Clinical studies of human papilloma vaccines in pre-invasive and invasive cancer

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Abstract

Cervical cancer is the second most common cause of cancer death in women worldwide. It is almost invariably associated with infection with human papilloma virus (HPV) particularly types 16 and 18. The ubiquitous expression of E6 and E7 oncogene products has been recognised as an attractive target for CTL-mediated immunotherapy. In-vivo expansion of an HPV oncogene product specific MHC class 1 restricted response has been demonstrated using intradermally administered live vaccinia virus HPV 16 and 18 E6/E7 gene construct (TA-HPV, Cantab Pharmaceuticals). Responses have been seen in 1/3 evaluable patients with advanced cervical cancer, and 3/12 CIN3 volunteers, and in 4/29 patients with early invasive cervical cancer. Rankin et al. Proceedings of 91st AACR Meeting, San Francisco, April 2000. In addition, the adoptive transfer of ex vivo HPV 16 or 18 positive autologous tumour lysate pulsed dendritic cells is currently being tested as an alternative means of expanding HPV specific CTL in advanced cervical cancer patients. So far an HLA-A*O201 restricted CD8 T cell response has been recorded in the single HLA-A*O201 patient whose tumour was shown to be HPV16 positive. It appears therefore feasible to induce HPV specific CTL responses in patients with cervical cancer using several vaccine strategies. However, further clinical trials are needed to determine the full anti-tumour potential of this vaccine based immunotherapy. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Cervical cancer remains the second commonest cause of cancer death in women, being responsible for 400 000 deaths worldwide every year [1]. There is now compelling evidence for the causative role of human papilloma infection (HPV) in the disease [2]. HPV DNA has been demonstrated in more than 99.7% of tumour biopsy specimens [3,4]. It appears that infection with HPV predisposes to cervical cancer in a multistage tumorigenic process. Whilst the HPV genome is episomal in early infection, in late pre-invasive and invasive cervical cancer the open reading frames encoding the E6 and E7 early proteins are integrated into the host cell genome [5]. This leads to expression of the key oncoproteins E6 and E7 capable of interfering with p53 and pRb functions. This has been demonstrated to be necessary for continued growth of the transformed malignant phenotype [6].

Over 80 types of HPV are described all of which are exclusively tropic to epithelial cells but it is the high risk types, specifically 16 and 18 which represent the key epidemiological risk factor for cervical malignancy [7]. Other high risk subtypes 31, 33, 45, 52, and 58 are largely responsible for the remainder of cervical cancers [1].

HPV viruses require proliferating cells for successful infection and in the cervix the virus therefore characteristically infects the basal and parabasal cells of the squamocolumnar junction. Replication and transcription of HPV DNA in early infection occur at low levels, increasing with late infection and accompanied by progressive cellular atypia and disturbed epithelial architecture corresponding to pre-invasive neoplasia or cervical intraepithelial neoplasia type 3 or CIN-3. HPV DNA can be demonstrated in pre-invasive lesions, the frequency increasing with the severity of the lesion, being...
demonstrated in 70% of women with CIN 1 and 70–100% of CIN 2-3 [8–10]. The key role of HPV infection in the development of CIN has been further supported by epidemiological evidence [11].

Detection and treatment of the precursor lesions have provided the basis for cervical screening programmes which have successfully reduced the incidence and mortality from cervical cancer in some Western countries [12–15]. However, 80% of cervical cancer deaths worldwide occur in countries where there are insufficient health care resources to treat invasive disease or establish cervical cancer screening programmes. Even with optimum treatment, 40% of patients treated for invasive cervical cancer are likely to relapse and die [16]. There is, therefore, a need for alternative strategies to reduce the global mortality and morbidity from cervical cancer.

Vaccine strategies targeting high risk HPV E6 and E7 oncogenes, if proven effective and affordable could prove an attractive, universally applicable option. This paper examines the rationale and the progress made so far based on four clinical studies employing two distinct HPV cervical vaccine strategies, one utilising a recombinant vaccinia virus encoding HPV 16 and 18 E6 and E7 oncoproteins; and the other autologous dendritic cells primed with autologous or allogeneic cervical tumour lysate, as an alternative source of HPV 16 or 18 antigens.

