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Treatment of Epstein-Barr-associated lymphomas in HIV

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The relationship between immunodeficiency and the increased frequency of non-Hodgkin lymphoma (NHL) was firstly noted nearly 35 years ago among transplanted patient, but it became more evident with the emergence of the human immunodeficiency virus (HIV) epidemic in the early 80's.[1] Since then, several epidemiological studies have assessed that HIV-infected individuals have 10 to 100-fold increased risks particularly for three types of NHL [i.e., primary central nervous system lymphoma (PCNSL); immunoblastic lymphoma and Burkitt's lymphoma (BL)]. The clinico-pathological spectrum of AIDS-related NHLs includes: 1) BL and BL like lymphomas; 2) diffuse large B cell lymphoma (DLBCL) with immunoblastic features; 3) primary central nervous system lymphoma (PCNSL); and 4) two novel and rare entities, primary effusion lymphoma (PEL) and plasmablastic lymphoma of the oral cavity. Hodgkin's disease (HD), is also seen with a higher frequency in HIV-infected persons,[2] but it is not included among AIDS–defining conditions.

Epstein-Barr virus (EBV), a widespread human γ-herpes virus, is a well-established risk factor for BL, HD, a subset of T cell lymphoma and various tumours of epithelial cell origin.[3] Furthermore, EBV-associated lymphoproliferative disorders are a well-recognized major complication of immune suppressions and, among HIV-infected individuals, the number of EBV-infected B cells tend to increase with worsening of the immune deficiency (from 10- to 20-fold, as compared with healthy persons).[3] EBV is identified in the neoplastic cells of approximately 50% of HIV–related lymphomas, although the detection of EBV varies considerably according to histological type. All three types of AIDS-associated NHL are related to EBV infection, but PCNSL show the strongest association with EBV, since it is present in nearly 100% of cases,[3] in contrast to an average prevalence of 40% in systemic NHL.[4] In people with HIV infection, PCNSL is associated with a severe degree of immunodeficiency (i.e., less than 50 CD4+ cell/mm) and it is thousand times higher in HIV-positive than in HIV-negative individuals.[5] Moreover, EBV infection occurs in almost all of HIV-associated HD and PEL, in 80% of DLBCL with immunoblastic features and in 35-70% of BL (Table 1).[6,7]

AIDS-associated, EBV-positive BL seems to be pathogenetically similar to sporadic BL, with chronic stimulation of the B-cell compartment of the immune system laying a crucial role. In fact, cases with *c-myc* translocation tends to occur in patients with a non severe deficit of the immune system, while mutations in the p53 gene have been detected in approximately 60% of BL in AIDS patients.[6] Recent advances in the elucidation of the mechanisms underlying the EBV-induced growth transformation and immune evasion have been instrumental in guiding the design of novel approaches for the treatment of EBV-associated malignancies.

Treatment

Although we are in the third decade of the AIDS epidemic, the optimal therapy for AIDS-associated NHL remains not completely defined yet. Patients who develop AIDS-associated NHL continue to have a poor prognosis, despite efforts to sort out the role of dose intensive chemotherapy regimens for the immunocompromised host. The rapid improvements in therapy for HIV infection make difficult the comparison and the clinical use of findings from clinical trials conducted at different times (Table 2).[8-11] To put this fact into prospective, it is usefully to consider some crucial steps in the treatment strategies for AIDS-related lymphomas

Non-Hodgkin's lymphoma

Data from phase-II trials on NHL in HIV-positive persons in the pre-HAART (higly active antiretroviral therapy) era suggested that second- and third-generation regimens (such as BACOD, ProMACE-CytaBOM and MACOP-B) had a better efficacy than

Table 1. EBV-associated lymphomas in HIV.

Histological type	Percent with EBV infection
Primary central nervous system lymphoma	Nearly 100%
Primary Effusion Lymphoma	Nearly 100%
DLBCL immunoblastic features	80%
Burkitt's Lymphoma	30-90%

DLBCL=Diffuse Large B Cell Lymphoma

standard regimen like CHOP. The introduction and the routinary use of granulocite colony stimulating factors (G-CSF) contributed to reduce myelosuppression and to allow the use of standard-dose CHOP or CHOP like regimen, with a significantly reduced frequency of neutropenia.[12] The introduction of HAART represented another crucial step in the treatment of AIDS–associated NHL - with substantial improvements in survival not recorded since then - although several aspects regarding their actual role need still to be defined. At this regard, the demonstration that patients with AIDS-associated NHL who responded to HAART had a better response to chemotherapy, and a longer survival than patients who failed (or who did not receive HAART), had substantial therapeutic implications.[13] On the basis of these results, it was suggested that AIDS-associated NHL should be treated with HAART starting at NHL diagnosis and in concomitance with chemotherapy. Interestingly, it was shown that, with the partial exception of performance status and B symptoms, the prognosis of AIDS-associated lymphoma in patients treated with HAART was not related to any previously reported prognostic factor, such as older age, CD4+ cell count less than 100 cells/mm^3, previous AIDS diagnosis, bone marrow involvement or extranodal disease (Table 3). Virological response to HAART was the only factor independently associated with a higher response rate to chemotherapy, with a statistically significant 6-fold increased probability of complete response and the likelihood of tumour regression increased 1.7-fold for each log10 decrease of plasma viraemia during therapy.[14]

