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EGR2 mutations define a new clinically aggressive subgroup of chronic lymphocytic leukemia

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Abstract

Recurrent mutations within *EGR2* were recently reported in advanced-stage chronic lymphocytic leukemia (CLL) patients and associated with a worse outcome. To study their prognostic impact, 2403 CLL patients were examined for mutations in the *EGR2* hotspot region including a screening (n=1283) and two validation cohorts (UK CLL4 trial patients, n=366; CLL Research Consortium (CRC) patients, n=490). Targeted deep-sequencing of 27 known/postulated CLL driver genes was also performed in 38 *EGR2*-mutated patients to assess concurrent mutations. *EGR2* mutations were detected in 91/2403 (3.8%) investigated cases, and associated with younger age at diagnosis, advanced clinical stage, high CD38 expression and unmutated IGHV genes. *EGR2*-mutated patients frequently carried *ATM* lesions (42%), *TP53* aberrations (18%), and *NOTCH1/FBXW7* mutations (16%). *EGR2* mutations independently predicted shorter time-to-first-treatment (TTFT) and overall survival (OS) in the screening cohort; they were confirmed associated with reduced TTFT and OS in the CRC cohort and independently predicted short OS from randomization in the UK CLL4 cohort. A particularly dismal outcome was observed among *EGR2*-mutated patients who also carried *TP53* aberrations. In summary, *EGR2* mutations were independently associated with an unfavorable prognosis, comparable to CLL patients carrying *TP53* aberrations, suggesting that *EGR2*-mutated patients represent a new patient subgroup with very poor outcome.

Introduction

Chronic lymphocytic leukemia (CLL), the most common adult leukemia in the Western world[1], is a malignancy of mature B lymphocytes that accumulate in the blood, bone marrow and other lymphoid tissues[2, 3]. Although treatment has undergone profound improvements in recent years[4-6], CLL shows a remarkable clinical variability which is likely to be reflective of a large biological heterogeneity[7, 8]. While numerous prognostic markers have been identified, the mutational status of the immunoglobulin heavy variable (IGHV) genes and certain, high-risk cytogenetic/genetic aberrations (i.e. 11q, 17p deletions and *TP53* mutations) have remained the strongest markers and are today applied in routine diagnostics[9-12].

More recently, whole-genome sequencing (WGS) and whole-exome sequencing (WES) studies have begun to unravel the molecular landscape of CLL, revealing a limited number of frequently mutated genes (e.g. *ATM*, *NOTCH1*, *SF3B1*, *TP53*)[13, 14] with a long tail of genes altered in <5% of cases (e.g. *CHD2*[15], *MED12*[16], *NFKBIE*[17], *POT1*[18], *RPS15*[19], *SETD2*[20], *XPO1*[21]). Though integration of molecular information has been proposed to improve classical risk stratification models[22-25], a substantial proportion of patients with a dismal clinical course will not be captured by these algorithms, hence indicating a need to identify additional molecular markers of disease aggressiveness.

Recurrent missense mutations within the *EGR2* (early growth response 2) gene, a versatile transcription factor involved in differentiation of hematopoietic cells[26-28], were recently reported in approximately 8% of advanced-stage CLL patients and appeared to be associated with a worse outcome[29]. Notably, *EGR2* mutations were predominantly observed within the three zinc-finger domains located in exon 2. EGR2 is activated through ERK phosphorylation upon B-cell receptor (BcR)

stimulation[26] and we have previously shown that *EGR2*-mutated CLL patients display altered expression of *EGR2* down-stream target genes compared to patients wild-type for *EGR2*, thus pointing to a pathogenic role for *EGR2* mutations through dysregulated BcR signaling[29]. In addition, global DNA methylation investigations linked abnormal EGR2 activity with aberrant hypomethylation of transcription factor binding sites in CLL[30].

In this study, we investigated the frequency, clinical and biological associations, and prognostic impact of *EGR2* mutations in a large well-characterized screening cohort (n=1283), two validation cohorts comprising untreated patients from the LRF UK CLL4 trial (n=366) and patient samples from the CLL Research Consortium (CRC, n=490), a Chinese CLL cohort (n=233) and Richter's syndrome (RS) patients (n=31). *EGR2* mutations were associated with younger age, more advanced disease and other molecular high-risk markers, and remained as an independent factor predicting poor outcome both in the screening and validation cohort. These findings suggest that *EGR2* mutations define a new, poor-prognostic subgroup of the disease.