2. Immune response to HPV

Precisely why most individuals are able to clear HPV infection is unknown. The importance of cell mediated immunity in clearing HPV presence once HPV integration has taken place is implied by the increased incidence of HPV lesions in individuals in whom cellular immune function is impaired, including HIV and renal transplantation patients [17–20]. In contrast, humoral immunodeficiency, characterised by a failure to produce antibodies does not increase susceptibility to the development of HPV lesions [21]. A higher prevalence of antibodies to HPV16 E6 and E7 can be observed in cervical cancer patients compared with healthy individuals (33 vs. 23%, respectively) [22] providing evidence of HPV dependent progression. Also, in the rabbit papilloma virus model, tumour development is associated with a humoral response to viral proteins but has no ability to induce regression of HPV lesions [23]. There is, therefore, no evidence to support a protective role for a humoral response against HPV E6 and E7. However, the cellular response to the virus appears to be important.

For both E6 and E7 proteins the immune response most likely to be effective against cervical cancer cells is the cytotoxic T lymphocyte (CTL) response. Generation of HPV specific CTLs requires the successful endogenous processing of the gene products of HPV oncogenes E6 and E7 by an antigen presenting cell and subsequent presentation to CD 8+ T cells. HLA-A*O201 class 1 restricted peptide CTL epitopes have been identified using peptide binding assays [24]. The rationale for HPV specific immunotherapy is supported by the observation that, peptide or recombinant vaccinia vaccines are effective as both prophylactic and therapeutic vaccines [25–28]. Therefore, our immunotherapeutic approach to patients with pre-invasive and invasive cervical cancer has been to develop vaccine strategies to induce specific CD8+ CTL responses the relevant class 1 specific peptide epitopes of HPV E6 and E7 proteins presented by tumours.

3. A recombinant vaccinia virus expressing HPV 16, 18 E6 and E7 proteins (TA-HPV)

3.1. Rationale and structure of TA-HPV vaccine

With the aim of inducing an anti-tumour CTL response in humans a recombinant vaccinia virus (designated TA-HPV) encoding modified forms of HPV 16 and 18 E6 protein sequences has been developed [29] by Cantab Pharmaceuticals, Cambridge, UK. HPV 16 and 18 are the most common of the oncogenic HPV strains and together account for between 70–85% of cervical tumours [30]. The recombinant was based on the Wyeth strain of vaccinia and modified to express the required oncogene products. Wyeth vaccinia vaccine has a long track record of safety, having been employed on a very large scale for smallpox vaccination in 14.2 million individuals in N America. Minor complications of a tender arm and lymphadenopathy were common but major complications such as progressive vaccinia were largely confined to children and the immunosuppressed e.g. those taking steroid therapy [31].

The use of a recombinant vaccinia vector carrying the E6/E7 ensures that the target antigens can be processed and presented by an antigen presenting cell for generation of MHC class 1 restricted CTL response [32]. Vaccination in mice confirmed that the CTL generated were capable of lysing targets cells infected with TA-HPV [29]. Vaccinia virus has a lytic life-cycle minimising the risk associated any potentially oncogenic insert. The construct also exhibited two further safety features.

1. Reduced neuro toxicity: in mice when compared to parent Wyeth strain, TA-HPV unexpectedly produced a lower fatality rate. At the highest TA-HPV dose used (107 pfu) 1/12 deaths occurred as compared to 10/12 deaths with the parent Wyeth
strain. This difference in the virulence was reproducible even when nude mice were infected instead.

2. Reduced carcinogenicity: The E7 ORF was mutated to eliminate its Rb protein binding site thus reducing oncogenic potential. Experiments with rat embryo fibroblasts confirmed a reduced capacity of the altered HPV16 E6/E7 coding sequences to mediate cell transformation.

3.2. Clinical trials with TA-HPV

To date three clinical studies [33–35] with TA-HPV have been completed in Europe in 49 patients with pre-invasive disease and cervical cancer. The phase 1 study [33] was performed in eight patients with advanced cervical cancer. For this study, 58 patients were screened the majority of whom were excluded because of their poor immune status as demonstrated by low peripheral blood CD4 counts. In these patients the presence of a low CD4 count was considered to pose a potential hazard for vaccinia vaccination and caution was necessary. The second study [34] included 12 volunteers with CIN3 who received two doses of TA-HPV administered 2 months apart as an adjuvant to conventional therapy. The third was a multicentre EORTC [35] phase 1 study in 29 patients with stage 1 and 2 invasive cervical cancer prior to surgery.

The major aims of these three studies were:
1. to assess the toxicity and safety of TA-HPV.
2. to evaluate the systematic immunological cytolytic T cell and antibody responses to HPV/18 E6/E7.

Clinical effectiveness could not be determined in these small studies since the vaccine was largely used in the adjuvant setting.

