The first clinical use of a live-attenuated *Listeria monocytogenes* vaccine: A Phase I safety study of *Lm*-LLO-E7 in patients with advanced carcinoma of the cervix

Paulo Cesar Maciag [a,1], Siniša Radulovic [b,2], John Rothman [a,*]

[a] *Advaxis Inc. 675 US 1 North, North Brunswick, NJ 08902, USA*
[b] *National Cancer Research Center, Pasterova 14, 11 000 Belgrade, Serbia*

ABSTRACT

Invasive carcinoma of the cervix (ICC) is the second most common cancer in women worldwide. *Lm*-LLO-E7 vaccine is a live-attenuated *Listeria monocytogenes* (*Lm*) that secretes the HPV-16 E7 antigen fused to a non-hemolytic fragment of the *Lm* protein listeriolysin O (LLO). In this Phase I trial, the safety of *Lm*-LLO-E7 was assessed in 15 patients with previously treated metastatic, refractory or recurrent ICC. Patients received 1 of 3 dose levels of *Lm*-LLO-E7 (1×10^9 CFU, 3.3×10^9 CFU or 1×10^{10} CFU) as an intravenous infusion, followed by a second dose 3 weeks later. All patients experienced a flu-like syndrome which responded to non-prescription symptomatic treatment. Severe (grade 3) adverse events related to *Lm*-LLO-E7 were reported in 6 patients (40%), but no grade 4 adverse events were observed. At the highest dose some patients had severe fever and dose limiting hypotension. By the end of the study protocol, 2 patients had died, 5 had progressed, 7 had stable disease and 1 qualified as a partial responder. This study shows for the first time that a live-attenuated *Lm* is safe to be administered to late stage ICC patients.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Invasive carcinoma of the cervix (ICC) is the second most common cancer in women worldwide with over 450,000 new cases and 230,000 deaths annually, most of them occurring in developing countries [1]. Persistent infection with high-oncogenic risk human papillomavirus (HR-HPV) types is recognized as a necessary, but not sufficient, cause of cervical cancer [2–4]. HPVs 16 and 18 are the most prevalent types in malignant lesions, accounting for over 70% of ICC and over 50% of high-grade precursor lesions [5]. Although the advent of prophylactic HPV vaccines may have a significant impact on the incidence of ICC in the future, the worldwide implementation of such vaccines remains a challenge, particularly in developing countries [6]. Additionally, these vaccines are not intended to treat pre-existing HPV infections and associated malignancies, which require therapeutic vaccines, mostly targeting the E6 and E7 HPV oncoproteins [7].

Listeria monocytogenes (*Lm*) is a food-borne gram-positive bacterium that can occasionally cause disease in humans. Listeriosis is an uncommon infection, primarily affecting elderly individuals, newborns, pregnant women and immunocompromised individuals [8]. In addition to strongly activating innate immunity and inducing a cytokine response that enhances antigen-presenting cell (APC) function, *Lm* has the ability to replicate in the cytosol of APCs after escaping from the phagolysosome, which requires the virulence factor listeriolysin O (LLO) protein [9,10]. This unique intracellular life cycle allows antigens secreted by *Lm* to be processed and presented in the context of both MHC class I and II molecules, resulting in potent cytotoxic CD8$^+$ and Th1 CD4$^+$ T-cell-mediated immune responses [10,11].

Lm has been extensively investigated as a vector for cancer immunotherapy in pre-clinical models [12–16]. *Lm*-LLO-E7 is a recombinant live-attenuated *Lm* that secretes the antigen HPV-16 E7 fused to a non-hemolytic LLO [16]. Previous studies have shown that genetically fusing an antigen to LLO enhances the immunogenicity of tumor-associated antigens, resulting in a better therapeutic efficacy against established tumors [15–17].

Immunization of mice with *Lm*-LLO-E7 induces regression of established tumors expressing E7 and confers long-term protection [16]. Moreover, it is able to overcome immunological tolerance and impair the development and severity of autochthonous tumors in an E7 transgenic mouse model [18]. The therapeutic efficacy of *Lm*-LLO-E7 correlates with its ability to induce E7-specific tumor-infiltrating CTLs, mature dendritic cells, reduce the number of

* Corresponding author. Tel.: +1 732 545 1590; fax: +1 732 545 1084.
E-mail addresses: maciag@advaxis.com (P.C. Maciag), sinisar@ncrc.ac.yu (S. Radulovic), rothman@advaxis.com (J. Rothman).
[1] Tel.: +1 732 545 1590; fax: +1 732 545 1084.
[2] Tel.: +381 11 20 67 434; fax: +381 11 20 67 294.

intratumoral regulatory CD4+ CD25+ T cells and inhibit tumor angiogenesis [19].

Lm has also a number of inherent advantages as a vaccine vector. The bacterium grows very efficiently *in vitro* without special requirements and it lacks LPS, which is a major toxicity factor in gram-negative bacteria, such as *Salmonella* [20]. Genetically attenuated *Lm* vectors also offer additional safety as they can be readily eliminated with antibiotics, in case of serious adverse effects and unlike some viral vectors, no integration of genetic material into the host genome occurs. Although the potential of *Lm* as a vaccine vector has been proven in pre-clinical studies, the feasibility of using live-attenuated *Lm* vectors as a cancer immunotherapeutic in humans has not been demonstrated yet. In a previous study, healthy volunteers were given orally escalating doses of an *actA/plcB*-deleted *Lm* strain without serious adverse effects or long-term sequelae [21]. However, no previous studies with *Lm* vectors have been done in cancer patients, who are the target population for *Lm*-LLO-E7 clinical use. Moreover, the highly attenuated *Lm*-LLO-E7 vector is given intravenously. We report herein the first clinical use and safety of a live-attenuated *Lm* vector to treat patients with advanced ICC, who had failed prior chemotherapy, radiotherapy, and/or surgery.

2. Materials and methods

2.1. Construction of the Lm-LLO-E7 vaccine

Construction of *Lm*-LLO-E7 was previously described [16]. Briefly, the prfA-defective *Lm* strain XFL-7, which is derived from the streptomycin-resistant wild-type *Lm* 10403S strain [22], was transformed with the multicopy plasmid pGG55. This plasmid contains an expression cassette with the HPV-16 E7 gene fused to a truncated *hly* gene that encodes the first 441 residues of LLO. Additionally, pGG55 contains a mutated copy of the *prfA* gene that partially restores the virulence of XFL-7 and it is required for plasmid retention *in vivo*. A chloramphenicol resistance gene in the plasmid allows *in vitro* growth of *Lm*-LLO-E7 under selective conditions. *In vivo* stability and clearance of *Lm*-LLO-E7 have been already described [23].

