ANTI-TUMOUR TREATMENT

Epstein-Barr virus-targeted immunotherapy for nasopharyngeal carcinoma

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Summary Epstein-Barr virus (EBV) is constantly present in undifferentiated and poorly-differentiated nasopharyngeal cancer. Thus, tumour-associated viral antigens are potential targets for immunotherapy. Recently, both preclinical and early clinical studies have shown that various strategies can enhance EBV-specific immunity. Moreover, significant anti-tumour effect has been observed, and was generally correlated with biological response. The present review discusses the rational for EBV-targeted immunotherapy and summarises the latest developments in this area.

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Introduction

Nasopharyngeal carcinoma (NPC) is an uncommon neoplasm in most parts of the world, with an annual incidence of less one per 100,000 habitants.1 However, this disease is endemic in southern China and Hong Kong, where incidence rates ranging from 15 to 50 per 100,000 have been reported.2 An intermediate incidence is seen in Alaska, Greenland, North Africa and the Mediterranean basin. In Tunisia, NPC is the most frequent head and neck cancer, with an age-adjusted incidence of 4.7 per 100,000 in men and 0.9 per 100,000 in women in the Southern region.3

Radiotherapy is the mainstay treatment of non metastatic NPC, achieving local control in 50–90% of cases.1 Recently, on the basis of randomised trials4–7 confirmed by two meta-analysis,8,9 concurrent platinum-based chemo-radiotherapy became the new standard of care, especially in patients with T3–T4 primary tumours and/or lymph node involvement (N1–N2–N3).10

Despite improvements in therapeutic results, local regional failure and distant metastasis are still occurring in a significant number of patients. Moreover, radiotherapy and chemotherapy often result in both acute side effects and long-term sequelae, with the latter being even more severe.
in younger patients. Xerostomia, trismus, endocrine dysfunction, cognitive impairment and radiation-induced secondary malignancies are examples of treatment effects which significantly affect patients’ quality of life, if not survival.

Therefore, it seems necessary to develop novel approaches, with the aim of improving outcome for refractory disease and, eventually, deescalating conventional cytotoxic therapies.

Epstein-Barr virus (EBV) is uniformly detected in patients with undifferentiated and poorly-differentiated NPC, regardless of geographical origin. On the other hand, there is now compelling evidence that cytotoxic T lymphocytes-based immunotherapy is effective in another entity of EBV-linked malignancies, namely post-transplant lymphoproliferative disorders (PTLD). The success of this therapy has prompted researchers to develop similar strategies for other EBV-positive tumours, such as NPC. We will discuss the rationale for virus-targeted immunotherapy and examine some of the most widely studied approaches in NPC.

**Epstein-Barr virus and nasopharyngeal carcinoma**

EBV is a DNA-virus that belongs to the herpesvirus family. The virus genome comprises more than 85 genes, some of which encodes for proteins that interact and/or have structural homologies with cytokines, cellular transduction signals or anti-apoptotic molecules (Table 1).

In addition to NPC, EBV is associated to at least four other types of malignancy in human: endemic Burkitt’s lymphoma, nasal T/natural killer cell lymphoma, Hodgkin’s lymphoma (with the exclusion of type 1) and PTLD. The implication of EBV in other neoplasms, such as leiomysarcomas in immuno-compromised individuals and gastric cancer, is strongly suspected but not yet firmly established.

Whereas infection of B lymphocytes begins with the attachment of the virus to the CD21 molecule on these cells, EBV penetration into nasopharyngeal epithelial cells may be explained by an IgA mediated endocytosis or by the presence of a B-cell antigenically-related surface protein.

All EBV-related malignancies involve the virus latent infection. According to the pattern of viral antigens expression, three types of latency (I, II and III) are described (Table 2). As opposed to type III, characterised by unrestricted expression of latent antigens-together with HLA molecules-, the absence of most EBNAs, all membrane proteins (LMPs) and HLA molecules in type I latency make tumour cells invisible to host anti-EBV immune surveillance. Latency II, associated with NPC (and Hodgkin’s lymphoma), involves a relatively limited expression of viral genes. Functions of latency proteins are summarized in Table 3.

