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High Frequency of Identical Clonal Immunoglobulin DNA in Pre-Treatment Tumor and Plasma from Untreated Patients with HIV-Associated Lymphoma: Prospective Multicenter Trial of the AIDS Malignancies Consortium (AMC 064)

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Abstract

Patients with HIV are at increased risk for developing B-cell lymphomas likely due in part to chronic antigen stimulation leading to clonal immunoglobulin (Ig) gene rearrangements. Clonal Ig DNA has been identified in the plasma in patients with lymphomas. However, next-generation sequencing (NGS)-based identification of circulating Ig clonotypes has not been well-characterized, particularly in HIV-related lymphomas. The AIDS Malignancies Consortium (AMC) enrolled 51 untreated patients with HIV-related B-cell lymphomas and analyzed paired tumor and plasma specimens for Ig clonotypes using an NGS-based approach (AMC064, NCT00981097). Lymphoma-specific clonotypes (>5% frequency) were identified in 83% (33/40) of the tumor specimens. Results from paired tumor and plasma specimens showed identical circulating clonotypes in the plasma from 97% (32/33) of patients. High International Prognostic Index (IPI) scores of 3–4 among patients with diffuse large B-cell lymphoma correlated with higher lymphoma molecules/million diploid genomes in the plasma compared with lower IPI scores of 0–2, median 77335 vs. 6876, $p = 0.005$. Further prospective studies are merited to

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Authorship Contributions

N.D.W. and R.F.A. designed the research project, collected and analyzed data, and wrote the first version of the manuscript.

S.L., S.S., J.Y.L., and M.F. analyzed data and wrote the manuscript.

A.N., D.H., and L.R. collected data and wrote the manuscript

Disclosure of Conflicts of Interest

N.D.W., S.L., J.Y.L., S.S., A.N., L.R., D.H., and R.F.A. declare no competing financial interests.

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determine whether plasma clonal Ig DNA has prognostic significance as a biomarker in HIV-related lymphomas and if the presence of lower frequency detection ($\leq 5\%$) may have similar implications.

Keywords

lymphoma; clones; Immunoglobulin; DNA; HIV

Introduction

B-cell lymphomas are characterized by the presence of clonal immunoglobulin (Ig) gene rearrangements. Determination of B-cell clonality in tumor tissue has been a clinically useful tumor marker for decades. Although the concept of circulating clonal Ig DNA is not new, previous tools to identify cell-free Ig gene rearrangements, such as multiplex PCR and capillary electrophoresis, have been limited by difficulties in quantification of clonal spikes and sensitivity.¹ We previously reported a 78% detection rate using multiplex primers in pretreatment plasma from 36 untreated patients with diffuse large B-cell lymphoma (DLBCL).² Using the same methodology, only 50% of pretreatment plasma specimens from 14 consecutive patients with AIDS-related lymphomas had detectable clonal Ig DNA.³ Ig gene capture with multiple parallel sequencing identified IgH DNA in the plasma among 8 of 14 subjects with B-cell non-Hodgkin lymphoma (NHL) in whom no tumor tissue was available.⁴ When clones are first identified in tumor tissue and then applied to the plasma using PCR, the sensitivity increases. Investigators from the NCI described an 86% (94/109) detection rate in the plasma using a next-generation sequencing (NGS)-based approach when sufficient tumor tissue was available, compared with a 37% (32/86) detection rate among plasma samples lacking corresponding tissue.⁵ The AIDS Malignancies Consortium (AMC) conducted a pilot study in a prospective group of untreated subjects with HIV-related lymphoma to evaluate paired tumor and plasma specimens for Ig clonotypes using high-throughput sequencing (AMC064, NCT00981097).