Methods

Patients

Peripheral blood samples from 1283 CLL patients with tumor content ≥60% (median 94%) were collected from collaborating institutions in the Czech Republic, France, Germany, Greece, Italy, the Netherlands, Sweden, and the United States and comprised the screening cohort. All CLL cases were diagnosed according to the iwCLL guidelines and displayed a typical CLL phenotype[31]. Over 76% of samples were collected before treatment and within a median of 7 months from time of diagnosis (*EGR2*-mutated cases, median 2 months). Clinical and biological characteristics of the screening cohort are summarized in Table 1; this cohort had a lower median age at diagnosis and a higher proportion of IGHV-unmutated cases compared to 'general' CLL cohorts, likely reflecting that several of the participating institutions are referral centers. Additionally, 366 CLL patients, entered into the multicenter trial UK LRF CLL4 (a randomized 1st-line comparison of chlorambucil, fludarabine and fludarabine plus cyclophosphamide) served as a first validation cohort (Supplemental Table S1). Details of the CLL4 treatment protocol have been previously reported[32]. Four hundred and ninety patients collected within the CRC served as a second validation cohort (Supplemental Table S2) with 81% samples obtained before treatment start. Finally, 233 cases from a Chinese CLL patient cohort (Supplemental Table S3) as well as 31 patients with RS were also screened for *EGR2* mutations. Written consent was obtained in accordance with the Declaration of Helsinki and with ethical approval obtained from the local ethics committees.

Analysis of *EGR2* mutations

The *EGR2* mutational hotspot region, covering the three zinc-finger domains located in exon 2, was screened as follows: Sanger sequencing was performed to analyze

1048 CLL and 31 RS patients; 622 cases were investigated using targeted next-generation sequencing (NGS), 171 patients were assessed by both techniques, and in 490 patients MassARRAY iPLEX assay was applied. In addition, for 41 patients in the screening cohort *EGR2* mutation status was derived from WES data[19]. Bidirectional Sanger sequencing was performed according to standard protocols (primers available upon request)[33]. For targeted NGS, a 500bp amplicon was bead-purified and library preparation was performed using the Nextera XT (Illumina, CA, USA) kit. Libraries were sequenced on the MiSeq instrument using v2 sequencing chemistry (Illumina). Applying standard settings, sequences were mapped using the alignment tool BWA (v.0.7.12)[34]. Variant calling was carried out using VarScan 2 (v.2.3.7)[35] with a minimum variant allele frequency (VAF) of 0.5% and variants were annotated using Annovar[36]. Samples from 366 patients enrolled in the UK LRF CLL4 trial were investigated by targeted NGS using a custom design TruSeq gene panel (Illumina) that included the entire coding region of *EGR2*[20]. 490 CRC samples were analyzed using the MassARRAY iPLEX assay (Agena, CA, USA) for recurrent mutations at EGR2 amino acid positions 356, 384 and 411/12; all mutated samples were validated by targeted NGS of a 210bp amplicon using MiSeq (Illumina). Samples with >5% VAF were considered mutated.

Analysis of concurrent mutations by targeted deep-sequencing

Thirty-eight *EGR2*-mutated patients (including 5 patients with VAF <5%) were analyzed using Haloplex technology (Agilent Technologies, CA, USA) according to the manufacturer's protocol. Probes targeting all coding exons or hotspot regions of 27 known CLL driver genes and/or genes previously reported in *EGR2*-mutated CLL[19, 29, 37-44] were designed using Agilent's SureDesign service (https://earray.chem.agilent.com/suredesign/home.htm, Supplemental Table S4).

Cluster generation and 125 cycle paired-end sequencing of the pooled library over one lane of the HiSeq 2500 instrument using v4 sequencing chemistry was performed (Illumina). Illumina sequencing adapters were removed using TrimGalore (v.0.3.7) and trimmed reads were aligned to the hg19 human reference genome (February 2009 assembly) using BWA (v.0.7.12). Variants were detected using VarScan2 with a VAF cutoff of 5% and a minimum 30 reads covering the variant was required. Non-synonymous single nucleotide variants (SNVs) and insertions/deletions (indels) that were not present in the 1000 genomes database were included for downstream analyses.

Statistical analysis

Mutational frequencies were assessed using two-sided, descriptive statistics. Overall survival (OS) was calculated from date of diagnosis or time from randomization (UK LRF CLL4) until last follow-up or death, while time-to-first-treatment (TTFT) was calculated from date of diagnosis until initial treatment. Kaplan-Meier analysis was performed to construct survival curves and the Cox-Mantel log rank test was used to determine differences between groups. Cox regression analysis was applied to compare the prognostic significance of *EGR2* mutations in relation to other prognostic markers. A significance level of $p<0.05$ was applied and all statistical analyses were performed using Statistica Software 13.0 (Dell Inc., OK, USA).