2.2. Clinical-grade Lm-LLO-E7 manufacturing

Lm-LLO-E7 was grown from a master cell bank according to Good Manufacturing Practices (GMP) conditions in a bioreactor. The biomass was washed and resuspended at concentrations of 1×10^9 CFU/ml, 3.3×10^9 CFU/ml and 1×10^{10} CFU/ml based on OD_{600} readings. Aliquots of 1.1 ml were prepared and stored at $-80\,^\circ$C for clinical use. Actual doses were calculated by counting colonies from serial dilutions in plate cultures in triplicates.

2.3. Pre-clinical toxicology

The maximum tolerated dose (MTD) and toxicity were determined in a single and multi-dose regimen by administering intravenously (IV) 0.1 ml of escalating doses of *Lm*-LLO-E7 corresponding to 2.8×10^5 CFU, 2.8×10^7 CFU and 2.8×10^9 CFU per mouse in 7–8 weeks old female BALB/c mice. In the multi-dose regimen, mice received *Lm*-LLO-E7 once a week for 4 consecutive weeks, in a total of 10 mice per group. Body weights were taken weekly and blood samples collected on day 28 from 5 animals per group for serum chemistry. Mice were sacrificed on the day of termination for gross necropsies and histopathological analysis. As control groups in these experiments, mice were given normal saline solution.

2.4. Study design, patient eligibility and enrollment

This was an open label, non-randomized and non-controlled study, conducted according to International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) standards. National and institutional approvals were obtained from each site conducting the study. A total of 15 patients were enrolled in the study: 7 patients from the Institute for Oncology and Radiology in Serbia; 6 patients at Cecetype in México; and 1 patient each from Hadassah Hebrew Medical University in Israel and the Fundación Rodolfo Padilla Padilla in México. Patients age 18 and older with histologically confirmed progressive, recurrent or advanced squamous cell carcinoma of the cervix who had failed chemotherapy, radiotherapy and/or surgery were eligible to participate in the study. Disease was staged according to the FIGO (International Federation of Gynecology and Obstetrics) system. Only immunocompetent patients with a positive response to delayed type hypersensitivity (DTH) screening panel, with one primary cancer and Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Karnofsky index >60%) were enrolled in the study. Pregnant women or at risk for pregnancy, drug abusers and HIV-positive patients were excluded as well as patients who received steroids or cancer treatments in the last 30 days, had prior biologic therapy or had a history of listeriosis. Two patients were granted eligibility exceptions to be enrolled into the study: patient 01-001 was enrolled with a creatinine value higher than allowed by inclusion/exclusion criteria and patient 02-006 had no measurable lesions at the time of study entry. HPV typing was done by PCR in archival paraffin-embedded or fresh tissue biopsies. HPV-16-positivity was an inclusion criterion at the beginning of the study and it was applied for the first 6 patients. However, this criterion was later excluded for the next 9 patients enrolled in the study.

After a screening visit, patients were admitted as in-patients for 5 days following each administration of *Lm*-LLO-E7 (Table 1). Groups of 5 patients received two vaccinations 21 days apart at nominal doses (based on OD_{600}) of 1×10^9 CFU, 3.3×10^9 CFU, or 1×10^{10} CFU that corresponded to actual doses of 5×10^8 CFU, 1.2×10^9 CFU, or 7.8×10^9 CFU (actual plate counting), respectively. The next scheduled cohort that would receive a nominal dose of 3.3×10^{10} CFU was not enrolled due to the presence of dose limiting toxicity (DLT) in the previous cohort. Each patient received an IV dose of ampicillin (500 mg) 5 days after treatment, followed by a 10-day oral course of ampicillin (500 mg QID). Follow-up visits were conducted to assess safety and efficacy 3 weeks and 3 months after the second dose (Table 1).

2.5. Lm-LLO-E7 preparation and infusion

Each vial of *Lm*-LLO-E7 contained 1.1 ml of frozen active material. To prepare the infusion solution, the material was thawed at 37 °C in a water bath and 1 ml withdrawn from the vial to be reconstituted in 250 ml of normal saline. The infusion solution was administered intravenously over 30 min.

2.6. Clinical and safety assessment

Physical examinations and hematologic (complete blood count with differential and platelets) and serum chemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, conjugated bilirubin, gamma-glutamyltransferase, lactate dehydrogenase, lipase, creatinine) standard safety laboratory tests were done in all patients (Table 1). The safety of *Lm*-LLO-E7 administration was assessed through changes from a pre-dose baseline on the injection site (swelling, irritation or other abnormalities), physical examination findings, vital signs or laboratory parameters.

Table 1
Lm-LLO-E7 Phase I study design.

Procedure		Days in the study																
	S[a]	1	2	3	4	5	8	15	22	23	24	25	26	29	36	43	79	111
Physical evaluation	X	X	X	X	X	X			X	X	X	X	X			X	X	X
Tumor assessment	X															X	X	X
Lm-LLO-E7 infusion[b]		X							X									
Antibiotics initiated						X						X						
Hematology	X	X					X	X	X					X	X	X	X	X
Serum chemistry	X	X					X	X	X					X	X	X	X	X
Microbiology			X	X						X	X							

[a] S, screening visit.
[b] Boxed area indicates the days of hospital stay.

All adverse events (AE) were recorded and an independent safety review panel assessed safety at each dosage step prior to escalation to the subsequent dosing level. Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Per the protocol, the trial was stopped when 2 patients in 1 group manifested DLT of hypotension, specifically defined in the protocol as a decrease in blood pressure sufficient to warrant therapeutic intervention.

2.7. Efficacy assessment

Regular CAT scans and tumor assessments were made according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (Table 1). Patients were included in the efficacy analysis only if they received the 2 doses of *Lm*-LLO-E7 and at least one post-dosing evaluation. Efficacy criteria included the following: tumor size, improvement in ECOG/Karnofsky performance status, and survival monitoring of the patients until the occurrence of the last death.

2.8. Microbiological assessment

Listeremia was evaluated by blood cultures on days 2 and 3 after each dose in all patients (Table 1). Stool and urine were analyzed for the presence of *Lm*-LLO-E7 to assess shedding. Two patients in the 3.3×10^9 CFU group (patients 04-004 and 04-005) and 3 patients in the 1×10^{10} CFU group (patients 01-005, 02-006, and 04-006) had fecal and urine samples tested.

