Systematic Analysis of Recurrent Copy Number Variants in Diffuse Large B-cell Lymphomas

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Accepted abstract:

Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common high grade lymphoma in the Western Hemisphere. The disease exhibits a wide spectrum of clinical aggressiveness, and is characterized by heterogeneity at the histologic, cytogenetic, and molecular levels. One goal of this study is to identify novel molecular prognostic biomarkers in DLBCL patients, and to integrate these with existing prognostic biomarkers. In a previous study using publicly available DLBCL datasets, we defined a set of 50 common copy number variations (CNAs) involving 36 genomic loci (ref 1), for which robust scoring criteria were established. Here, we present the initial results of an attempt to systematically validate these CNAs using formalin fixed paraffin embedded tissue blocks from an additional group of DLBCL patients.

Methods: Patients diagnosed with *de novo* DLBCL and treated with R-CHOP from 2003 to 2009 at the Beth Israel Deaconess Medical Center (BIDMC) were identified through retrospective review of electronic medical records (EMR). Sections of one formalin fixed paraffin embedded block from each patient were submitted for custom array comparative genomic hybridization (aCGH) at Cancer Genetics Inc., and each sample was scored according to the established criteria for the presence/absence of each of the 50 CNAs (ref 1). Patient survival data was obtained through the BIDMC EMR. Kaplan-Meier survival analysis was performed using the R statistical software. Bonferroni correction was employed to adjust for multiple testing (cutoff p-value < 0.001).

Results: To date, the 50 CNAs were successfully assayed in samples from 45 DLBCL patients with survival data. In univariate analyses, the presence of four of these CNAs were associated with poor survival at a p-value of <0.05. One of the four CNAs (loss at 1p13.1) was significantly associated with poor outcome, after adjusting for multiple testing ($p = 1.43 \times 10^{-5}$).

Conclusions: Our ongoing systematic analysis of common copy number variations in DLBCLs identified several biomarkers potentially informative about patient survival. Further validation with additional cases and correlation with immunostains for cell of origin studies (ABC versus GCB subtypes) are in progress. The utility of each potentially useful novel biomarker will be assessed via multivariate analyses along with prognostic clinical variables.

Introduction:

Diffuse large B-cell lymphomas (DLBCLs) exhibit a wide spectrum of clinical and biological heterogeneity. Copy number alterations (CNAs) in DLBCLs are likely important for tumor biology, and may predict patient prognosis. Using three publically available datasets, members of our group previously defined a robust scoring criteria for 50 recurrent CNAs involving 36 minimal common genomic regions in DLBCL (ref. 1). The purpose of this study is to assess the performance of the 50 CNAs as prognostic biomarkers in another cohort of DLBCL patients.

Methods:

Patients diagnosed with DLBCL at the Beth Israel Deaconess Medical Center (BIDMC, Boston, MA) from 2003-2011 were identified by review of institutional electronic medical records (EMR). Selected formalin fixed paraffin embedded blocks of tumor were sent to Cancer Genetics Inc. (CGI, Rutherford, NJ) for array comparative genomic hybridization using a custom targeted oligonucleotide array. Calls for the 50 CNAs for each sample were made according to previously published criteria (ref. 1). Overall survival data was obtained through the BIDMC EMR and a public domain database (Ancestry.com). Univariate Cox proportional hazard (CPH) analysis was performed for each of the 50 CNAs using R (version 3.2.2). No analyses were performed using other clinical (e.g. R-IPI) or other molecular/immunohistochemical data for the partial dataset. The research was approved by the BIDMC and Dana Farber Cancer Institute Institutional Review Boards.

