The molecular characterisation of human tumour antigens recognised by T cells has provided new impetus for immunisation of patients bearing tumours expressing well-defined antigens. After evaluating the immunogenicity of the new, molecularly characterised antigens in vitro, several clinical studies were conducted to assess the in vivo immunogenicity and the clinical efficacy of vaccines including these antigens. The findings generated by trials based on the administration of peptides or DNA-encoding antigens are discussed to highlight the limits of this therapeutic approach; however, this approach has resulted in some complete and durable regressions, although still in an unsatisfactory small number of cases (5–25%). The recent use of dendritic cells loaded ex vivo with tumour antigens suggests that a high frequency of tumour-specific immune responses can be achieved. Possible means of overcoming the clinical limits and improving the outcome of previous studies are also discussed.

**Key words:** cancer, melanoma, T cells, tumour escape, vaccination

### Introduction

Vaccination is a procedure aimed at eliciting a specific (adaptive) immunological reaction against the antigen(s) contained in the administered vaccine. While it is known that prophylactic vaccines are given to healthy individuals and can be effective in blocking the onset of an infectious disease, anticancer vaccines have been tested by and large on diseased subjects bearing a growing neoplasm. In fact, the only available and effective antitumour prophylactic vaccine in humans is that against the hepatitis B virus (HBV), an important determinant of liver cancer. The difference between these two vaccination approaches (i.e. prophylactic versus therapeutic) is particularly relevant in cancer because, up until now, only therapeutic vaccines have been given to metastatic patients whose immune system may be already altered by the presence of growing tumour cells [1, 2]. Based on interesting pre-clinical findings, however, additional preventive vaccines are being proposed in selected subgroups of subjects under high genetic or environmental risks [3].

Although vaccination of cancer patients has been attempted in the past, it is only during the last 10 years that the great progress in basic immunology and molecular biology has provided this therapeutic approach with a more scientifically sound basis.

Cancer vaccines have been prepared under various formulations, which can be grouped as follows: (i) cellular; (ii) anti-idiotypic; (iii) peptide/protein based; (iv) glycolipid; and (v) nucleic acid based. Different types of immunological adjuvants have also been used, including the more classical ones [e.g. incomplete Freund’s adjuvant (IFA), alum, Bacille Calmette–Guérin (BCG) and QS-21], selected cytokines [e.g. interleukin (IL)-2, IL-12, granulocyte–macrophage colony-stimulating factor (GM-CSF)], dendritic cells (DCs) and heat shock proteins (HSPs).

### Pre-clinical studies

Mechanisms of antitumour immunisation and its effectiveness have been evaluated in animal models. However, the majority of studies published in the early 1990s have adopted the prophylactic approach, which involves immunisation of healthy animals followed by challenge with antigenically related, immunogenic transplantable tumours. These experiments showed that vaccines made by irradiated syngeneic tumour cells, particularly when modified ex vivo by insertion of cytokine genes [4] or with tumour-derived T-cell peptide epitopes [5], were able to confer a protective immunity against the challenge of the same tumour. However, subsequent experiments were unable to reproduce such a high frequency of tumour rejections, even with the same tumour models, when tumour-bearing animals were used to mimic more closely the clinical situation [6].

Studies with animal models, however, have altogether resulted in a wealth of useful information on the following: (i) the molecular basis of tumour antigen immunogenicity and presentation (particularly on the role of DCs); (ii) the subsets of T cells involved in different steps of antitumour response; and (iii) mechanisms of tumour escape from immune reactions [6–8], leading to the design of new vaccines and schedules of immunisation in cancer patients.

### Clinical trials of vaccination

A number of vaccination trials were performed during the last two decades using different types of vaccine formulations aimed at targeting different tumour antigens mostly recognised by antibodies rather than by T cells. Results of such studies are summarised...
below, while more detail is provided for the last generation of clinical trials involving the use of peptide-based, T-cell activating anticancer vaccines.

