Immunologic recovery in survivors following chemotherapy for AIDS-related non-Hodgkin lymphoma

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The late effects of chemotherapy on immunologic parameters in AIDS-related non-Hodgkin lymphoma (NHL) have not been described. From a cohort of 105 consecutive patients treated with infusional chemotherapy and highly active antiretroviral therapy (HAART), 68 survived more than 3 months following the end of chemotherapy. Their lymphocyte subsets and plasma HIV viral loads were measured at regular intervals for 2 years and values compared with baseline. During chemotherapy, there were statistically significant falls in CD4 (helper T), CD8 (cytotoxic T), and CD19 (B) cell populations but no changes in the CD56 (natural killer [NK]) cell population. Among the 68 survivors, there were statistically significant increases in CD4, CD8, CD19, and CD56 cell populations during the first year of follow up, compared with the values at the start of chemotherapy. During the second year of follow up, there were further statistically significant rises in CD4 and CD19 cell populations, compared with the values at 12 months after chemotherapy. During 244 years of follow-up since chemotherapy in these 68 survivors, 7 second primary tumors and 8 opportunistic infections were diagnosed. Chemotherapy and concomitant HAART for AIDS-related NHL does not cause prolonged suppression of lymphocyte subsets. These data should provide reassurance regarding the long-term consequences of chemotherapy in these individuals. (Blood. 2008;111:3986-3990)

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Methods

Patients

All patients diagnosed with HIV-related high-grade NHL at our institution between 1999 and 2006 were treated with a combination of infusional CDE (cyclophosphamide, doxorubicin, and etoposide) chemotherapy,15 and HAART (highly active antiretroviral therapy) comprising a combination of at least 3 antiretroviral agents including a nucleoside analog backbone combined with a protease inhibitor and or a nonnucleoside reverse transcriptase inhibitor. All patients had histologically confirmed aggressive high-grade NHL and full staging at diagnosis. The immunologic analysis here focuses on individuals surviving for more than 3 months after completing the course of chemotherapy, and this study was approved by the Chelsea and Westminster institutional review board. Informed consent was obtained in accordance with the Declaration of Helsinki.

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Follow-up and immune subsets

Following completion of chemotherapy, patients were seen at least once a month for the first year and thereafter at least once every 3 months. Lymphocyte subsets and plasma HIV-1 viral load were measured at the start of chemotherapy, after 1 and 3 months on chemotherapy, at the completion of chemotherapy, 1 month after completing chemotherapy, and thereafter at 3, 6, 12, 18, and 24 months after chemotherapy. Opportunistic infection prophylaxis was stopped following chemotherapy when the CD4 cell count achieved appropriate levels, while all patients continued on their HAART.

Total lymphocyte and subset analysis was performed using whole blood stained with murine antihuman monoclonal antibodies to CD4 (T helper cells), CD8 (cytotoxic T-cell marker), CD19 (B cells), and CD16/56 (natural killer cells) (TetraOne; Beckman Coulter, High Wycombe, United Kingdom) and were evaluated on an Epics XL-MCL (Beckman Coulter) multiparametric 4-color flow cytometer. Plasma viral loads (Quantiplex HIV-1 RNA 3.0; Chiron, Haidel, United Kingdom) were recorded with a lower limit of detection of 50 copies/mL.

Statistical methods

Median and interquartile ranges for lymphocyte subsets are described, and the variation of lymphocyte subsets in subjects over time is analyzed by nonparametric variables between groups. We used Mann-Whitney U test to compare nonparametric variables between groups.

Results

Patient characteristics

Between January 1999 and December 2006, 105 patients with high-grade HIV-associated NHL were treated with CDE chemotherapy and HAART. The characteristics at presentation of the entire cohort and the study group of 68 survivors are shown in Table 1. As expected, the survivors had statistically significantly lower risk IPI scores whether measured as composite IPI risk groups or individual variables Eastern Cooperative Oncology Group (ECOG) performance score, stage, elevated serum lactate dehydrogenase (LDH) level, although not number of extranodal sites). In addition, CD4 cell count at NHL diagnosis was significantly higher in the survivors.

Survival data

Overall, 42 patients have died and 63 survive at a median follow up of 3.8 years, and the 5-year overall survival is 59% (95% CI: 49% to 68%). Twenty-two patients died during the planned cycles of chemotherapy, 14 from progressive lymphoma, 7 from sepsis (4 during their first 2 cycles of chemotherapy), and 1 from a cerebrovascular accident. Seventeen patients have died of recurrent lymphoma, including 8 with leptomeningeal disease recurrence. Three patients have died while in remission of lymphoma; the causes of these deaths are as follows: 1 gastric cancer second primary malignancy, 1 esophageal cancer second primary malignancy, and 1 cardiac event.