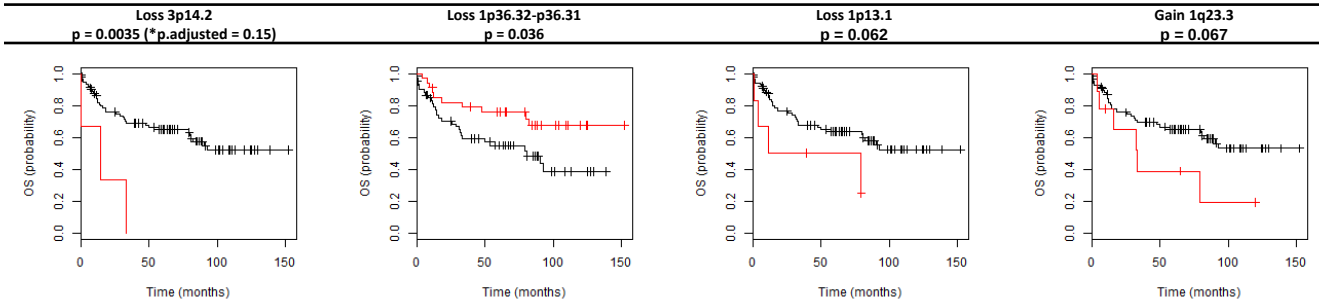
Results: Individual CNAs may be associated with overall survival in this partial dataset (n=95)

This partial dataset contains overall survival data for 95 DLBCL patients and calls for 48 recurrent CNAs for each patient (there were no calls for 2 of the 50 CNAs). The average length of follow-up was 83 months (~6.9 years). No individual CNA was significantly associated with survival after adjusting for multiple testing (Bonferroni correction, number of tests=48). Individual CNAs potentially associated with survival are presented in Table 1.

Table 1. CNAs with univariate CPH model p-value <0.10 (not adjusted for multiple testing). Only CNAs that have been called more than once are listed. The most significant CNAs are listed first.

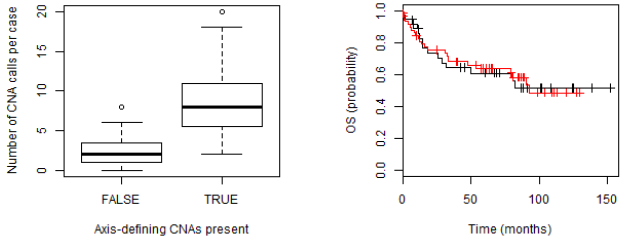
Copy number alteration	Hazard ratio (95% CI)	Univariate CPH p-value (unadjusted)	Number of times called (% of dataset)
Loss 3p14.2	5.0 (1.5-17)	0.0085	3 (3.2%)
Loss 1p36.32-p36.31	0.48 (0.22-0.95)	0.036	24 (25%)
Loss 1p13.1	2.6 (0.92-7.4)	0.072	6 (6.3%)
Gain 1q23.3	2.2 (0.93-5.3)	0.074	9 (9.5%)
Gain 11q23.3	2.0 (0.93-4.1)	0.079	15 (16%)
Loss 15q15.2 and 15q21.1	1.9 (0.89-4.2)	0.097	14 (15%)

Figure 1. Kaplan Meier survival curves for the top four CNAs from Table 1. Log-rank p-values are provided. Black = CNA absent. Red = CNA present.



Results: CDKN2A-TP53-RB1-E2F axis-defining CNAs are associated with the number of CNA calls per case, but not with overall survival in this partial dataset (n=95)

Figure 2. A. Boxplot of number of CNAs per case versus CDKN2A-TP53-RB1-E2F axis status (as defined in Ref. 2). Wilcoxon test p-value = 1.0×10^{-10}. B. Kaplan Meier survival curve for CDKN2A-TP53-RB1-E2F axis status (Black = axis-defining CNAs absent, n=36; Red = one or more axis-defining CNAs present, n=59). Log-rank p-value = 0.94.



Conclusions:

Recurrent copy number alterations in DLBCL may be a useful source of prognostic biomarkers. Additional clinical, somatic mutation, DNA copy number, and cell of origin data (by immunohistochemistry) will be obtained on these and additional cases. Integrated and multivariate analyses will be performed on the full dataset.

References:

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Disclosures: D.X., P.M., R.J., and R.D. have nothing to disclose. A.G. and J.H. are employed by and hold stock/stock options in Cancer Genetics Inc.