Methods

The primary objective of this prospective pilot study was to compare Ig DNA in paired diagnostic tissue and plasma from 50 subjects with HIV-related lymphomas. Eligibility criteria included patients with HIV and an untreated aggressive B-cell lymphoma, including DLBCL, plasmablastic, Burkitt, or Hodgkin lymphoma (HL); available diagnostic material from fresh frozen tissue or formalin-fixed paraffin embedded (FFPE) tissue age ≤ 18 years. NGS with the ImmunoSEQ-MRD™ platform of the diagnostic tissue was undertaken to amplify heavy chain (IGH) variable, diversity, and joining, and Ig kappa chain (IGK) gene segments from genomic DNA using universal primer sets⁶ (Adaptive Biotechnologies Corp, South San Francisco, CA). The minimum input DNA threshold to analyze for lymphoma clonotypes was defined as 15 ng. Amplified products were sequenced and analyzed using standardized algorithms for clonotype determination⁶. Clones were characterized in a qualitative and quantitative fashion. Presence of mutations in clones is indicative of somatic hypermutation. The frequency of the clonotype was determined by calculating the number of

sequencing reads for the particular clonotype divided by the total number of sequencing reads in the sample. Frequencies >5% were defined as the minimal threshold to characterize a clone as a tumor-specific clonotype. The tumor specific clonotypes were subsequently quantified in pretreatment plasma.

An exploratory analysis examined the association between the proportion of patients with clonal Ig DNA findings from the plasma for each of the clinical and pathologic correlates (histologic subtype, Ki67 staining index, and International Prognostic Score [IPI] score [NHL only]) using a two-tailed Fisher test. The study was conducted in accordance with Good Clinical Practice guidelines, as provided by the International Conference on Harmonization and principles of the Declaration of Helsinki. Institutional review boards approved the study protocol at the respective sites, and all patients provided written informed consent.

Results/Discussion

Fifty-one eligible patients registered to the study and tissue/plasma samples were received from 48. 79% of patients were male. Ethnicity/race for the participants included White, non-Hispanic (48%), Black/African American (31%), White/Hispanic (8%), Asian (4%), Hispanic/not-reported/unknown race (4%), Unknown/not reported ethnicity/white (2%), Unknown/not-reported ethnicity and race (2%). The lymphoma histologies included 27 DLBCL, 7 Burkitt, 7 HL, 5 plasmablastic, 1 primary effusion, and 1 B-cell lymphoma, unclassifiable. 81% of patients had stage III/IV disease. The median age was 47 (range 23–69). Additional high risk features among patients with aggressive B-cell lymphoma (HL patients excluded) included 73% with elevated LDH, 83% with Ki67 proliferative rates >80%, and high-intermediate (32%) and high-risk (12%) IPI scores.

Tissue sources among 43 patients with tumor specimens included 31 FFPE, 9 unstained slides, 3 fresh frozen tissue, and 1 punch biopsy. Four of the FFPE samples did not have sufficient DNA (although paired unstained slide was also available for one), leaving 40 patients with evaluable tumor samples. Lymphoma clonotypes were identified in 83% of patients with evaluable tumor specimens, including 24/27 FFPE, 7/9 unstained slides, 2/3 frozen tissue, and 0/1 punch biopsy. By histology clonal Ig DNA was detectable in the tumor from 19/23 DLBCL, 4/4 Burkitt, 3/6 HL, 5/5 plasmablastic, and 1/1 primary effusion lymphoma and 1/1 unclassified BCL. A median of 11,725 lymphoma molecules/million diploid genomes (range 4.8 – 363,200) were detected in the tumor samples. The number of high-frequency lymphoma specific clonotypes identified in the tumor samples ranged from 0–6 per sample (Figure 1). The gene rearrangements observed included 17 IGH-VDJ, 24 IGH-DJ, and 55 IGK receptors across the cohort. Identical Ig clonotypes were identified in the plasma in 97% (32/33) of patients with clonal Ig DNA in the tumor. High-frequency lymphoma-specific clonotypes (>5% frequency) were detected in 53% of the plasma samples (17/32), including 10 DLBCL, 4 burkitt lymphoma, 2 HL, and 1 plasmablastic lymphoma. Among 5 subjects with plasma but no matching tumor sample, 4 had clonotypes detected with the number of clones per patient being 1, 2, 4, and 5. DLBCL patients with IPI scores of 3–4 had higher lymphoma molecules/million diploid genomes in the plasma than those with scores of 0–2 (median (IQR): 77335 (8535-121200) vs. 6876 (116-23150), $p =$