Results

EGR2 mutations and their association with patient characteristics

The overall prevalence of *EGR2* mutations in this study was 3.8% (91/2403 patients). In detail, *EGR2* mutations were detected in 50/1283 CLL patients (3.9%) of the screening cohort, in 12/366 patients (3.3%) of the UK CLL4 trial cohort, and in 18/490 of the CRC cohort (3.7%; Figure 1A and Supplemental Table S5). Further screening revealed that 9/233 (3.9%) patients in the Chinese cohort, and 2/31 (6.5%) RS patients carried an *EGR2* mutation (Figure 1A and Supplemental Table S5).

All mutations represented heterozygous missense mutations except for a recurrent in-frame 3-bp insertion identified in 3 cases. The majority of *EGR2* mutations (85/91, 93%) were localized to the DNA-binding sites of the 3 zinc-finger domains, and predominantly affected codons E356, H384 and D411 (Figure 1B). These domains are highly conserved between orthologues in different species (Supplemental Figure S1). The somatic nature of *EGR2* mutations affecting codons E356, H384, D411, and E412 has been previously confirmed[13, 14, 19, 29]. With the exception of R318Q, all identified amino acid substitutions were predicted damaging with a SIFT score <0.05[45].

For 49 *EGR2*-mutated samples from the screening cohort and all patients from the two validation cohorts, information on allelic frequency was available from deep-sequencing and the median VAFs were 38.9%, 36.3% (UK CLL4 trial) and 39% (CRC cohort), respectively (range, 5.6–62%). Additionally, 33 patients were found to exhibit low-frequency *EGR2* variants at the aforementioned hotspot codons with a VAF ranging from 0.5%-5%. However, only 14/33 (42%) of these variants could be verified in an independent experiment (Supplemental Table S6). These low-frequency *EGR2* mutations were excluded from subsequent survival analyses.

We next evaluated the correlation between clinico-biological characteristics and *EGR2* mutation status in all 1283 patients included in the screening cohort (Table 1). Compared to wild-type cases, *EGR2*-mutated patients were significantly younger (57 vs. 62 years; P=0.0042), more often presented with an advanced Binet stage at diagnosis (Binet B/C 56% vs. 30%; P=0.0005), carried unmutated IGHV genes (81% vs. 61%; P=0.0041) and del(11)(q22) (33% vs. 18%; P=0.0161), as well as expressed high levels of CD38 (67% vs. 27%; P<0.0001).

Co-existing mutations and clonal dynamics of *EGR2*-mutated CLL

Through targeted enrichment, we investigated 27 known CLL driver genes and/or genes previously reported in *EGR2*-mutated patients[19, 29, 37-44] in 38 *EGR2*-mutated CLL patients (including 5 cases with VAF <5%). Overall, a mean coverage of 4099 reads per targeted region was achieved with at least 500 reads in 95% and 1000 reads in 90% per nucleotide base of the targeted regions (Supplemental Table S7), hence allowing reliable detection of co-existing mutations. A total of 92 nonsynonymous alterations were detected (Supplemental Table S8); 15 cases harbored one, 8 showed two, 5 patients had three, and a single patient presented with four additional gene mutations (Figure 1C), while no additional mutations were detected in the remaining 9 *EGR2*-mutated cases. Mutations occurred most frequently in *ATM* (12/38, 31.6%), *TP53* (7/38, 18.4%), and *SF3B1* (4/38, 10.5%). Of note, 4/12 *ATM*-mutated cases had two *ATM* mutations and another 5 cases showed del(11q), resulting in a high frequency of multiple *ATM* aberrations in *EGR2*-mutated CLL (9/12, 75%). Alterations affecting the NOTCH signaling pathway were found in 7 patients (18.4%; *NOTCH1* (n=3), *FBXW7* (n=3), and *SPEN* (n=1); Figure 1C). Similar findings were observed when comparing identified mutation frequencies with published WES data from 964 *EGR2*-wildtype patients[13, 14], showing a significant

enrichment of *ATM* (31.6% vs. 10.9%, P<0.001, two-sided Fisher's exact), *TP53* (18.4% vs. 5.4%, P=0.005, two-sided Fisher's exact), and *FBXW7* (7.9% vs. 1.8%, P=0.036, two-sided Fisher's exact) mutations.

To gain insights into the clonal dynamics of *EGR2*-mutated CLL patients, we studied the VAF derived from WES data before fludarabine, cyclophosphamide, and rituximab (FCR) treatment and at relapse in 5 patients with available samples from both time points. A sixth patient was sampled one year after diagnosis and again 7 years later, however remaining untreated at both time points. All samples at both times had been negatively selected for $CD5^+/CD19^+$ cells to ensure a high tumor content (>95%). In all 6 patients, the clone harboring an *EGR2* mutation expanded during the clinical course and became the dominant clone at relapse or at follow-up (Figure 1D).