4. Methods used in clinical studies

4.1. Vaccination technique and environmental monitoring

TA-HPV was provided by Cantab Pharmaceuticals, and administered by scarification using a percutaneous multiple puncture technique (for vaccination schedules see Table 1). For the phase 1 study patients were required to remain in an isolation facility in order to undertake environmental monitoring of recombinant virus release. It was necessary to monitor the presence of infectious vaccinia at the vaccination site, in the scab and in genital swabs by culture and indirect immunofluorescence before and after vaccination for environmental reasons and to detect systemic spread of the vaccinia virus.

For the latter two studies the vaccination was administered on an outpatient basis. The vaccination site was allowed to dry and then covered with an absorbent, occlusive dressing which was changed regularly until scab dehiscence. Patients or household contacts who were either taking chronic steroid therapy, suffering from eczema, receiving immunosuppressive therapy, or had history of any immunodeficiency or household exposure to children under 5 years, were deemed to be at risk of generalised vaccinia and were therefore excluded from the study.

5. Measurement of immune response to TA-HPV

5.1. Serological response

Vaccinia-specific IgG was detected by ELISA using

<table>
<thead>
<tr>
<th>Type of patients</th>
<th>No of patients</th>
<th>Previous vaccinia</th>
<th>Dose/schedule of TA-HPV</th>
<th>Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced [33] cervical cancer (prior to radical surgery)</td>
<td>29</td>
<td>9/29 7 not known</td>
<td>Two vaccinations $2.5 \times 10^5$ pfu/ml 4 weeks apart by dermal scarification</td>
<td>Mild swelling and ulceration grade 2 at vaccination site less local reaction with second vaccination. No serious toxicities.</td>
</tr>
<tr>
<td>CIN3 [34] volunteers (prior to laser therapy)</td>
<td>12</td>
<td>2/12</td>
<td>Two vaccinations $2.5 \times 10^5$ pfu/ml 4 weeks apart by dermal scarification (10/12) or one vaccination (2/12)</td>
<td>12/12 local erythema/scab formation less local reaction with second vaccination. No serious toxicities.</td>
</tr>
<tr>
<td>8/8 0.5–1.0 cm vaccination lesion 2–4 days post vaccination scab separated days 10–14. No systemic and no serious toxicities and no short or medium term complications (&gt;6 months).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1
Vaccination schedule and toxicities of TA-HPV in clinical trials [33–35]
Wyeth strain cell lysates as antigen. Mock infected cell lysates were used as the control antigen. HPV 16 and 18 E6 and E7 specific antibodies were assayed using a radio immuno precipitation assay. All samples were assayed by Dr Simon Stacey, University of Manchester.

5.2. HPV-specific cytotoxic T cells

Blood samples were collected to provide samples before and after vaccination (days 0, 5, 7, 14, 28, 56, 84 and 112 in the phase 1 study, to establish an EBV transformed B-lymphoblastoid cell line (B-LCL) from each patient, and determine HLA types. Peripheral blood mononuclear cells (PBMC) were cryopreserved in liquid nitrogen. Recombinant adenovirus vectors containing HPV 16 E6 /E7 sequences (Rad 101) and HPV 18 E6/E7 sequences (Rad 102) were constructed under the control of human cytomegalovirus mIE promoter [36]. In order to restimulate the HPV-specific CTL in vitro, PHA induced autologous lymphoblasts were infected with Rad 101 and 102, irradiated and cultured with PBMC and cultured in the presence of interleukin 7 for 7 days. PBMC were restimulated and exposed to IL2 on day 10 and CTL assays performed on day 14. CTL cultures were assayed against autologous and allogeneic 51Cr-labelled B-LCL [37,51]. Target B-LCL cells were either uninfected or infected with TA-HPV or Wyeth strain vaccinia virus. The assay was modified for the last study. The stimulator cells employed being autologous monocytes cultured for 3 days with GM-CSF rather than PHA lymphoblasts. These were then transfected with Rad 101 and 102 to restimulate HPV specific CTL.

6. Results

6.1. Safety and toxicity

6.1.1. Environmental safety

In the phase 1 study [33] careful patient monitoring in the infectious diseases isolation unit revealed that live virus was only recoverable from the vaccination site and the scab and dressings in immediate contact with the vaccination site. No virus was recovered from throat and genital swabs, suggesting the virus did not spread systemically. Absence of environmental spread of recombinant virus meant that patients in the subsequent clinical studies did not require isolation and were vaccinated on an out patient basis and the use of an occlusive dressing was sufficient, provided that patients deemed to be at risk of generalised vaccination and patients in household contact with at risk individuals, were excluded from the study.

