on ALK with the most potent derivatives, adaphostin and NSC 689857, having IC_{50} values of 23 and 10 μM, respectively (*data not shown*). UCN-01, a 7-hydroxy staurosporine derivative, reported to have induced disease stability in a patient with ALK-positive ALCL in a phase I clinical trial,[9] was also tested. UCN-01 inhibited ALK in the presence of 30 μM ATP (IC_{50}=5 μM), however no inhibition was observed at 300 μM ATP (*data not shown*). The low potency of UCN-01 and its lack of specificity for NPM/ALK transformed cells in proliferation assays (*data not shown*) suggest that UCN-01 does not target ALK. Therefore, potent and specific ALK inhibitors still need to be developed. The ALK-ELISA is a robust and accurate method suitable for middle and high-throughput screening that can be applied to the discovery of ALK inhibitors.

Rosalind Helen Gunby, Carmen Julia Tartari,* Francesca Porchia,* Arianna Donella-Deana,° Leonardo Scapozza,# Carlo Gambacorti-Passerini*@*

**Department of Experimental Oncology, National Cancer Institute, Milan, Italy; °Department of Biological Chemistry and CRIBI, National Research Centre, Institute of Neuroscience, University of Padua, Italy; #Section des Sciences Pharmaceutiques, University of Geneva, Switzerland; @McGill University, Montreal, Canada; ^Department of Internal Medicine, University of Milan Bicocca, Milan, Italy*

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Key words: ALCL, ALK, kinase assay, inhibitor screening.

Correspondence: Rosalind Helen Gunby, Department of Experimental Oncology, National Cancer Institute, via Venezian 1, 20133 Milan, Italy. Phone: international +39.02.23902689. Fax: international +39.02.23903237. E-mail: rosalind.gunby@istitutotumori.mi.it

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Malignant Lymphomas

Highly active antiretroviral therapy and outcome of AIDS-related Burkitt's lymphoma or leukemia. Results of the PETHEMA-LAL3/97 study

Short, intensive cycles of chemotherapy have resulted in improved survival in Burkitt's lymphoma/leukemia (BL) in adults.[1] The prognosis of patients with immunodeficiency virus (HIV)-associated BL is considered to be poor, but these patients have seldom been treated with BL-specific protocols.[2] However, a study (PETHEMA-LAL3/97) in which patients with BL were treated regardless of their HIV status failed to find differences between HIV-infected and immunocompetent individuals.[3] Furthermore, patients who received highly active antiretroviral therapy (HAART) seemed to have a slightly better disease-free survival than those who did not (p=0.051). We extended the follow-up analysis to elucidate the role of HAART in the survival of HIV-infected patients included in the PETHEMA-LAL3/97 protocol.

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We present the longer-term results of the multicenter PETHEMA LAL3/97 study on Burkitt's lymphoma/leukemia carried out by the Spanish PETHEMA group. The diagnostic criteria, characteristics of treatment, response criteria and follow-up procedures were reported in the original analysis.[3] Briefly, patients over 15 years old with newly diagnosed advanced BL (leukemic disease, lymphoma in stages III-IV or in stage II with a bulky mass) were treated, irrespectively of HIV status, with eight cycles of chemotherapy including alternating combinations of cytarabine, methotrexate, cyclophosphamide, ifosfamide, doxorubicin, teniposide, vincristine and dexamethasone. Triple drug HAART,including at least one protease inhibitor and two nucleoside reverse transcriptase inhibitors,[4] was recommended from diagnosis for HIV-positive patients if they were not receiving it already and was continued thereafter. The present analysis includes an extended follow-up of the 14 original HIV-positive patients reported[3] and 5 additional patients included in the protocol but previously excluded from analysis because of insufficient follow-up at that time. Overall survival (OS) and disease-free survival (DFS) were censored in July 2004 or date of last contact. Virological response to HAART was defined as having

Table 1. Characteristics of 18 HIV-positive adult patients with Burkitt's leukemia/lymphoma according to response to HAART.

