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Tailored approaches grounded on immunogenetic features for refined prognostication in chronic lymphocytic leukemia

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ABSTRACT

Chronic lymphocytic leukemia patients with differential somatic hypermutation status of the immunoglobulin heavy variable genes, namely mutated or unmutated, display fundamental clinicobiological differences. Considering this, we assessed prognosis separately within mutated and unmutated chronic lymphocytic leukemia in 3015 patients, hypothesizing that the relative significance of relevant indicators may differ between these two categories. Within Binet-A mutated chronic lymphocytic leukemia patients, besides *TP53* abnormalities, trisomy 12 and stereotyped subset #2 membership were equivalently associated with the shortest time-to-first-treatment and a treatment probability at 5- and 10-years after diagnosis of 40% and 55%, respectively; the remaining cases exhibited 5-year and 10-year treatment probability of 12% and 25%, respectively. Within Binet-A unmutated chronic lymphocytic leukemia patients, besides *TP53* abnormalities, del(11q) and/or *SF3B1* mutations were associated with the shortest time-to-first-trearment (5- and 10-year treatment probability: 78% and 98%, respectively); in the remaining cases, males had a significantly worse prognosis than females. In conclusion, the relative weight of indicators that can accurately risk stratify early-stage chronic lymphocytic leukemia patients differs depending on the somatic hypermutation status of the immunoglobulin heavy variable genes of each patient. This finding highlights the fact that compartmentalized approaches based on immunogenetic features are necessary to refine and tailor prognostication in chronic lymphocytic leukemia.

INTRODUCTION

Despite mounting evidence for the existence of distinct biological variants of chronic lymphocytic leukemia (CLL), the 2016 update of the WHO classification still considers CLL as a single, homogeneous entity, thus contrasting other hematological malignancies (e.g. diffuse large B cell lymphoma, DLBCL) which are segregated in different subgroups, based on the integration of genetic, morphological, immunophenotypic and clinical features[1].

Since the introduction of the Rai and Binet clinical staging systems in the 1970s[2,3], it became increasingly evident that the clinical heterogeneity in CLL is linked to and reflects the underlying biological heterogeneity. Hence, several initiatives focused on identifying biomarkers that would refine prognostication especially for cases who present with early stage disease, representing nowadays the great majority of patients (80-85%)[4-12]. Consequently, numerous prognostic indices have been proposed, however, none has been adopted in every-day clinical practice[13]. This is partially due to the fact that different variables have been assessed in each evaluated cohort while the actual routine diagnostic and monitoring practice varies between different institutions. Moreover, most reported cohorts were rather small, thus inherently limited in their capacity to both encompass the remarkable clinicobiological heterogeneity of CLL and reveal possible interactions and interdependencies among the evaluated prognosticators.

The clonotypic B cell receptor immunoglobulin (BcR IG) is a unique molecular signature for every CLL clone, present from its genesis and remaining unaltered throughout the course of the disease, thus sharply contrasting other tumor-derived biomarkers[14-19]. Seminal studies from the late 1990s have established that the somatic hypermutation (SHM) status of the immunoglobulin heavy variable (IGHV) gene expressed by the clonotypic BcR IG is a robust prognostic and predictive biomarker for CLL, stratifying patients into two non-interchangeable categories with different clinical behavior[20,21]. More specifically, CLL with a significant SHM load ("mutated" CLL, M-CLL) generally follow an indolent clinical course, whereas CLL carrying no or few mutations ("unmutated" CLL, U-CLL) generally have an aggressive disease and an overall inferior response to chemoimmunotherapy[22-24].

This subclassification into M-CLL and U-CLL reflects fundamental clinicobiological differences extending from the genomic and epigenomic to the transcriptomic and proteomic levels, alluding to distinct ontogeny and evolution patterns, including response to treatment, for

the two patient categories[14,24-27]. That said, within both M-CLL and U-CLL, a sizeable proportion of cases exhibit a clinicobiological behavior that deviates from the expected, thus highlighting that the heterogeneity of CLL persists even within a given SHM category[28-31]. The paradigmatic example is offered by CLL subset #2, defined by the expression of stereotyped IGHV3-21/IGLV3-21 BcR IG, within which M-CLL cases follow aggressive clinical course similar to U-CLL[30,32,33].

Notably, other established prognosticators such as cytogenetic aberrations or recurrent gene mutations are asymmetrically distributed within M-CLL or U-CLL[10,34-36]. On these grounds, it is not unreasonable to think that definitive conclusions about the precise clinical implications of any given biomarker should be drawn only after considering the SHM status of the clonotypic BcR IG.

In this manuscript, we followed a compartmentalized approach where we assessed the prognostic impact of traditional and novel prognostic parameters separately within M-CLL and U-CLL in a large multi-institutional cohort of well characterized CLL patients, based on the hypothesis that not all variables would carry equal weight within the two SHM categories. Considering that the key challenge at the time of diagnosis is determining if, and consequently when, early stage/asymptomatic patients will require treatment, we focused on identifying a robust prognostication scheme for time-to-first-treatment (TTFT) in these separate disease categories.

METHODS

Patient cohort

Overall, 2366 general practice patients with CLL diagnosed following the 2008 iwCLL diagnostic criteria[37] from 10 European institutions were included in this multicenter retrospective study (Supplemental Table 1). The external validation cohort comprised of 649 Binet A cases evaluated at the Munich Leukemia Laboratory (n=508) and from a Scandinavian population-based study (n=141) (Supplemental Table 2). Ethical approval was granted by the local review committees and informed consent was collected according to the Helsinki Declaration.

Methods

Detailed information about the methodologies used to analyze the prognostic markers and definitions about stereotyped subsets and the criteria for subset assignment are provided in the Supplemental Material. In short: (i) mutational screening was performed for the following genes: *NOTCH1*, entire exon 34 or targeted analysis for del7544-45/p.P2514Rfs*4; *TP53*, exons 4–8 but also exons 9–10 for some centers; *SF3B1*, exons 14–16; *BIRC3*, exons 6–9 and *MYD88*, exons 3 and 5 or targeted analysis for p.L265P. The great majority (80%) of cases included in this study were screened for the aforementioned recurrent mutations by Sanger sequencing. In the cases remaining cases (20%) next generation sequencing (NGS) was applied and only those clones with higher than 10% variant allele frequency /VAF >10%) were considered; (ii) FISH was performed in 1825 (77%) cases using probes for the 13q14, 11q22, 17p13 regions and trisomy 12 (cut-off: 5%) while results were interpreted following Döhner's hierarchical model[38]; and (iii) sequence analysis and interpretation of IGHV-IGHD–IGHJ rearrangements (including BcR IG stereotypy) was performed as described previously[39].