Based on the findings of a high percentage (15–20%) of patients with central nervous system (CNS) involvement at presentation, routine CNS prophylaxis is now considered standard practice in the management of AIDS-associated NHL.[15]

There are no standard options for the treatment of relapsed or refractory NHL, although some investigations suggest that second-line chemotherapy can be beneficial. One regimen combined etoposide with continuous infusion ifosfamide and mesna or ESHAP (etoposide, methylprednisolone, cytarabine and cisplatine). The overall response rate was 59%, with 42% of patients achieving a complete response and 17% of patients achieving partial remission. In patients treated with highest dosages, the complete response rate was 62%. The median duration of the response was 12.5 weeks (range: 4–78 weeks), with a median survival of 16 weeks (range: 2–168 weeks).[16]

The introduction of HAART, by restoring the deficit of the immune system in HIV-positive patients has allowed to investigate the effect of high dose-intensive therapy, such as a high dose therapy (HDT) with peripheral blood stem cells transplantation (PBCST). This approach, at present, is the gold standard treatment in relapsed or refractory lymphoma in the general population. Findings from some small case series regarding second line treatments in selected HIV-positive patients with NHL suggested that HDT with PBCST is feasible, with rapid haematological recovery and acceptable toxicity. Re et al have implemented a program of PBCST mobilization and collection with subsequent HDT and transplantation as savage therapy for HIV-positive patients with relapsed or refractory lymphomas. Prompt haematological recovery was observed and all patients responded to treatment, while HIV viral load remained undetectable in patients receiving HAART before and after transplantation. Interestingly, no HCV reactivations were seen. The authors concluded that adequate number of CD34+ cells can be collected in most patients with AIDS-NHL, and that, in selected patients, HDT and transplantation is feasible with rapid haematological recovery and acceptable toxicity.[17]

Table 2. Selected regimens and outcome for AIDS-related systemic lymphomas.

Regimen	No. of patients	CD4 + (median)	CR rate (%)	OS/RR (mo)	Author/reference
Low or standard dose of m-BACOD	175	100 52 standard	41 low	8/8	Kaplan et al., 1997[8]
Low or standard dos of CHOP + HAART	53	119 37 standard	35 low	Not available	Ratner et al., 1999[9]
Infusional CDE	21	87	62	18/not available	Sparano et al., 1994[10]
Dose adjusted EPOCH	23	255	79	72/83	Little et al., 1999[11]

Table 3. Adverse prognostic factors in HIV-related lymphomas

More frequent	Less frequent
CD4 +cell < 100/µl	Bone marrow involvment
Prior AIDS diagnosis	Age > 40 yars
Low Performace Status	High level of LDH
	Extranodal disease

Interest has also been generated in the potential role of anti-CD20 monoclonal antibody, rituximab, in the treatment of HIV-related NHL. Spina and colleagues reported the results of a clinical investigation conducted on 41 HIV-positive patients with CD20+ AIDS-NHL. These patients received a combination of rituximab chemotherapy with CDE (cyclophosphamide; doxorubycin; etoposide) every four weeks for up to six cycles and HAART. Overall, 38 patients were evaluable for response and toxicity: 76% of them achieved a complete response, 5% had partial remission, whereas in 18% of patients a disease progression was registered. After a median follow-up of 12 months, only three patients out of the 29 who had a complete response (10%) relapsed and 32 out of 41 patients were alive. The results showed that the combination of rituximab plus CDE was be very active, with a complete response rate higher than that reported since then in AIDS-NHL (i.e., 45%–65%), and that a significant increase of overall survival (70% at 2 years vs a median of 7–18 months) could be achieved. Thus, the association of rituximab + chemotherapy should be strongly recommended as a front line treatment for HIV-positive patients with CD20+ NHL.[18]