Characteristic	*HAART-responders n=7*	*HAART-non responders n=11*
No HAART treatment	–	9
HAART prior to diagnosis	4	1
HAART from diagnosis	3	1
Age (years)	42	34
Median (range)	(29-65)	(23-57)
LDH (U/L)	1024	699
Median (range)	(100-3553)	(307-9130)
Albumin (g/L)	35	35
Median (range)	(33 – 46)	(26 – 44)
CD4 (×10³/mL)*	600	177
Median (range)	(195 – 1000)	(49 – 371)
Viral load (copies×10³)*	<0.2	515
Median (range)	(<0.2 – 29)	(3.3 - >103)
Male/Female N (%)	6 (86)/1 (14)	9
Performance status N (%) Zubrod 0-1/Zubrod >1	4 (57)/3 (43)	6 (55)/5 (45)
L3ALL (>20% BM blasts) N(%)	3 (43)	2 (18)
Bulky disease N (%)	2 (29)	3 (27)
CSF involvement N (%)	2 (29)	1 (9)

*HAART: highly active antiretroviral therapy. *Significantly different p<0.01.*

total HIV RNA loads below the limit of detection in serum (<80 copies/mL). According to response to HAART, the association with complete remission (CR) and survival was explored respectively by the χ^2 and the log-rank test. Nineteen HIV-infected patients were enrolled between January 1998 and June 2003; (84%) were male and the median age of the whole group was 41 years (range 23-65). The baseline clinical characteristics of patients stratified according to response to HAART are presented in Table 1. The diagnosis of HIV infection was made at the time of diagnosis of BL in 10 patients and between 1 and 13 years prior to diagnosis of the malignancy in the other 9 patients; five of them had had prior AIDS-defining conditions. Nine patients (47%) did not receive or discontinued HAART, 5 patients had started HAART before the diagnosis of BL (4 of them were in complete virological response at the beginning of chemotherapy). Thirteen patients (68%) achieved a CR, 2 had resistant disease and 4 died during induction because of infection in the neutropenic period. Seventeen induction cycles were evaluable for toxicity: there were 4 infections, 2 episodes of mucositis and 1 of hepatoxicity grade > 2. The median duration of neutropenia was 10 days (range 2-25) and that of thrombocytopenia 10 days (3-23). The CR rate was 70% for HAART users (7/10) and 71% for HAART responders (5/7). The CR rate of the HIV-negative patients of the same age and treated during the same time period was 77% (31/40). No toxic deaths were reported during consolidation although toxicity

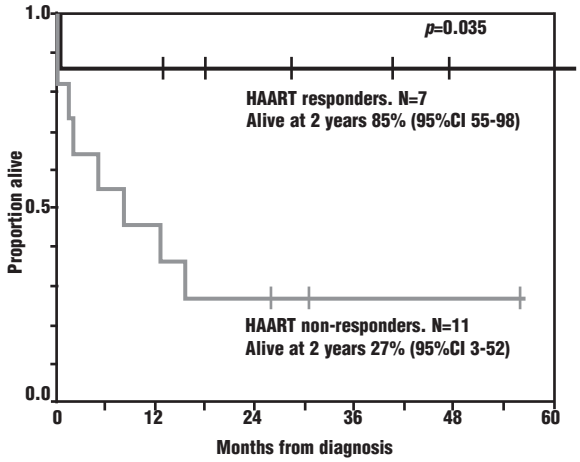

Figure 1. Kaplan-Meier plot of overall survival of the 19 patients according to HAART response.

necessitated discontinuation of treatment in 3 patients. In 34 consolidation cycles evaluable for toxicity 3 episodes of infection grade > 2 and 4 of mucositis grade > 2 were reported. Four patients relapsed while on therapy. Six patients (32%) completed the eight scheduled cycles. The 2-year OS probability was 46% (95%CI, 28%-64%) for the 19 patients (median follow-up 31 months) and the 2-year DFS for the 13 patients achieving CR was 71% (95%CI, 45%-94%). Lack of statistical differences from the 40 HIV-negative patients persisted (2-year OS 55%, 95%CI 38%-64% and 2-year DFS 57%, 95%CI 23%-75%). The OS of HAART responders (85% at 2 years; 95%CI, 55%-98%) and non-responders (27% at 2 years; 95%CI, 3%-52%) differed significantly (p=0.035) (Figure 1). Among 5 HIV-positive patients who achieved a CR and responded to HAART, no deaths or relapses have been observed (median follow-up 38 months, range 12-68), whereas there have been 3 relapses and 1 death due to an opportunistic infection among the non-responding patients (p=0.06).