2.9. Detection of HPV-specific T cells by IFNγ-ELISpot

Peripheral blood (10 ml) was collected from patients and peripheral blood mononuclear cells (PBMCs) isolated by ficoll-hypaque gradient centrifugation. Blood samples were withdrawn in the visits before and after vaccination. PBMCs were stored frozen and at the end of the study shipped to a central laboratory for ELISpot assay. Briefly, PBMCS were thawed and seeded in duplicates at 2.5×10^5 cells per well in a pre-coated IFNγ-ELISpot 96-well plate (Mabtech) in RPMI 1640 medium supplemented with 10% human serum. In the experimental wells, samples were either incubated with a pool of 22 15-mer peptides (short peptides) or a pool of 5 28-mer peptides (long peptides), overlapping by 11 residues and spanning the entire HPV-16 E7 antigen. The final concentration was 1 µg/ml for each of 15-mer peptides or 2 µg/ml for each of the 28-mer peptides. Anti-CD3 antibody (0.1 µg/ml) was used as a positive control in the assays to assess T cell and ELISpot quality. The ELISpot assay was done according to the manufacturer's instructions and spots

were counted under a dissection microscope with digital assistance. Specific spots were calculated by subtracting the mean number of spots + 2 × SD of the medium only control from the mean number of spots in experimental wells. Specific T cell response to HPV-16 E7 was considered positive when specific T cell frequencies were greater than 1 in 10^4 PBMCs. Positive responses induced by the vaccine were considered positive only when the frequency of specific responses increased by at least 3-fold compared to the baseline sample [24].

2.10. Statistical analyses

Comparisons of means were done by ANOVA and Student's *t*-tests for independent samples. For disease stage comparison, the non-parametric Kruskal–Wallis test was used. Safety and efficacy results were analyzed using descriptive statistics only. Survival rates in the cohorts were compared by the log-rank test. All analyses were conducted with Statistical Package for the Social Sciences® 15.0 software. Statistical significance was based on a value of $P \leq 0.05$.

3. Results

3.1. Pre-clinical toxicity evaluation of Lm-LLO-E7

The MTD for IV administration of *Lm*-LLO-E7 in BALB/c female mice was 2.8×10^7 CFU. In the group that received 2.8×10^9 CFU, splenomegaly was observed in 2 out of 5 mice on day 7 after injection and it was defined as an AE. No deaths occurred at the doses tested. A dose of 2.8×10^7 CFU corresponds to a human dose of 7.8×10^{10} by body mass or 7.5×10^9 by surface area, a range that is consistent with the clinical doses used in this clinical trial. Importantly, an IV dose of 2.8×10^7 CFU of *Lm*-LLO-E7 was also tolerated by immunodeficient SCID mice, with no deaths observed. In contrast, the MTD for the 10403S strain was 1×10^3 CFU in the SCID mouse.

Toxicity was also evaluated in a multi-dose regimen in BALB/c female mice. After 4 weekly doses, a weight loss that averaged 1.2 g was observed only in the group given 2.8×10^9 CFU (Supplementary Fig. 1A). All mice receiving the highest dose had piloerection following injections on day 14 and 21. On day 28, 1 mouse (20%) in this group had highly increased levels of the liver enzyme aspartate aminotransferase (AST), in contrast to none in the other groups (Supplementary Fig. 1B). The most common necropsy finding was also splenomegaly in the group given the highest dose. All mice treated with *Lm*-LLO-E7 had histologic alterations in the liver, characterized by multifocal, random accumulations of

mixed inflammatory cells including macrophages, lymphocytes, and neutrophils. In the group given 2.8×10^7 CFU, infiltration was minimal, with approximately 5–20 cells per foci and less than 10 foci per section in all 9 animals evaluated. Kidneys were variably affected in this group and 7 out of 9 mice exhibited tubular alterations, including dilation, epithelial attenuation and/or regeneration. Active neutrophilic pyelonephritis was present in 1 animal, consistent with an active infection with Lm-LLO-E7. Despite these findings, the levels of serum creatinine did not increase in these mice, indicating a preservation of kidney function. Overall, these pre-clinical results indicate that Lm-LLO-E7 is highly attenuated and the dosages used in this human trial are within the expected safety range for this agent and associated with transient and subtle alterations within the liver and kidneys consequent to acute infection.

3.2. Antibiotic sensitivity

An important safety characteristic of bacterial vectors is the possibility of readily eliminating the recombinant bacteria with antibiotics, if necessary. An antibiotic sensitivity assay showed that Lm-LLO-E7 was susceptible to the lowest tested concentration of 9 commonly used antimicrobial agents (penicillin, ampicillin, amoxicillin/clavulanate, gentamicin, tetracycline, erythromycin, trimethoprim/sulfamethoxazole, vancomycin and ciprofloxacin) (Supplementary Table 1). As expected, Lm-LLO-E7 was resistant to the highest tested concentration of streptomycin and chloramphenicol.

3.3. Characteristic of patients and disease history

A total of 15 patients with advanced ICC were enrolled in the study and information on patients, prior therapy, disease stage, and dosage groups is shown in Table 2. The mean age for all patients was 52.1 years, ranging from 35 to 72 years. No significant differences in the mean age and disease severity were observed between the cohorts. A majority (53.3%) of the study population was Caucasian and the remainder (46.7%) were Latino/Hispanic. Most patients (60%) were post-menopausal. The median disease duration between initial diagnosis and progression and enrollment was 2 years and no significant correlation was found with clinical outcome. Thirteen patients (86.7%) had a history of failed platinum-based chemotherapy, 13 (86.7%) had failed radiotherapy, and 6 had failed surgery (40%) (Table 2). All patients received the 2 scheduled Lm-LLO-E7 doses and 7 patients completed all follow-up visits, none of them in the 3.3×10^9 cohort. A total of 8 patients were discontinued from the study before completing all the scheduled visits due to disease progression (6 patients), investigator decision or death (1 patient each).

3.4. Clinical trial results

All 15 patients experienced AE during the period of the study and the most commonly reported AE were pyrexia (100%), vomiting (60%), chills, headache and anemia (53.3% each), nausea and tachycardia (46.7% each), and musculoskeletal pain (26.7%). The number of patients with moderate and severe (grades 2 and 3) AE attributed to Lm-LLO-E7 is shown in Table 3. No drug-related grade 4 AE were reported in this study and none of the patients experienced an injection site reaction or other acute reaction to administration. In the first hours following the Lm-LLO-E7 injection, all the patients exhibited an increase in the body temperature (Fig. 1), which in some patients was also associated with decreased blood pressure and tachycardia, with no significant differences observed across treatment groups. The hypotension and tachycardia were likely to be a consequence of the pyretic response to the vaccine. Additionally,

Table 2
Clinical data of patients enrolled for Lm-LLO-E7 vaccination.

