Cancer Genetics 207 (2014) 19–30

Acquired chromosomal anomalies in chronic lymphocytic leukemia patients compared with more than 50,000 quasi-normal participants

Cathy C. Laurie [a,*], Cecelia A. Laurie [a], Stephanie A. Smoley [b], Erin E. Carlson [c], Ian Flinn [d], Brooke L. Fridley [c,e], Harvey A. Greisman [f,g], John G. Gribben [h], Diane F. Jelinek [i,j], Sarah C. Nelson [a], Elisabeth Paietta [k], Dan Schaid [c], Zhuoxin Sun [l], Martin S. Tallman [m], Richard Weinshilboum [n], Neil E. Kay [i], Tait D. Shanafelt [i]

[a] *Biostatistics Department, University of Washington, Seattle, WA, USA;* [b] *Department of Laboratory Medicine and Pathology, Cytogenetics Laboratory, Mayo Clinic, Rochester, MN, USA;* [c] *Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA;* [d] *Sarah Cannon Research Institute, Nashville, TN, USA;* [e] *University of Kansas Medical Center, Kansas City, KS, USA;* [f] *Department of Laboratory Medicine, University of Washington, Seattle, WA, USA;* [g] *PhenoPath Laboratories, Seattle, WA, USA;* [h] *Barts Cancer Institute, Queen Mary, University of London, London, UK;* [i] *Division of Hematology, Mayo Clinic, Rochester, MN, USA;* [j] *Department of Immunology, Mayo Clinic, Rochester, MN, USA;* [k] *The North Division of Montefiore Medical Center, Bronx, NY, USA;* [l] *Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA;* [m] *Memorial Sloan-Kettering Cancer Center, New York, NY, USA;* [n] *Division of Clinical Pharmacology, Mayo Clinic, Rochester, MN, USA*

Pretherapy patients with chronic lymphocytic leukemia (CLL) from US Intergroup trial E2997 were analyzed with single nucleotide polymorphism microarrays to detect acquired chromosomal anomalies. The four CLL-typical anomalies (11q-, +12, 13q-, and 17p-) were found at expected frequencies. Acquired anomalies in other regions account for 70% of the total detected anomalies, and their number per participant has a significant effect on progression-free survival after adjusting for the effects of 17p- (and other covariates). These results were compared with those from a previous study of more than 50,000 participants from the GENEVA consortium of genome-wide association studies, which analyzed individuals with a variety of medical conditions and healthy controls. The percentage of individuals with acquired anomalies is vastly different between the two studies (GENEVA 0.8%; E2997 80%). The composition of the anomalies also differs, with GENEVA having a higher percentage of acquired uniparental disomies and a lower percentage of deletions. The four common CLL anomalies are among the most frequent in GENEVA participants, some of whom may have CLL-precursor conditions or early stages of CLL. However, the patients from E2997 (and other studies of symptomatic CLL) have recurrent acquired anomalies that were not found in GENEVA participants, thus identifying genomic changes that may be unique to symptomatic stages of CLL.

Keywords Chromosomal aberration, chromosomal mosaic, chronic lymphocytic leukemia, cancer precursor condition, cytogenetics

Received July 10, 2013; received in revised form January 3, 2014; accepted January 11, 2014.

* Corresponding author.
E-mail address: cclaurie@u.washington.edu

Chronic lymphocytic leukemia (CLL) is one of the most common hematological cancers in adults, with an incidence that increases with age (1). CLL is defined by a total blood lymphocyte count greater than 5×10^9 cells/L and a specific

2210-7762/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cancergen.2014.01.004

immunophenotype (2). The clinical course of disease is highly variable. There is strong evidence that CLL is nearly always preceded by monoclonal B cell lymphocytosis (MBL), defined by the presence of a low concentration of clonal B cells with an immunophenotype characteristic of CLL (3). People with MBL are asymptomatic, but have an estimated 1–2% annual risk of progression to CLL (4). The mechanisms of disease progression from MBL to CLL, and from indolent to aggressive CLL are still largely unknown.

Many studies of CLL patients, using chromosome banding or interphase fluorescence in situ hybridization (FISH), have shown four or five common anomalies involving copy number variation (5,6). For example, Döhner et al. (7) found 55% of patients with del(13q14), 18% with del(11q22), 16% with 12q trisomy, 7% with del(17p13), and 6% with del(6q21). The 17p and 11q deletions were associated with a poor prognosis, whereas the 13q deletions were associated with a better prognosis. Microarray technologies also have been used to detect acquired chromosomal anomalies in CLL patients (8–13). In addition to the five common abnormalities, these studies detected a variety of deletions, duplications, and acquired uniparental disomies (aUPDs). Genomic complexity, defined by the number or length of anomalies per patient, has been associated with shorter progression-free survival (PFS) (10) and time to treatment (14). Genomic sequencing studies of CLL patients have shown that the recurrence of mutations in specific genes is low (15) and that the total number of point mutations in coding sequences is lower than in many other cancers (16,17). Although chromosome banding studies have found that balanced translocations in CLL are rare, sequencing studies have shown that some patients have multiple rearrangements associated with copy number variation in a phenomenon known as chromothripsis (18), which has been associated with a poor prognosis (19).