Statistical analysis

We assessed the prognostic impact on TTFT of the following variables: age at the time of diagnosis; gender; CD38 expression; cytogenetic aberrations [del(17p), del(11q), del(13q) and trisomy 12 (+12)]; mutations within the *TP53, SF3B1, NOTCH1, BIRC3* and *MYD88* genes; and immunogenetic features, including borderline germline identity (GI: 97-97.99%) and assignment to stereotyped subsets #1, #2 and #4.

The following methodology was applied separately for M-CLL and U-CLL patients. The chi-square test was used to assess the homogeneity of all categorical prognostic variables within the different Binet stages (A, B and C). In order to evaluate homogeneity and detect significant differences within each categorical variable, the Bonferroni correction was applied to adjust for multiple comparison error and the significance level was set to $p<0.017$. TTFT was evaluated from the diagnostic date until the date of initial treatment; untreated cases were censored at the time of last follow-up. Survival curves were constructed using the Kaplan-Meier method, and the log-rank test was applied to determine statistically significant differences between survival proportions.

The univariable Cox regression model was used to determine the most important prognostic factors. Multivariable Cox regression analysis was subsequently applied to evaluate the simultaneous effect of the important variable on TTFT. For the multivariable Cox analysis we considered only those cases with available data for all the factors included in the model (n=918 for M-CLL and n=384 for U-CLL), on the grounds that imputing the values of the biomarkers could introduce substantial bias. The same rule was applied for the Binary recursive partitioning. That said, no major differences were observed between the entire cohort and the proportion of cases included in the Multivariable Cox analysis (Supplemental Table 5); this suggests that the patients included in the multivariable analysis were representative of the entire cohort.

The proportional hazard assumption was assessed for both the univariable and multivariable analysis. The stability of the multivariable Cox model was internally validated using a bootstrapping procedure. Harrell's C index was also calculated to further assess the discriminatory power of the multivariable analysis when (a) all important prognostic variables were included in the model, and (b) the resultant prognostic index was included in the model as the sole prognostic variable (C=1 indicates perfect discrimination; C=0.5 equates to chance)[40].

Binary recursive partitioning, based on the development of conditional inference trees, was used to further validate the results of Cox regression analysis[41]. The patients were initially divided in subgroups (terminal nodes) with different survival behavior. An amalgamation algorithm was subsequently applied to merge the terminal nodes that did not exhibit a significant difference in survival. All tests were two-sided and statistical significance was attained when the p-value was <0.05. Statistical analyses were performed using the

Statistica Software 10.0 (StatSoftInc, Tulsa, OK) and R-3.2.2. Details are provided in the Supplemental Material.

RESULTS

Prognostic impact of clinical staging within M-CLL and U-CLL

The median follow-up time for the entire cohort was 7.1 years (range, 0.1-33.1) with a median TTFT of 6.4 years (95% CI: 0.01-20.2 years, Supplemental Figure 1). As expected, in both M-CLL and U-CLL, Binet A patients exhibited significantly longer TTFT compared to Binet B and C cases (Figure 1). Most likely, this reflects the sharp clinicobiological differences between Binet A and Binet B/C patients while also underscoring the current indications for treatment that broadly overlap with the criteria for Binet stage B and C assignment (Supplemental Tables 3, 4). In keeping with previous reports, M-CLL cases (n=1364, 58%) exhibited significantly ($p<0.0001$) longer TTFT compared to U-CLL cases (n=1002, 42%) (Supplemental Figure 2A). Significant differences ($p<0.0001$) between M-CLL versus U-CLL remained when the analysis was restricted to Binet A cases (Supplemental Figure 2B).

Prompted by these findings and also considering that Binet A cases predominated in both M-CLL and U-CLL (90% and 67%, respectively), we focused our attention on 1900 Binet A cases (M-CLL: n=1224; U-CLL: n=676). We refrained from investigating the impact of biomarkers within Binet B/C cases since the limited number of cases within each subgroup would not allow firm conclusions to be drawn.

Analysis for TTFT within early stage M-CLL

Univariable Cox-regression analysis within M-CLL Binet-A cases (n=1224) revealed that male gender, CD38 positivity, +12, subset #2, TP53abn and borderline IGHV gene germline identity (GI: 97-97.9%) were associated with significantly shorter TTFT (Table 1). Bordeline GI remained significant in the univariable analysis even when subset #2 cases were excluded. *TP53abn*, +12, subset #2 membership and male gender retained independent adverse prognostic significance in the multivariable analysis (n=918, Table 1). Of note, *TP53abn*, +12 and subset #2 membership identified 3 groups of almost mutually exclusive cases (altogether: n=150, 16% of all Binet A M-CLL) (Figure 2A) with no statistical differences in

TTFT (median TTFT for *TP53abn*, +12, subset #2: 5.4, 7.1 and 4.1 years respectively, p=0.19; Figure 2B).

To further validate the results of the Cox-regression analysis, we applied recursive partitioning models within a conditional inference framework. *TP53abn*, +12 and subset #2 membership were again identified as the most important predictors since the 3 groups defined by these predictors displayed similar TTFT that was significantly shorter from the remaining Binet A M-CLL (Figure 3A).

Analysis for TTFT within early stage U-CLL

Univariable Cox regression analysis revealed that *TP53abn*, *SF3B1* mutations, del(11q) and male gender had a shorter TTFT within Binet-A U-CLL (n=676), while +12 had a longer TTFT (Table 1). *TP53abn*, *SF3B1* mutations, del(11q) and male gender retained significance in the multivariable analysis (n=384, Table 1). *TP53abn* and/or *SF3B1* mutations and/or del(11q) [*TP53abn*/*SF3B1mut*/del(11q)] were positive in 146 cases (42% of the evaluated cohort with available data for all 3 parameters, Figure 2C) and exhibited a similar TTFT (median TTFT for *TP53abn*, *SF3B1mut*, del(11q): 1.8, 2.8 and 2.1 years, respectively, p=0.47; Figure 2D). The co-occurrence of these aberrations was not found to aggravate the prognosis when compared to single aberrations alone (Supplemental Figure 3). Male gender was associated with a shorter TTFT (median TTFT: 3.9 years, 95% CI: 0.1-5.9 years) amongst non-*TP53abn*/*SF3B1mut*/del(11q) U-CLL cases (Supplemental Figure 4).