Site	Patient	Age	Primary staging	Date of diagnosis	HPV type	Date of study entry	Disease at study entry	Previous therapies
Cohort 1, nominal dose: 1×10^9 CFU[a], actual dose: 5×10^8 CFU								
Serbia	01-001	63	IIIB	April 2001	16	April 2006	Metastatic, unresectable	Cisplatin, radiation
Serbia	01-002	38	IB	January 2005	16	April 2006	Metastatic	Cisplatin, radiation, surgery
Serbia	01-003	70	IIB	July 2004	16	May 2006	Metastatic, recurrent and unresectable	Radiation
Serbia	01-004	35	IIA	August 2005	16	October 2006	Metastatic	Cisplatin, 5FU[b], radiation, surgery
Mexico	04-001	56	IIB	August 2000	16	August 2006	Unresectable	Carboplatin
Cohort 2, nominal dose: 3.3×10^9 CFU, actual dose: 1.2×10^9 CFU								
Mexico	03-001	43	IIIB	October 2005	16	December 2006	Metastatic	Cisplatin, 5FU, radiation
Mexico	04-002	72	IB2	May 2001	16	October 2006	Recurrent	Carboplatin, radiation, surgery
Mexico	04-003	56	IIIB	August 2004	16	November 2006	Recurrent and unresectable	Cisplatin, radiation
Mexico	04-004	55	IB2	January 2005	31	December 2006	Recurrent	Carboplatin, radiation, surgery
Mexico	04-005	61	IIB	December 2004	51, 68	December 2006	Unresectable	Carboplatin, radiation
Cohort 3, nominal dose: 1×10^{10} CFU, actual dose: 7.8×10^9 CFU								
Serbia	01-005	36	IB2	April 2004	Negative	February 2007	Metastatic, recurrent and unresectable	Cisplatin, surgery
Serbia	01-006	38	IIIB	December 2005	16	January 2007	Metastatic and unresectable	Cisplatin, radiation
Serbia	01-007	68	IIB	July 2005	16	February 2007	Metastatic	Cisplatin, radiation
Israel	02-006	46	IB1	January 2004	Unknown	March 2007	Unknown[c]	Evorel, radiation, surgery
Mexico	04-006	61	IIB	January 2003	Unknown	February 2007	Recurrent	Carboplatin, gemcitabine, radiation

a Colony-forming units.
b Fluorouracil.
c Patient 02-006 had metastatic and recurrent disease before having surgical procedures to remove the lesions. At enrollment, no detectable disease was present.

Table 3
Summary of drug-related adverse events (common terminology criteria for adverse events grading) during the study.

Dose group	Lm-LLO-E7 nominal dose (CFU[a])							
	1×10^9 $n = 5$		3.3×10^9 $n = 5$		1×10^{10} $n = 5$		Total $n = 15$	
CTCAE[b] grade	2	3	2	3	2	3	2	3
No. of subjects with grade ≥2 drug-related adverse events	5	1	3	4	5	1	13	6
Pyrexia	3	0	1	2	3	1	7	3
Chills	3	0	0	0	4	0	7	0
Vomiting	2	0	1	0	2	0	5	0
Nausea	2	0	0	0	1	0	3	0
Hypotension[c]	0	0	0	0	3	0	3	0
Headache	1	0	0	0	2	0	3	0
Fatigue	0	1	0	0	0	0	0	1
Somnolence	0	0	0	0	1	0	1	0
Asthenia	0	0	0	0	1	0	1	0
Increased liver enzymes	0	0	1	2	0	0	1	2
Gamma-glutamyltransferase (GGT)	0	0	0	2	0	0	0	2
Lactate dehydrogenase (LDH)	0	0	0	1	0	0	0	1
Alkaline phosphatase (ALP)	0	0	1	0	0	0	1	0

[a] Colony-forming units.
[b] Common Terminology Criteria for Adverse Events version 3.0.
[c] Event met dose-limiting toxicity criteria.

we did not observe significant differences either in the frequency or severity of the adverse effects after the first or second dose. In most patients, these AE had a short duration and were within normal limits at 12 h post-dose (Fig. 1), responding to treatment with analgesics and antipyretics when necessary. Patients 01-004 and 01-007 had moderate and severe fevers, respectively, which were present at 48 h post-dosing. Per the protocol, these patients were given antibiotic on the third day after the infusion rather than waiting until day 5.

A total of 9 (60%) patients experienced severe adverse (grade 3) events and in 6 (40%) patients they were considered related to Lm-LLO-E7, including pyrexia in 3 patients, fatigue in 1 patient, and increased gamma-glutamyltransferase (GGT) levels in 2 patients (Table 3). Overall, changes in physical examination were considered not clinically significant and the majority of them attributed to underlying disease. Additionally, there were no improvements in either ECOG performance status or Karnofsky index over time.

Two deaths occurred during the 111 days protocol period of the study, although they were considered unrelated to the administration of Lm-LLO-E7 (Table 4). One patient (04-004) was withdrawn from the study on day 43 due to disease progression prior to her death on day 87. The other patient (04-005) died during active study participation on day 36 due to renal failure, metabolic acidosis and cardiac arrest 2 weeks after receiving her second dose of Lm-LLO-E7. Although normal at screening, this patient had a clinically significant increase in creatinine

(5.1 mg/dl) and urea (46.7 mg/dl) prior to her first dose on day 1, associated with bilateral hydronephrosis and dilated ureters. These levels remained high for this patient after immunization, with levels of creatinine between 3.2 mg/dl and 5.8 mg/dl. The urea concentration was also high on the day of the first dose (47 mg/dl), increasing on day 15 and remaining at the same levels thereafter (74–80 mg/dl). This patient evolved with severe or life-threatening AE that included elevated serum amylase, anemia, metabolic acidosis, renal failure, cardiorespiratory arrest and death.

3.5. Laboratory assessment

A majority of patients had abnormal clinical chemistry and hematology values during the study, most of them attributed to the underlying disease or prior therapy and not directly related to Lm-LLO-E7. Due to the liver tropism of Lm, liver function was carefully followed in all patients. Clinically significant changes to chemistry parameters were observed in 6 (40%) patients, including elevated levels of serum GGT, alanine aminotransferase (ALT), urea and creatinine.

Abnormal liver function tests (LFT) were observed only on day 8 or later. Two patients in the 3.3×10^9 CFU (40%) and 1 patient in the 1×10^{10} CFU groups (20%), but none in the 1×10^9 CFU group, were reported to have elevated liver enzymes in the serum. On average the levels of liver enzymes were higher 1 week after the first Lm-
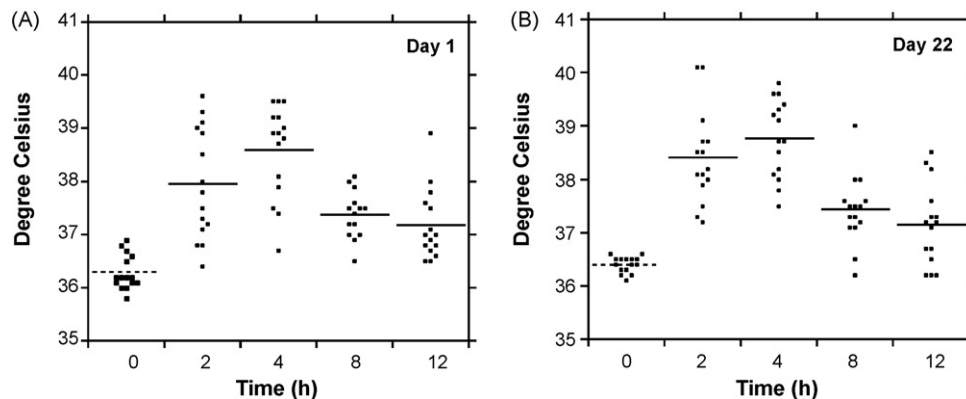

Fig. 1. Fever in patients treated with Lm-LLO-E7. Body temperature variation in the first 12 h following the first Lm-LLO-E7 dose on day 1 (A) and the second dose on day 22 (B). Horizontal bars indicate the mean value for all patients in each time point.