Although NPC is associated with global cellular immunity suppression, an EBV-specific immune response is well documented. However, the frequency of anti-EBV specific T cells in NPC patients is usually lower than in healthy carriers of the virus. Nonetheless, in patients attaining clinical remission, EBV specific immunity increases to levels similar to those of healthy seropositive individuals, thus supporting a direct role of the tumour in immunity suppression.

NPC is characterised by a unique histological pattern, where a rich lymphocyte infiltrate is intimately associated to malignant epithelial cells. The function of these

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<td>EBV gene</td>
<td>Human homologue</td>
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<tr>
<td>BCRF1</td>
<td>Interleukin 10</td>
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<tr>
<td>BDLF2</td>
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<td>BHRF1</td>
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<td>BARF</td>
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<th>Table 2</th>
<th>Latency patterns in EBV-related malignancies</th>
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<td>Latency type</td>
<td>Viral genes expressed</td>
</tr>
<tr>
<td>Type I</td>
<td>EBNA-1</td>
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<td>Type II</td>
<td>EBNA-1, LMP-1, LMP-2</td>
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<td>Type III</td>
<td>All EBNAs (EBNA-1, EBNA-2, EBNA-LP, EBNA-3A, 3B and 3C), LMP-1, LMP-2</td>
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tumour-infiltrating lymphocytes remains largely unknown. Although in vitro studies suggested a cytotoxic activity against EBV infected cells, specificity and effectiveness of this function have not been confirmed in vivo. Moreover, NPC cells can escape cytotoxic T lymphocytes (CTL) control in vivo, and several mechanisms, such as the expression of Fas ligand, have been suggested to explain this immune evasion.

Immunotherapy strategies

Two distinct approaches are being developed to treat NPC. Adoptive immunotherapy consists to bypass the antigen presentation step by directly activating effector cells, whereas active immunotherapy aims to enhance tumour antigens recognition by the immune system (Table 4).

Adoptive immunotherapy

The example of post-transplant lymphoproliferative disorders

PTLD represent a heterogeneous group of haematological malignancies of B phenotype that develop in the setting of iatrogenic immunosuppression after bone marrow or solid organ transplantation. These neoplasms have an aggressive clinical behaviour, and are constantly associated with EBV infection. The observation of tumour regression in approximately 25% of patients after simple reduction of immunosuppression gave rational for anti-EBV adoptive immunotherapy. Initial studies included bone marrow recipients, in whom PTLD are generally of donor origin. Papadopoulos et al. first reported complete remission in patients with PTLD who received unmanipulated donor T lymphocytes. Subsequently, another group demonstrated the effectiveness of donor EBV-specific lymphocytes infusions for the prevention or the treatment of PTLD.

By contrast to bone marrow recipients, PTLD arising after solid organ transplantation are of recipient origin, making the safety and efficacy of allogenic therapy uncertain. In this setting, autologous CTLs, generated before transplantation and infused back to the patient during therapeutic immunosuppression, significantly reduce viral genome load and prevent the onset of PTLD. The procedure for generating EBV specific CTLs is schematically summarised in Figure 1.

Thus, PTLD are considered as a model of EBV-related tumours in which virus-targeted immunotherapy is feasible.
and efficient. It is important, however, to underline the high immunogenicity of these neoplasms, mainly due to the expression of EBNA3 antigens. Despite the lack of EBNA3 expression in type II latency, immunotherapy techniques targeting other viral antigens are beginning to show real promise in NPC.

Allogeneic CTL therapy in nasopharyngeal cancer

The first observation suggesting clinical efficacy of this approach was reported by Comoli et al.28 In a heavily pre-treated NPC patient with intra-cranial disease progression, the infusion of EBV-specific CTLs from a HLA-identical sibling resulted in a minor tumour regression followed by a 3-month stabilisation. Simultaneously, peripheral anti-LMP2 specific CTLs increased to a level similar to healthy EBV seropositive controls. Biopsy taken after therapy showed a marked increase in CTLs infiltrate in the tumour, but no allogeneic CTLs were detected.

