Lung Cancer (2006) **52**, 189—197

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/lungcan

Recombinant GM-CSF plus autologous tumor cells as a vaccine for patients with mesothelioma

Alex Powell[a,c,*], **Jenette Creaney**[a], **Steven Broomfield**[a], **Ivonne Van Bruggen**[a], **Bruce Robinson**[a,b]

[a] *School of Medicine and Pharmacology, Sir Charles Gairdner Hospital Perth, Western Australia*
[b] *Western Australian Institute of Medical Research, Western Australia*
[c] *Department of Medical Oncology, Sir Charles Gairdner Hospital, Perth, Western Australia*

Received 29 July 2005; received in revised form 6 January 2006; accepted 9 January 2006

KEYWORDS
MM;
Vaccine;
Granulocyte macrophage colony stimulating factor;
Delayed type hypersensitivity;
Western blot;
Tumor lysate

Summary Treatments evaluated for malignant mesothelioma (MM), including chemotherapy, radiotherapy and surgery are of limited efficacy. Immunotherapy has shown some promise in MM but optimal vaccination conditions are yet to be defined. Autologous tumour vaccines have the advantage of containing both 'self'- and 'neo'-tumor antigens but they are not commonly used in any cancer, and never in MM. We therefore evaluated the effect of an autologous MM tumor cell lysate, given s.c. with recombinant granulocyte-macrophage colony stimulating factor (GM-CSF), on anti-tumor immunity in patients with MM.

Patients and methods: An autologous tumor lysate vaccine was manufactured from surgically resected tumor and administered subcutaneously together with GM-CSF. Induction of tumor specific cellular immunity was assessed by delayed type hypersensitivy (DTH) skin testing using autologous tumor tissue and of humoral immune responses to shared MM antigens by western blotting of patients' sera against a panel of allogeneic human MM cell lines. CT scanning was used to evaluate tumor progression.

Results: Twenty-two patients were enrolled onto the trial. Of these five developed positive delayed type hypersensitivity skin tests and five showed evidence of altered antibody specificities by western blotting. A total of seven patients developed at least one type of anti-MM immune response. On an intention-to-treat basis the median survival of all patients was 11.5 months, and the 1- and 2-year survival rates were 50% and 27%, respectively. Complete or partial CT responses were not seen, however seven patients had stable disease for the duration of the trial. Vaccination was safe with no severe adverse reactions.

Conclusion: Vaccination with autologous MM tumor cell lysate with GM-CSF induced tumor specific immunity in 32% of patients, was safe and was associated with stable disease but no major tumour regressions.

* Corresponding author at: School of Medicine and Pharmacology, 4th Floor G Block, Sir Charles Gairdner Hospital, Nedlands 6009, Perth, Western Australia. Tel.: +61 8 93862111; fax: +61 8 93868475.
E-mail address: powellhollywood@westnet.com.au (A. Powell).

0169-5002/$ — see front matter.
doi:10.1016/j.lungcan.2006.01.007

1. Introduction

Malignant mesothelioma (MM) is a tumour that arises from the pleural surfaces or peritoneum. It is a disease that is relentlessly progressive and there are no known curative treatments [1]. Historically, median survival of untreated patients has been 9—13 months [2,3]. The incidence of MM is rising and the peak incidence may not occur for another 10—20 years [4,5]. Standard anti-cancer therapies are of limited value. Chemotherapy can result in overall response rates of up to 42% with one randomized trial suggesting improvement in life expectancy of 3 months [6]. Radiotherapy is limited to temporary relief of local symptoms. Surgery is not a standard procedure though it may be an option for select patients presenting with early stage disease. Therefore new therapeutic approaches are needed for this disease.

The fact that some patients with MM have tumours that regress spontaneously [7] or respond to immunotherapy suggests the immune system can respond to MM under some circumstances. Animal studies have also demonstrated immunoreactivity to MM and tumour responses to different immunotherapies [8,9]. Clinical immunotherapy trials in MM patients have included intrapleural administration of interleukin 2 (IL2) or gamma interferon, systemic alpha interferon alone or with chemotherapy, intrapleural administration of lymphokine activated killer (LAK) cells with IL2 and intra-tumoral administration of granulocyte macrophage colony stimulating factor (GM-CSF) [10,11,12]. The response rates and median survival of these studies along with the evidence of anti-MM immune reactivity have encouraged us and others to further evaluate immunotherapies in MM.

