REVIEW ARTICLE

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Tumour vaccines: a new immunotherapeutic approach in oncology

Abstract Substantial progress has been made in vaccine development in recent years for the treatment of malignant diseases. New technologies have fostered the identification of potentially immunogenic tumour antigens that can be used to activate the patient's immune system to specifically recognize and destroy human tumour cells. More detailed insights into the process of intracellular protein degradation, processing and cell surface presentation have allowed immunogenic peptide domains to be identified that can be used in vaccine trials. Still a matter of intensive debate is the question of the most optimal presentation of tumour-derived proteins or peptides to the immune system to achieve a maximum response. In this area, major progress has been made by using dendritic cell or even naked DNA-based vaccines. The generation of new tools such as HLA–tetramer complexes now allows researchers to monitor more closely the expansion of peptide-specific T cells under the process of vaccination. On the basis of these advances, a couple of vaccine trials have been performed that have increased our knowledge of vaccine development and provided indications that the concept of tumour-specific vaccination might be valid. However, we are still at the beginning of this process and should always remember that only well-designed, prospective clinical trials can define the optimal use of tumour vaccines in oncology.

Keywords Tumour vaccine · Serological analysis of antigens by recombinant expression cloning · Cytotoxic T cells · Dendritic cells · HLA–tetramer

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Introduction: existence of autologous immune responses against human tumours

It is now more than 200 years since Edward Jenner made his observation that benign and self-limiting cowpox could be used to protect an individual against a virulent and sometimes lethal smallpox infection. This strategy ultimately resulted in the eradication of smallpox as an infectious threat. Rubella and rabies are also largely under control, and polio may soon be eradicated [78]. Many tumour immunologists aim for the kind of success enjoyed by their colleagues in the fields of infectious diseases.

Historically, tumour immunologists have sought to increase immune responses against tumours by increasing their immunogenicity. William Coley first tried to provoke an immune attack of tumours over 100 years ago by injecting live cultures of *Streptococcus erysipelas* into growing tumour nodules. Since then, attempts to increase the immunogenicity of tumours have included the creation of "oncolysates" made from tumour cells infected with viruses such as vaccinia and the use of irradiated tumours mixed with *Corynebacterium parvum* or Calmette-Guérin bacillus (BCG) [75, 90, 106]. With the development of molecular biology, the approach to a tumour-specific vaccine has radically changed and is characterized by the attempt to understand tumour immunology on a molecular level. Central to this approach is the cloning and characterization of the antigens that are recognized by immune cells [23, 85, 102]. These antigens can then be used in their DNA, recombinant or synthetic form to develop vaccines.

Mechanisms of antigen-specific immune responses: antigen uptake, processing and presentation

The response of the immune system to antigens derived from cancer cells involves a number of distinct receptor–ligand links. The major players are the major histo-

compatibility complex (MHC) molecules class I and II, which are expressed as professional antigen-presenting molecules on the surface of so-called antigen-presenting cells (APC). The MHC is encoded by a gene segment of approximately four mega-bases on the short arm of chromosome 6 in humans and includes at least 200 genes [98]. The complex is divided into three regions – class I (HLA-A, -B, -C, -E, -H, -G and -F genes), class II (HLA-DR, -DP and -DQ genes) and class III (including genes encoding complement and tumour necrosis factor, TNF). These genes control immune responses to pathogens, graft acceptance or rejection and tumour surveillance. Almost 20 years ago, MHC class II molecules were found to interact with CD4+ helper T cells, and MHC class I molecules with CD8+ cytotoxic T cells [93]. Subsequently, the CD4 and CD8 antigens on T cells were themselves shown to be receptors for MHC molecules, and mutational analysis established that the binding sites for CD4 [13, 46] and for CD8 [66, 86] mapped to structurally similar regions of the constant domains of MHC class II and class I molecules, respectively. The observation that the binding sites for CD4 and CD8 on MHC molecules were separate from the peptide-binding domain of MHC molecules, and therefore from the site of interaction with the T cell receptor (TCR), suggested that a single MHC molecule could be bound simultaneously by both TCR and CD4 or CD8, increasing the overall avidity of the interaction. However, the interaction between MHC and TCR is not stable, but transient. Many reports have demonstrated that as few as 100 peptide-MHC complexes [16, 27] serially engage and trigger as many as 18,000 TCR [101].