Immunologic effects during chemotherapy

During the course of chemotherapy and 1 and 3 months following completion of the chemotherapy, patients had lymphocyte subsets and HIV plasma viral load measured. From the initial cohort of 105 patients, 68 survived to the 3-month postchemotherapy time point, thus the number of subjects at each time point diminishes and this may confound interpretation of results. During chemotherapy, there were statistically significant declines in the CD4 (helper T cell), CD8 (cytotoxic T-cell), and CD19 (B cell) cell populations in the peripheral blood but no significant change in the CD56 (natural killer cell) population (Table 2). At diagnosis of lymphoma, 62 (59%) of 105 patients were on HAART and the remaining patients all started HAART with their chemotherapy. At the start of chemotherapy, only 27% patients had an undetectable HIV plasma viral load. This value rose steadily through the course of chemotherapy to 69% at the completion of chemotherapy and on to 79% 3 months after completing chemotherapy. This rise in the proportion of patients with undetectable viral loads will be mainly because 41% of the cohort were antiretroviral naive and started HAART with their chemotherapy.

Late immunologic effects following chemotherapy

Lymphocyte subset and plasma HIV viral load data are available for 68 patients 3 months after completing chemotherapy, and further measurements of these factors has been undertaken at 6 (n = 64), 12 (n = 54), 18 (n = 47), and 24 (n = 43) months after...
completing chemotherapy. For the analysis of late immunologic toxicity among these survivors, only these 68 patients are included to eradicate any bias. During the first year of follow-up from the end of chemotherapy, there were statistically significant rises in CD4, CD8, CD19, and CD56 cell counts and the undetectable rate rose from 69% to 81%. The rises in cell counts were most marked during the first 3 months of follow-up, and the median rise in cell counts over the first year was 97 cells/mL for CD4, 318 cells/mL for CD8, 137 cells/mL for CD19, and 32 cells/mL for CD56. During the second year of follow-up, further statistically significant rises were documented for CD4 and CD19 cell counts but not for CD8 or CD56 cell counts (Table 3; Figure 1). Data are available for 43 patients at 24 months after chemotherapy, and for these patients the CD4 cell count rose from 165/mL (IQR: 62-252) at the start of chemotherapy to 270/mL (IQR: 174-445) 24 months after completing chemotherapy.

**Delayed toxicity following chemotherapy**

Sixty-eight patients were monitored during late follow-up (more than 3 months after completing chemotherapy). The total follow-up for these patients is 244 patient years. Seven patients developed second primary tumors (2 Kaposi sarcoma [K5], 1 each anal squamous cancer, esophageal cancer, renal cell cancer, skin basal cell cancer, and skin squamous cell cancer), and a further 3 patients developed cervical intraepithelial neoplasia (CIN). During this period of follow-up, 7 patients developed 8 AIDS-defining opportunistic infections: 3 nonpulmonary tuberculosis (TB), 1 each cerebral toxoplasmosis, cryptococcosis, visceral leishmaniasis, pneumocystis carinii pneumonia, and esophageal candidiasis (last 2 diagnoses in the same patient).

**Discussion**

Dramatic and prolonged T-cell depletion following the administration of chemotherapy for AIDS-related NHL was described in the pre-HAART era. We describe the use of infusional CDE chemotherapy with HAART in 105 patients with AIDS-associated aggressive high-grade NHL and the early and delayed immunologic sequelae of this treatment. The early recovery of lymphocyte subsets within 3 months of the end of chemotherapy to prechemotherapy levels and the continued rise of these parameters during prolonged follow-up are reassuring, suggesting that combination chemotherapy with HAART can be safely delivered without long-term immune suppression. This pattern of lymphocyte subset recovery following chemotherapy is similar, although probably swifter than that seen in the immunocompetent population. Moreover, during a median follow-up of 3.8 years there were relatively few AIDS-defining opportunistic infections.