Primary central nervous system lymphomas

In contrast to the protective effect of HAART on incidence of PCNSL, survival after a diagnosis of PCNSL in the HIV/AIDS setting remains poor even in the late HAART era.[19-20]The therapeutic antiviral approach used among immunocompetent patients are applied to HIV-positive patients with PCNSL, but the outcomes were generally less positive, with higher frequency of toxicity and poorer results. Patients with PCNSL respond to whole brain irradiation and a substantial proportion of them can be expected to have a complete tumour eradication. However, these patients have a high probability of subsequently develop and die for opportunistic infections and/or recurrent lymphomas. The best therapeutic regimen is presently constituted by the association of chemotherapy (methotrexate and high dose citarabin) plus whole brain irradiation. As it regards HAART, we have already observed that HAART offered a survival advantage only to a restricted group of PCNSL patients, i.e., to naïve patients exposed to HAART after PCNSL diagnosis. Timely integration of HAART with conventional chemotherapy or radiotherapy represents the current optimal therapeutic approach for patients with AIDS-associated primary brain lymphoma.[20]

The detection of Epstein-Barr virus (EBV)-DNA in cerebrospinal fluid (CSF) by means of the polymerase chain reaction (PCR) has been revealed, in retrospective studies, to be a good marker of AIDS-associated PCNSL. However, the technique's usefulness in the management of AIDS patients with focal brain lesions is still unknown. The clinical usefulness of testing CSF obtained by lumbar puncture for the presence of EBV-DNA as a minimally invasive approach to the diagnosis of AIDS-PCNSL was studied in patients with focal brain lesions. Cerebrospinal fluid was collected from 101 patients, and the presence of EBV-DNA in lumbar CSF turned out to be a sensitive and highly specific diagnostic marker of AIDS-PCNSL. EBV-DNA detection in CSF may allow a minimally invasive diagnosis in a large percentage of patients with brain lymphomas.[21]

Hodgkin's disease

Most HIV-positive patients with HD have advanced disease, and they are treated with combination chemotherapy regimens like MOPP (mechlorethamine, vincristine,procarbazine and dacarbazine), and more recently with ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine). However, the optimal therapy for HD in HIV-positive patients has not been defined yet and the complete response rate remains lower than that seen in the general population. Errante et al reported the results of a trial with epirubicin, bleomycin, vinblastine and prednisone (EBVP) chemotherapy in combination with antiretroviral therapy, G-CSF and Pneumocystis carinii pneumonia (PCP) prophylaxis. Most patients (83%) had advanced stages of HD, and 90% had B symptoms. A high overall response rate of 91% was observed with a CR rate of 74%. The toxicity was moderate, with a grade 3–4 leucopenia and thrombocytopenia of 32 and 10%, respectively. The median survival was 16 months, with a survival rate of 32% and a disease-free survival of 53% at 36 months (22). Recently, the Stanford V regimen with concomitant HAART was evaluated in the treatment of HIV-associated HD. The findings suggested that such treatment was feasible and highly active in the setting of HIV-related HD.[23]

New treatment modalities for EBV-associated lymphomas

The recent advances in our understanding of the mechanisms by which EBV induces cell transformation and escapes host immune control provide the rational background to design new strategies of intervention for EBV-related malignancies.

Immunotherapy

Most of EBV-associated malignancies constitute ideal targets for T cell-based immunotherapy approaches. In fact, immunogenic EBV antigens are expressed only on tumor cells and not in normal cells, providing thus the rationale for a tumor-specific treatment. In addition, the lymphoblastoid cell lines generated by infecting normal peripheral blood B cells with EBV function as excellent antigen-presenting cells, allowing the induction/reactivation in vitro of EBV-specific CTLs, which may be easily cultured in large numbers. Finally, most patients are immune to EBV, and because the virus persists in latent form, the EBV-specific CTL precursors persist lifelong at high frequency.

Initial treatment of patients who developed PTLD after allogeneic stem cell transplant with unmanipulated donor mononuclear cells resulted in high response rates.[24-25] The success of the adoptive transfer of polyclonal EBV-specific CTLs for the prophylaxis and treatment of patients with PTLDs provided an important basis to verify the efficacy of CTL-based therapies also in other EBV-related diseases such as HL and NPC. Although promising results were reported [26-27] this proved to be a highly challenging task due to low immunogenicity of LMP-1 and LMP-2 compared to the EBNAs expressed in PTLDs.

Virus –targeted therapies

Both LMP-1- and LMP-2-responsive elements were used to induce the transcription of the herpes simplex virus thymidine kinase (HSV-TK) in tumor cells (28-29). These approaches successfully sensitized LMP-1 or EBNA-2-expressing cells to gancyclovir-dependent killing in vitro. Nevertheless, the clinical applicability of these strategies is limited by the limited number of EBV-related malignancies that express EBNA-2, whereas the expression of LMP-1 is highly heterogeneous.

Conclusions

Despite significant efforts to improve the outcome of AIDS-NHL, the optimal treatment for AIDS-associated NHL remains still to be fully clarified. Such neoplasm continues to limit the life expectancy of HIV-positive individuals more significantly than any other single AIDS-defining condition, except for multifocal encephalopathy. The etiologic role of EBV has significant implications from the diagnostic and therapeutic view point and needs to be carefully considered.

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