0.005, Wilcoxon rank sum test) (Figure 2). Clinical and pathologic characteristics were similar among those with and without detectable lymphoma-specific clonotypes in the plasma except for higher prevalence of stage IV disease and elevated serum LDH among patients with detectable clones ($P = 0.037$ and <0.001 , respectively, two-sided Fisher's exact test).

Discussion

To our knowledge, this is the first prospective study evaluating circulating Ig clonotypes in patients with HIV-related B-cell lymphomas. Our tumor sequence detection rate of 83% is similar to that described in two previously published reports of NGS-based minimal residual disease assessment in patients with DLBCL of 83% and 86%, respectively.^{5,7} Roschewski et al.'s study included 24 subjects with HIV although separate detection rates were not detailed for the HIV population.⁵ A recent report described a 71% detection rate among 17 patients with Hodgkin lymphoma⁸. Low numbers of HL patients in our study preclude comparisons. The very high detection rate seen with Burkitt and plasmablastic lymphoma is not altogether surprising in light of the association of high burden of disease with identification of clones in plasma. Our study provides proof of concept that detection is feasible across the spectrum of aggressive B-lineage histologies.

The number of clonotypes identified per patient ranged from 0–6 with 59% of patients having 2 or more clones. This may reflect the high prevalence of aberrant somatic hypermutation and clonal evolution in HIV-associated lymphomas, similar to that of B-cell NHL without HIV.⁹ An advantage of the ImmunoSEQ-MRD platform is that it comprehensively captures the Ig landscape including clonal evolution often present in diagnostic specimens.⁶ The number of clonotypes seen in our study is similar to two previous studies in DLBCL with 2 lymphoma-specific clones identified in 44% and 53% of subjects, respectively.^{5,7}

Detection in the plasma is maximized when high-frequency lymphoma clonotypes are first identified in the tumor. However, our results demonstrate that high-frequency lymphoma clonotype identification in the plasma alone is feasible in a smaller subset of patients and may be useful in certain circumstances when tumor is unavailable or inadequate. In our series, clonotypes were detected in 4/5 pre-treatment plasma specimens without matched tumor. In the NCI study, clonal Ig DNA was identified in 32/86 (37%) pre-treatment plasma samples without paired tumor and interim monitoring for these clones predicted relapse.⁵

Conclusions

Identification and quantification of tumor-specific clonotypes in the plasma is feasible in patients with HIV. These findings support the development of future studies to assess the prognostic significance of this biomarker in assessing treatment response and predicting early relapse.

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Key Points

- Clonal Ig DNA is detectable in pre-treatment plasma from patients with HIV-related lymphomas

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Figure 1.
Number of lymphoma clonotypes per patient

Figure 2. Quantitative analysis of clonotype level in plasma at diagnosis
Level of lymphoma molecules/million diploid genomes in the plasma at diagnosis depicted in tumors with low and high International Prognostic Index (IPI) scores.

Table 1

Patient characteristics (n = 48).

Age (years) , mean (SD)	46.3 (11.5)
Range	(23–69)
Gender	
Male	38 (79)
Female	10 (21)
Ethnicity/Race	
White	23 (48)
Black or African American	15 (31)
Asian	2 (4)
Hispanic	6 (13)
Unknown	2 (4)
Histology	
Diffuse large B-cell lymphoma	27 (56)
Burkitt lymphoma	7 (15)
Primary effusion lymphoma	1 (2)
Plasmablastic lymphoma	5 (10)
Hodgkin lymphoma	7 (15)
B-cell lymphoma, unclassifiable	1 (2)
Ann Arbor stage	
I	4 (8)
II	5 (10)
III	6 (13)
IIIS	2 (4)
IV	31 (65)