Table 4
Day and reason for early discontinuation, response to treatment and survival of patients treated with *Lm*-LLO-E7.

Patient no.	Last day in study	Reason for withdraw	RECIST[a]	Change in tumor load (%)	Study day at death	Days alive (January 2009)
Cohort 1, nominal dose: 1×10^9 CFU[b], actual dose: 5×10^8 CFU						
01-001	111		SD[c]	17.5	367	
01-002	79	PD[d]	PD	142.5	118	
01-003	111		PD	30.0	347	
01-004	111		SD	−20.0		838
04-001	43	PD	SD	11.0	381	
Cohort 2, nominal dose: 3.3×10^9 CFU, actual dose: 1.2×10^9 CFU						
03-001	79	PD	SD	−19.4	323	
04-002	43	PD	PD	34.4	535	
04-003	79	ID[e]	PR[f]	−32.5		806
04-004	43	PD	PD	81.3	87	
04-005	22	Death	NE[g]		36	
Cohort 3, nominal dose: 1×10^{10} CFU, actual dose: 7.8×10^9 CFU						
01-005	111		SD	7.4	178	
01-006	111		PD	56.3	194	
01-007	111		SD	−20.8		707
04-006	111	PD	SD	17.8	542	
02-006	43	LFU[h]	NE		NE	

[a] Response evaluation criteria in solid tumors.
[b] Colony-forming units.
[c] Stable disease.
[d] Progressive disease.
[e] Investigator decision.
[f] Partial response.
[g] Not evaluable.
[h] Lost to follow-up. Patient data was not included for efficacy evaluation.

LLO-E7 dose and decreased thereafter, with a second small peak 1 week following the second dose (Fig. 2A and B and Supplementary Fig. 2A–C). One patient in the 3.3×10^9 CFU group had severe elevation in GGT 7 days after receiving her first and second *Lm*-LLO-E7 infusion. Another patient had severe elevation of ALT and GGT immediately before the second dose, which persisted at moderate levels until day 43. As the increase in liver enzymes was either transient or not clinically significant in these patients, no medical intervention was necessary. On average, no significant changes or clear patterns were observed with creatinine and urea concentrations (Fig. 2C and Supplementary Fig. 3).

Additionally, 8 (53.3%) patients had clinically significant changes to hematology parameters associated with anemia, such as decreased hematocrit, hemoglobin concentration and red blood cells count. Analysis of hemoglobin did not show a clear correlation between *Lm*-LLO-E7 infusion and decreased hemoglobin levels (Fig. 2D and Supplementary Fig. 4). One patient had severe anemia on day 111 visit, which was considered to be likely unrelated to
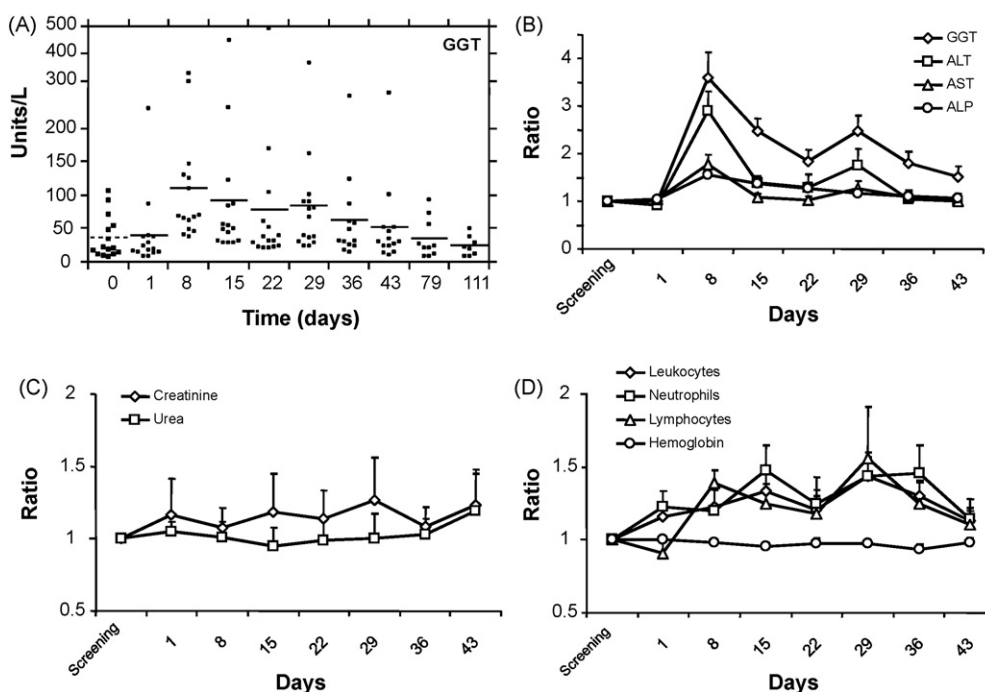

Fig. 2. Laboratory parameters in patients treated with *Lm*-LLO-E7. (A) Serum GGT levels from individual patients in different time points. Overtime variation in liver enzymes (B), creatinine and urea (C) and hematologic parameters (D) is shown as the mean ratio of values in each time point to the reference screening values for each patient. Vertical bars represent the standard error of the mean (SEM) for each time point.

the study vaccine. No clinically significant drug-related leukocytosis, granulocytosis or lymphocytosis was observed in the patients (Fig. 2D).

3.6. Dose limiting toxicity events

Patients in the first 2 dose levels of 1×10^9 CFU and 3.3×10^9 CFU experienced a tolerable safety profile, whereas DLT occurred in 3 patients in the 1×10^{10} CFU group, resulting in study discontinuation per the protocol. All of these events were episodes of grade 2 diastolic hypotension within hours after the *Lm*-LLO-E7 infusion that required therapeutic intervention. In all patients the hypotension was successfully controlled with IV fluids and supporting medication. One patient experienced the event during the first dose of study medication, associated with moderate fever. The remaining 2 patients had the DLT event during the second dose, associated with moderate and severe pyrexia. One patient (01-007) required antibiotics for complete resolution of the fever.