**Cellular vaccines**

Early studies of vaccination of tumour-bearing subjects were conducted based on the assumption that human tumour cells express antigens recognised by the immune system. At that time, however, such evidence was limited to the recognition of neoplastic cells by antibodies that defined several well-expressed or overexpressed components of the tumour cell membrane, such as gangliosides in melanoma or neuroblastoma and oncogenic/oncosuppressive proteins (Her2/NEU or p53) in breast and colon cancers, respectively [9, 10]. However, after controversial results following phase II studies, phase III prospective randomised trials of vaccination with irradiated tumour cells are only now being performed. Meanwhile, phase III vaccination trials of stage III (AJCC) melanoma patients with the GM2 ganglioside and adjuvants have failed to show a clinical benefit in patients given these vaccines when compared with the control arm receiving high-dose interferon (IFN)-α2b [11]. Molecularily and biologically ill-defined autologous or allogeneic cancer cells, or their subcellular fractions, were also used for vaccination and resulted in some interesting results in phase II studies, although such promises have yet to be fulfilled [12]. However, it should be noted that one of these vaccines, Melacine® (Corixa, Seattle, WA), which includes lysates of melanoma cells and the bacterial-derived adjuvant Detox, has been compared in a phase III randomised trial with chemotherapy in patients with metastatic stage IV melanoma. The two treatments resulted in similar clinical outcomes, although Melacine® showed no relevant side-effects [13]. For this reason, Melacine® has been approved in Canada, but not elsewhere, for the treatment of metastatic melanoma patients. A phase III prospective study with Melacine® in stage II melanoma patients failed to show any significant effect, although analysis of these patients according to their HLA allele has revealed a statistically significant impact of HLA-A2 and HLA-C3 on relapse-free survival that warrants further study [14].

However, a cellular vaccine made of autologous irradiated tumour cells admixed with BCG was shown to increase overall survival in adjuvant setting of Duke’s B, but not C, colorectal cancer patients [15]. Cellular vaccines, made either of autologous or allogeneic virus-modified cancer cells, have been prepared in an attempt to increase their immunogenicity. Phase II and III trials were conducted in melanoma and colon cancer, with patients monitored essentially through delayed type hypersensitivity (DTH) reactions, but the results were rather disappointing [16, 17].

**Gene-modified cancer cell vaccines**

Following pre-clinical studies in vitro and with animal models that suggested an increased immunogenicity and better antitumour response of vaccines made from cytokine-gene modified neoplastic cells [4], a series of phase I–II trials were conducted using this approach in human melanoma, prostate, pancreas and renal cell carcinomas. Results have been disappointing in terms of both immunological and clinical responses, irrespective of the type of cytokine gene (IL-2, -4, -7, -12, IFN-γ, GM-CSF) transduced into tumour cells used as vaccine [18]. However, phase II trials of vaccination with GM-CSF gene-transduced tumour cells (GVax) are still ongoing and a recent study with transduced autologous tumour cells in non-small-cell lung cancer (NSCLC) patients resulted in three complete responses (CRs) out of 26 evaluable subjects treated, a finding that certainly warrants further investigation [19].

A different approach to cellular anticancer vaccines has been proposed that uses hybrids between autologous tumour cells, which provide antigens, and DCs, which confer an antigen-presenting function to the hybrid cells. Such a vaccine was shown to induce four CRs, two partial responses (PRs) and two stable diseases (SDs) in 17 treated patients with metastatic renal cell carcinoma [20]. However, a similar vaccine was less effective in metastatic melanoma patients, casting doubt on the value of this approach [21]. Another interesting approach is that of anti-idiotypic vaccines based on immunisation with anti-idiotype antibodies of antibodies recognising a tumour antigen, i.e. antibodies that mimic the given antigen. This approach has been adopted in melanoma, colon and breast carcinoma patients resulting in humoral and T-cell immune responses to the antigens recognised by the first antibody, although the clinical response rate remains low [22].

