy of SANTAVAC™ to each cancer type would be required. Furthermore, a significant number of different SANTAVAC™ vaccine preparations differing in distinct compositions would need to be tested as either preventive or therapeutic vaccines. Additionally, cohorts of human subjects in clinical trials may be stratified by gender, age, race, life styles, or concomitant therapies. It is therefore expected, due to the significant heterogeneity between clinical trials, that some trials may yield positive results (from weak to pronounced delay of tumor growth depending on the tumor's requirement for vasculature, total tumor growth arrest, or even its destruction) while others do not, suggesting that SANTAVAC™ development and clinical trials testing its efficacy be started simultaneously in numerous countries following local regulatory requirements for the treatment and prevention of different tumors in the context of different adjuvants, vaccination regimes, and patient cohorts. Delaying the development of this universal antigen composition for cancer vaccines may result in millions of lost lives.

To support this strategy, the SANTAVAC™ developer and its committed partners currently provide the final composition of SANTAVAC™ to scientific organizations at no cost for use in vaccine development activities followed by clinical trials in accordance with the local regulations. The set of clinical trials are expected in the near future to provide data regarding the efficacy of SANTAVAC™-based cancer vaccines on human.

CONFLICT OF INTEREST

PGL declares that SANTAVAC™ preparation is protected by Eurasian, Japanese, Korean, and European patents and the U.S. patent is pending. The SANTAVAC™ trademark is protected in Russia and protection is pending in the E.U., U.S., China, Korea, and Japan.

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REFERENCES

[1] Lollini P-L, Cavallo F, Nanni P, Formi G. Vaccines for tumour prevention. Nat Rev Cancer 2006; 6: 204-16.
[2] Boehm T, Folkman J, Browder T, O'Reilly MS. Anti-angiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 1997; 390: 404-7.
[3] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182-6.
[4] Folkman J. What is the evidence that tumors are angiogenesis dependent?. J Natl Cancer Inst 1990; 82: 4-6.
[5] Pluda JM. Tumor-associated angiogenesis: mechanisms, clinical implications, and therapeutic strategies. Semin Oncol 1997; 24: 203-18.
[6] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407: 249-57.
[7] Copier J, Dalgleish A. Overview of tumor cell-based vaccines. Int Rev Immunol 2006; 25: 297-319.
Antigen Composition for Developing Cancer Vaccines

[8] Old LJ. Cancer vaccines: an overview. Cancer Immun 2008; 8 (Suppl 1): 1.

[9] Chiang CLL, Benencia F, Coukos G. Whole tumor antigen vaccines. Semin Immunol 2010; 22: 132-43.

[10] Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. Circ Res 2007; 100: 158-73.

[11] Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ Res 2007; 100: 174-90.

[12] St Croix B, Rago C, Velculescu V, et al. Genes expressed in human tumor endothelium. Science 2000; 289: 1197-202.

[13] Khodarev NN, Yu J, Labay E, et al. Tumour-endothelium interactions in co-culture: coordinated changes of gene expression profiles and phenotypic properties of endothelial cells. J Cell Sci 2003; 116: 1013-22.

[14] Bhati R, Patterson C, Livasy CA, et al. Molecular characterization of human breast tumor vascular cells. Am J Pathol 2008; 172: 1381-90.

[15] Hellebrekers DMEI, Castermans K, Viré E, et al. Epigenetic regulation of tumor endothelial cell anergy: silencing of intercellular adhesion molecule-1 by histone modifications. Cancer Res 2006; 66: 10770-7.

[16] Hellebrekers DMEI, Jair K-W, Viré E, et al. Angiostatic activity of DNA methyltransferase inhibitors. Mol Cancer Ther 2006; 5: 467-75.

[17] Hellebrekers DMEI, Melotte V, Viré E, et al. Identification of epigenetically silenced genes in tumor endothelial cells. Cancer Res 2007; 67: 4138-48.