3.7. Microbiology assessment

Blood cultures were assessed on the second and third days after each dose in all patients. One patient in the 1×10^9 CFU group was positive for *Lm*-LLO-E7 (35 CFU/ml) on the second day after her first dose, but negative on day 3 culture. No other positive blood cultures were observed in the study. Urine and stool microbiological analysis was done in 5 patients and no samples were positive for the presence of *Lm*-LLO-E7. These results indicate that *Lm*-LLO-E7 was rapidly cleared from the blood, with a low probability of clinically significant listeriosis caused by the vaccine. Additionally, since no *Lm*-LLO-E7 was detected in the urine or feces of the tested patients, it is unlikely that shedding of the vaccine vector occurred.

3.8. Detection of HPV-16 E7-specific T cell responses after vaccination with Lm-LLO-E7

Although PBMCs were prepared from all patients, most samples were not suitable for ELISpot analysis because of low yield and poor viability after thawing the cells. ELISpot results from these samples were inconsistent and not interpretable. Pre- and post-vaccination samples with high viability and interpretable ELISpots were obtained from 3 patients (01-001, 01-003 and 01-004) only. Positive and specific T cell response to HPV-16 E7 antigen induced by the vaccine was observed after the second dose on day 26 in patient 01-004 (Fig. 3). Unfortunately, results from samples after day 26 were not available from this patient to evaluate the dura-

Fig. 3. IFNγ-ELISpot for patients 01-004 before and after two immunizations with *Lm*-LLO-E7. The E7 short peptide pool contains 22 15-mer peptides and the long peptide poll contains 5 28-mer peptides spanning the entire E7 protein. The hachured line in the graph shows the value for a response to be considered positive (1/10,000 T cell frequency).

tion of the T cell response. On the other hand, no E7-specific T cell responses were detected after vaccination in patients 01-001 (on day 43) and 01-003 (on days 26, 43 and 111) (Supplementary Fig. 5).

3.9. Efficacy and survival

Although this study was not designed to evaluate the *Lm*-LLO-E7 efficacy, tumor assessment using the RECIST criteria and survival data were collected. Two patients were not eligible for efficacy evaluation (Table 4), as 1 patient (04-005) died on day 36 while on the study and the other (02-006) was enrolled with no measurable disease after her third surgery. Stable disease was reported in 7 (53.8%) and progressive disease in 5 (38.5%) of the 13 evaluable patients (Table 4). An unconfirmed partial tumor response was observed in 1 patient (7.7%), with a 32% reduction in tumor load (Table 4). This patient (04-003) had a response of all 3 target lesions and one of them completely disappeared. However, no confirmatory scan 1 month post-response was done in this patient as required under the RECIST criteria. In addition, a complete response of a non-target lesion was reported for another patient who had stable disease. Overall, a reduction in total tumor size was observed in 4 patients (30.8%), with no dose–response effect observed.

Eleven (73.3%) deaths have been reported so far from the patients enrolled in this study, 2 of them during the protocol period of the study and 9 during the survival follow-up. A total of 4 patients died in each of the first 2 cohorts and 3 in the third cohort, with 1 patient lost to follow-up (Table 4). Three (20%) patients are still alive, 1 from each cohort (Table 4). Overall, the median survival was 347 days for all the patients and no significant difference in survival was observed among the cohorts.

4. Discussion

To our knowledge, this is the first clinical report demonstrating safety and feasibility on using a live-attenuated *Lm* vaccine in patients with cancer. Intravenous administration of *Lm*-LLO-E7 was consistently associated with a flu-like syndrome in all patients, characterized by pyrexia, chills, headache, tachycardia, hypotension, nausea and vomiting. These AE were acute and transient in most patients, resolving in the first 12 h after infusion. While well tolerated in the lower two dosage groups, dose limiting diastolic hypotension was observed in the highest dosage group.

Such a flu-like syndrome is expected to occur after infusion of a bacterial vector, most likely due to a potent induction of innate immune responses. In fact, this self-limited flu-like syndrome observed in this study is consistent with reports of food-borne gastroenteritis due to *Lm*, where the most common symptoms include fever, diarrhea, nausea, headache, and pain in joints and muscles [25].

Importantly, no evidence of listeriosis or meningitis was observed and most AE, including dose limiting hypotension, responded to symptomatic treatment without the need for antibiotics. In 2 patients, ampicillin was given per protocol on day 3 after infusion due to persistent fever, instead of starting 5 days after each dose. Interestingly, these 2 patients experienced reductions in their tumor burdens and are both currently alive. Although it is not possible to make any inferences regarding reaction to the vaccine, patient immunocompetency and efficacy on this study, future trials with larger samples might be able to address these issues.

The activation of innate immunity by *Lm* is well-characterized in animal models and it plays an essential role in the clearance of the bacteria and control of the infection at early stages [10]. Infection of macrophages by *Lm* results in secretion of cytokines and chemokines such as interleukin (IL)-1, IL-6, IL-12, IL-18, tumor
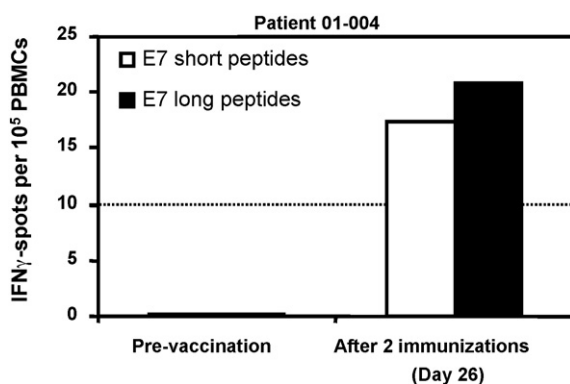
necrosis factor α, IL-8 and the monocyte chemoattractant protein-1 (MCP-1), among others [26]. In particular, interleukin (IL)-1 and IL-6 are potent inducers of fever [27] and might be associated with the pyrexia observed on *Lm*-LLO-E7 infusion. In a clinical trial in patients with metastatic melanoma, infusion of an attenuated *Salmonella typhimurium* strain [20] caused a similar flu-like syndrome in patients receiving higher doses, along with increased levels of IL-6, IL-1β and TNF-α in the serum. The levels of cytokines peaked between 4 and 6 h after *Salmonella* infusion and increased as the dose escalated [20].

In mice, circulating *Lm* is removed from the blood stream primarily by splenic and hepatic macrophages [28]. In the liver, the foci of infection are characterized by granulocyte and mononuclear cell infiltration, with resolution of the lesions within 2 weeks [29]. Despite the *Lm*-LLO-E7 attenuation, similar lesions were observed in the liver after a multi-dose regimen in mice, indicating that some degree of hepatotoxicity might also be observed in humans. In fact, most patients had a minimal or mild increase in the levels of liver enzymes after the first dose of *Lm*-LLO-E7, relative to their screening visit values. After the second dose, the levels of liver enzymes also increased, but much less when compared to the first dose. One possible explanation is that the duration of liver infection by *Lm*-LLO-E7 is shorter after the second dose, possibly due to acquired immunity elicited by the vaccine vector.