6.1.2. Patient toxicities (see Table 1)

No serious toxicities were observed in the 49 patients vaccinated whether with one vaccination or two (for schedule see Table 1). Toxicities were largely confined to local erythema, swelling and scab formation at the vaccination site. Systemic toxicity was unusual and limited to transient malaise and headache. There have been no medium and long term toxicities, the longest period of patient follow up being 7 years.

6.2. Evaluation of immunological response

6.2.1. Serological response (see Table 2)

In the phase 1 and the CIN3 study all patients developed IgG antibody to vaccinia virus consistent with the clinical impression of vaccination ‘take’. The kinetics of the antibody response to vaccinia in the phase 1 study was suggestive of a secondary response associated with a history of previous vaccination (see Table 1). Serological response to vaccinia was evident in 18/29 of patients in the EORTC study [35].

In the phase 1 [33] study antibody response to one or more HPV protein was seen 2–4 weeks post vaccination in 3/8 patients and 27/29 patients when measured by radio-immuno precipitation in the EORTC study. No antibody response to HPV proteins was demonstrated in the CIN3 patients. The significance of these antibody responses is not clear.

6.2.2. Specific HPV CTL response (see Table 2)

Of the eight patients in the phase 1 study [33] only three were evaluable for assessment of antigen specific MHC class 1 restricted CTL response. In one patient a MHC restricted HPV CTL response to HPV 18 was detected at 9 weeks post vaccination which was no longer evident at 14 and 20 weeks. This patient is still alive 6 years after vaccination. It is however not possible to conclude that the favourable outcome is entirely related to the vaccination, as she also received palliative radiotherapy and chemotherapy. The patient had an HPV 16 positive cervical cancer and there was also evidence of HPV 16 specific lysis as compared to uninfected control B-LCL targets. However, this response is more difficult to interpret as non-specific background lysis from targets expressing Wyeth vaccinia virus obscured measurement of the degree of specific lysis to HPV 16. This maybe due to natural killer activity obscuring MHC class 1 specific lysis, so the CTL assays in the EORTC study [35] included K562 cells to reduce this effect.

In the CIN 3 study 3/10 patients exhibited a CTL response to HPV 18, however, this may be an under representation of HPV specific CTL responses, as HPV 16 responses were not tested for technical reasons. Importantly, neither pre-existing CTL responses nor vaccinia induced responses were boosted by vaccination.
### Table 2
Immunological response to TA-HPV in clinical trials [33–35]

<table>
<thead>
<tr>
<th>Patients studied</th>
<th>No of patients</th>
<th>Tests of immunological status</th>
<th>Serological response</th>
<th>Response to E6/E7</th>
<th>CTL response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced cervical cancer [33]</td>
<td>8</td>
<td>(1) CD4 count 350/mm [3]; (2) Normal immunoglobulin levels; (3) Antibody response to polysaccharide pneumococcal antigens (Pneumovax 11); (4) Lymphocyte transformation to PHA, Con-A, OKT3</td>
<td>8/8</td>
<td>3/8</td>
<td>1/3 evaluable HPV 18 E6/E7</td>
</tr>
<tr>
<td>5/8 recurrent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN3 [34] volunteers</td>
<td>12</td>
<td>(1) CD4 count 400/mm [3] (2–4) as above</td>
<td>5/5</td>
<td>0/5</td>
<td>3/12 evaluable HPV 18 E6/E7 HPV 16 was not tested.</td>
</tr>
</tbody>
</table>
with TA-HPV. In the EORTC study [35] three patients with early cervical cancer had a specific CTL response to HPV 16 and 18 at 8 weeks, and one to HPV18 at 4 weeks. As in the phase 1 study, responses were seen only at one time point (most commonly at 8 weeks). There is at present no evidence to suggest a boosting effect for two doses of vaccine similar to the findings using vaccinia in animal models [38,39].

