Formulation and evaluation of oral microparticulate ovarian cancer vaccines

Suprita A. Tawde, Lipika Chablani, Archana Akalkotkar, Cherilyn D’Souza, Periasamy Selvaraj, Martin J. D’Souza

A Vaccine Nanotechnology Laboratory, Department of Pharmaceutical Sciences, Mercer University, College of Pharmacy and Health Sciences, 3001 Mercer University Drive, Atlanta, GA 30341, USA

B Department of Pathology and Laboratory Medicine, Emory University, 7309 Woodruff Memorial Research Building, 101 Woodruff Circle, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 16 April 2012
Accepted 25 May 2012
Available online xxx

Keywords:
Microparticles
Ovarian cancer
Oral delivery
M-cells
ID8 cells
Spray drying

ABSTRACT

Ovarian cancer is the fifth most leading cause of cancer related deaths in women in the US. Customized immunotherapeutic strategies may serve as an alternative method to control the recurrence or progression of ovarian cancer and to avoid severe adverse effects of chemotherapy. In this study, a microparticulate vaccine using whole cell lysate of a murine ovarian cancer cell line, ID8 was prepared with the use of a spray dryer. These particles were designed for oral delivery using enteric polymers such as methacrylic copolymer, Eudragit® FS30D and hydroxyl propyl methyl cellulose acetate succinate. These particles were targeted for uptake via microfold cell (M-cell) in Peyer’s patches of small intestine using M-cell targeting ligand, Aleuria aurantia lectin. The interleukins (ILs) such as IL-2 and IL-12 were added to the vaccine formulation to further enhance the immune response. The particles obtained were of 1.58 ± 0.62 μm size with a charge of 12.48 ± 2.32 mV. The vaccine efficacy was evaluated by administering the particles via oral route to C57Bl/6 female mice. At the end of vaccination, mice were challenged with live tumor cells. Vaccinated mice showed significant (around six-fold) retardation of tumor volume in comparison to non-vaccinated animals for 3 weeks after the tumor challenge (p < 0.001). The serum IgG antibody levels were found to be elevated in case of vaccinated animals in comparison to non-vaccinated group (p < 0.05). Analysis of IgG1 titers (indicative of Th2 response) and IgG2a titers (indicative of Th1 response) showed a mixed Th1 and Th2 immune response in case vaccine alone and Th2 response in case of vaccine with interleukins group. Moreover, CD8+ T-cell, CD4+ T-cell and B-cell populations in different lymphatic organs were elevated in case of vaccinated mice. Thus, whole cell lysate vaccine microparticles formulated by spray drying could trigger humoral as well as cellular immune response when administered orally. Such vaccine could potentially be an effective treatment for patients with residual tumor or high tumor-relapse probability.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Ovarian cancer is the most lethal gynecological cancer in the US. Only about 10% of ovarian cancers are usually found in early stages. Patients with epithelial tumors, which account for approximately 90% of ovarian cancer, generally have poor overall survival [1]. First-line treatment for advanced cancer involves surgery followed by chemotherapy. However, cancer relapses within relatively short periods of time even after treatment [2]. Therefore, alternative approaches, immunotherapy is being investigated to prevent relapse of cancer.

Several vaccines are underway in clinical trials and most of them have not progressed beyond phase I/II studies. Even though antigen-specific response is obtained with different approaches of antigen specific immunization, there is no consistency in clinical benefit [3,4]. In this study, we have investigated whether oral vaccination with microparticles containing the ovarian cancer antigens can prevent/retard ovarian cancer growth. A murine ovarian cancer cell line, ID8 was used as a source of antigens as it correlates closely to human ovarian cancer cell lines in signaling pathways and results in development of tumor in mice models similar to human ovarian cancer [5]. Despite of advancement in vaccine technology, the whole cell lysate vaccine still remains a very promising approach as it can overcome the demerits associated with a single antigen/epitope vaccine. Whole cell lysate provides a pool of tumor-associated antigens (TAs) which can induce both CD8+ and CD4+ T-cells [6]. Therefore, we proceeded with a whole cell lysate of ID8 cells to prepare the vaccine for this study.