On the other hand, toxicity to the kidneys was variable in the pre-clinical studies of *Lm*-LLO-E7, with subtle tubular alterations without altering the levels of creatinine in the serum. Most patients in this study had previous treatment with platinum chemotherapy, which is associated with nephrotoxicity and hematotoxicity [30–32]. Nevertheless, on average the creatinine and urea concentration did not change significantly after treatment with *Lm*-LLO-E7. During the study, some patients had elevated levels of urea and creatinine which were attributed to pre-treatment conditions, such as hydronephrosis and ureter obstruction by tumor mass. Similarly, no significant differences in hematologic parameters could clearly be associated to *Lm*-LLO-E7 treatment. Moreover, *Lm*-LLO-E7 was rapidly cleared from the blood and no shedding in fecal or urinary samples was observed in any of the 5 patients tested for this purpose.

The toxicity of *Lm*-LLO-E7 was significantly milder when compared to the adverse effects attributed to salvage chemotherapy [33,34], although it is associated with more severe adverse events when compared to other therapeutic HPV vaccines, as expected from a live-attenuated bacterial vector. Vaccination of ICC patients with a live recombinant vaccinia virus expressing modified forms of the HPV-16 and -18 E6 and E7 proteins is well-tolerated and related with only mild to moderate local toxicity, and no serious systemic adverse events [35]. Similarly, in Phase I studies using autologous dendritic cells loaded with the E7 antigen [36], E6 and E7 long peptides [24] or E7 fused to the BCG heat shock protein 65 [37], no significant toxicity is associated with the vaccines administration. On the other hand, the toxicity observed with *Lm*-LLO-E7 is very similar to adverse events reported in patients with metastatic melanoma after infusion of an attenuated *S. typhimurium* strain [20]. In this study, 33% and a 100% of the patients in the cohorts receiving the two highest doses (3×10^8 CFU/m^2 and 1×10^9 CFU/m^2, respectively) experienced grade 3 adverse events, such as fever, hypotension, nausea, vomiting, anemia and elevated liver enzymes [20].

Although this Phase I safety study was not designed to assess efficacy, tumor masses could be evaluated by RECIST criteria in 13 of 15 treated patients. Overall, stable disease was reported in 7 patients and an unconfirmed partial tumor response in 1 patient (04-003) by the end of the study. However, the small sample size and lack of an appropriate control group preclude us from make any conclusions about the therapeutic efficacy of *Lm*-LLO-E7. More-over, the patients enrolled in the study had advanced ICC and failed prior therapies, decreasing the probability of experiencing clinical responses. Additionally, some patients were discontinued from the study before completing all follow-up evaluations.

Patient 04-003 had refractory ICC with prior cisplatin and radiotherapy before enrolling in this study. This patient was withdrawn early from the study at the investigation site because of a significant reduction in tumor size (>30%) and complete disappearance of a target lesion following treatment with *Lm*-LLO-E7. This patient received additional therapy, consisting of 6 courses of carboplatin and paclitaxel followed by radical hysterectomy, resulting in the disappearance of all observable lesions. This patient remained apparently tumor free for 16 months, although it was not possible to ascribe the response to any single therapeutic procedure. Noteworthy, some studies have shown that previous immunotherapy may improve the patient's response to following chemotherapy [38,39], an interesting possibility to be investigated in subsequent studies with *Lm*-LLO-E7.

Therapeutic vaccines to cervical cancer have been extensively investigated and the viral etiology of this cancer makes it an attractive target for immunotherapies [7,40]. Several strategies have been used with promising clinical results, including vaccination with HPV peptides or proteins, dendritic cell vaccines, DNA vaccines, chimeric virus-like particles and viral vectors [7,40]. However, no clear correlation between peripheral immune responses to tumor-associated antigens and clinical outcome has been observed in most cancer vaccines studies. One caveat in the present study is the lack of immunological data from most patients treated with *Lm*-LLO-E7 and the ability of this vaccine to induce potent and long-term T cell immune responses to HPV-16 E7 in humans has yet to be demonstrated. In this study, the immunological evaluation of E7-specific T cell responses was seriously compromised by the quality of the PBMC samples. Interpretable ELISpot data, before and after vaccination, was obtained from 3 patients only. An E7-specific T cell response induced by *Lm*-LLO-E7 could be detected just in patient 01-004, but not in the other 2 patients. Nevertheless, it is also noteworthy that all 4 patients who experienced tumor reductions were positive for HPV-16, including patient 01-004. Although these results suggest that *Lm*-LLO-E7 can induce an immune response to HPV-16 E7, confirmation in a larger number of patients with high quality samples and standardized procedures is required. A phase II controlled trial is planned to evaluate the safety, efficacy and immunogenicity of *Lm*-LLO-E7 in a large cohort of patients with pre-invasive cervical cancer.

In this *Lm*-LLO-E7 Phase I trial, the overall median survival was 347 days. As this trial was a non-controlled study, no inferences can be made regarding differences in survival. Nonetheless, this result was encouraging if we take into consideration that historically the survival of patients with previously treated metastatic, refractory or recurrent ICC is very poor, with a median survival time of 6–7 months [33,34,41].

Overall, *Lm*-LLO-E7 infusion was safe and well-tolerated in end stage ICC patients who had failed multiple modes of prior therapy, reaching dose limiting toxicity in the group receiving the highest dose of 1×10^{10} CFU. Importantly, AE in all groups responded to treatment when needed with no further consequences. As all patients had advanced progressive disease and most had failed more than one therapeutic regimen, there were a number of AE attributable to progressive disease and prior therapy. The only patient who died while enrolled in the trial succumbed to progressive renal failure that resulted in metabolic acidosis and cardiac failure, probably unrelated to *Lm*-LLO-E7 administration. These results show that cancer immunotherapy based on live-attenuated *Lm*, and possibly other bacterial vectors as well, is feasible and can become an alternative treatment in the future.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.vaccine.2009.04.041.