We have previously described the depression of CD4 and CD19 cells during chemotherapy and HAART treatment in both lymphoma (including 10 patients who are also included in this study) and Kaposi sarcoma. This has also been documented during chemotherapy in other studies, however the findings are often complicated by the initiation of HAART with the chemotherapy in antiretroviral-naive patients, while patients already on HAART continue with it. Moreover, none of the reports compare the pre chemotherapy and postchemotherapy lymphocyte subsets and HIV viral loads in the survivors, and so all these analyses are biased by patients who fail to complete therapy. Five studies have reported changes in CD4 cell counts during chemotherapy, including 4 where patients received concomitant HAART. In 3 papers, the fall in CD4 cell count from the start to end of chemotherapy is described in 36,1 10,2 and 20 2 patients with declines in median or mean CD4 cell counts recorded of −47 cells/mL, −106 cells/mL, and −61 cells/mL, respectively. One paper describes a median rise in CD4 cell count from the start of chemotherapy to its maximal value of +216 cells/mL, although it is not clear when the maximum CD4 cell count was recorded in relationship to the chemotherapy.

The fifth paper describes therapy with the DA-EPOCH (dose-adjusted etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone) chemotherapy regimen used at the NCI that requires the interruption of HAART for the entire duration of chemotherapy. During DA-EPOCH chemotherapy, the CD4 count fell by a median of 189 cells/mm³ by the end of chemotherapy.

With regard to HIV viral load, the first 3 trials found a significant decrease during chemotherapy of −1.61 log₁₀ copies/mL and −2 log₁₀ copies/mL and −2.8 log₁₀ copies/mL during chemotherapy with HAART. The study describing the maximal responses at uncertain timing reports a decline in median HIV viral load from 29 000 copies/mL at the start of chemotherapy to less than 500 copies/mL. In contrast, DA-EPOCH therapy without concomitant HAART was associated with a median rise in viral load during chemotherapy of +0.83 log₁₀ copies/mL. A further paper describes the maintenance of virologic response during the administration of both rituximab plus CDE (R-CDE) and CHOP
### Table 3. Changes in HIV plasma viral load and lymphocyte subsets after chemotherapy for study group of 68 patients who survived more than 3 months after completing chemotherapy (chemo)

<table>
<thead>
<tr>
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<th>First year following chemo</th>
<th>Second year following chemo</th>
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<tr>
<td></td>
<td>Median change</td>
<td>Median change</td>
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<tr>
<td></td>
<td>at end of chemo (IQR)</td>
<td>at 6 months after chemo (IQR)</td>
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<tr>
<td>Median value at start of chemo (per mL)</td>
<td>127 (66 to 268)</td>
<td>127 (66 to 268)</td>
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<tr>
<td>CD4 cell count</td>
<td>178 (66 to 269)</td>
<td>178 (66 to 269)</td>
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<tr>
<td>CD8 cell count</td>
<td>693 (462 to 984)</td>
<td>440 (66 to 269)</td>
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<tr>
<td>CD19 cell count</td>
<td>3.5 (0-33)</td>
<td>3.5 (0-33)</td>
</tr>
<tr>
<td>CD56 cell count</td>
<td>59 (29 to 116)</td>
<td>59 (29 to 116)</td>
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One potential shortcoming of the analysis of delayed immunologic effects in this cohort is that inevitably only those patients who survived lymphoma can be studied. Thus the rapid immune cell recovery following chemotherapy may reflect favorable outcome and it is possible that under these circumstances the immune cells may be contributing an antilymphoma effect. These data present evidence that in those individuals who survive lymphoma, the immune subsets with the strongest mechanistic antitumor data (NK and CD8 cells) are maintained, although we have not measured specific antitumor cytotoxic T lymphocytes (CTLs).

The delayed follow-up for the 68 patients who survived at least 3 months after completing chemotherapy is 244 patient years.

Figure 1. Graph of median lymphocyte subset cell counts during and following chemotherapy for 68 patients who survived longer than 3 months after completing chemotherapy.
During this follow-up, 7 developed second primary tumors. The development of Kaposi sarcoma in 2 patients may relate to the chemotherapy-induced lymphocyte suppression, as CD4 cell nadir, CD8 cell nadir,\(^21\) and B-cell nadir,\(^22\) but not natural killer cell nadir,\(^23\) correlate with the risk of developing KS. The 5 non–AIDS-defining cancers observed in this study follow-up period have been shown to occur at increased frequency in people with HIV,\(^11\) although a direct correlation with immunologic parameters has not been established.\(^24\)

This analysis confirms that chemotherapy and concomitant HAART for AIDS-related NHL do not cause prolonged suppression of lymphocyte subsets and will alleviate clinicians’ fears that this treatment may compromise long-term immunologic function in people with AIDS.

### Authorship

Contribution: All authors contributed to the long-term follow-up and patient care of this large cohort and analyzed and provided data; M.B., B.G., T.P., and M.N. were the clinicians in overall charge and all authors conceptualized and approved the study; T.P., J.S., and M.B. wrote the final paper, which was reviewed by all the authors.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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### References

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