Clinical impact of *EGR2* mutations

In the screening cohort (1178 cases with available clinical data), the median follow-up time for patients who remained alive was 87.5 months (interquartile range, 49.1 to 138.3 months). Patients with mutated *EGR2* (VAF >5%) had a significantly worse TTFT (median, 7.8 vs. 38.5 months; HR 1.86, 95% CI 1.35-2.57, P<0.001; Figure 2A) and OS as compared with *EGR2*-wild-type patients (median, 74.7 vs. 127.2 months; HR 2.03, 95% CI 1.41-2.92, P<0.001; Figure 2B). No survival difference was observed neither between patients with *TP53*abn and *EGR2* mutations (P=0.900; Figure 2C/D), nor between *EGR2*-mutated patients with or without concomitant *ATM* lesions (P=0.665; Supplemental Figure S2). Notably, within the subgroup of 691 U-CLL patients, *EGR2*-mutated cases (n=39) showed a significantly inferior OS than wild-type cases (P=0.009; Supplemental Figure S3). Similarly, among the 164

patients with *TP53*abn, a high-risk group defined by a concomitant *EGR2* mutation with a shorter OS was identified (P=0.023; Supplemental Figure S4).

In multivariate analysis, *EGR2* mutations remained an independent negative prognostic marker both for TTFT (HR 1.44, 95% CI 1.01 to 2.06; P=0.047) and OS (HR 1.72, 95% CI 1.12 to 2.65, P=0.014; Table 2), when including *EGR2* mutation status, age, gender, Binet stage, IGHV mutational status, del(11q), and *TP53*abn in the model. This independent effect on shorter TTFT and OS remained significant also when including additional molecular markers to the model such as *NOTCH1* and *SF3B1* mutations (Supplemental Table S9).

In the UK CLL4 trial cohort (n=366), the median follow-up time for patients who remained alive was 145 months (interquartile range, 127.7 to 157 months). Among the 12 *EGR2*-mutated cases treated within the CLL4 trial, 7 patients were randomly assigned to the fludarabine plus cyclophosphamide arm, 5 patients received chlorambucil and none fludarabine treatment. In univariate analysis, *EGR2* mutations were significantly associated with a reduced median OS from time of randomization of 24.6 versus 75.7 months for mutated vs. wild-type patients, respectively (HR 1.71, 95% CI 1.30-2.25, P=0.004; Figure 3A/B). Multivariate analysis confirmed *EGR2* mutation status as an independent risk factor for OS (HR 1.95, 95% CI 1.02-3.73, P=0.043; Table 3).

In the CRC cohort (486 cases with available clinical data), the median follow-up time for patients who remained alive was 64.9 months (interquartile range, 35.6-103.7 months). In univariate analysis, *EGR2* mutations were significantly associated with a shorter TTFT (median, 35.8 vs. 55.7 months; HR 2.08, 95% CI 1.21-3.57, P=0.007; Figure 3C) and reduced median OS of 98.3 versus 152.9 months for mutated vs. wild-type patients, respectively (HR: 2.22, 95% CI 1.03-4.77, P=0.036; Figure 3D).

Lack of cytogenetic/molecular data in a substantial proportion of CRC patients precluded testing of the above multivariate model. Nevertheless, multivariate analysis including age, gender, IGHV and *EGR2* mutation status, confirmed *EGR2* as an independent risk factor for TTFT (HR 1.92, 95% CI 1.11 – 3.32, P=0.020), while only a borderline significance was seen for OS (HR 1.90, 95% CI 0.88 – 4.12, P=0.10; Supplemental Table S10), probably due to relatively few events and shorter median follow-up time in this cohort.

Discussion

Missense mutations within the *EGR2* gene were recently reported in progressive and/or relapsing CLL patients and hence indicated to be associated with a worse clinical outcome[29]. Here, by investigating large well-characterized cohorts, we not only confirm and significantly extend this observation, but also reveal that *EGR2*-mutated CLL patients display distinctive clinicobiological features and a rapidly progressive disease course. Indeed, survival analysis in our screening cohort demonstrated a significant, negative prognostic impact of *EGR2* mutations with markedly short TTFT and OS, similar to patients with *TP53*abn, that remained as an independent negative factor in multivariate analysis. While *EGR2* mutations were confirmed as a high-risk marker of short TTFT and OS in the CRC cohort, they were also shown to independently predict short OS from time of randomization in the UK CLL4 trial cohort. Importantly, the negative prognostic impact of *EGR2* mutations was also evident in aggressive subgroups, such as U-CLL and patients with *TP53* aberrations, which displayed a particularly short OS. Taken together, our data supports that *EGR2* mutations define a new subgroup of patients with a particularly dismal outcome.