Oral vaccine delivery is an attractive mode of immunization because of its ease of administration, low manufacturing costs and patient compliance. However, for an oral vaccine to be effective, it must withstand gastro-intestinal degradation. For this purpose,
enteric polymers such as methacrylic copolymer Eudragit® FS 30 D and hydroxypropyl methyl cellulose acetate succinate (HPMCAS) were used. Ano et al. have reported a formulation of oral vaccine of inactivated Vibrio cholerae by spray drying using Eudragit® FS30D [7]. This polymer was found to be a good candidate for controlled release of antigens and resulted in vibrioidal antibody titers. We prepared the particles utilizing this polymer along with other excipients by using an optimized microparticulate formula for oral vaccine delivery by spray drying [8]. To target our vaccine formulation to M-cells in the Peyer’s patches of the intestine, M-cell targeting agent, Aleuria aurantia lectin (AAL) was used in the formulation [9,10]. In addition, inclusion of immunostimulatory molecules such as IL-2 and IL-12 was evaluated in order to enhance the overall potency of the formulated vaccines [11,12]. It has been observed that specific immune response by tumor cell vaccines is often impaired due to deficiency of co-stimulatory signals. Vaccines prepared with cancer cells secreting IL-2 or IL-12 have resulted in better immune response when compared to non-secreting cell lines. IL-2 has been efficiently used in immunotherapy against cancers [13]. Moreover, higher anti-tumor effects were reported when IL-2 was delivered as an adjuvant in a slow-releasing depot form rather than a free form [11,13]. Incorporation of IL-2 in particle addresses this issue seen with free form of IL-2.

In the present study, we demonstrate the efficacy of oral vaccine formulations which was evaluated in vivo in mouse tumor model, using the ID8 murine ovarian cancer cell line as a solid tumor model.

2. Materials and methods

2.1. Materials

ID8 cell line was kindly provided by Dr. Katherine Roby, Kansas University Medical Center, Kansas City, KS. Six to eight week-old C57BL/6 female mice were purchased from Charles River Laboratories, Wilmington, MA. HPMCAS was purchased from ABOAT, FMC Biopolymers, Philadelphia, PA. Eudragit® FS 30 D was generously gifted by Evonik industries, Parsippany, NJ. Mouse plasma was obtained from Biochemed, Winchester, VA. AAL was obtained from Vector Labs, Inc., Burlingame, CA. Recombinant mouse interleukins, IL-2 (5 × 10^6 units/mg) and IL-12 (1 × 10^5 units/mg) were purchased from Peprotech Inc., Rocky Hill, NJ. Flow cytometry cell markers were purchased from ebioScience, San Diego, CA. Goat anti-mouse HRP-IgG and anti-IgG subtypes were purchased from Bethyl Laboratories, Montgomery, TX and Sigma, St. Louis, MO respectively. All other materials used were of analytical grade.

2.2. Preparation of whole cell lysate

The murine ovarian cancer ID8 cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite (Sigma, St. Louis, MO) [14]. The confluent cells were washed with cold phosphate buffered saline (PBS). The flasks were then treated with hypotonic buffer (10 mM Tris and 10 mM NaCl) and subjected to five 15 min freeze–thaw cycles at temperatures of −80 °C and 37 °C respectively to obtain the cell lysate [15,16].

2.3. Characterization of the lysate

The protein content of lysate was analyzed using Bio–Rad DC™ protein assay. The lysate was screened for the presence of the only known antigen, SP17 by western blot analysis [17]. Briefly, 25 μg of lysate protein was resolved by SDS-PAGE gel electrophoresis and subjected to overnight wet-transfer on to a PVDF membrane. After blocking, the membrane was treated with primary mouse anti-SP17 antibody (kindly provided by Dr. Chiriva-Internati, Texas Tech University Health Science Center, Lubbock, TX), followed by incubation with a horseradish peroxidase-conjugated secondary antibody. Finally, the membrane was incubated with Enhanced Chemiluminescence (abcam, Cambridge, MA), then exposed to imaging film.