**Protein/peptide-based cancer vaccines**

New molecular techniques have allowed the definition of the molecular structure of antigenic proteins; peptides containing stretches of 8–10 amino acids are recognised by cytotoxic T cells in the context of class I HLA. These peptide epitopes can be expressed on tumour cells where they can be identified by immunohistochemistry or PCR. Studies were therefore initiated to evaluate the immunogenicity and clinical effects of the administration of these well defined peptides, mostly in melanoma patients. It should be noted, however, that most of the early antigens discovered are normal proteins to which the body may be tolerant or weakly responsive.

**In vitro or ex vivo immunogenicity**

Before their use in patients, several of these peptide antigens had been tested in vitro or ex vivo to evaluate their ability to induce a specific, HLA-restricted T-cell response by using lymphocytes obtained from the blood or metastatic lesions of the patient. Such studies were mainly performed with the melanoma peptides Melan-A/MART-127, 35, gp100, tyrosinase and MAGE-3 recognised by HLA-A2, but other peptides [e.g. carcino-embryonic antigen (CEA) in colon cancer] recognised in the context of HLA-A2 or of other HLA alleles were then evaluated. After in vitro stimulation with peptides pulsed onto antigen-presenting cells (APC), T cells recognising Melan-A/MART-1 and able to kill Melan-A/MART-1+ melanoma cells were obtained in almost 100% of patients [23]. However, studies on the frequency in which patients’ T cells recognise the many different melanoma antigens indicate the existence of a hierarchy, where the Melan-A/MART-1
antigen is most frequently recognised, followed by gp100; whereas tyrosinase was seen much less frequently and the MAGEs were only recognised in a negligible number of cases [24]. More recently, the availability of the HLA/peptide tetramer staining technique, which allows the enumeration ex vivo of T cells bearing T cell receptors (TCR) recognising a given antigenic peptide, led to confirmation of previous in vitro data [25, 26]. Although the immunogenicity of tumour-associated antigen (TAAs) was initially defined by class I HLA-restricted epitopes, recently several class II HLA-restricted peptide epitopes have been characterized (reviewed in [27]).

Thus, at least some TAAs, despite encompassing ‘normal’ proteins, appear to stimulate the host’s immune system during tumour growth and to drive an expansion of both T and B cell immune repertoires leading to a state of systemic immunity, although only in a limited number of cases.

**In vivo immunogenicity, toxicity and clinical efficacy**

Based on this information, the first clinical studies of phase I and II were initiated essentially with melanoma peptides made available using a formulation suitable for injection into tumour-bearing subjects. In several phase I trials conducted in different centres, no major toxicities were reported after administration of 100–1000 µg peptides/patient, although local erythema, induration and pain or grade 1–2 chills and fever occurred in 20–50% of cases, mostly related to the adjuvants (such as IFA or QS21, etc.) given with the peptides. In some melanoma patients showing a clinical response, skin depigmentation occurred probably caused by T (or even B?) cell-mediated recognition of antigens shared between melanoma cells and normal melanocytes [28].

In this first wave of studies, a variety of peptides were given to metastatic subjects together with different common adjuvants. In addition, at that time (from 1995 to 1998), evaluation of the immune response was essentially based on T-cell proliferation and cytotoxicity assays characterised by both low specificity and sensitivity.