This comprehensive analysis identified *EGR2* mutations in 3.8% of 2403 investigated patients with similar mutation frequencies in the different cohorts analyzed (range, 3.3-3.9%), although different techniques for mutation screening were used. This relatively low mutation rate reflects the known genetic heterogeneity in CLL, with only a handful of genes mutated in 10-20% of cases, but with a long list of gene mutations occurring in less than 5% of CLL cases[13, 14]. Notably, no difference was observed between European/American and Chinese CLL patients with respect to their *EGR2* mutation frequencies as suggested for other known CLL drivers such as *SF3B1*[46]. *EGR2* mutations were less often found in subsets carrying stereotyped BcR (4/326=1.2%, P=0.027) in contrast to *SF3B1*, *NOTCH1*, *TP53*, and *NFKBIE* mutations that recently were reported to be enriched in specific stereotyped subsets[17, 47-49].

EGR2 mutations were often detected with a high mutant allele burden (median VAF 38.9%; Supplemental Table S11), indicating that these aberrations occur early in CLL development[29]. From our targeted NGS panel, we noted that patients with *EGR2* mutations frequently displayed concurrent mutations in DNA damage response, i.e. *ATM* and *TP53,* and in NOTCH signaling pathway, i.e. *NOTCH1* and *FBXW7*, indicating that aberrations within these pathways are important contributors to the evolution of the aggressive phenotype in *EGR2*-mutated patients. In fact, the majority of *EGR2*-mutated patients (75%) showed multiple *ATM* aberrations, which is considerably higher than reported in *EGR2* wild-type, *ATM*-mutated CLL patients (30-40%)[50-52]. Bi-allelic *ATM* inactivation is known to be associated with a shorter TTFT and OS[50]. Collectively, our data indicate an accumulation of several distinct poor-prognostic markers in *EGR2*-mutated CLL.

Although most *EGR2* mutations were deemed to be clonal, we observed a minor proportion of patients (n=14) with a low *EGR2* mutation burden (<5% VAF, confirmed by independent experiments). Survival analysis revealed a trend for shorter OS in these low-burden cases compared with wild-type patients (P=0.11; Supplemental Figure S5). Furthermore, serial sampling in 5 treated CLL cases revealed an expansion of the *EGR2*-mutated subclone over the clinical course and at relapse (Figure 1D; Supplemental Figure S6). Interestingly, one additional case that remained untreated also showed an expansion of the *EGR2*-mutated clone. This preferential selection of molecularly defined subclones under pressure of chemotherapy is similar to other poor-prognostic markers such as *TP53* in CLL[53] and *BCOR* in myelodysplastic syndromes[54]. Larger studies are now warranted to further analyze the potential clinical impact of low-frequency *EGR2* mutations as was recently shown for *TP53* and *NOTCH1*[53, 55].

Similar to the pivotal studies[29, 30], *EGR2* mutations were clustered in DNA binding domains pointing to a pathogenic role for the hotspots in codons E356, H384 and D411. The functional role of mutated *EGR2* in leukemogenesis is however still poorly understood. We recently showed that mutations in the *EGR2* DNA binding domain affect cell cycle behavior and lead to altered transcriptional activity and dysregulated BcR signaling[29]. Oakes *et al.* also identified altered EGR2 activity as a mediator for hypomethylation of distinct transcription factor binding sites[30]. Whether these effects are restricted to mutations localized in the three sites within the EGR2 DNA binding domain remains to be addressed. Nevertheless, considering the potential role of mutated EGR2 in altering BcR signaling, it will be particularly relevant to study the efficacy of BcR inhibitors in this patient subgroup.

In summary, our novel data highlight *EGR2* mutations as an adverse prognostic biomarker for CLL. *EGR2* appears to identify a subgroup of CLL patients with a particularly dismal outcome similar to patients with *TP53*abn. Upon confirmation of our current data in other cohorts, in particular in patients treated with novel agents (e.g. ibrutinib, venetoclax), *EGR2* mutation analysis should be considered for inclusion in the current work-up of CLL to identify high-risk patients.

Conflict of interest

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Supplementary Information for this article is accessible through the Leukemia website

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Figure legends

Figure 1: Frequency, localization and dynamics of *EGR2* mutations. (A) Frequency of *EGR2* mutations in investigated cohorts. (B) Localization of mutations identified in *EGR2*. (C) Co-existing mutations in 38 *EGR2*-mutated CLL patients. (D) Clonal dynamics of six *EGR2*-mutated CLL patients. The first 5 patients received FCR therapy between the first (blue bars) and second (green bars) time point sample, while the last patient remained untreated at the second time point (7 years between the samples).