[18] Unger RE, Oltrogge JB, Von Briesen H, et al. Isolation and molecular characterization of brain microvascular endothelial cells from human brain tumors. In Vitro Cell Dev Biol Anim 2002; 38: 273-81.

[19] Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. FASEB J 2003; 17: 1159-61.

[20] Hart MN, Sadewasser KL, Cancilla PA, DeBault LE. Experimental autoimmune type of vasculitis resulting from activation of mouse lymphocytes to cultured endothelium. Lab Invest 1983; 48: 419-27.

[21] Matsuda M. Experimental glomerular tissue injury induced by immunization with cultured endothelial cell plasma membrane. Acta Pathol Jpn 1988; 38: 823-39.

[22] Ludewig B, Oehsenbein AF, Odermatt B, Paulin D, Hengartner H, Zinkernagel RM. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. J Exp Med 2000; 191: 795-804.

[23] Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298: 850-4.

[24] Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 2003; 100: 8372-7.

[25] Maker AV, Phan GQ, Attia P, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol 2005; 12: 1005-16.

[26] Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005; 23(25): 6043-53.

[27] Finn OJ. Cancer vaccines: between the idea and the reality. Nat Rev Immunol 2003; 3(8): 630-41.

[28] Lokhov PG, Balashova EE. Tumor-induced endothelial cell surface heterogeneity directly affects endothelial cell escape from a cell-mediated immune response in vitro. Hum Vaccin Immunother 2013; 9(1): 198-209.

[29] Balashova EE, Lokhov PG. Proteolytically-cleaved fragments of cell surface proteins stimulate a cytotoxic immune response against tumor-activated endothelial cells in vitro. J Cancer Sci Ther 2010; 2: 126-31.

[30] Lokhov PG, Balashova EE. Universal cancer vaccine: an update on the design of cancer vaccines generated from endothelial cells. Hum Vaccin Immunother 2013; 9(7): 1549-52.

[31] Lokhov PG, Balashova EE. Cellular cancer vaccines: an update on the development of vaccines generated from cell surface antigens. J Cancer 2010; 1: 230-41.

[32] Lokhov PG. Method for producing an antitumoral vaccine based on surface endothelial cell antigens. EU2140873, 2015.

[33] Lokhov PG. Method for producing an antitumoral vaccine based on surface endothelial cell antigens. JP10-1290641, 2013.

[34] Lokhov PG. Method for producing an antitumoral vaccine based on surface endothelial cell antigens. JP154641, 2012.

[35] Lokhov PG. Method for producing an antitumoral vaccine based on surface endothelial cell antigens. EU9327, 2007.

[36] Lokhov P, Balashova E, Dashtiev M. Cell proteomic footprint. Rapid Commun Mass Spectrom 2009; 23: 680-2.

[37] Lokhov PG, Balashova EE. Method for testing cell culture quality. EU9326, 2007.

[38] Kumar S, West DC, Ager A. Heterogeneity in endothelial cells from large vessels and microvessels. Differentiation 1987; 36: 57-70.

[39] Lang I, Pabst MA, Hiden U, et al. Heterogeneity of microvascular endothelial cells isolated from human term placenta and macrovascular umbilical vein endothelial cells. Eur J Cell Biol 2003; 82(4): 163-73.

[40] Shreeminivas R, Ogawa S, Cozzolino F, et al. Macrophage and microvascular endothelium during long-term hypoxia: alterations in cell growth, monolayer permeability, and cell surface coagulant properties. J Cell Physiol 1991; 146(1): 8-17.

[41] Hewett PW. Identification of tumour-induced changes in endothelial cell surface protein expression: an in vitro model. Int J Biochem Cell Biol 2001; 33(4): 325-35.
[42] Swerlick RA, Lee KH, Wick TM, Lawley TJ. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. J Immunol 1992; 148(1): 78-83.

[43] Swerlick RA, Lee KH, Li LJ, Sepp NT, Caughman SW, Lawley TJ. Regulation of vascular cell adhesion molecule 1 on human dermal microvascular endothelial cells. J Immunol 1992; 149(2): 698-705.