The clinical results of these trials are summarised in Table 1. It is evident that in only half of the eight studies listed, in which measurable metastases were used to assess a clinical response, the response rate exceeded 20%, including durable CRs. However, the clinical response rate rarely corresponded with an induction of a measurable T-lymphocyte reaction in the blood of vaccinated subjects. For example, none of the melanoma patients who displayed a CR or PR after vaccination with the MAGE-3-A1 peptide showed a detectable increase in anti-peptide cytotoxic T lymphocytes (CTLs) [34]. Likewise, no vaccine-specific CTLs could be detected in the blood of melanoma patients given the melanoma peptide gp100(09)-2M and who had responded clinically [29]. Whether or not the lack of T-cell response in the blood of vaccinated patients indicates a lack of, or a weak, immunisation or simply reflects the concentration of these effectors within the tumour lesions remains to be established. The opposite, i.e. a high in vitro CTL response of peripheral blood lymphocytes (PBLs) of vaccinated patients that is not associated with a clinical response, has also been reported [6, 40, 41].

Table 1 also summarises the results of trials in which cytokines, particularly GM-CSF, were used as immunological adjuvants to recruit APC at the vaccination site. However, up until now this approach has failed to improve significantly the clinical outcome in comparison with trials in which traditional adjuvants, such as IFA, were used [37]. The same thing applies to concomitant use of IL-12, although the addition of this cytokine appears to increase the frequency of detectable T lymphocyte responses in vaccinated patients [33]. It is of note that a recent randomised study comparing three different adjuvants, IFA, GM-CSF and QS-21, given with the same peptide (tyrosinase) to melanoma patients, revealed that QS-21 and GM-CSF are superior to IFA in eliciting a T-cell response in more than 50% of cases, and that the clinical response rate rarely corresponded with an induction of measurable metastases were used to assess a clinical response, the response rate exceeded 20%, including durable CRs. However, the clinical response rate rarely corresponded with an induction of a measurable T-lymphocyte reaction in the blood of vaccinated subjects. For example, none of the melanoma patients who displayed a CR or PR after vaccination with the MAGE-3-A1 peptide showed a detectable increase in anti-peptide cytotoxic T lymphocytes (CTLs) [34]. Likewise, no vaccine-specific CTLs could be detected in the blood of melanoma patients given the melanoma peptide gp100(09)-2M and who had responded clinically [29]. Whether or not the lack of T-cell response in the blood of vaccinated patients indicates a lack of, or a weak, immunisation or simply reflects the concentration of these effectors within the tumour lesions remains to be established. The opposite, i.e. a high in vitro CTL response of peripheral blood lymphocytes (PBLs) of vaccinated patients that is not associated with a clinical response, has also been reported [6, 40, 41].

Table 1 presents the peptide-based vaccination of cancer patients: phase I/II trials

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Peptide vaccine</th>
<th>Adjuvant</th>
<th>No. of patients</th>
<th>Clinical response</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Gp100(209)-2M</td>
<td>IFA + IL-2</td>
<td>31</td>
<td>1 CR, 12 PR</td>
<td>42</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>MART-127,35</td>
<td>IFA</td>
<td>18</td>
<td>1 PR</td>
<td>5</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Gp100 + MART-127,35 + tyrosinase</td>
<td>GM-CSF or IL-12</td>
<td>51</td>
<td>5 CR, 6 PR</td>
<td>21</td>
<td>–*</td>
</tr>
<tr>
<td></td>
<td>Gp100</td>
<td>IFA + IL-12 or GM-CSF</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>MART-127,35</td>
<td>IFA</td>
<td>25</td>
<td>&gt;DFS</td>
<td>NA</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Gp100(210M) + tyrosinase</td>
<td>IFA ± IL-12</td>
<td>48</td>
<td>&gt;DFS</td>
<td>NA</td>
<td>[33]</td>
</tr>
<tr>
<td></td>
<td>MAGE-3-A1</td>
<td>None</td>
<td>25</td>
<td>3 CR, 4 PR</td>
<td>28</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>Tyrosinase</td>
<td>GM-CSF</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>HSPC-96</td>
<td>None</td>
<td>28</td>
<td>2 CR, 3 SD</td>
<td>–</td>
<td>[36]</td>
</tr>
<tr>
<td>Melanoma and others</td>
<td>NY-ESO-1</td>
<td>± GM-CSF</td>
<td>12</td>
<td>3 SD</td>
<td>25</td>
<td>[37]</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>K-Ras/12</td>
<td>GM-CSF</td>
<td>48</td>
<td>&gt;OS</td>
<td>NA</td>
<td>[38]</td>
</tr>
<tr>
<td>CIN</td>
<td>HPV-16/E7 + KSS-PADRE</td>
<td>IFA</td>
<td>18</td>
<td>3 CR, 6 PR</td>
<td>50</td>
<td>[39]</td>
</tr>
</tbody>
</table>