Autologous tumour vaccines have been shown in animal models to generate tumor specific immunity and tumor regression [13,14]. Autologous tumor lysate vaccines have been tested in clinical trials involving patients with malignant melanoma and prostate cancer with evidence of tumor specific immunity being induced and tumor regression in some patients [15,16]. Most of these trials employed GM-CSF as an 'adjuvant' to the vaccine with the aim of helping recruitment and differentiation of antigen presenting cells (dendritic cells). None of these published clinical trials reported tumor growth at the site of inoculation or clinically significant auto-immunity.

1.1. Use of autologous tumor vaccines rather than defined, shared antigens

An ideal vaccine needs to be immunogenic, i.e. it requires relevant tumour antigens which may be known or unknown. These can be manufactured ex vivo using known antigenic peptides (e.g. prostate specific membrane antigen, glycoprotein melanoma associated antigen, gp100 and the widely shared tumour antigens MAGE-1 and MAGE 3). There are at present no candidate MM antigens that could be manufactured ex vivo. There remain concerns that the use of these common antigens, because they are 'self' antigens, will not be able to generate strong anti-tumor responses because the potential T-cell repertoire is partially tolerant to these antigens. Also, autoimmunity is more likely to develop in patients treated using such antigens. In contrast, unknown neo-tumour antigens, e.g. mutations, because they are 'non-self' should have a potentially normally reactive, untolerized T-cell repertoire available to respond to the vaccine.

Autologous neo-antigens can only be obtained from autologous whole tumour cells. Allogeneic tumour cell lines rely on the induction of immune reactivity to shared, i.e. self-tumor antigens. One limitation to using autologous tumor cells is that it is harder to measure specific anti-tumor responses accurately, in contrast to the use of defined shared antigens, where assays like tetramer analysis of specific T-cells and ELISPOT analysis of specific T-cell function can be undertaken. We rely on the DTH test to assess cellular immunity to meosthelioma. Western blot analysis of anti-tumor reactivity is used to measure anti-MM humoral immunity to antigens that are shared with other MM cells.

1.2. Use of GM-CSF?

GM-CSF activates antigen presenting cells (APCs) which in turn take up, process and present tumour antigens to local draining lymph node sites. It has been used with some success as an adjuvant with cancer vaccines in other cancer trials. We had previously shown that GM-CSF with autologous tumor induced anti-MM immunity in murine MM models. When present within tumors, GM-CSF can powerfully boost anti-tumor immune responses in murine studies and when delivered intratumorally in humans with MM, some responses can be seen [12].

We hypothesized that a vaccine created out of the patient's own tumor cells (autologous) mixed with recombinant GM-CSF would generate immune responses to MM cells.

The primary outcome of the study was to evaluate the capacity of a vaccine manufactured from autologous tumor tissue administered with GM-CSF to increase tumor specific immunity in patients with MM. Tumour stabilization or regression and changes in overall survival represent secondary outcomes that were also studied.

2. Patients and methods

2.1. Patients

Eligible patients were those with pleural MM that was surgically accessible whereby at least one cubic centimeter of tissue could be procured. Patients also had to have adequate end-organ function as manifest by a white cell count $>3 \times 10^9$/L, haemoglobin >10 g/dL, platelet count $>100 \times 10^9$, liver function tests <1.5 times the upper limit of normal and creatinine <150 μmol/L. Other eligibility criteria included an ECOG score of <2, life expectancy of >3 months, and ability to give informed consent. Exclusion criteria included previous chemotherapy within the last 8 weeks, immunosuppressed or HIV positive patients, patients on dexamethasone, previous investigational therapies, or previous immunotherapy.

2.2. Vaccine production

After consent was given, tumor specimens were obtained from patients either via debulking pleurectomy or resection of palpable chest wall masses. Tumor specimens obtained