For a long time, CD8+ cytotoxic T cells were thought to be the prime cellular compartment controlling and attacking malignant cells. The preferential attention paid to anti-tumour responses by CD8+ T cells stems from two facts [25, 73]: firstly, most tumours are positive for MHC class I but negative for MHC class II; and secondly, CD8+ cytotoxic T lymphocytes (CTL) are able to induce tumour killing upon direct recognition of peptide antigens presented by the tumour's MHC class I molecules [72]. This preference had been demonstrated by numerous adoptive transfer studies in which CD8+ T cell lines and CD8+ clones specific for tumour antigens that have been stimulated in vitro were able to mediate anti-tumour immunity when transferred back into tumour-bearing hosts [79]; furthermore, immunization strategies using either adjuvant or dendritic cells (DC) with pure tumour peptides resulted in productive anti-tumour immunity that was restricted by MHC class I [24, 57, 65].

However, recent evidence in cell-based vaccine models indicates that CD4+ T cells can mediate a number of anti-tumour effector pathways either in conjunction with CD8+ cytotoxic T cells or independent of them. Many studies on the role of CD4+ T cells have demonstrated their ability to provide help for the induction of cytotoxic CD8+ T cells [10]. This can happen via the secretion of stimulatory lymphokines or by an indirect mechanism where the APC is activated by the CD4+ T cell [55]. This activation process is dependent on the interaction between CD40 ligand and CD40 on the CD4+ T cell and the APC, which appears critical in stimulating the APC to present antigens to, and costimulate the priming of, CD8+ CTL precursors (pCTL) [5, 80, 89]. Beyond their ability to provide help for the priming of tumour-specific CD8+ CTL, evidence is accumulating that tumour-specific CD4+ T cells can orchestrate additional effector functions in anti-tumour immunity [1]. Independently of CD8+ T cells, CD4+ T cells that are specific for tumour antigens are critical in orchestrating the production of nitric oxide and superoxides by macrophages and are also critical in recruiting and activating eosinophils to mediate complete anti-tumour responses [33].

In conclusion, most people in the field of tumour immunology now agree that successful immunity to cancer will require the activation of both tumour-specific CD4+ and CD8+ T cells. Combined approaches simultaneously stimulating CD4+ and CD8+ T cells are therefore thought to be the most effective.

Rationale for the development of cancer vaccines

Clinical observations in cancer patients with spontaneously regressing tumours have always supported the hypothesis of a pre-existing anti-tumour response in a subset of patients. Several tumour entities are now defined in which this phenomenon can be observed with a frequency ranging between 5% and 15% [11, 26]. T lymphocytes have been discovered to be the prime component of this anti-tumour response, since tumour-specific CTL clones were able be raised from patients suffering from melanoma [103], renal cancer [63] and breast cancer [9]. In addition, tumour-specific pCTL clones can be detected in a variety of cancer patients with an increase in number after antigen-dependent vaccination applied in form of protein, peptide or DNA vaccines [60]. Since the induction and expansion of tumour-antigen specific CTL clones was correlated in some patients with a clinical response and tumour regression, tumour immunologists initially focused on T cells for the development of cancer vaccines.

The field of cancer vaccine development is currently developing rapidly in different areas. The present review cannot deal with all advances made in the entire field over the past years. We will therefore concentrate on three areas in which progress has been profound and the contribution to further vaccine development outstanding:
1. Characterization of (new) tumour antigens
2. Advances in vaccine development
3. Advances in monitoring vaccine trials

Recent advances in the characterization of tumour antigens: prerequisite for successful vaccine development

T cell-defined tumour antigens

The first approach was established by Boon and colleagues [102] and makes use of antigen-loss tumour cell variants transfected with cDNA isolated from tumour tissue and cytotoxic CD8+ anti-tumour T cell clones (CTL). The second approach was introduced by Rammensee and colleagues based on a biochemical strategy using acid elution of antigenic peptides bound to MHC class I molecules from tumour cells [23]. These strategies have helped to define several new human tumour antigens at the molecular level, most notably in malignant melanoma. The difficulty involved in expanding specific T cell clones for the majority of tumour entities is a major obstacle for the general application of these strategies.