References

[1] Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2(9):533–43.
[2] Munoz N, Bosch FX. HPV and cervical neoplasia: review of case-control and cohort studies, vol. 119. IARC scientific publications; 1992. p. 251–61.
[3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189(1):12–9.
[4] Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24(Suppl. 3). S3/1–10.
[5] Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24(Suppl. 3). S3/26–34.
[6] Herzog TJ, Huh WK, Downs LS, Smith JS, Monk BJ. Initial lessons learned in HPV vaccination. Gynecol Oncol 2008;109(2 Suppl):S4–11.
[7] Hung CF, Ma B, Monie A, Tsen SW, Wu TC. Therapeutic human papillomavirus vaccines: current clinical trials and future directions. Expert Opin Biol Ther 2008;8(4):421–39.
[8] Schlech 3rd WF. Foodborne listeriosis. Clin Infect Dis 2000;31(3):770–5.
[9] Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. Microbes Infect/Institut Pasteur 2007;9(10):1176–87.
[10] Pamer EG. Immune responses to *Listeria monocytogenes*. Nature Rev Immunol 2004;4(10):812–23.
[11] Ikonomidis G, Paterson Y, Kos FJ, Portnoy DA. Delivery of a viral antigen to the class I processing and presentation pathway by *Listeria monocytogenes*. J Exp Med 1994;180(6):2209–18.
[12] Maciag PC, Seavey MM, Pan ZK, Ferrone S, Paterson Y. Cancer immunotherapy targeting the high molecular weight melanoma-associated antigen protein results in a broad antitumor response and reduction of pericytes in the tumor vasculature. Cancer Res 2008;68(19):8066–75.
[13] Shahabi V, Reyes-Reyes M, Wallecha A, Rivera S, Paterson Y, Maciag PC. Development of a *Listeria monocytogenes* based vaccine against prostate cancer. Cancer Immunol Immunother 2008;57(9):1301–13.
[14] Paterson Y, Maciag PC. *Listeria*-based vaccines for cancer treatment. Curr Opin Mol Ther 2005;7(5):454–60.
[15] Singh R, Dominiecki ME, Jaffee EM, Paterson Y. Fusion to Listeriolysin O and delivery by *Listeria monocytogenes* enhances the immunogenicity of HER-2/neu and reveals subdominant epitopes in the FVB/N mouse. J Immunol 2005;175(6):3663–73.
[16] Gunn GR, Zubair A, Peters C, Pan ZK, Wu TC, Paterson Y. Two *Listeria monocytogenes* vaccine vectors that express different molecular forms of human papilloma virus-16 (HPV-16) E7 induce qualitatively different T cell immunity that correlates with their ability to induce regression of established tumors immortalized by HPV-16. J Immunol 2001;167(11):6471–9.
[17] Sewell DA, Shahabi V, Gunn 3rd GR, Pan ZK, Dominiecki ME, Paterson Y. Recombinant *Listeria* vaccines containing PEST sequences are potent immune adjuvants for the tumor-associated antigen human papillomavirus-16 E7. Cancer Res 2004;64(24):8821–5.
[18] Souders NC, Sewell DA, Pan ZK, Hussain SF, Rodriguez A, Wallecha A, et al. *Listeria*-based vaccines can overcome tolerance by expanding low avidity CD8$^+$ T cells capable of eradicating a solid tumor in a transgenic mouse model of cancer. Cancer Immun 2007;7:2.
[19] Hussain SF, Paterson Y. What is needed for effective antitumor immunotherapy? Lessons learned using *Listeria monocytogenes* as a live vector for HPV-associated tumors. Cancer Immunol Immunother 2005;54(6):577–86.
[20] Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, et al. Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. J Clin Oncol 2002;20(1):142–52.
[21] Angelakopoulos H, Loock K, Sisul DM, Jensen ER, Miller JF, Hohmann EL. Safety and shedding of an attenuated strain of *Listeria monocytogenes* with a deletion of *actA/plcB* in adult volunteers: a dose escalation study of oral inoculation. Infect Immun 2002;70(7):3592–601.
[22] Bishop DK, Hinrichs DJ. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of in vitro stimulation on lymphocyte subset requirements. J Immunol 1987;139(6):2005–9.
[23] Verch T, Pan ZK, Paterson Y. *Listeria monocytogenes*-based antibiotic resistance gene-free antigen delivery system applicable to other bacterial vectors and DNA vaccines. Infect Immun 2004;72(11):6418–25.
[24] Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res 2008;14(1):169–77.
[25] Ooi ST, Lorber B. Gastroenteritis due to *Listeria monocytogenes*. Clin Infect Dis 2005;40(9):1327–32.
[26] Geginat G, Grauling-Halama S. Innate immunity. In: Liu D, editor. Handbook of *Listeria monocytogenes*. Boca Raton: Taylor & Francis; 2008. p. 397–426.
[27] Netea MG, Kullberg BJ, Van der Meer JW. Circulating cytokines as mediators of fever. Clin Infect Dis 2000;(Suppl. 5):S178–84.
[28] Aichele P, Zinke J, Grode L, Schwendener RA, Kaufmann SH, Seiler P. Macrophages of the splenic marginal zone are essential for trapping of blood-borne particulate antigen but dispensable for induction of specific T cell responses. J Immunol 2003;171(3):1148–55.
[29] Mandel TE, Cheers C. Resistance and susceptibility of mice to bacterial infection: histopathology of listeriosis in resistant and susceptible strains. Infect Immun 1980;30(3):851–61.
[30] Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 2008;73(9):994–1007.
[31] Lokich J, Anderson N. Carboplatin versus cisplatin in solid tumors: an analysis of the literature. Ann Oncol 1998;9(1):13–21.
[32] McKeage MJ. Comparative adverse effect profiles of platinum drugs. Drug Saf 1995;13(4):228–44.
[33] Moore DH, Blessing JA, McQuellon RP, Thaler HT, Cella D, Benda J, et al. Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a gynecologic oncology group study. J Clin Oncol 2004;22(15):3113–9.
[34] Bookman MA, Blessing JA, Hanjani P, Herzog TJ, Andersen WA. Topotecan in squamous cell carcinoma of the cervix: a phase II study of the gynecologic oncology group. Gynecol Oncol 2000;77(3):446–9.
[35] Kaufmann AM, Stern PL, Rankin EM, Sommer H, Nuessler V, Schneider A, et al. Safety and immunogenicity of TA-HPV, a recombinant vaccinia virus expressing modified human papillomavirus (HPV)-16 and HPV-18 E6 and E7 genes, in women with progressive cervical cancer. Clin Cancer Res 2002;8(12):3676–85.
[36] Santin AD, Bellone S, Palmieri M, Ravaggi A, Romani C, Tassi R, et al. HPV16/18 E7-pulsed dendritic cell vaccination in cervical cancer patients with recurrent disease refractory to standard treatment modalities. Gynecol Oncol 2006;100(3):469–78.
[37] Maciag PC, Paterson Y. Technology evaluation: HspE7 (Stressgen). Curr Opin Mol Ther 2005;7(3):256–63.
[38] Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, et al. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res 2008;14(14):4526–31.
[39] Ramakrishnan R, Antonia S, Gabrilovich DI. Combined modality immunotherapy and chemotherapy: a new perspective. Cancer Immunol Immunother 2008;57(10):1523–9.
[40] Brinkman JA, Hughes SH, Stone P, Caffrey AS, Muderspach LI, Roman LD, et al. Therapeutic vaccination for HPV induced cervical cancers. Dis Markers 2007;23(4):337–52.
[41] Garcia AA, Blessing JA, Vaccarello L, Roman LD. Phase II clinical trial of docetaxel in refractory squamous cell carcinoma of the cervix: a gynecologic oncology group study. Am J Clinical Oncol 2007;30(4):428–31.