2.4. Preparation of vaccine microparticles

The vaccine formulation was prepared by spray drying technique [8–10,18–24]. Briefly, HPMCAS and Eudragit® FS 30 D were dissolved in an alkaline solution, followed by addition of chitosan glycol. Mouse plasma was added to the polymeric solution at pH 7.0 as a source of amound. Trehalose, tween 20 and AAL were added to the solution, followed by addition of the lysate (5%, w/w). In case of vaccine with interleukins formulations, 4 × 10^5 U of IL-2 and 8 × 10^5 U of IL-2 were added to this feed mixture [11,12]. This aqueous solution was spray dried using Buchi B-191 Mini Spray Dryer (Buchi Corporation, New Castle, DE).

2.5. Characterization of microparticles

Microparticles were visualized using by scanning electron microscope (JEOL JSM 5800LV, Tokyo, Japan) at EM Core facility, Emory University, Atlanta. The particles were characterized for size and charge, using laser particle counter (Spectrex PC-2000) and Malvern zeta sizer (ZEN 1600) respectively. Loading efficiency was determined by extracting the proteins in PBS and analyzing them using Biorad DC™ protein assay in comparison to placebo particles.

2.6. Immunization

The immunogenicity of microparticulate vaccine was evaluated using C57BL/6 female mice model. Animal experiments were carried out as per approved protocols by Mercer University’s Institutional Committee for the care and Use of Laboratory animals. Animals (n = 8) were administered with microparticles as one prime dose followed by one booster after 1 week and thereafter by 8 boosters with an interval of 2 weeks. Briefly, 5 mg of microparticles were suspended in citrate buffer (pH 4.0) and administered orally using oral gavage. Three different formulations such as placebo, vaccine and vaccine with interleukins were evaluated for this purpose.

2.7. Tumor challenge study

One week after the last vaccination, the mice were challenged s.c. with 1 × 10^5 live ID8 cells [25]. The cells were suspended in incomplete media and injected into the right back flank of mice. Tumor development was monitored using digital vernier calipers. The mice were euthanized whenever the tumor ulcerated or tumors exceeded a size of 15 mm in any of the perpendicular diameters. The tumor volume (V) was determined by using the formula, \[ V = \frac{1}{2}(\text{length})(\text{width})^2 \] [26,27].

2.8. Assessment of humoral (B-cell mediated) immune response in serum

The blood samples were collected prior to each dose of vaccination. Serum was analyzed by ELISA. Briefly, overnight coating of the lysate (100 μg/well) was done in a 96 well plate. The plate was blocked with non-fat dry milk. After washing, the plate was incubated with 1:10 dilution of serum samples. HRP-tagged secondary anti-mouse goat IgG was then added to each well and incubated for 1 h. TMB substrate reagent (3,3′, 5,5′-tetramethyl benzidine) (BD OptEIA™, BD Biosciences, CA) was added and the plate was
again incubated for 30 min. The reaction was stopped by addition of 4 N H2SO4. The plate was read using microplate reader (BioTek instruments Inc., Winooski, VT) at 450 nm. In case of IgG subtypes analysis, serum samples were analyzed in dilution of 1:50. The plate was then incubated with goat anti-mouse IgG subtype IgG1 or IgG2a, followed incubation with HRP-conjugated anti-goat IgG. Thereafter, same procedure as described above for IgG titers analysis was followed.

2.9. Determination of T-cell and B-cell based immune response in lymphatic organs

A separate group of mice was vaccinated in similar way as described in Section 2.6. At the end of vaccination, mice were euthanized and lymphatic organs such as the spleen, lymph nodes (axillary and inguinal) and bone marrows were harvested. The single cell suspension of these organs were stimulated for 5 days at 37°C with mitomycin-treated tumor cells in a ratio 10:1 with 10U/ml of recombinant murine IL-2 [28]. At the end of 5-days, the cells were washed with Hank’s balanced salt solution and labeled with anti-mouse CD4 PE, anti-mouse CD8a FITC and anti-mouse/human CD45R (B220) FITC (for B-cells). The cells were analyzed for the specific cell populations by flow cytometric analysis using BD accuritrC flow cytometer.