Antibody response to tumour antigens

The search for autologous antibodies recognizing antigens specifically expressed by tumour cells was initiated in the 1960s [68, 69]. At this time, studies were hampered by technical limitations, since polyclonal patient serum usually demonstrated a strong reaction with normal tissue antigens and did not show tumour specificity. The development of the hybridoma technology by Köhler and Milstein [47] raised the hope that monoclonal antibodies could uncover tumour-specific antigens in humans. This technique allowed the characterization of a large variety of new antigens which might be used as targets for immunotherapeutic approaches. However, only a minority of these antigens seem to evoke an immune response in cancer patients and could be used as surrogate markers [68]. To date, the clinical significance of B cell responses to tumour antigens remains unknown. While the presence of p53 antibodies is associated with a poor prognosis [74, 108], the clinical significance of anti-HER-2/neu antibodies cannot yet be determined [20, 41, 107]. More patients need to be analysed in order to determine whether the development of antibodies to tumour antigens is associated with clinically relevant features or might be used for diagnosis and prognosis.

Characterization of tumour antigens by serological analysis of antigens by recombinant expression cloning

To overcome the limitations of the T cell-based methods, a new strategy using autologous serum for the characterization of tumour antigens was recently introduced by Pfreundschuh and colleagues [85]. This novel technique, serological analysis of antigens by recombinant expression cloning (SEREX), allows for the direct molecular definition of new tumour antigens that elicit an immunoglobulin G (IgG) antibody response in tumour patients [15, 88]. By screening procaryotically expressed tumour-derived cDNA libraries with autologous sera, several new antigens in different tumour entities have been identified, as have the known MAGE-1 and tyrosinase antigens, which were originally defined by their T cell reactivity [84, 99]. One of the first antigens characterized by SEREX was a renal-specific carbonic anhydrase. The identification of this enzyme by the SEREX technology proved the old concept that non-mutated, cellular antigens which are amplified or overexpressed can serve as immunogenic antigens. Several new antigens (Galectin 9, Aldolase A and translation initiation factor eIF-4γ) discovered recently have confirmed this observation [28, 100]. The most fascinating group of antigens is the so-called cancer testis (CT) antigen group [15, 92]. These antigens are expressed by a variable proportion of different tumour types, but are highly restricted in their expression pattern in normal tissues, with testis being the sole or predominant site [70]. Three antigens in this category, MAGE [103], BAGE [9] and RAGE [63], were initially identified as targets for cytotoxic T cells. A variety of new CT antigens (HOM-MEL-40, NY-ESO-1, SCP1) have now been discovered using SEREX analysis [15]. As no mutations, rearrangements or amplifications have been observed for the genes encoding these CT antigens, the most likely explanation for their expression as tumour antigens is gene activation or de-repression [70].

The high titre of autologous, tumour-specific antibodies detected by SEREX implies that cognate CD4+ helper T cell immunity must be present and operative in antibody-positive patients [10]. Stockert and colleagues [92] recently evaluated the antibody response of tumour patients against a variety of SEREX-defined tumour antigens. Significant antibody titres of 1:25,000 and higher were demonstrated in melanoma patients against the NY-ESO-1 antigen, suggesting strongly the existence of a CD4+ T cell-mediated B cell expansion. For NY-ESO-1, putative class II epitopes have recently been characterized in vitro and need to be confirmed for their relevance in vivo [38]. The co-existence of CD4+ T cells and a B cell response has been clearly demonstrated for the HER-2/neu protein in patients with breast cancer [19]. In addition, it has been shown that CD4+ T cell lines and clones cultured from tumour-infiltrating lymphocytes recognize epitopes that are products of the same tyrosinase gene that was shown to encode class I-restricted peptides recognized by CD8+ T cells [64, 109]. These results, together with the observation that classical tumour antigens defined by T cell responses such as MAGE-1 and tyrosinase can also be detected by the serological approach, suggest that an integrated immune response against tumour antigens may exist that involves both CD8+ and CD4+ T cells as well as B cells. Knuth and colleagues have demonstrated a CD8+-specific CTL response against peptides of the NY-ESO-1 antigen in one melanoma patient who initially presented with high titre an-