[44] Lee KH, Lawley TJ, Xu YL, Swerlick RA. VCAM-1-, ELAM-1-, and ICAM-1-independent adhesion of melanoma cells to cultured human dermal microvascular endothelial cells. J Invest Dermatol 1992; 98(1): 79-85.

[45] Thompson PL, Dessureault S. Tumor cell Vaccines. In: Shurin MR, Smolkin YS, eds. Immune-Mediated diseases from theory to therapy. New York: Springer 2007, pp. 345-55.

[46] De Gruijl TD, Van Den Eertwegh AJM, Pinedo HM, Scheper RJ. Whole-cell cancer vaccination: From autologous to allogeneic tumor- and dendritic cell-based vaccines. Cancer Immunol Immunother 2008; 57(10): 1569-77.

[47] Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res 2009; 15: 5323-37.

[48] Lang JM, Andrei AC, McNeel DG. Prioritization of cancer antigens: keeping the target in sight. Expert Rev Vaccines 2009; 8: 1657-61.

[49] Balashova EE, Lokhov PG. Proteolytically-cleaved fragments of cell-surface proteins from live tumor cells stimulate anti-tumor immune response in vitro. J Carcinog Mutagen 2010; 1: 1-3.

[50] Balashova EE, Dashtiev MI, Lokhov PG. Proteomic footprinting of drug-treated cancer cells as a measure of cellular vaccine efficacy for the prevention of cancer recurrence. Mol Cell Proteomics 2012; M111.014480-M111.014480.

[51] Lokhov PG. Tumor vaccine, a method for producing a tumor vaccine and a method for carrying out anti-tumor immunotherapy. US20110027322, 2011.

[52] Lokhov PG. A method for producing a tumor vaccine. JP5172864, 2013.

[53] Lokhov PG, Balashova EE. Design of universal cancer vaccines using natural tumor vessel-specific antigens (SANTAVAC). Hum Vaccin Immunother 2015; 11(3): 689-98.

[54] Lokhov PG, Balashova EE. Allogeneic antigen composition for preparing universal cancer vaccines. J Immunol Res 2016; 2016: Article ID 5031529.

[55] Takeichi N, Economou GC, Boone CW. Accelerated regeneration of trypsin-treated surface antigens of simian virus 40-transformed BALB/3T3 cells induced by X-irradiation. Cancer Res 1976; 36: 1258-62.

[56] Glick MC, Kimhi Y, Littauer UZ. Glycopeptides from surface membranes of neuroblastoma cells. Proc Natl Acad Sci USA 1973; 70: 1682-7.

[57] Anglilieri LJ, Dermietzel. Cell coat in tumor cells - effects of trypsin and EDTA: a biochemical and morphological study. Oncology 1976; 33: 17-23.

[58] Baumann H, Doyle D. Effect of trypsin on the cell surface proteins of hepatoma tissue culture cells. Characterization of a carbohydrate-rich glycoprotein released from a calcium binding membrane glycoprotein. J Biol Chem 1979; 254: 3935-46.

[59] Weiss L. The effects of trypsin on the size, viability and dry mass of sarcoma 37 cells. Exp Cell Res 1958; 14(1): 80-3.

[60] Barnard PJ, Weiss L, Ratcliffe T. Changes in the surface properties of embryonic chick neural retina cells after dissociation. Exp Cell Res 1969; 54(3): 293-301.

[61] Kraemer PM. Regeneration of sialic acid on the surface of Chinese hamster cells in culture. I. General characteristics of the replacement process. J Cell Physiol 1966; 68(1): 85-90.

[62] Kraemer PM. Sialic acid of mammalian cell lines. J Cell Physiol 1966; 67(1): 23-34.

[63] Kemp RB, Jones BM, Cunningham I, James MC. Quantitative investigation on the effect of puromycin on the aggregation of trypsin-and versene-dissociated chick fibroblast cells. J Cell Sci 1967; 2(3): 323-40.