2.10. Statistical analysis

Serum IgG subtype titers and flow-cytometry results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Tumor volume measurements and serum IgGs were analyzed by two-way ANOVA followed by Bonferroni multiple comparisons. All statistical analyses were performed using GraphPad Prism5 (trial version 5.04, GraphPad Software, Inc., La Jolla, CA). For each test, p value less than 0.05 was considered significant. Data are expressed as mean ± standard error.

3. Results and discussion

3.1. Preparation and characterization of the lysate

The lysate obtained was a turbid extract and the total protein concentration of lysate was 1.56 ± 0.5 mg/ml. The only protein which has been studied is the sperm protein (SP17, molecular weight 15 kD) which is expressed by ID8 cells [17]. It is one of the cancer/testis antigens, a sub-class of TAAs. This protein has been extensively studied and it is found to be expressed or over-expressed in multiple myeloma and ovarian cancers. In a study by Chiriva-Internati et al., this protein has been evaluated as an ovarian cancer vaccine in murine models, where the mice were immunized intramuscularly with SP17 in ten doses of vaccination. It has shown to be a promising approach prophylactically as well as therapeutically. Moreover, human SP17 shows 70% homology with murine SP17 and is expressed in ovarian cancer. Thus, SP17 may be a promising antigen for immunotherapy in ovarian cancer [17]. Therefore, we performed western blot analysis for SP17 on the lysate prepared. We could trace the presence of this protein as shown in Fig. 1(A). The presence of SP17 in the lysate ensured that the protein cocktail obtained was antigenic when given as a vaccine.

3.2. Preparation and characterization of vaccine microparticles

One of the major challenges of oral vaccination is the avoidance of immune tolerance. However, this issue can be avoided by entrapping vaccine antigen into particles [29–31]. This can be due to the efficient and enhanced uptake of particulate antigen by M cells in the Peyer’s patches by phagocytosis, when compared to minimal antigen uptake in solution form by pinocytosis. Moreover, protein antigens taken up as particles are more potent in activating antigen presenting cells (APCs) than soluble antigens [32–34]. Orally delivered vaccines, especially particulate antigens are sampled by M-cells in the Peyer’s patches of the intestine. Further these antigens are processed by professional APCs such as dendritic cells and macrophages that reside in the Peyer’s patches [35].

The morphological characterization of particles showed crumpled, collapsed and irregular shaped microparticles as shown in Fig. 1(B). Eudragit® FS30D microparticles have shown to result in such a shape upon spray drying by others as well [7]. The production yield was 72.58 ± 3.41% (w/w). The particles obtained were of 1.58 ± 0.62 μm size with a charge of 12.48 ± 2.32 mV. There was no significant change in size and charge upon loading these particles with the lysate and interleukins. The loading efficiency of the particles was found to be 92.68 ± 4.77% (w/w).

3.3. Immunization suppresses tumor growth

In case of mice treated with placebo particles, the tumor developed rapidly. However, vaccinated mice showed around six-fold tumor suppression when compared to non-vaccinated/placebo group. The tumor volume measurements obtained are shown in Fig. 2. However, there was no significant difference between vaccine and vaccine with interleukin groups in terms of tumor volumes. This can be due to the interleukin concentration used in vaccine which was not high enough to retard tumor growth more than that seen with vaccine alone. Higher concentration of interleukins might be needed to show additional tumor suppression.