Similar to M-CLL, we applied recursive partitioning (Figure 3B) which highlighted *TP53abn*, del(11q) and male gender as the most important variables within Binet-A U-CLL. *SF3B1* mutations did not reach significance, potentially due to the low number of cases with isolated *SF3B1* mutations at the stage of the final nodes. In short, the evaluated U-CLL Binet-A cohort was allocated to subgroups with differing TTFT, from shorter to longer, as follows: males with *TP53abn*; males with del(11q)/females with *TP53abn* and/or del(11q); males without *TP53abn*/del(11q); and, females without *TP53abn*/del(11q).

Two SHM categories of CLL patients, two prognostic indices for TTFT

Based on the above, we developed two prognostic indices for assessing TTFT, tailored to each SHM category. Within M-CLL, we defined 4 groups based on their clinicobiological profiles at the time of diagnosis: (i) *very high risk*: Binet C with identical 5- and 10-year

treatment-probability (TP) of 92%; (ii) *high risk*: Binet B, 5y-TP and 10y-TP: 64% and 84%, respectively; (iii) *intermediate risk*: Binet A with one of the following: *TP53abn* and/or +12 and/or subset #2 membership, 5y-TP and 10y-TP: 40% and 55%, respectively - of note, among 18 non censored cases with no treatment indication for more than 10 years after diagnosis, 5 (30%) carried *TP53abn*; and (iv) *low risk*: non-*TP53abn*/+12/subset#2 Binet A, 5y-TP and 10y-TP: 12% and 25%, respectively (Figure 4A). Harrell's C index was calculated for the multivariable Cox model with the prognostic index above being the sole predictor and found equal to 0.745 (se = 0.013).

In U-CLL, we could define 5 groups: (i) *very high risk*: Binet C with 5- and 10-year TP of 100%; (ii) *high risk*: Binet B, 5y-TP and 10y-TP: 90% and 100%, respectively; (iii) *intermediate risk*: Binet A with one of the following: *TP53abn* and/or *SF3B1mut* and/or del(11q), 5y-TP and 10y-TP: 78% and 98%, respectively; (v) *low risk*: non-*TP53abn*/*SF3B1mut*/del(11q) male Binet A, 5y-TP and 10y-TP: 65% and 85%, respectively; and, (iv) *very low risk*: non-*TP53abn*/*SF3B1*mut/del(11q) female Binet A, 5y-TP and 10y-TP: 45% and 65%, respectively (Figure 4B). Harrell's C index was calculated for the multivariable Cox model with the prognostic index being the sole predictor and found equal to 0.753 (se = 0.013).

Internal validation

In order to validate the results mentioned above, bootstrapping procedure was performed. Within early stage M-CLL patients showed that the average number of predictors included in the multivariable Cox model was 3.2 with three variables exhibiting selection percentages greater than 60%, i.e. *TP53abn*, +12 and subset #2 (Table 1). Within early stage U-CLL patients, bootstrapping showed that the average number of predictors considered significant in the multivariable Cox model was 3.5. Four variables exhibited selection percentages greater than 60%, i.e. *TP53abn*, *SF3B1*mut, del(11q) and male gender.

External validation

Application of the above mentioned indices to the validation cohort (n=649) led to the following observations: (i) in M-CLL, *TP53abn*, +12 and subset #2 membership (intermediate risk for M-CLL) exhibited similar TTFT, constituting a group (16% of all Binet cases also in the validation cohort) with almost identical 5y-TP (43%) and 10y-TP (60%) to those observed in the training cohort (40% and 55% respectively) (Figure 5A); (ii) similarly, in U-CLL no

difference was observed in TTFT for Binet A cases carrying *TP53abn* and/or *SF3B1mut* and/or del(11q) (intermediate risk for U-CLL) who exhibited similar 5y-TP and 10y-TP to that of the training cohort (74% versus 78% and 92% versus 98%, respectively; p-values: non significant) (Figure 5B); (iii) amongst the remaining non-*TP53abn*/*SF3B1mut*/del(11q) U-CLL Binet A cases, the difference between male and female patients did not reach statistical significance (p=0.2) (Supplemental Figure 5A, 5B).

DISCUSSION

CLL constitutes a rather unique case amongst cancers in that the great majority of patients are asymptomatic at diagnosis and classified as early stage, thus not requiring immediate treatment[42]. However, most patients will progress and meet the criteria for treatment initiation albeit at a variable time from diagnosis[37,42]. Therefore, accurate prediction of the TTFT is of major importance for both patients and physicians having to address the challenge of living with and managing this (largely) invisible and incurable disease. In support of this argument, solid prognostication is increasingly recognized as both a priority and an unmet need by CLL patients themselves, who would benefit from accurate prognostic information in order to make educated choices about their life and, perhaps, also participate more actively in their care.

Several prognostic models have been developed for CLL based on combinations of biomarkers and host-derived features, however, none has been adopted in everyday clinical practice[13] e.g. for deciding about the follow-up strategy amongst untreated patients. These indices were mainly based on Cox-regression models, where each respective training/validation cohort was considered as a single group. Herein, we followed a novel approach evaluating prognosis separately within M-CLL and U-CLL focusing on TTFT in the largest ever series studied to this purpose. We report that within early stage M-CLL, *TP53abn*, +12 and assignment to stereotyped subset #2 identified a subgroup of patients with uniformly shorter TTFT compared to the remaining early Binet A M-CLL. Similarly, within U-CLL the presence of *TP53abn*, del(11q) and *SF3B1* mutations was found to be associated with the shortest TTFT, whereas the remaining U-CLL female patients had a significantly longer TTFT compared to males.