Figure 2: Clinical impact of *EGR2* mutations in 1178 CLL patients from the screening cohort. (A) Time-to-first-treatment and (B) overall survival in the screening cohort according to *EGR2* mutation status. (C) Time-to-first-treatment and (D) overall survival in the screening cohort according to the established hierarchy for genomic aberrations[9] and *EGR2* mutation status. Patients with *TP53*abn and concomitant *EGR2* mutation are grouped into the *TP53*abn group (TTFT, n=7; OS, n=8).

Figure 3: Clinical impact of *EGR2* mutations in 366 patients from the UK LRF CLL4 trial and 486 patients from the CRC cohort. (A) Overall survival in the validation cohort according to *EGR2* mutation status. (B) Overall survival in the validation cohort according to the established hierarchy for genomic aberrations[9] and *EGR2* mutation status. Patients with *TP53*abn and concomitant *EGR2* mutation are grouped into the *TP53*abn group (n=1). (C) Time-to-first-treatment and (D) overall survival in the CRC validation cohort according to *EGR2* mutation status.

Table 1: Comparison of clinical and biological characteristics between *EGR2* mutated and wild-type CLL patients within the screening cohort (n=1283).

	***EGR2* wild-type n=1233**	***EGR2* mutated n=50**	**P-value**
Age			
Median (years)	62.1	57.4	
<55 years - no. (%)	278 (25%)	17 (35%)	.0042
>71 years - no. (%)	194 (17%)	5 (10%)	
No information	105	1	
Sex			
Female - no. (%)	393 (34%)	19 (40%)	.3490
Male - no. (%)	769 (66%)	28 (60%)	
No information	71	3	
Binet stage			
A - no. (%)	674 (70%)	17 (44%)	.0005
B/C - no. (%)	290 (30%)	22 (56%)	
No information	269	11	
Need of treatment			
Yes - no. (%)	726 (68%)	42 (91%)	.0010
No - no. (%)	334 (32%)	4 (9%)	
No information	173	4	
CD38^{+}			
High (>30%) - no. (%)	161 (27%)	20 (67%)	< .0001
Low (≤30%) - no. (%)	438 (73%)	10 (33%)	
No information	634	20	
IGHV			
Mutated – (<98% identity) – no. (%)	460 (39%)	9 (19%)	.0041
Unmutated (≥98% identity) – no. (%)	710 (61%)	39 (81%)	
No information	63	2	
del(13)(q14)			
Absent - no. (%)	665 (64%)	35 (81%)	.0194
Present - no. (%)	374 (36%)	8 (19%)	
No information	194	7	
del(11)(q22)			
Absent - no. (%)	852 (82%)	29 (67%)	.0161
Present - no. (%)	187 (18%)	14 (33%)	
No information	194	7	
+12			
Absent - no. (%)	927 (89%)	36 (84%)	.2587
Present - no. (%)	112 (11%)	7 (16%)	
No information	194	7	
***TP53*abn**			
Absent - no. (%)	993 (86%)	41 (84%)	.6394
Present - no. (%)	161 (14%)	8 (16%)	
No information	79	1	
***NOTCH1* mutation**			
Absent - no. (%)	812 (91%)	42 (91%)	.8773

Present - no. (%)	84 (9%)	4 (9%)	
No information	337	4	
***SF3B1* mutation**			
Absent - no. (%)	804 (90%)	38 (84%)	.2703
Present - no. (%)	93 (10%)	7 (16%)	
No information	336	5	
Sampled prior to treatment			
Yes – no. (%)	665 (76%)	35 (80%)	.5480
No – no. (%)	215 (24%)	9 (20%)	
No information	353	6	

Recurrent genomic aberrations were classified according to the Döhner classification[9]. A two-sided student's t-test was used to assess differences in age at diagnosis, while a Chi-square test was applied to evaluate all other variables in *EGR2* wild-type versus mutated cases. *TP53*abn, *TP53* aberrations (i.e. mutations and or deletions)**.**

Table 2: Multivariate Cox proportional hazard analysis of time-to-first-treatment (TTFT, cases, n=898; events, n=624) and overall survival (OS, cases, n=863; events, n=371) in the screening cohort.