3.4. Immunization generates humoral immune response and orally administered interleukins influence Th1/Th2 pathway

In order to determine B-cell response, the serum samples collected before each dose were analyzed by ELISA. As shown in Fig. 3(A), at end of vaccination, immunized mice showed elevated
IgG titers as compared to non-vaccinated ones (p < 0.05). Mice treated with vaccine microparticles with interleukins showed even further elevated response as compared to mice receiving placebo microparticles (p < 0.0001). When serum IgG1 titers were analyzed, there was an elevation in titers in mice treated with vaccine alone when compared to placebo group (p < 0.01) as shown in Fig. 3(B). The response was even further elevated in case of vaccine with interleukins group when compared to placebo (p < 0.001). In case of IgG2a titers, the titers were elevated in case of vaccine alone when compared to placebo (p < 0.01) as shown in Fig. 3(C). Thus, both subtypes of IgGs indicate that mixed Th1 and Th2 immune response was generated in case of vaccine alone and Th2 type response was triggered in case of vaccine with interleukin group. The reason behind the difference in the immune response can be attributed to IL-12 effect in the Peyer’s patches which has been reported to be a possible strategy for targeted manipulation of immune responses to oral vaccines. Such response was reported by Marinaro et al., when IL-12 was given orally to mice orally immunized with tetanus toxoid and cholera toxin and the effect was compared with intraperitoneal administration of IL-12. They found that oral IL-12 resulted in the Th2 response to oral vaccine, while intraperitoneal IL-12 caused Th1 response [12].

3.5. Immunization generates T-cell and B-cell based immune response

Lymphatic organs such as spleen and lymph nodes were analyzed for T-cell populations. The CD8+ as well as CD4+ T-cell populations were found to be elevated in vaccinated mice when compared to placebo group as shown in Fig. 4. The CD4+ T-cell population was found to be elevated in case of spleen cells of vaccine with interleukin group (Fig. 4(C)) when compared to spleen cells of vaccine alone (p < 0.01). On the other hand, B-cell population was determined in spleen, lymph nodes and bone marrow. In case of B-cell population in spleen cells (Fig. 5(A)), there were elevated levels in vaccine with interleukins group when compared to
Fig. 4. Immunization with vaccine microparticles generates CD8+ and CD4+ T-cell based immune response: CD8+ T-cells population in (A) spleen, (B) lymph nodes, CD4+ T-cell population in (C) spleen, (D) lymph nodes; (E) CD8+ T-cells population in spleen determined by flow cytometry, FL1-H being the count detected by filter FL1 for FITC-tagged CD8+ T-cells (shown in gate P5). The results are summarized in (A) indicating the elevated CD8+ T-cell population in vaccinated mice when compared to non-vaccinated mice (p < 0.005); (F) CD8+ T-cells population (gate P1) in lymph nodes determined by flow cytometry. The results are summarized in (B) indicating the elevated CD8+ T-cell population in mice receiving vaccine alone (p < 0.001) and in mice receiving vaccine with interleukins (p < 0.01) when compared to non-vaccinated mice (G) CD4+ T-cells population (gate P7) in spleens determined by flow cytometry. The results are summarized in (C) indicating the elevated CD4+ T-cell population in mice receiving vaccine alone (p < 0.05) and in mice receiving vaccine with interleukins (p < 0.001) when compared to non-vaccinated mice (H) CD4+ T-cells population (gate P3) in lymph nodes determined by flow cytometry. The results are summarized in (D) indicating the elevated CD4+ T-cell population in vaccinated mice when compared to placebo treated/non-vaccinated mice (p < 0.001); *p < 0.05, **p < 0.01, ***p < 0.001.

placebo (p < 0.001) and vaccine alone (p < 0.01). B-cell populations in lymph node cells as shown in Fig. 5(B) were found be elevated in case of vaccinated groups when compared to non-vaccinated mice (p < 0.001). B-cells in bone marrows (Fig. 5(C)) were found to be elevated in case of vaccine with interleukins group than placebo as well as vaccine alone (p < 0.001).

The overall results obtained are summarized in Table 1. When data was analyzed for vaccinated and non-vaccinated

Table 1
Summary of results obtained from in vivo study of ovarian cancer vaccine microparticles when administered orally to mice.