7. Summary of other related observations

TA-HPV vaccine appears to be safe with the ability to induce specific class 1 specific HPV specific CTL response against HPV16 and 18 in patients with pre-invasive and invasive cancer. Using a similar protocol, specific CTL to HPV 16 and 18 E6 and E7 were detected in peripheral blood of 6/10 CIN3 subjects who had not been vaccinated, but these were not found in normal subjects [40]. These CTL were able to lyse autologous targets when HPV proteins were presented by multiple HLA alleles, including A*0201, A24 and B7. Similarly HLA-A*0201 restricted HPV 16 E7 [11–20] CTL have been also demonstrated in peripheral blood (4/5 patients), draining lymph nodes (3/4 patients) and tumour (1/3 patients) [41]. These HPV CTL were found to have a higher frequency in tumour and lymph nodes than in peripheral blood. A number of mechanisms have been proposed to explain the failure to develop effective endogenous anti-cancer immunity including loss of MHC expression [42] and production of immunosuppressive molecules such as IL10 [43]. More fundamental mechanisms whereby tumours induce tolerance of T cells specific to the tumour associated antigens may be important [44]. Nevertheless, CIN3 is a condition which exhibits natural regression in 25–30% of patients [45] and immunological tolerance mechanisms may be reversible. Thus CIN3 maybe a good model to determine whether TA-HPV is capable of inducing a specific CTL response powerful enough to eradicate the condition. This is currently being tested in clinical trial using more sensitive assays for CTL measurement such as HLA/HPV tetramers [46].

8. Autologous dendritic cells primed with tumour lysate as an anti cancer vaccine

8.1. Rationale

An alternative way of generating powerful anti-cancer immune response may be to generate large numbers of autologous antigen-loaded dendritic cells for vaccination. These powerful, highly specialised antigen presenting cells are uniquely capable of priming naïve T cells in vivo and in vitro [47]. The key feature of these cells is a combination of stimulatory and co-stimulatory signals presented via MHC class 1 and 2 and costimulatory molecules necessary for the generation of primary antigen specific CTL responses. Tumour antigen peptide-primed immature monocyte derived DC can produce a significant anti-tumour effect against established tumours [48,49] in a variety of animal models and in humans with metastatic melanoma [50]. In a murine tumour expressing HPV16 E7 (the C3 sarcoma) immature DC pulsed with HPV 16 E7 peptide resulted in sustained complete eradication of tumour in 80% of mice. Using human DC it has proved possible to induce class 1 specific responses to targets expressing HPV antigens [51]. Anti-tumour responses have been noted in humans with the use of tumour antigen-primed mature and immature DC [50,52]. Consequently, this approach is currently being tested in a phase 1B trial using HPV tumour lysate as the source of the antigen. An initial cohort of patients treated with immature DC will be followed with by a cohort treated with mature DC. Maturation of DC may offer the advantage of a phenotype with optimum migratory capacity to lymph nodes to prime T cells in lymph nodes, optimum Th1 lymphokine [53] production capacity and a stable state which is not susceptible to the cancer-associated tolerogenic influences such as interleukin 10 [54]. The manufacture of a DC vaccine however is complex and the optimum dose, timing and coordination of antigen loading and vaccination is essential.

8.2. Patients and methods

To date seven patients of the first cohort with advanced cervical cancer (5/7 with recurrent gross tumour) have been vaccinated. They have received up to six subcutaneous vaccinations of $10^6–10^7$ immature DC antigen loaded with allogeneic or autologous sonicated HPV positive tumour lysate. Despite the fact that all patients had a CD4 count >400, only 2/7 exhibited a significant DTH response to 1 Pasteur Merieux recall antigen. The study is ongoing and the primary aims are to (1) assess toxicity; (2) monitor specific CTL response to HPV antigen; (3) note any anti-tumour activity.

8.3. Preliminary results

To date 39 immature DC vaccinations have been administered to seven patients. One patient developed a capillary leak syndrome and required treatment with steroid therapy. The remaining patients have complained of no severe toxicity. Three have experienced grade 1 toxicities including mild pyrexia, malaise and nausea. No significant DTH response has been seen to HPV antigen.

A specific HPV class 1 response has been seen in 1 patient with advanced metastatic disease. In this patient
the frequency of HPV 16 E7 [11–20] CTL rose to 2.2% 1 week after the first of six vaccinations. Subsequent vaccinations produced no increase in specific CTL. Within 2 weeks of completing six vaccinations the tumour was found to progress.

8.4. Observations

To date the emphasis has been on induction of a MHC class 1 specific CTL response with no obvious anti-tumour effect. It remains to be seen whether the vaccine can be optimised with the use of mature DC for the vaccine for reasons described above.

9. Conclusion

E6 and E7 offer attractive targets for specific immunotherapies for HPV associated malignancies. Our studies have shown that it is feasible to induce HPV specific CTL responses in patients with cervical cancer and pre invasive disease with a variety of vaccines including TA-HPV and HPV antigen loaded dendritic cells. Ongoing and further clinical trials will hopefully optimise these approaches and attempt to relate the level and kinetics of immune response to anti-tumour effect, in order to assess the true anti-tumour potential of such immunotherapy.

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References