*CIN, cervical intraepithelial neoplasia; CR, complete response; DFS, disease-free survival; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFA, incomplete Freund’s adjuvant; NA, not applicable; PR, partial response; SD, stable disease.

*Personal communication by Dr Alexander Knuth (Department of Medical Oncology, Krankenhaus Nordwest, Frankfurt, Germany).
response, despite the widespread use of IFA in previous vaccination trials [42].

More recently, vaccinations with peptides were performed in subjects with tumours other than melanoma, such as pancreatic and cervical cancers, by immunising with peptides from the oncogenic protein K-Ras, known to be mutated in 90% of pancreatic cancers, and of HPV that is expressed in cervical intraepithelial carcinoma (CIN). Table 1 shows the results of these trials, which appear promising, since an increased overall survival was observed in pancreatic cancer [38] and a 50% response rate was reported in CIN [39]. These latter studies, although conducted in a limited number of patients, are important because they show that other antigens, in addition to those found in melanoma, have the potential to generate a T-cell response that may translate into a clinical benefit. It should also be noted that these two studies were conducted in subjects with a limited tumour burden, a clinical situation which may help the body to mount an efficient immune response against cancer antigens included in the vaccine.

Dendritic cell-based vaccines

Meanwhile, basic studies in immunology both in vitro and in animal models had identified DCs as the most potent APC which appear indispensable for the efficient activation of naive T cells in the lymph nodes [43]. When recruited at a vaccination site, DCs can internalise and process TAA and then travel to the lymph nodes where DCs can present TAAs to T cells [43]. DCs have been shown to elicit a tumour protective immunity even when administered to tumour-bearing mice [44]. A further potential advantage of the use of DCs is that they can also be loaded, in addition to peptides and proteins, with tumour cell lysates containing all the possible TAA repertoire, or even with tumour-derived RNA, without the need to isolate and molecularly characterise the peptides recognised by T cells [43].

Table 2. Results of antigen-loaded autologous dendritic cell vaccination in patients with metastatic disease

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Vaccine</th>
<th>No. of patients</th>
<th>Clinical response</th>
<th>%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>MART-1 + gp100 + tyrosinase + MAGE-3-A2/A1</td>
<td>32</td>
<td>3 CR, 5 PR</td>
<td>25</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>MAGE-3.A1</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>MART-1 + tyrosinase + gp100</td>
<td>28</td>
<td>2 CR, 1 PR</td>
<td>17</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>MART-1 + tyrosine + gp100 + MAGE-3</td>
<td>18</td>
<td>3 CR</td>
<td>17</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>MAGE-1 or -3, MART-1 + gp100 + tyrosinase</td>
<td>14</td>
<td>1 MR, 6 SD</td>
<td>0</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>Lysate</td>
<td>11</td>
<td>1 PR, 3 SD</td>
<td>10</td>
<td>[50]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PAP/GM-CSF</td>
<td>31</td>
<td>0*</td>
<td>0</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>PSA/A2</td>
<td>33</td>
<td>2 CR, 6 PR</td>
<td>22</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td>PSA/RNA</td>
<td>13</td>
<td>3 CR*</td>
<td>23</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>Mouse PAP</td>
<td>21</td>
<td>6 SD</td>
<td>0</td>
<td>[54]</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Modified CEA/A2 + KLH + Fer3L</td>
<td>12</td>
<td>2 CR, 2 SD</td>
<td>17</td>
<td>[55]</td>
</tr>
<tr>
<td>Paediatric tumours (NB, PTEN)</td>
<td>Lysate + KLH</td>
<td>10</td>
<td>4 CR, 1 PR</td>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>

*Complete clearance of circulating tumour cells.