	TTFT			OS		
Variable	**Hazard ratio**	**95% Confidence interval**	**P-value**	**Hazard ratio**	**95% Confidence interval**	**P-value**
EGR2 mutation status	1.44	1.01 – 2.06	.047	1.72	1.12 – 2.65	.014
Age	1.04	0.89 – 1.22	.611	2.20	1.78 – 2.72	< .001
Gender	1.11	0.94 – 1.31	.230	1.32	1.05 – 1.65	.016
Binet stage	NA	NA	NA	2.26	1.82 – 2.81	< .001
IGHV mutation status	4.18	3.41 – 5.12	< .001	3.28	2.54 – 4.25	< .001
del(11q)(q22)	1.05	0.87 – 1.27	.620	0.84	0.64 – 1.09	.190
*TP53*abn	1.31	1.06 – 1.62	.013	1.66	1.26 – 2.18	< .001

NA, not applicable. *TP53*abn, *TP53* aberrations (i.e. mutations and or deletions).

Table 3: Multivariate Cox proportional hazard analysis of overall survival in the UK LRF CLL4 patients (cases, n=297; events, n=231)

Variable	Hazard ratio	95% Confidence interval	P-value
EGR2 mutation status	1.95	1.02 – 3.73	.043
Age	1.72	1.32 – 2.24	< .001
Gender	1.44	1.04 – 1.99	.029
Binet stage	1.21	0.86 – 1.71	.271
IGHV mutation status	2.22	1.64 – 2.99	< .001
del(11q)(q22)	1.46	1.07 – 1.99	.018
*TP53*abn	4.85	2.90 – 8.11	< .001

*TP53*abn, *TP53* aberrations (i.e. mutations and or deletions).