Classification of CLL patients based on the SHM status of the clonotypic BcR IG into M-CLL and U-CLL categories offers robust prognostic information, differing from other prognostic markers (e.g. genetic aberrations) that may evolve overtime[15,17,18,43]. Of note, studies by us and others have documented an asymmetric distribution of certain genetic aberrations in patients with distinct immunogenetic features extending from the M-CLL or U-CLL categorization to different stereotyped subsets[32,44,45]. This has prompted speculations that particular modes of immune signaling initiated by specific BcR IG may trigger different pathways of clonal evolution leading to the emergence of distinct disease variants.

For these reasons, the BcR IG appears an obvious starting point for developing a biologically-orientated prognostication scheme for CLL, as in our present study, offering the possibility to dissect the precise impact of a given biomarker within a particular immunogenetic category (e.g. M-CLL or U-CLL). For example, within M-CLL, +12 was found to define a subgroup of patients with a TTFT similar to that of patients harboring *TP53abn*, whereas, in contrast, +12 was associated with favorable outcome within U-CLL (Figures 5C, 5D). Of note, mutations within the *NOTCH1* gene had no impact on survival among U-CLL cases with +12 (Supplemental Figure 6). These findings may explain: (i) why +12 is considered as an intermediate-risk aberration in prognostic indices where CLL is evaluated as one group regardless of the SHM status[38]; and, (ii) the contradictory results reported in different cohorts with different relative proportion of M-CLL and U-CLL patients, regarding the significance of a given indicator that can show an asymmetric distribution within each SHM group.

Our initial results based on Cox-regression analysis were validated internally, being highly reproducible in an independent validation cohort. In particular, the median TTFT for the subgroups of patients with the shortest TTFT in both SHM categories, namely *TP53abn*/+12/#2 and *TP53abn*/*SF3B1mut*/del(11q) for M-CLL and U-CLL respectively, was almost identical between the validation and the training cohort. Interestingly, the latter exhibited significant differences in terms of the biological background compared to the training cohort (Supplemental Table 2): this may be taken as further evidence for the robustness of our approach, since similar results were obtained across cohorts with differing patient composition. Cox-regression results were further confirmed through the application of an alternative statistical approach, namely binary recursive partitioning, which offers a

different framework, thereby conveying a hierarchical order of importance and classification for the evaluated prognostic parameters.

Admittedly, despite providing a robust risk stratification scheme, the herein proposed prognostic indices will not solve the problem of outliers, while they also overlook the potential effect of other variables with proven prognostic significance e.g. cytogenetic complexity or methylation signatures. Thus, they cannot be considered the last word in biomarker-orientated risk stratification for TTFT, highlighting the need for further studies, while providing the conceptual frame of compartimentalization. It should be further highlighted that our approach is built upon the pivotal role of IGHV SHM status in the prognostic setting, which appears to be less important in the era of novel agents, namely BTK and BCL2 inhibitors, where response rates seem to be similar between M-CLL and U-CLL[46,47].

Recently, the International Prognostic Index for patients with CLL (CLL-IPI) was developed for assessing OS[7], providing a robust prognostic classification scheme as it included well characterized patients followed in the context of clinical trials. A caveat of CLL-IPI concerns the fact that the evaluated patients had been treated with various regimens in the context of different clinical trials. Moreover, CLL-IPI does not allow the identification of distinct groups within each SHM category. For example, following the CLL-IPI score, a young (<65 years), early-stage patient, negative for *TP53abn* and belonging to M-CLL would never be characterized as very-high risk. Relevant to note, therefore, clinically aggressive stereotyped subset #2 would be overlooked by the CLL-IPI as it largely falls into this category of M-CLL cases lacking *TP53abn*, with 60% of patients also aged below 65. This is not trivial, considering that subset #2 accounts for up to 5% of all CLL requiring treatment and, therefore, is equal in size to the CLL-IPI very high risk group with very limited if any overlap[30].

A final, more general concern is that CLL-IPI was developed based on the analysis of cases treated prior to the introduction of novel therapies, which are likely to change the treatment expectations and OS in CLL, thus eventually creating the need for novel predictive schemes[48].

Our study identified subset #2 membership and *SF3B1* mutations as prognostically important biomarkers for early-stage M-CLL and U-CLL, respectively. In contrast, other recurrent gene mutations such as *NOTCH1* or *BIRC3* failed to reach significance even in univariable analysis. Interestingly, *SF3B1* mutations are remarkably enriched within subset #2 (~50% of cases

harbor a mutation), however, their impact within this very aggressive subset remains equivocal[30,32]. Overall, these results emphasize the value of investigating IG sequences for stereotyped subset #2 membership (easily determined through the use of a free online tool available at: http://bat.infspire.org/arrest/assignsubsets/[49]) and searching for *SF3B1* mutations in routine clinical practice as this would enable a more accurate assessment of prognosis (TTFT) at diagnosis of CLL.

In conclusion, we propose a novel approach to prognostic assessment in CLL grounded on the fact that not all CLL are equal, but instead that M-CLL and U-CLL categories are fundamentally different regarding their ontogeny and molecular landscape, at least for early-stage patients (Figure 7). Our results support that compartmentalizing CLL with the BcR IG as the starting point allows accurate prognostication in early-stage CLL. This further highlights that the relative weight of well established prognostic indicators differs based on the immunogenetic features of each individual case. From a broader perspective, such compartmentalized approaches might prove relevant in other B cell lymphomas as well e.g. diffuse large B cell lymphoma where different biomarkers are emerging as prognostically relevant for the activated B cell or the germinal center subtype, respectively[50], that display distinct immunogenetic features and signaling signatures[50].

References

1.Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375-2390.

2.Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48(1):198-206.

3.Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46(2):219-234.

4.Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? Nat Rev Clin Oncol. 2011;8(1):38-47.

5.Gentile M, Mauro FR, Rossi D, et al. Italian external and multicentric validation of the MD Anderson Cancer Center nomogram and prognostic index for chronic lymphocytic leukaemia patients: analysis of 1502 cases. Br J Haematol. 2014;167(2):224-232.

6.Haferlach C, Dicker F, Weiss T, et al. Toward a comprehensive prognostic scoring system in chronic lymphocytic leukemia based on a combination of genetic parameters. Genes Chromosomes Cancer. 2010;49(9):851-859.

7.International CLLIPIwg. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016;17(6):779-790.