<table>
<thead>
<tr>
<th>In vivo study</th>
<th>Organ/tissue analyzed</th>
<th>Placebo</th>
<th>Oral vaccine</th>
<th>Oral vaccine + interleukins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume suppression</td>
<td>Tumor</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IgG titers at the end of vaccination</td>
<td>Serum</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IgG subtype IgG1 titers (Th2 type response)</td>
<td>Serum</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IgG subtype IgG2a titers (Th1 type response)</td>
<td>Serum</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B-cell population</td>
<td>Bone marrow</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD8+ T-cells</td>
<td>Spleen</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD4+ T-cells</td>
<td>Spleen</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lymph nodes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* p < 0.05, results expressed in comparison to placebo group.
** p < 0.01 results expressed in comparison to placebo group.
*** p < 0.001 results expressed in comparison to placebo group.
**** p < 0.0001, results expressed in comparison to placebo group.
* p < 0.05 results expressed for comparison between vaccine and vaccine with interleukins groups.
** p < 0.01 results expressed for comparison between vaccine and vaccine with interleukins groups.
*** p < 0.001, results expressed for comparison between vaccine and vaccine with interleukins groups.

Please cite this article in press as: Tawde SA, et al. Formulation and evaluation of oral microparticulate ovarian cancer vaccines. Vaccine (2012), http://dx.doi.org/10.1016/j.vaccine.2012.05.073
mice, humoral response study showed higher antibody titers in vaccinated mice. Vaccine alone resulted in mixed Th1 and Th2 response, while vaccine with interleukins showed predominant Th2 response. The B-cell population was found to be elevated in bone marrow and spleen cells of vaccine with interleukins group than vaccine alone, while it was higher in lymph nodes in case of vaccine alone as well as vaccine with interleukins groups when compared to placebo. We found CD8+ and CD4+ T-cells were expanded in mice treated with vaccine with and without interleukins when compared to non-vaccinated mice. However, when the comparison was done between vaccine with and without interleukins groups, B-cell populations in bone marrow and spleen along with CD4+ T-cells in spleen were found to be elevated in case of interleukins group. The overall stimulation of humoral and cellular response upon vaccination indicates the efficacy of oral vaccine microparticles. Moreover, the immune stimulation in terms of humoral and cellular response obtained correlated with tumor volume retardation.

However, the anti-tumor effect was expected to be enhanced by interleukins, which was not observed in this study. The interleukin concentration used might need to be increased to result in enhanced anti-tumor activity. Another reason can be the activation of regulatory T-cells (T-regs) by IL-2, which can be a demerit for vaccine efficacy. It has been reported that low-dose of IL-2 can result in expansion of T-regs. The overall effect of T-helper cells and NK-cells versus Tregs in response to IL-2 depends on IL-2 concentration, dosing time and duration of exposure, which ultimately determines anti-tumor activity [13].

4. Conclusion

We have demonstrated the efficacy of vaccine microparticles containing whole cell lysate of ID8 ovarian cancer cells in retarding tumor growth in murine models. Thus, the oral microparticulate vaccine described provides a promising approach in terms of cost-effectiveness, ease of production and patient compatibility. This study was performed in prophylactic setting to check the efficacy of particles, which forms the basis for further studies to evaluate therapeutic efficacy of the particles in tumor bearing animals.

Acknowledgements

Authors acknowledge Dr. Ray Green, Mercer University, Atlanta, GA for his generous help with SDS-PAGE analysis, Dr. Chiriva Maurizio and Leonardo Mirandola, Texas Tech University Health Sciences Center, TX for their kind guidance regarding Western blot analysis and Dirk Anderson, BD Accuri Cytometers, MI for his generous help with flow cytometry data analysis. A grant support from NIH (R01CA138993 to P.S.) is gratefully acknowledged.

Conflict of interest: The authors report no conflict of interest.
References

[1] Berkowitz Z, Rim SH, Peipins LA. Characteristics, survival associated with ovari-

an cancer diagnosis as first cancer and ovarian cancer diagnosed subsequent


[2] Stuart GC. First-line treatment regimens and the role of consolidation


promotes survival and resistance to chemotherapeutics in a mouse ovarian


oral vaccine candidate based on the microencapsulation by spray-drying of


[8] Chablani J, Tawde SA, D’Souza MJ. Spray-dried microparticles: a potential vehi-

cle for oral delivery of vaccines. J Microencapsul. in press.