1Personal communication by Dr James Mulé (University of Michigan Medical Centre, Ann Arbor, Michigan).

CEA, carcino-embryonic antigen; GM-CSF, granulocyte–macrophage colony-stimulating factor; KLH, key limpet hemocyanin; PAP, prostate acid phosphatase; PSA, prostate specific antigens; PSMA, prostate-specific membrane antigens.
Vaccination in patients bearing virus-induced tumours. In fact, an omen and colon cancer patients. Immunisation with HSPPC-96 ova peptides to T cells [59]. We have then used autologous HSP peptides to be presented on the cell surface by MHC molecules; and neck and penile cancers by human papilloma virus (HPV); T-cell responses is instead clearly increased when DCs are used.

Vaccination against virus-induced tumours
Several human tumours are caused by viruses: cervical, head and neck and penile cancers by human papilloma virus (HPV); Burkitt’s lymphoma by Epstein–Barr virus (EBV); and liver cancer by HBV/HCV. Since these viruses introduce strongly immunogenic proteins in the body during infection, a clinically significant immune response could develop or be induced by vaccination in patients bearing virus-induced tumours. In fact, an interesting clinical result was obtained by the group of J. Weber (University of South California), who vaccinated CIN-bearing women with HPV peptides and IFA, a treatment that resulted in 50% response rate [39] (Table 1). It should also be noted that virus-induced tumours are to date the only setting where a prophylactic cancer vaccine may provide significant protection against cancer, as exemplified by the anti-HBV and, perhaps, by some ongoing anti-HPV vaccines, the latter being made from virus-like particles [57].

Vaccination with heat-shock proteins
Heat-shock proteins are a family of proteins of differing in molecular weight which have been conserved during evolution as they perform crucial functions in maintaining the homeostasis of normal cells. These proteins also have the following important immunological functions: (i) the chaperoning of intracellular peptides to be presented on the cell surface by MHC molecules; (ii) the delivery of peptides to the outside of the cell by interacting with Toll-like specific receptors on APC; and (iii) the promotion of differentiation and maturation of DCs [58]. Animal studies demonstrate that HSP families of molecular weights 60, 70, 96 and 110, although endowed with different cell homeostatic functions and located in different subcellular compartments, can present tumour peptides and generate strong, individually specific T-cell responses that may prevent or even induce regression of a variety of mouse tumours [58].

In humans, we have shown that HSP 70, extracted from human melanoma lines and tumour samples, can present known melanoma peptides to T cells [59]. We have then used autologous HSP peptide complex 96 (HSPPC-96) to vaccinate metastatic melanoma and colon cancer patients. Immunisation with HSPPC-96 weekly for 4 weeks (first cycle) and then bi-weekly \( \times 4 \) (second cycle) has resulted in induction/augmentation of antitumour specific T-cell response and clinical response in 50% and 18% of melanoma patients, respectively [36], while 60% of colon cancer patients developed a tumour-specific T-cell response associated with an increased disease-free survival [60].

Recombinant vaccines
A plethora of papers has been published on the use of viral or non-viral vectors to bring DNA encoding TAA into the body. One potential advantage of this approach is generating a T-cell response against multiple peptide epitopes including those seen in the context of class II HLA. However, owing to complex regulatory and safety issues, few clinical trials of recombinant vaccines used to treat cancer patients have been completed or published. Rosenberg et al. [61] used adenovirus 5 to administer genes encoding MART-1 or gp100 into metastatic melanoma patients; the vector was given either alone or followed by IL-2. Only one of 16 evaluable patients experienced a CR, and antibody reactions were detected against viral proteins but not against the transgene product.