tibodies directed against MAGE-1, tyrosinase and NY-ESO-1 [37]. This is the first evidence of the co-existence of an antibody and CD8+-dependent T cell response against an antigen originally detected using the SEREX approach. More importantly, there seems to be a strong correlation between antibody and T cell response in patients with NY-ESO-1-positive tumours [39]. In a recent NY-ESO-1-based vaccine trial, a high percentage of patients with NY-ESO-1 antibody also had detectable CD8+ T cell responses to known HLA-A2-restricted NY-ESO-1 peptides. Antibody and CD8+ T cell responses to NY-ESO-1 only occurred in patients with NY-ESO-1-expressing tumours, and CD8+ T cell responses to NY-ESO-1 were not detected in patients without NY-ESO-1 antibody. Humoral immunity to NY-ESO-1 in the absence of CD8+ T cells to known HLA-A2-restricted NY-ESO-1 peptides was observed in only one patient, clearly demonstrating the close link between antibody and cellular immune response at least for this SEREX-defined protein.

As the number of SEREX-defined tumour antigens is expected to increase rapidly in the near future, the importance of tumour-specific antibody responses in tumour patients will be further strengthened. Antibody response to new tumour antigens might lead to the development of new screening methods for the detection of cancers and will increase the number of tumour antigens recognized by both the humoral and cellular part of the immune system.

Vaccine development: recent advances in the induction of tumour-specific immune responses

The use of the SEREX methodology has boosted the field of potentially useful tumour antigens and contributed more than 1600 antigens, of which a proportion might be used for vaccine purposes. However, the most imminent question that remains is how the antigen should be delivered to the immune system in an optimal way in order to achieve the strongest cytotoxic response. A wide variety of approaches are currently being tested, and no final comment can be made at the moment. One of the most promising approaches is the use of tumour antigen-derived proteins or peptides in combination with DC [43, 52, 97].

Dendritic cell-based vaccines

DC form the link between the innate and the acquired immune system by presenting antigens and by their expression of pattern recognition receptors that detect foreign molecules in their local environment [34, 59]. They were first described as the morphologically distinct Langerhans cells in the skin and have since been shown to be the most efficient APC specialized to initiate T cell immunity; as such, they can be regarded as nature's adjuvant [3, 31, 50]. DC have the capacity in vivo to migrate from tissues, where they encounter an-