Figure 1

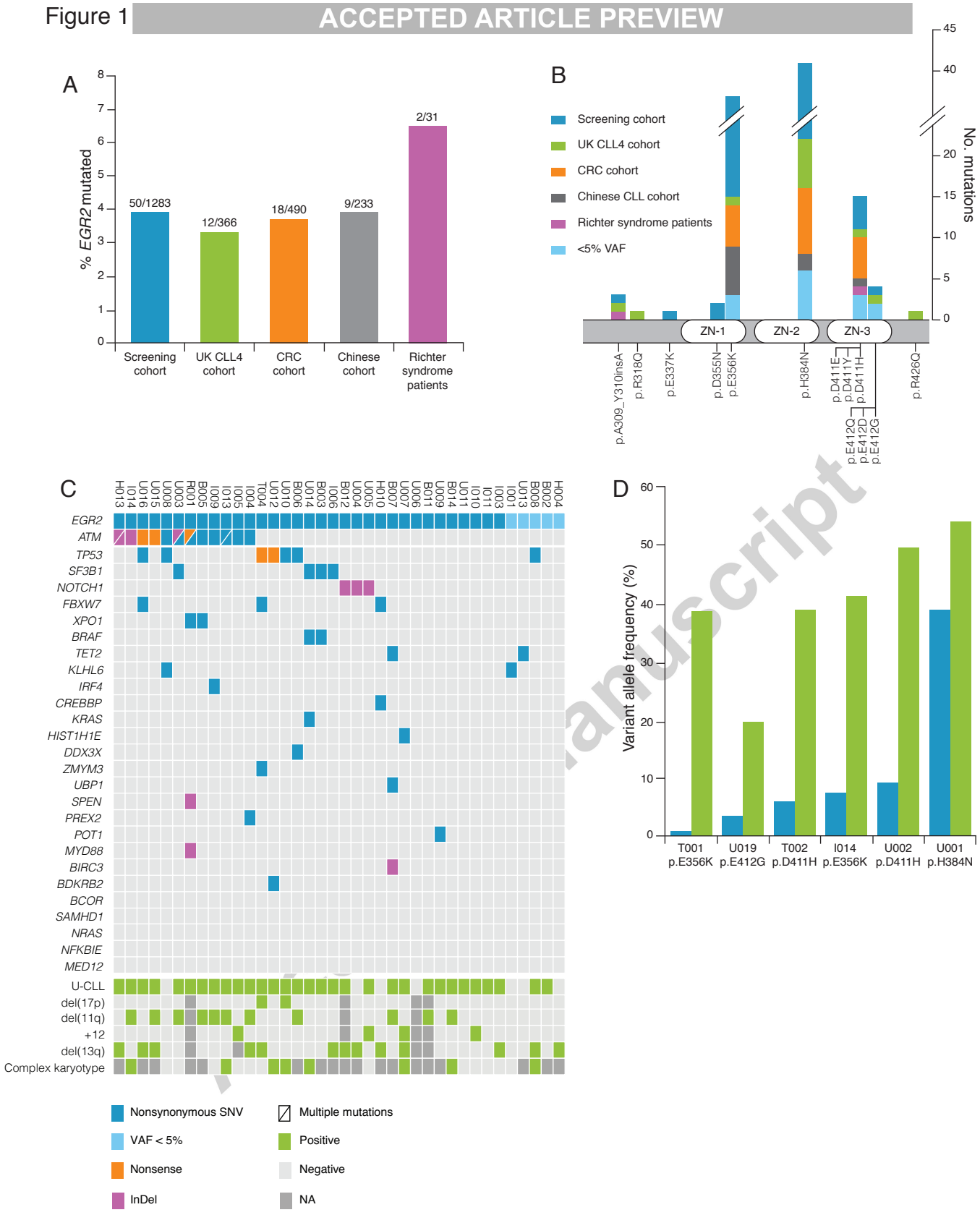

Figure 2

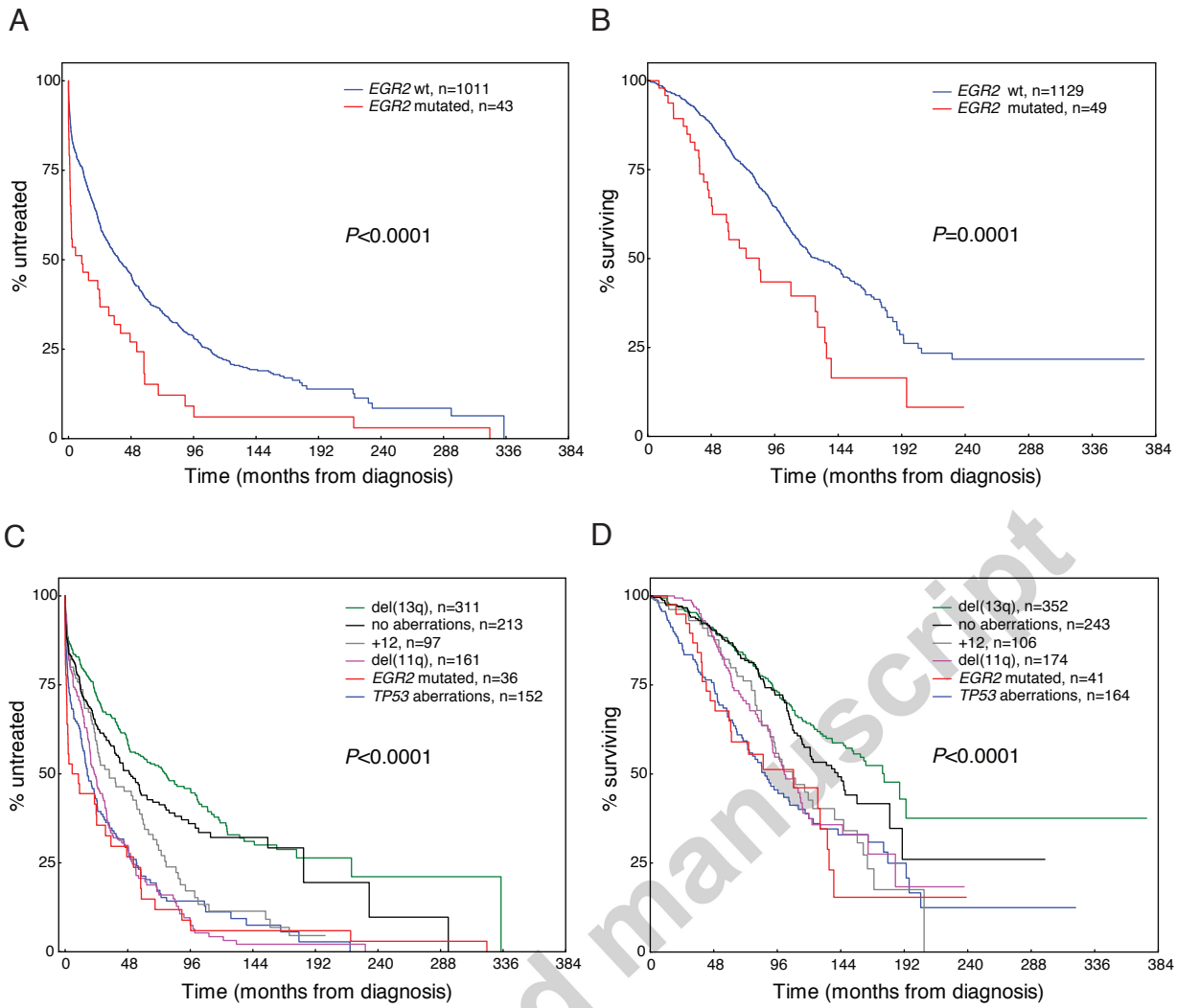

Figure 3