8.Morabito F, Cutrona G, Gentile M, et al. Definition of progression risk based on combinations of cellular and molecular markers in patients with Binet stage A chronic lymphocytic leukaemia. Br J Haematol. 2009;146(1):44-53.

9.Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood. 2002;100(4):1177-1184.

10.Rossi D, Rasi S, Spina V, et al. Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood. 2013;121(8):1403-1412.

11.Shanafelt TD, Jenkins G, Call TG, et al. Validation of a new prognostic index for patients with chronic lymphocytic leukemia. Cancer. 2009;115(2):363-372.

12.Wierda WG, O'Brien S, Wang X, et al. Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood. 2007;109(11):4679-4685.

13.Baliakas P, Mattsson M, Stamatopoulos K, Rosenquist R. Prognostic indices in chronic lymphocytic leukaemia: where do we stand how do we proceed? J Intern Med. 2016;279(4):347-357.

14.Fabbri G, Dalla-Favera R. The molecular pathogenesis of chronic lymphocytic leukaemia. Nat Rev Cancer. 2016;16(3):145-162.

15.Sutton LA, Rosenquist R. Deciphering the molecular landscape in chronic lymphocytic leukemia: time frame of disease evolution. Haematologica. 2015;100(1):7-16.

16.Sutton LA, Rosenquist R. The complex interplay between cell-intrinsic and cell-extrinsic factors driving the evolution of chronic lymphocytic leukemia. Semin Cancer Biol. 2015;34:22-35.

17.Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell. 2013;152(4):714-726.

18.Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015;526(7574):525-530.

19.Stevenson FK, Caligaris-Cappio F. Chronic lymphocytic leukemia: revelations from the B-cell receptor. Blood. 2004;103(12):4389-4395.

20.Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840-1847.

21.Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94(6):1848-1854.

22.Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016;127(2):208-215.

23.Rossi D, Terzi-di-Bergamo L, De Paoli L, et al. Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. Blood. 2015;126(16):1921-1924.

24.Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. Blood. 2016;127(3):303-309.

25.Kulis M, Heath S, Bibikova M, et al. Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. Nat Genet. 2012;44(11):1236-1242.

26.Marincevic M, Cahill N, Gunnarsson R, et al. High-density screening reveals a different spectrum of genomic aberrations in chronic lymphocytic leukemia patients with 'stereotyped' IGHV3-21 and IGHV4-34 B-cell receptors. Haematologica. 2010;95(9):1519-1525.

27.Ntoufa S, Vardi A, Papakonstantinou N, et al. Distinct innate immunity pathways to activation and tolerance in subgroups of chronic lymphocytic leukemia with distinct immunoglobulin receptors. Mol Med. 2012;18:1281-1291.

28.Baliakas P, Hadzidimitriou A, Sutton LA, et al. Clinical effect of stereotyped B-cell receptor immunoglobulins in chronic lymphocytic leukaemia: a retrospective multicentre study. Lancet Haematology. 2014;1(2):74-84.

29.Agathangelidis A, Vardi A, Baliakas P, Stamatopoulos K. Stereotyped B-cell receptors in chronic lymphocytic leukemia. Leuk Lymphoma. 2014;55(10):2252-2261.

30.Baliakas P, Agathangelidis A, Hadzidimitriou A, et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. Blood. 2015;125(5):856-859.

31.Xochelli A, Baliakas P, Kavakiotis I, et al. Chronic Lymphocytic Leukemia with Mutated IGHV4-34 Receptors: Shared and Distinct Immunogenetic Features and Clinical Outcomes. Clin Cancer Res. 2017;23(17):5292-5301.

32.Jeromin S, Haferlach C, Dicker F, Alpermann T, Haferlach T, Kern W. Differences in prognosis of stereotyped IGHV3-21 chronic lymphocytic leukaemia according to additional molecular and cytogenetic aberrations. Leukemia. 2016;30(11):2251-2253.

33.Baliakas P, Mattsson M, Hadzidimitriou A, et al. No improvement in long-term survival over time for chronic lymphocytic leukemia patients in stereotyped subsets #1 and #2 treated with chemo(immuno)therapy. Haematologica. 2018;103(4):e158-e161.

34.Baliakas P, Hadzidimitriou A, Sutton LA, et al. Recurrent mutations refine prognosis in chronic lymphocytic leukemia. Leukemia. 2015;29(2):329-336.

35.Jeromin S, Weissmann S, Haferlach C, et al. SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia. 2014;28(1):108-117.

36.Baliakas P, Hadzidimitriou A, Agathangelidis A, et al. Prognostic relevance of MYD88 mutations in CLL: the jury is still out. Blood. 2015;126(8):1043-1044.

37.Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111(12):5446-5456.

38.Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910-1916.

39.Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood. 2012;119(19):4467-4475.

40.Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361-387.

41.Hothorn T, Hornik K, Zeileis A. Unbaised Recursive Partitioning: A Conditional Inference Framework. Journal of Computational and Graphical Statistics. 2006;15(3):651-674.

42.Hallek M. Chronic lymphocytic leukemia: 2015 Update on diagnosis, risk stratification, and treatment. Am J Hematol. 2015;90(5):446-460.

43.Ljungstrom V, Cortese D, Young E, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. Blood. 2016;127(8):1007-1016.

44.Rossi D, Spina V, Bomben R, et al. Association between molecular lesions and specific B-cell receptor subsets in chronic lymphocytic leukemia. Blood. 2013;121(24):4902-4905.

45.Sutton LA, Young E, Baliakas P, et al. Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukemia harboring stereotyped B-cell receptors. Haematologica. 2016;101(8):959-967.

46.Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naive and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood. 2015;125(16):2497-2506.

47.Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016;374(4):311-322.

48.Lamanna N, O'Brien S. Novel agents in chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2016;2016(1):137-145.

49.Bystry V, Agathangelidis A, Bikos V, et al. ARResT/AssignSubsets: a novel application for robust subclassification of chronic lymphocytic leukemia based on B cell receptor IG stereotypy. Bioinformatics. 2015;31(23):3844-3846.

50.Karube K, Enjuanes A, Dlouhy I, et al. Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. Leukemia. 2018;32(3):675-684.