tigens, to the T cell areas of lymphoid tissues, where immune responses begin [3, 35, 49]. They reside in tissues as immature cells, specialized to capture and process antigens, and, after antigen capture, they mature in response to inflammatory stimuli characterized by an upregulation of MHC and T cell costimulatory molecules [3]. DC then migrate to the draining lymph node (LN) and stimulate antigen-specific T cells to initiate an immune response [36]. The use of DC as a cellular adjuvant is a promising approach in immunotherapy of infectious disease and cancer [3]. The development of simple methods to isolate DC precursors from blood and the expansion of these cells in vitro to yield potent APC has enabled their use in cancer immunotherapy [56, 71]. Numerous animal models have conclusively demonstrated that ex vivo-generated DC pulsed with protein antigen are useful for the immunotherapy of infectious diseases and cancer [22, 58]. In addition, initial clinical studies indicate that tumour antigen-pulsed DC might be effective in the immunotherapy of cancer patients [18, 54, 61, 62]. As reported by Nestle and co-workers [62], vaccination with peptide- or tumour lysate-loaded DC resulted in a positive delayed-type hypersensitivity to peptide-loaded DC in 11 out of 15 patients and in an objective response in 5 out of 16 evaluable patients. Responses included regression of metastases in skin, soft tissue, lung and pancreas, suggesting that DC-mediated vaccination mediated a systemic response effective in inducing tumour regression in lesions in several organs. In a second study [83], peptide administration without DC failed to elicit clinically significant responses. These were observed only when high doses of interleukin (IL)-12 were added to the peptide vaccination. The vaccination approach using peptide-pulsed DC has also been transferred to haematological diseases, such as B cell non-Hodgkin's lymphoma (NHL) and multiple myeloma [32, 77]. Vaccination of NHL patients with DC loaded with the tumour-specific idiotype protein resulted in objective tumour regression, including one complete response [32]. A feasibility study was recently performed on DC-based anti-idiotype vaccination after autologous peripheral blood stem cell transplantation in multiple myeloma patients [77]. This study demonstrated that, despite high-dose chemotherapy, patients mounted a strong anti-keyhole limpet hemocyanin (KLH) immune response and, more importantly, some patients showed an anti-idiotype CTL response.

In conclusion, despite a large body of evidence strongly supporting the existence of DC-mediated anti-tumour effects, many issues related to DC need to be understood in more detail to allow successful manipulation of the immune system. During recent years, the number of DC subsets has increased rapidly. DC originating from different precursors in different tissues have different functions, and some of them do not activate the immune system after antigen uptake, processing and presentation, but induce tolerance. It is essential for the homeostasis of the immune system that

DC tolerize developing thymocytes [105, 111], as otherwise autoimmune disease could develop. The fine-tuned processes controlling the differentiation of DC in activating or tolerizing APC need to be analysed in more detail and will help to establish better-designed vaccine trials based on the use of antigen- or peptide-pulsed DC.

DNA-based vaccines

A fascinating new approach in the area of developing strategies for vaccine delivery is based on the use of naked DNA as vaccine [30, 81]. If DNA-based vaccines prove to be effective, their simplicity in terms of production and use will become a hallmark of vaccine development. Standard DNA vaccines consist of the specific gene or genes of interest cloned into a bacterial plasmid engineered for optimal expression in eukaryotic cells [29]. Essential features include a strong promoter for optimal expression in mammalian cells, an origin of replication allowing for growth in bacteria, a bacterial antibiotic resistance gene and incorporation of polyadenylation sequences to stabilize mRNA transcripts.

Notably, DNA vaccines also contain specific nucleotide sequences that play a critical role in the immunogenicity of these vaccines. This specific motif consists of an unmethylated cytosine–phosphate–guanosine (CpG) dinucleotide with optimal flanking regions composed of two 5′ purines and two 3′ pyrimidines [48, 87]. Such motifs are normally unmethylated and are 16- to 20-fold more common in microbial than in mammalian DNA [14, 76]. Functionally, these motifs (CpG oligodeoxynucleotides, ODN) can directly stimulate multiple types of immune cells, including monocytes/macrophages, DC, B cells and T cells. The ability of CpG ODN to directly induce professional APCs such as DC to secrete cytokines such as IL-12, TNF and interferon (IFN) is critical in their striking enhancement of cellular immune responses [40, 44, 45, 87]. The use of plasmid DNA as a vaccine can address all critical issues simultaneously: (a) it provides an immunogenic antigen, (b) it is processed via MHC class I and II, (c) it stimulates immunological memory and (d) it contains an adjuvant [96]. DNA vaccines have been extensively studied, especially in the field of lymphoma [6, 53, 91]. For this purpose, DNA plasmids encoding the variable regions of the tumour-specific idiotype have been developed and studied in animal models. In a study published by Syrengelas and Levy [94], a DNA vaccine encoding for the lymphoma-specific idiotype followed by the coding sequence for human granulocyte–monocyte colony-stimulating factor (GM-CSF) was compared with conventional methods using the respective protein for immunization. In this study, the DNA vaccine was shown to be equivalent in its protective activity against tumour outgrowth after challenge. However, when the mechanisms of protection are analysed in more detail, current evidence suggests that the tumour-protective effects of GM-CSF-based DNA vaccine constructs can be largely attributed to idiotype-specific humoral immunity. Replacing the GM-CSF moiety by the C fragment of tetanus toxin [42] or chemokines such as INF-inducible protein 10 or monocyte chemotactic protein 3 [7, 8] generated superior protection against a large tumour challenge (20 times the minimum lethal dose), based mainly on a strong T cell response. The chemokine approach was even more active than the best available protein vaccines strengthening again the potential of DNA vaccines.