Here we describe genomic profiling, using single nucleotide polymorphism (SNP) microarrays, of 214 CLL patients enrolled in E2997, a randomized, phase III intergroup clinical trial led by the Eastern Cooperative Oncology Group (ECOG) (20). This trial evaluated treatment with a combination of fludarabine and cyclophosphamide (FC) compared with fludarabine alone (FL). FC provides the backbone of a widely used chemotherapy for CLL, which now commonly includes rituximab to constitute a regimen designated as FCR. Previously, the prognostic significance of a large panel of laboratory factors was evaluated in this trial, including *IGHV* mutational status, *TP53* mutations, and FISH assays of the five copy number alterations (CNAs) described above (21). The occurrences of del(17p13) and del(11q22) were each associated with reduced PFS, whereas mutational status of *IGHV* and *TP53* had no significant effect independent of del(17p13).

The blood cell chromosomal anomalies that we discuss in this paper were acquired during the lifetime of the individual. Ideally, acquired and inherited anomalies are distinguished using paired tumor–normal samples. However, in many studies (such as E2997), normal control tissues were not genotyped. An alternative method of identifying acquired anomalies is detection of a mosaic mixture of normal and abnormal cells in a single tissue, assuming that the abnormality was acquired during development. This approach works well using the B-allele frequency (BAF) and log *r* ratio (LRR) metrics from Illumina microarrays (22–24), provided that a single anomaly constitutes a significant fraction

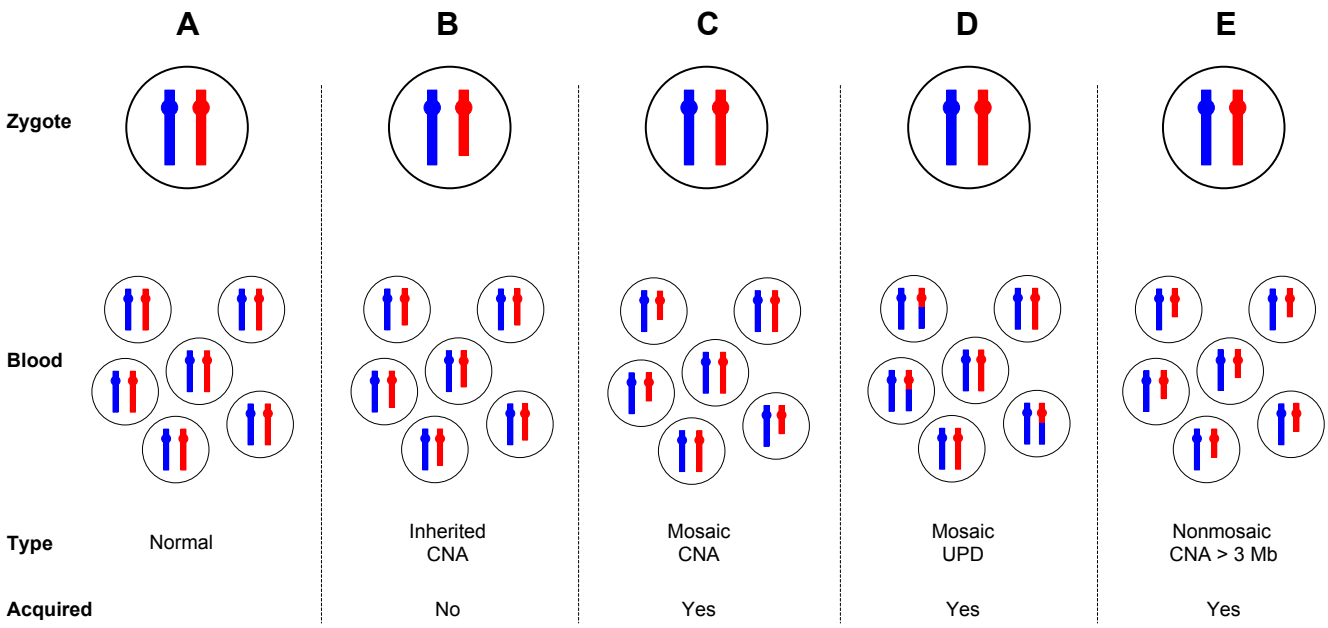

Figure 1 Classification of chromosome anomalies. (A) Normal individuals have biparental disomy in the zygote (fertilized egg) and in somatic cells such as blood. (B) Individuals with an inherited CNA have the abnormality in the zygote and somatic cells. (C) and (D) Individuals with a mixture of normal cells and abnormal cells in the soma are assumed to have developed from a normal zygote and, therefore, the abnormality is classified as acquired. In (C), the abnormality is a CNA and in (D) a segmental UPD. (E) Individuals with a large (>3Mb) non-mosaic CNA in somatic cells are considered to have an acquired anomaly (derived from a normal zygote) because CNAs > 3 Mb are very rarely inherited (see text). Each cell is shown with a pair of homologous autosomes, one maternal (red) and one paternal (blue).