Table 1. Univariable and multivariable analysis for TTFT within Binet A M-CLL and U-CLL. Harrell's C-index (standard error in parenthesis) was calculated as 0.745 (se=0.013) and 0.753 (se=0.013) for the M-CLL and the U-CLL, respectively. Internal bootstrap validation for the multivariable analysis. Means of the HR, 95% CI and percentage of selection within 1000 bootstrap samples are displayed per predictor.

	M-CLL/Binet A								
	Univariable analysis (n=1224)			Multivariable analysis (n=918)			Internal bootstrap validation		
							Parameters (mean)		Selection
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	percentage
Male	1.352	1.063-1.718	0.013	1.354	1.007-1.820	0.044	1.406	1.044-1.894	57%
Age >65 years	0.976	0.769-2.237	0.842	-	-	-			
CD38 expression	2.017	1.474-2.760	<0.0001	1.058	0.678-1.652	0.801	1.048	0.666-1.648	8.4%
idel(13q)	0.761	0.573-1.010	0.059	-	-	-			
+12	2.428	1.691-3.487	<0.0001	2.565	1.665-3.952	<0.0001	2.669	1.769-4.031	98.2%
del(11q)	2.010	0.944-4.281	0.071	-	-	-			
TP53abn	3.347	2.175-	<0.0001	2.685	1.607-4.485	0.0001	3.064	1.864-5.047	95.6%

		5.151							
NOTCH1	2.107	0.993-4.447	0.052	-	-	-			
SF3B1	1.856	0.913-3.771	0.088	-	-	-			
MYD88	1.551	0.680-3.538	0.296	-	-	-			
GI:97-97.99%	1.571	1.069-2.308	0.021	0.988	0.568-1.717	0.966	0.940	0.529-1.679	3.4%
IGHV3-23	1.025	0.719-1.461	0.89	-	-	-			
IGHV3-21*	1.466	0.605-3.555	0.396	-	-	-			
Subset #2	3.384	1.594-7.181	0.001	3.268	1.155-9.244	0.025	3.885	1.353-12.267	62%
Subset #4	0.658	0.309-1.398	0.277	-	-	-			

	U-CLL/Binet A								
	Univariable analysis (n=672)			Multivariable analysis (n=384)			Internal bootstrap validation		
							Parameters (mean)		Selection
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	percentage
Male	1.341	1.106-1.626	0.028	1.610	1.229-2.109	0.0005	1.630	1.239-2.146	93.8%
Age >65 years	0.845	0.701-1-020	0.079	-	-	-			
CD38 expression	1.144	0.832-1.575	0.405	-	-	-			
del13q**	0.902	0.729-1.117	0.347	-	-	-			
+12	0.751	0.568-0.993	0.045	0.898	0.632-1.274	0.547	0.912	0.640-1.299	10.4%
del(11q)	1.640	1.292-2.082	<0.0001	1.432	1.071-1.916	0.0151	1.475	1.098-1.983	69.1%
TP53abn	1.613	1.213-2.146	0.001	2.357	1.548-3.589	<0.0001	1.475	1.098-1.983	69.1%
NOTCH1	1.142	0.874-1.493	0.328	-	-	-			
SF3B1	1.892	1.335-2.682	<0.0001	1.624	1.129-2.337	0.008	1.682	1.159-2.442	73.4%
BIRC3	1.206	0.532-2.732	0.652	-	-	-			
GI:100%	1.231	0.993-1.524	0.056	-	-	-			
IGHV1-69	1.190	0.959-1.457	0.112	-	-	-			
Subset #1	1.382	0.939-2.033	0.099	-	-	-			

CD38 expression: positivity >30%, idel(13q): isolated deletion of chromosome 13q, +12: trisomy 12, del(11q): deletion of chromosome 11q, *TP53abn*: deletion of chromosome 17p (del(17p)) and/or *TP53* mutation, GI: germline identity, IGHV3-23: usage of IGHV3-23, IGHV3-21*: usage

of IGHV3-21 non subset#2, subset #2: assignment to stereotyped subset #2, subset#4: assignment to stereotyped subset #4, **: due to the low number of cases with available data for all FISH detected abnormalities, deletion of chromosome 13q was used instead of idel(13q) for the U-CLL cohort.

Figure 1. Kaplan Meier curves for time-to-first-treatment (TTFT). (A) In Binet A, B and C M-CLL patients; and, (B) Binet A, B and C U-CLL patients.

Figure 2. Subgroups of patients with similar prognosis within M-CLL and U-CLL. (A) *TP53abn*, trisomy 12 (+12) and stereotyped subset #2 assignment define three almost non-overlapping groups within early stage M-CLL; (B) Binet A M-CLL cases with *TP53abn*, trisomy 12 (+12) or assignment to stereotyped subset #2 display similar TTFT; (C) Distribution of *TP53abn*, *SF3B1* mutations and del(11q) in Binet A U-CLL; (D) Binet A U-CLL cases carrying *TP53abn*, *SF3B1* mutations or del(11q) exhibit similar TTFT.

Figure 3. Application of binary recursive partitioning in M-CLL and U-CLL. (A) Decision tree for Binet A M-CLL based on binary recursive partitioning and the subsequent application of an amalgamation algorithm. Trisomy 12 (+12), *TP53abn* and subset #2 membership were found to be the most significant factors as determined by the partitioning algorithm. The Binet A population is split in 4 terminal nodes. The amalgamation algorithm applied subsequently merged 3 of them in a larger final node. In particular, +12 was considered as the covariate with the strongest association to TTFT. Amongst patients lacking +12, *TP53abn* was the covariate with the strongest association to TTFT and so on. After applying the amalgamation algorithm, patients with +12 and/or *TP53abn* and/or assignment to subset #2 were grouped into a larger node, resulting in two terminal nodes. The splitting is performed from right to left, following the criterion of strongest factor association with TTFT. The right branch represents the presence of a particular factor and the left branch the absence of that factor. The p-value corresponds to a log-rank scores based test. The Kaplan-Meier curves estimate the TTFT of patients within each terminal node and *n* represents the number of patients per node. (B) Decision tree for Binet A U-CLL based on binary recursive partitioning and the subsequent application of an amalgamation algorithm. Male gender, *TP53abn* and del(11q) were the most significant factors as determined by the partitioning algorithm. The Binet A population was split into 6 terminal nodes. The amalgamation algorithm applied merged 3 of the terminal nodes into a larger final node. Gender was deemed to be the covariate with the strongest association to TTFT. Amongst male patients, *TP53abn* was the covariate with strongest association to TTFT. Amongst female patients, del(11q) was the

covariate with strongest association to TTFT and so on. After applying the amalgamation algorithm, male patients without *TP53abn* and with del(11q), and female patients with del(11q) and/or *TP53abn* were grouped into a larger node. The final number of terminal nodes was 4. The splitting is performed from top to bottom, following the criterion of strongest factor association with TTFT. The right branch represents the presence of a particular factor and the left one the absence of that factor. The p-value corresponds to a log-rank scores based test. The Kaplan-Meier curves estimate the TTFT of patients within each terminal node and *n* represents the number of patients per node.