Recent advances in monitoring tumour-specific immune responses: prerequisite for meaningful clinical trials

The next question in vaccine development after having defined the antigen and the route of application to be used is how to monitor a patient's immune response in an optimal fashion after immunization. The development of peptide–HLA tetrameric complexes in 1996 [2] heralded a new era in the study of antigen-specific T cells and their role in viral infections and malignancy. For the first time, it became possible to identify antigen-specific T cells directly from peripheral blood by labelling them with fluorochrome-tagged complexes of an HLA heavy chain folded around a known epitope peptide, representing the natural target of the T cells in vivo. This assay provides a reproducible way of quantifying specific T cells and following them over time. In the field of monitoring viral infections, peptide-loaded tetramers have helped to discover that the magnitude of the virus-specific T cell response was much greater than previously thought, particularly in the acute stages of infection [67]. In acute Epstein-Barr virus (EBV) infection, for example, almost 50% of all circulating CD8+ T cells were found to be directed to a single peptide derived from the EBV lytic cycle [95]. Recently, initial studies in patients with melanoma have demonstrated, for the first time, the quantification and phenotyping of tumour-specific CTL in metastatic tumours directly ex vivo [82]. Data published by Lee and co-workers [51] showed that tumour antigen-specific T cell responses develop in many patients with metastatic melanoma, accounting for more than 2% of the total CD8+ T cells. MHC/peptide tetramers allow antigen-specific T cells to be directly sorted and analysed without in vitro stimulation and expansion. This enabled the group to address the native functional state of the circulating tumour antigen-specific CD8+ T cells in single patients, demonstrating that these cells were non-cytolytic in vivo. Moreover, these cells failed to produce cytokines even after phorbol 12-myristate 13-acetate (PMA) and ionomycin, thereby demonstrating functional T cell anergy, which might be an important mechanism of immune evasion in cancer. Taken together, there is increasing evidence that potentially tumour-reactive T cells in the peripheral circulation of cancer patients are functionally inactive. In addition, this study

supported recent findings that the frequency of tumour antigen-specific T cells in cancer patients has been grossly underestimated, because only cells that retain their functional capacities in vivo and in vitro were detected by previously used methods [21].

Romero and co-workers used a different approach and analysed the functional status of CD8+ T cells isolated from tumour-infiltrated lymph nodes (TILN). They showed that tumour-specific CTL are often present in high numbers in TILN, are antigen experienced and are capable of massive expansion when exposed to the appropriate cytokines, generating highly tumoricidal CTL populations. In their study, the frequency of Melan-A-specific CTL in freshly isolated TILN ex vivo was found to be as high as 1 in 30 CD3+ CD8+ cells. Given that they only analysed two known tumour epitopes and considering that many more epitopes are yet unknown, it seems likely that tumour-specific CTL may account for a substantial proportion of the total CD3+ CD8+ cells present in TILN. It is also noteworthy that peptide-specific CTL populations purified from TILN by flow cytometry sorting were functionally extremely effective in killing both peptide-pulsed cells and autologous tumour cells. In contrast to previous methods used for generating CTL for adoptive immunotherapy, tetramer-based sorting methods do not require peptide stimulation for generation and thus may circumvent the production of low-avidity CTL due to prolonged exposure to cognate peptide [110]. Clearly, isolation of peptide-specific CTL using tetramers may provide new opportunities in adoptive immunotherapy. Tetramers might therefore be used as very effective tools in monitoring clinical trials and for the isolation of highly cytotoxic T cell clones.