[64] Pitelka DR, Kerkof PR, Gagne HT, Smith S, Abraham S. Characteristics of cells dissociated from mouse mammary glands. I. Method of separation and morphology of parenchymal cells from lactating glands. Exp Cell Res 1969; 57(1): 43-62.

[65] De Luca C. The use of trypsin for the determination of cellular viability. Exp Cell Res 1965; 40(1): 186-8.

[66] Kraemer PM. Regeneration of sialic acid on the surface of Chinese hamster cells in culture. II. Incorporation of radioactivity from glucosamine-1-14C. J Cell Physiol 1967; 69(2): 199-207.

[67] Van Der Pol L, Tramper J. Shear sensitivity of animal cells from a culture-medium perspective. Trends Biotechnol 1998; 16(8): 323-8.

[68] Lau JY, Tchao R. Stressed polystyrene causes increased membrane sensitivity of adherent cells to fluid shear force: technical note. Eur Cell Mater 2007; 14: 40-3.

[69] McQueen A, Bailey JE. Influence of serum level, cell line, flow type and viscosity on flow-induced lysis of suspended mammalian cells. Biotechnol Lett 1989; 11: 551-6.

[70] McQueen A, Meilhoc E, Bailey JE. Flow effects on the viability and lysis of suspended mammalian cells. Biotechnol Lett 1987; 9: 831-6.

[71] Tchao R. Fluid shear force and turbulence-induced cell death in plastic tissue culture flasks. In Vitro Toxicol 1996; 9(1): 93-100.

[72] Corsini E, Gelati M, Calatozzolo C, et al. Immunotherapy with bovine aortic endothelial cells in subcutaneous and intracerebral glioma models in rats: Effects on survival time, tumor growth, and tumor neovascularization. Cancer Immunol Immunother 2004; 53: 955-62.

[73] Okaji Y, Tsuino NH, Kitayama J, et al. Vaccination with autologous endothelium inhibits angiogenesis and metastasis of colon cancer through autoimmunity. Cancer Sci 2004; 95: 85-90.

[74] Chen X-Y, Zhang W, Zhang W, et al. Vaccination with viable human umbilical vein endothelial cells prevents metastatic tumors by attack on tumor vasculature with both cellular and humoral immunity. Clin Cancer Res 2006; 12: 5834-40.
Antigen Composition for Developing Cancer Vaccines

[75] Scappaticci FA, Nolan GP. Induction of anti-tumor immunity in mice using a syngeneic endothelial cell vaccine. Anticancer Res 2003; 23: 1165-72.

[76] Wei YQ, Wang QR, Zhao X, et al. Immunotherapy of tumors with xenogeneic endothelial cells as a vaccine. Nat Med 2000; 6: 1160-6.

[77] Kyte JA. Cancer vaccination with telomerase peptide GV1001. Expert Opin Investig Drugs 2009; 18: 687-94.

[78] Shaw VE, Naisbitt DJ, Costello E, et al. Current status of GV1001 and other telomerase vaccination strategies in the treatment of cancer. Expert Rev Vaccines 2010; 9: 1007-16.

[79] Kim SJ. Biological markers useful in cancer immunotherapy. WO2014196841, 2014.

[80] Carmon L. Antigen specific multi epitope vaccines. WO2008035350, 2008.

[81] Carmon L. Antigen specific multi epitope vaccines. US20100074925, 2010.

[82] Carmon L. Antigen specific multi epitope vaccines. EP2089423, 2016.

[83] Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 1990; 265: 15286-93.

[84] Singh R, Bandyopadhyay D. MUC1: a target molecule for cancer therapy. Cancer Biol Ther 2007; 6(4): 481-6.

[85] Middleton G, Silcokcs P, Valle J, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. Lancet Oncol 2014; 15(8): 829-40.

[86] Rivalland G, Loveland B, Mitchell P. Update on Mucin-1 immunotherapy in cancer: a clinical perspective. Expert Opin Biol Ther 2015; 15(12): 1773-87.