Despite this encouraging report, the use of allogeneic CTLs has not been further evaluated in NPC, which is in contrast with the extensive study of this approach in bone marrow-related PTLD. Possible reasons for this include: (1) the need for a HLA-matched donor; (2) the risk of CTLs rejection by the patient’s immune system; (3) the short-term persistence of infused allogeneic CTLs. Nevertheless, an attractive strategy to circumvent these obstacles would be to use best-matched HLA allogeneic CTLs from a frozen bank of well-characterised EBV-specific CTL lines.29

This approach has shown promising results in solid organ transplantation-related PTLD,30 but studies in the setting of NPC are still lacking.

Autologous CTL therapy in nasopharyngeal cancer

Chua et al. firstly demonstrated the feasibility of autologous EBV-specific CTL transfer in NPC patients.31 The procedure was well tolerated, and CTL transfer significantly increased the frequency of EBV-specific CTL precursors and reduced blood viral load. However, no objective clinical response was documented.

More recently, Straathof et al. reported their preliminary experience in 10 NPC patients treated with autologous EBV-specific CTLs.32 Six patients had refractory disease, while the four others received CTLs as adjunctive treatment after attaining clinical remission with chemoradiation for locally advanced NPC. No significant toxicity was observed, except swelling at tumour site in one case, which could not be definitely attributed to therapy. Among six patients with refractory NPC, two had complete responses, and remained in remission over 11–23 months after treatment; one had a partial remission that persisted for 12 months; one had stable disease for more than 14 months; and two had no response. All four patients treated in remission remained disease-free, with a follow-up ranging from 19 to 27 months. While viral genome load was significantly reduced after CTL therapy in six patients, the frequency of peripheral EBV-specific T cells remained unchanged, although a transient increase in LMP2-specific T cells was observed in four patients among eight tested. Thus, no correlation between immunological response and clinical activity could be clearly demonstrated. The authors stipulated that antigens other than LMP2 may have been involved, or that T cell expansion was restricted to sites of tumour antigen presentation.

Another feasibility study of CTL therapy was performed by Conoli et al. All 10 included patients were diagnosed with NPC that had progressed after radiotherapy and two or more lines of chemotherapy. In this heavily pre-treated cohort, a partial remission was observed in two patients and a stabilisation in four others, giving an encouraging disease control rate of 60%. Progression-free interval ranged from 3 to 15 months. CTL infusions were well tolerated, with the exception of mild inflammatory reactions at the tumour site in two cases. In contrast to the findings by Straathof et al., an enhancement in EBV specific immunity was uniformly observed, with a significant increase in LMP-2 specific cells in four patients, of whom three had clinical benefit.33

Perspectives

A phase I trial is currently performed under the hospice of Baylor College of Medicine (Houston, TX, USA) in patients diagnosed with relapsed or primarily refractory NPC. In this study, two monoclonal antibodies that are directed to non-overlapping epitopes on human CD45 (Leukocyte Common Antigen) will be administered with the aim of creating a profound lymphocyte depletion prior to EBV-specific CTL infusions.34 It will be interesting to determine the duration of immunological response and to compare it to previous studies.

Considering that viral proteins expressed during latency II are moderately immunogenic, it would be interesting to determine whether EBV specific immunity can be enhanced by the induction of EBV lytic cycle. Preclinical data demonstrated the capacity of gamma-irradiation35 or cytotoxic drugs such as cisplatin and fluorouracil36 to induce EBV lytic cycle, making the combination of CTL therapy with chemotherapy and radiotherapy a potential area of investigation.

Active immunotherapy

Active immunotherapy, also known as vaccination, consists to deliver selected tumour-associated antigens to cancer patients in order to induce an immune response that may result in the eradication of malignant cells. Currently, two modalities are being developed in NPC: dendritic cells (DCs) vaccination and viral vectors-introduced peptides.

Figure 1: Generation of EBV-specific cytotoxic T cells (adapted from Ref. 23, with permission). PBMC, peripheral blood mononuclear cells; LCL, lymphoblastoid cell lines; CTLs, cytotoxic T cells.
Dendritic cells therapy

DCs are professional antigen-presenting cells that have a crucial role in the activation of naïve CD4+ and CD8+ T cells. Immature autologous DCs can be generated ex vivo by cultivating peripheral blood monocytes in the presence of interleukin-4 and granulocyte-monocyte colony stimulating factor. Then, DCs are matured using tumour necrosis factor-alpha or other agents, pulsed with tumour antigens and finally administered to the patient.