Figure 4. Prognostic index fot time-to-first-treatment (TTFT) for M-CLL and U-CLL. (A) Prognostic index for TTFT within M-CLL: (i) Very high risk: Binet C; (ii) High risk: Binet B; (iii) Intermediate risk: Binet A with at least one of the following: *TP53abn* or +12 or assignment to subset #2 and; (iv) Low risk: non-*TP53abn*/+12/#2 Binet A; (B) Prognostic index for TTFT within U-CLL: (i) Very high risk: Binet C; (ii) High risk: Binet B; (iii) Intermediate risk: Binet A with at least one of the following: *TP53abn* or +*SF3B1* mutations or del(11q) membership; (iv) Low risk: non-*TP53abn*/*SF3B1*mut/del(11q) male Binet A and; (v) Very low risk: non-*TP53abn*/*SF3B1*mut/del(11q) female Binet A.

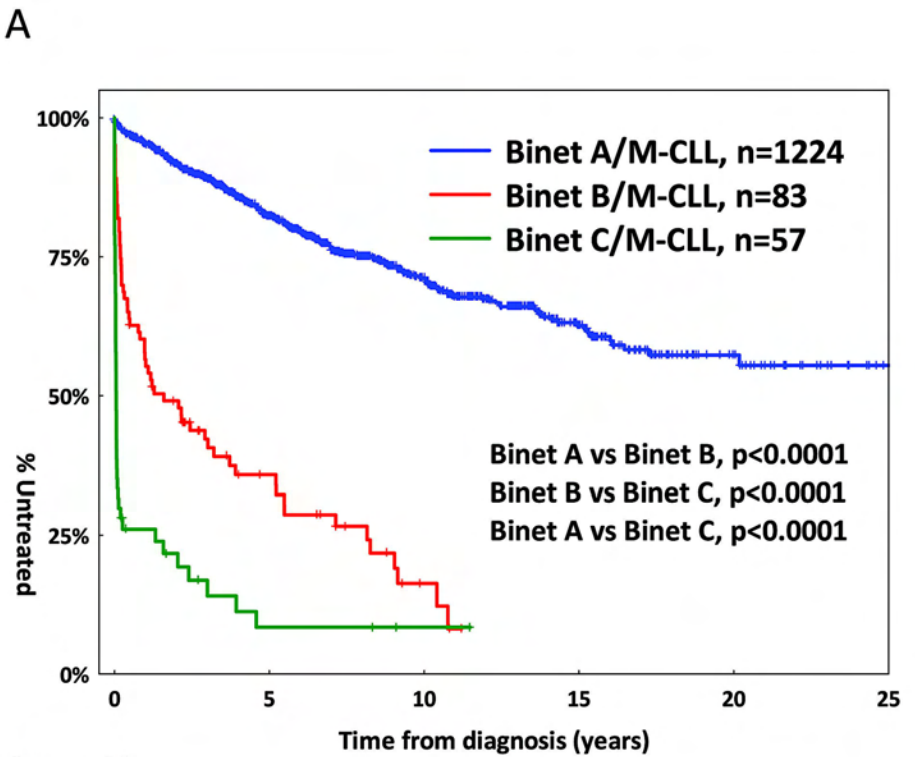
Figure 5. Kaplan Meier curves for time-to-first-treatment (TTFT) in the validation cohort. (A) Within M-CLL, Binet A cases positive for *TP53abn*, trisomy 12 (+12) and stereotyped subset #2 assignment display similar TTFT; (B) No difference regarding TTFT among Binet A U-CLL cases carrying *TP53abn*, *SF3B1* mutations or del(11q) in the validation cohort.

Figure 6. Kaplan Meier curves for time-to-first-treatment (TTFT) for M-CLL and U-CLL cases carrying trisomy 12 (+12). (A) +12 is an unfavorable prognosticator in M-CLL; (B) +12 is associated with more indolent clinical course in U-CLL.

Figure 7. Prognostic algorithm regarding treatment probability for CLL. 5y-TP: Treatment probability 5 years from diagnosis; 10y-TP: Treatment probability 10 years from diagnosis; U-CLL: CLL with unmutated IGHV genes; M:CLL: CLL with mutated IGHV genes; *TP53abn*: deletion of chromosome 17p (del(17p)) and/or *TP53* mutation; +12:trisomy 12, del(11q): deletion of chromosome 11q; *SF3B1*mut: *SF3B1* mutation; #2: assignment to stereotyped

subset #2. The pie refers to the entire cohort with each slice indicating the patients' proportion according to Binet clinical staging.

Figure 1


A
100%
75%
50%
25%
0%
% Untreated
0
5
10
15
20
25
Time from diagnosis (years)
Binet A/M-CLL, n=1224
Binet B/M-CLL, n=83
Binet C/M-CLL, n=57
Binet A vs Binet B, p<0.0001
Binet B vs Binet C, p<0.0001
Binet A vs Binet C, p<0.0001
Patients at risk
Binet A 1224 700 308 124 36 5
Binet B 83 20 5
Binet C 57 10 1