M. Oral microparticulate vaccine for melanoma using M-cell targeting. J Drug


delivery of antigens to the gut-associated lymphoid tissues: 2. Ex vivo evalua-
tion of lectin-labelled albumin microbubbles for targeted delivery of antigens


vaccine composed of liposome-encapsulated haemagglutinin/neuraminidase

and IL-2 or GM-CSF. II. Induction of TH1 and TH2 responses in mice. Vaccine


Oral but not parenteral interleukin-12 redirects T helper 2 (Th2-type) responses

to an oral vaccine without altering mucosal IgA responses. J Exp


[13] Huang J, Le D, Thorpe PE. Enhancing the potency of a whole-cell breast cancer

vaccine in mice with an antibody-IL-2 immunocytokine that targets exposed


increases cell proliferation, survival and tumorigenicity in a mouse model of


[15] Hashemi SM, Hassan ZM, Soudi S, Shahabi S. The effect of vaccination with the

lysate of heat-shocked tumor cells on nitric oxide production in BALB/c mice


[16] Danfeng X. Induction of protective antitumor activity of tumor lysate-pulsed

dendritic cells vaccine in RM-1 prostate cancer model. J Med Coll PLA


M, et al. Cancer testis antigen vaccination affords long-term protection


http://dx.doi.org/10.1371/journal.pone.0010471.

[18] Lai YH, D’Souza MJ. Microparticle transport in the human intestinal M cell


[21] Yeboah KG, D’Souza MJ. Evaluation of albumin microspheres as oral delivery

system for Mycobacterium tuberculosis vaccines. J Microencapsul


[22] Gayakwad SG, Bejugam NK, Akhavein N, Uddin NA, Oettinger CE, D’Souza MJ.

Formulation and in vitro characterization of spray-dried antisense oligonu-

cleotide to NF-KappaB encapsulated albumin microbubbles. J Microencapsul


[23] Uddin AN, Bejugam NK, Gayakwad SG, Akhavein N, D’Souza MJ. Oral delivery

of gastro-resistant microencapsulated typhoid vaccine. J Drug Target


containing plasmid DNA encoding hepatitis-B surface antigen. J Drug Target,
in press.


of apoptotic tumor cells with replication-incompetent HSV augments the
182–93.


Direct vaccination with tumor cells killed with ICPI4-deficient

HSV120 elicits effective antitumor immunity. Cancer Biol Ther 2006;5(7):
867–74.

[27] Sharma RK, Srivastava AK, Yolcu ES, Malekoejdi K, Schabowski RH, Madireddi S,
et al. SA-4-IBBL as the immunomodulatory component of a HPV-16 E7 protein

based vaccine shows robust therapeutic efficacy in a mouse cervical cancer


Decreased cytotoxic T cell activity generated by co-administration of PSA vac-
cine and CpG ODN is associated with increased tumor protection in a mouse


Enhanced and prolonged cross-presentation following endosomal escape

eosynogenic antigens encapsulated in biodegradable nanoparticles. Immunology


vaccine strategy for overcoming mucosal tolerance? Expert Rev Vaccines


poly[(lactic acid)] nanoparticle vaccine vehicle from intestinal mucous to sub-


[32] O’Hagan DT, Palin KJ, Davis SS, O’Hagan DT. Enhanced secretory IgA and

systemic IgG antibody responses after oral immunization with biodegradable


[33] Challacombe SJ, Rahman D, Jeffery H, Davis SS, O’Hagan DT. Enhanced

secretory IgA and systemic IgG antibody responses after oral immunization

with biodegradable microparticles containing antigen. Immunology


[34] O’Hagan DT, Palin K, Davis SS, Artursson P, Sjoholm L. Microparticles as poten-