Other studies were carried out in colorectal cancer patients with poxviruses acting as a platform for presenting antigens to T cells. A canarypox vaccinia-based vector (ALVAC), in particular, was used to introduce the CEA gene and genes coding for T-cell co-stimulatory molecules (e.g. B7.1, GM-CSF) into the patients’ tissues by the group of J. Schlom (NCI, Bethesda, MD). Several trials were conducted to test the in vivo immunogenicity and clinical effectiveness of this vaccination approach. Altogether clinical responses were confined to stabilisation and T cell-specific responses were induced or weakly increased in a limited number of subjects (reviewed in [62]). The same applies to a study of vaccination with rVaccine-PSA in prostate cancer patients [63]. Thus, at present one cannot conclude that recombinant vaccines are more effective in the treatment of cancer than their peptide- or DC-based counterparts.

Vaccination, however, can also be performed by using plasmid or ‘naked’ DNA coding for different TAAs that can thus be directly transfected in vivo into APCs without the interference of the immune response that can be caused by the viral proteins of the vector. The adjuvant activity of bacterial cytosine-phosphate-guanosin (CpG) DNA sequences has been shown to help in activating and/or amplifying the immune response [64] and is being tested in clinical studies.

Tumour escape mechanisms
A major reason for the relative ineffectiveness of vaccination in controlling human tumour growth lies in the many mechanisms devised by neoplastic cells to avoid recognition and destruction by the immune system. These mechanisms include changes in the phenotype or gene expression of tumour cells and dysfunction of the immune cells [7]. The first group encompasses down-regulation of expression of the HLA–peptide complex (the target of T cells), expression of molecules inhibiting other effectors, like natural killer (NK) lymphocytes, or the release of factors (e.g. vascular endothelial growth factor) that can impair the activity of
DCs. The host’s T or NK lymphocytes can then become functionally crippled by molecules released within the tumour microenvironment or directly by neoplastic cells. A clear example of this is the expression of the molecule FasL by tumour cells, which may kill incoming activated lymphocytes, either T or NK, that express Fas. This phenomenon, defined as ‘tumour counterattack’ has been recently clarified by our group showing that FasL is not expressed on the tumour cell membrane but rather released in the microenvironment through microvesicles, like melanosomes in melanoma cells [65].

Conclusions and perspectives

There are now many different molecularly defined vaccines that can be used in cancer patients and more will become available in the near future. Each of them has advantages and disadvantages, all of which should be taken into consideration before selecting the most appropriate vaccine according to the clinical condition of patient that will be treated. Certainly vaccination is likely to have a better chance of being beneficial in a clinical setting characterised by subjects with a limited tumour burden and, therefore, with a functional immune system. The vaccine itself, whether made from peptides or DNA, should include multiple classes I and II HLA epitopes to avoid immunoselection and to maintain the immune response over time, be given along with an efficient adjuvant and possibly through the administration of autologous, mature DCs. Such vaccines can now be constructed based on the results obtained from pre-clinical studies in the last 2–3 years. In addition, we now have different in vitro assays that can be reliably used to assess the T-cell response in vaccinated patients, i.e. HLA/peptide tetramer staining and ELISPOT, which may quantify changes, if any, of frequency and function of T cells recognising the given TAA.

The different immune escape mechanisms are by no means alternative and, therefore, they represent a formidable barrier to a clinically relevant destruction of tumour cells. We are convinced that, without a detailed knowledge of the many aspects of tumour escape and/or immune inefficiency and an intensification of research efforts to cope with them, even a potentially strong vaccine may fail to impact on the patients’ clinical conditions. Thus, though the clinical response rate of vaccination trials conducted so far remains unsatisfactory, numerous new findings summarised in this review suggest that these results could be significantly improved in the near future.

Acknowledgements

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