A feasibility trial of LMP2 peptide-loaded DCs was reported by Lin et al. DCs were given by intranodal inguinal injections in 16 patients with refractory NPC. Therapy was well tolerated, with minor side effects occurring in four patients. Nine (56%) patients showed a significant immune response, as assessed by LMP2-specific CD8+ T cell frequency. Moreover, a radiographically-documented tumour regression was observed in two patients, with a progression-free interval of 10 months and more than 12 months, respectively.

In the light of this report, and similar studies in other tumours, DCs therapy may be considered as one of the most attractive approaches in cancer immunotherapy. Given the moderate immunogenicity of NPC-associated viral antigens, it seems interesting to explore the activity of DCs pulsed with tumour lysates rather than limited epitopes. On the other hand, the hypothesis that DCs may be more efficient towards minimal residual disease could not be tested without conducting well-designed randomised trials in patients with early disease. Unfortunately, significant barriers, such as cost and the lack of standardisation, are still limiting the development and the availability of DCs therapy.

Viral-vector introduced peptides

Using six HLA A2-restricted epitopes derived from LMP-1, Duraiswamy et al. constructed a recombinant poxvirus that encodes the corresponding protein. Human fibroblasts infected with the engineered virus were efficiently recognized by LMP1-specific CTLs from HLA A2 healthy individuals. In HLA A2/Kb transgenic mice, a LMP1-specific T cell immunity was elicited by intraperitoneal injection of the vaccine. Moreover, immunisation significantly afforded protection against LMP1-expressing induced thymoma. Finally, a therapeutic effect could be clearly demonstrated in mice with established tumours: while a complete and sustained remission was observed in recombinant virus-vaccinated mice, all animals that received non engineered poxvirus died rapidly from tumour progression.

Considering the potential risks of poxvirus in human, the same group has developed a similar approach using a replication-incompetent adenovirus. In this model, the DNA sequence inserted into virus genome encoded multiple HLA class I-restricted CTL epitopes from LMP1 and LMP2. Similarly to the previous study, the authors reported significant immune responses as well as prophylactic and therapeutic anti-tumour efficiency. Importantly, immunogenic epitopes were restricted through HLA alleles common in different ethnic groups, including NPC endemic regions.

Encouraging results with recombinant virus vaccination in patients with colorectal and prostate cancer have been published, and further evaluation of this approach in NPC is awaited.

Future needs and challenges

As with any cancer, a major obstacle in promoting novel therapies for NPC is the traditional lack of interaction between basic scientists and clinicians. Thus, it is necessary to develop translational research programmes as an integral part of head and neck oncology units. This will permit not only improving current immunotherapy but also tailoring treatment to the patients most likely to benefit. For example, a polyepitope vaccine based on selected immunogenic peptides restricted by each patient’s HLA system would be a promising individualized approach.

Since neither specific anti-EBV cells assessment nor cytokine release measurement have been considered as reliable predictors of clinical benefit, alternative tools for monitoring the immune response are required to serve as surrogate markers for immunotherapy efficacy. Finally, a better understanding of cancer cells immune evasion mechanisms and tumour–host interaction at the tumour microenvironment level would help understanding the algorithm of immune responsiveness and design strategies to overcome resistance to therapy.

To achieve these goals, there is a need to both sophisticated technology and highly qualified personnel. However, it should be emphasized that more than 90% of all new NPC cases worldwide occur in developing countries. Because of restricted resources, research programmes have not been identified as a priority cancer care issue in many high- and intermediate-risk regions. Thus, the establishment of an international collaborative partnership is essential to promote exchange of NPC-related expertise and to facilitate access to populations with higher prevalence of the disease.

Conclusion

Despite an abundant preclinical and clinical research, cancer immunotherapy has not yet entered into standard practice. Among head and neck cancer, NPC is characterised by a strong association to EBV, thus giving rationale for viral antigens-targeted immunotherapy. Preliminary clinical data have shown that both CTL and DC-based therapies are well tolerated and can induce significant immune response that is associated with clinical benefits in some patients. Recombinant-virus vaccination has shown promising activity in mouse models, and feasibility studies in NPC patients are planned.

Although these data are encouraging, there is still a need for technique standardisation and strategies to circumvent tumour’s immune evasion before large-scale clinical assessment can be undertaken.

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