Conclusions and future directions for research

As outlined in the present review, the progress in vaccine development is fast and diverse. New technologies such as the SEREX method provide researchers and clinicians with a vast number of potentially useful tumour antigens. The major problem at the moment is the lack of comparability between the individually chosen approaches. Since all tumour antigens can be administered in multiple formats and by many routes, results obtained in single-institution studies cannot be compared with each other [62, 104]. Multi-centre studies are therefore needed in which patients are vaccinated according to standardized protocols. Since in most vaccines trials the patients who are currently enrolled have advanced disease, immunological parameters instead of tumour responses have to be the prime objectives. To achieve this goal, standardization of immunological assays is of high priority to allow the comparison of different approaches or even the same approach performed at different institutions. So far, no standard procedure exists, and most vaccine centres use their own protocol for measuring T cell responses by cytokine release, cytotoxicity assay, ELISPOT and other techniques. Thus, despite a large body of work in this area, many tumour vaccine protocols tested to date have not proven effective or yielded large amounts of useful information [17, 104]. The tetramer technology as described above might constitute a first step towards developing tools to monitor clinical trials that can be applied under standardized conditions in a larger number of laboratories.

An open question at the moment is the search for the most suitable malignancy to be used as a model for the development of basic vaccine strategies. Since the first tumour antigens were discovered in melanoma [102] and a pre-existing T cell immune response can be demonstrated in some patients [51], most clinical trials are currently performed in this area. Nevertheless, haematological malignancies may be better suited for the early development of vaccine approaches. For most lymphomas, effective first-line treatments exist that allow a substantial reduction of tumour cells to a state of minimal residual disease (MRD). MRD can be monitored by sensitive quantitative molecular assays, since individual molecular tumour markers, e.g. individual immune receptor rearrangements, can frequently be defined. Preventing the regrowth of lymphoma cells by specifically activating the immune system is feasible, as demonstrated recently by Bendandi and co-workers [4] in follicular lymphoma (FL). In this trial, all patients were in complete remission after chemotherapy, but their original malignant clone could still be detected by polymerase chain reaction (PCR). However, after idiotype vaccination, 8 of 11 patients lacked cells from the malignant clone in their blood as determined by PCR and sustained their molecular remissions. Tumour-specific cytotoxic CD8+ and CD4+ T cells were uniformly found (19 of 20 patients). Vaccination was thus associated with clearance of residual tumour cells from blood and long-term disease-free survival. Their analysis of molecular response rate provides definitive evidence for an anti-tumour effect of lymphoma-specific vaccination. In addition, this study supports an initial report in multiple myeloma [77] indicating that most patients are able to generate a tumour-specific T cell response capable of clearing residual tumour cells from the blood after tumour cytoreduction by chemotherapy.

The latest development in this area is the combination of donor lymphocyte infusion (DLI) with idiotype vaccine strategies in immunoglobulin-positive NHL. As DLI is based on the capability of the infused leukocytes to detect and destroy the malignant clone, this immune response can be boosted by pre-immunizing the donor with the patient's idiotype to already generate a strong T cell response in the immunocompetent donor and to transfer these primed T cells to the host [12]. However, standardized trials are required to prove the feasibility of this approach before the concept of tumour-specific lymphocyte infusion can enter the field of clinical medicine.

Researchers have to be aware that the present euphoria in the field offers a unique opportunity to test the fascinating concept of tumour-specific vaccination in a variety of malignancies. However, as discussed before, spontaneous tumour regression is known in some tumour entities and can be correlated with specific effector mechanisms such as the pre-existence of CTL. Vaccine trials therefore always have to be interpreted with caution, keeping in mind that some studies deal with a biased patient cohort selected on the basis of immune competence [17]. These patients are the ones who have a good chance of tumour regression and superior performance anyway. Thus only prospective randomized trials will present us with the true picture of the value of cancer treatment by tumour-specific vaccination.

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