Short, intensive chemotherapy including high-dose methotrexate, cytarabine and anthracycline has become the standard treatment for BL. The original report of the PETHEMA-LAL3/97 trial confirmed the feasibility of such approach in HIV-positive patients,[3] with these patients' CR rates, DFS and OS remaining comparable to those of immunocompetent patients after extended follow-up. Despite the apparent benefit of HAART response in HIV-related lymphomas treated with intermediate intensity regimens,[5,6] there may be concerns about the toxicity of combining antiretroviral treatment with high-dose chemotherapy. The protocol was not designed to detect specific interactions of antiretroviral and chemotherapeutic agents but the high proportion of patients not completing the eight scheduled cycles strongly suggested increased toxicity. However such events were counterbalanced by increased efficacy. The present subanalysis demonstrates, for the first time, that response to HAART prolongs OS in HIV-infected patients treated with a specific BL protocol.

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Key words: Burkitt's lymphoma; Burkitt's leukemia, HIV infection; HAART treatment, intensive chemotherapy.

Correspondence: Albert Oriol, Servei d'Hematologia Clínica, Institut Català d'Oncologia, Hospital Universitari Germans Trias i Pujol, Ctra. Canyet s/n, 08916 Badalona, Barcelona, Spain. Phone: international +34.9.34978987. Fax: international +34 .9.34978995. E-mail: aoriol@ns.hugtip.scs.es

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Lymphoproliferative Disorders

Detection of tumor-derived antigen receptor DNA by polymerase chain reaction in the serum of patients with B-cell chronic lymphocytic leukemia

Tumor-derived DNA is detectable in the serum of patients. In this study, tumor derived and mononuclear cell DNA from 50 patients with B-cell chronic lymphocytic leukemia (B-CLL) was amplified by polymerase chain reaction (PCR) and analyzed using polyacrylamide electrophoressis and Genescan analysis. DNA was demonstrated in 86% of serum samples. Genescan analysis has significantly enhanced the sensitivity and specificity of detection of tumor-derived DNA.

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The detection of clonality using polymerase chain reaction (PCR) amplification of antigen receptor gene rearrangements in chronic lymphoproliferative disorders aids the diagnosis and detection of minimal residual disease (MRD).[1] The discovery of tumor-derived DNA in the circulation of cancer patients raised the possibility of a new strategy for non-invasive cancer detection and monitoring.[2-3] The mechanism by which DNA is released into the serum is not fully understood but is thought to be derived from apoptosis of cancerous and/or normal cells.[4] Previous studies have reported that rearranged immunoglobulin heavy chain gene (IgH) DNA was detectable in approximately 50% of plasma/serum samples of patients with B-cell malignancies.[5-7] These studies analyzed only the Fr3 region of the Ig gene, with resolution of PCR products on denaturating gels. This insensitive approach has been superseded by the development of automated fragment analyzers which provide reproducible sizing of fluorescent PCR products.

This study investigated whether rearranged antigen receptor DNA could be detected in the serum of patients with B-cell chronic lymphocytic leukemia (B-CLL), or non-Hodgkin's lymphoma (B-NHL) patients without peripheral blood (PB) or bone marrow (BM) involvement, using fluorescent Ig PCR. Additionally, we quantified absolute values of $CD5^{+}19^{+}$ B cells and serum lactate dehydrogenase (LDH) levels as measurements of tumor load and/or activity.

Peripheral blood samples were collected from 50 patients with B-CLL at the Haematology Outpatient's Clinic, Belfast City Hospital. DNA was extracted from peripheral blood mononuclear cells (PBMC) and serum using the QIAmp DNA blood kit (Qiagen). DNA was amplified for Ig gene rearrangements, as previously described.[8,9] After amplification, products were separated on a non-denaturating polyacrylamide gel. For Genescan analysis, primers were replaced with 5-carboxyfluorescein (FAM). PCR products (1-2 µL) were mixed with 15 µL formamide, and 0.3 µL of size standard (Applied Biosystems). Lymphocyte subset analysis was carried out on a Becton Dickinson FACScan using two-or three-color whole blood labeling with directly conjugated antibodies.

A clonal IgH rearrangement was detected in serum DNA of 43 out of the 50 (86%) patients (Table 1). PCR products were first run on polyacrylamide gel and 25 of the 50