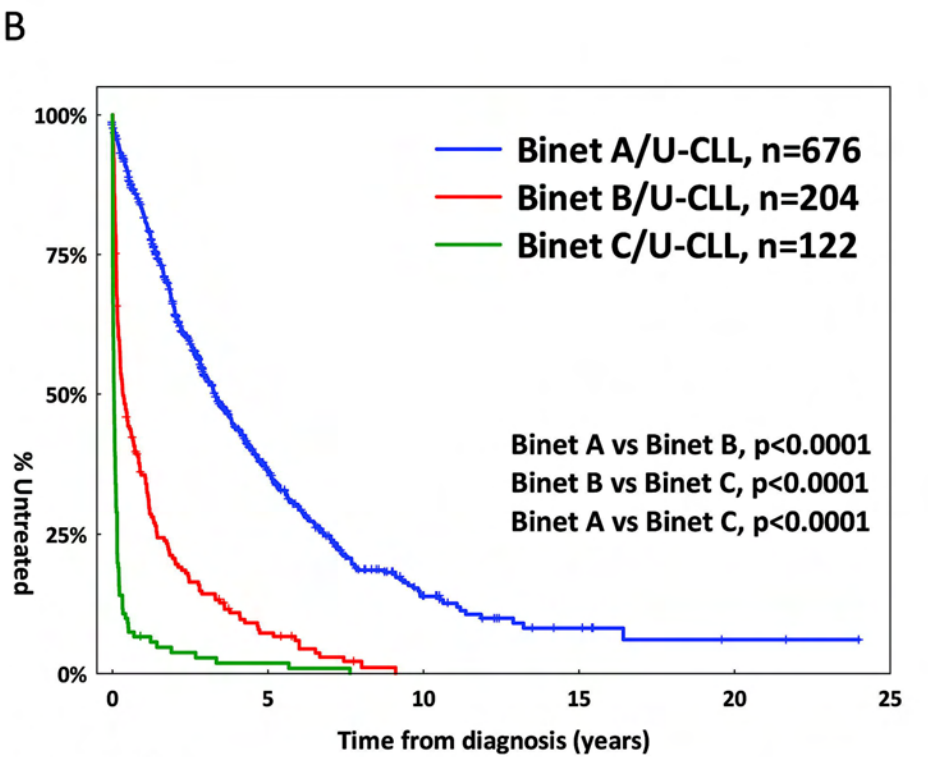

B
100%
75%
50%
25%
0%
% Untreated
0
5
10
15
20
25
Time from diagnosis (years)
Binet A/U-CLL, n=676
Binet B/U-CLL, n=204
Binet C/U-CLL, n=122
Binet A vs Binet B, p<0.0001
Binet B vs Binet C, p<0.0001
Binet A vs Binet C, p<0.0001
Patients at risk
676 185 19 6 1
204 10
122 2

Figure 2

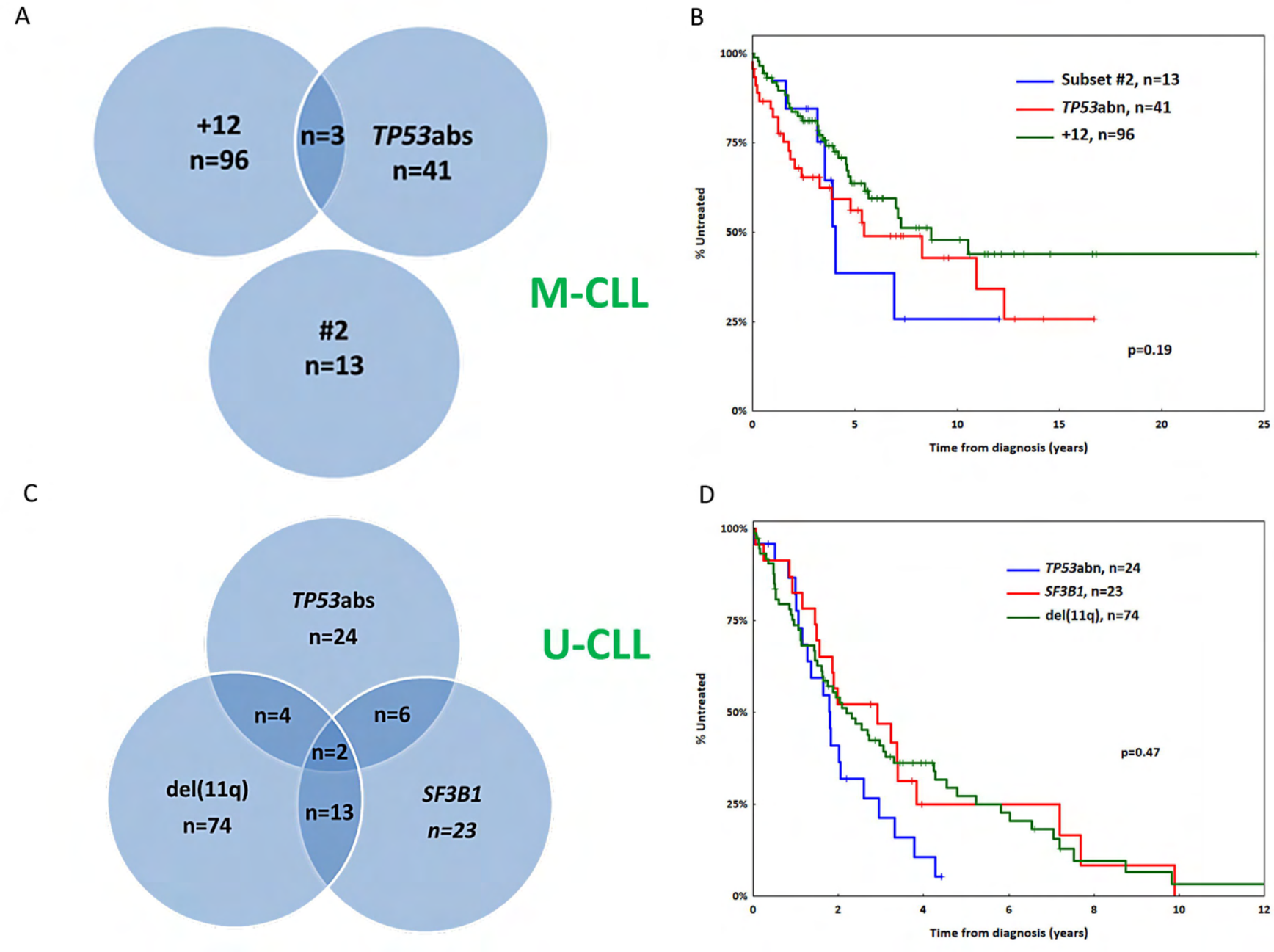
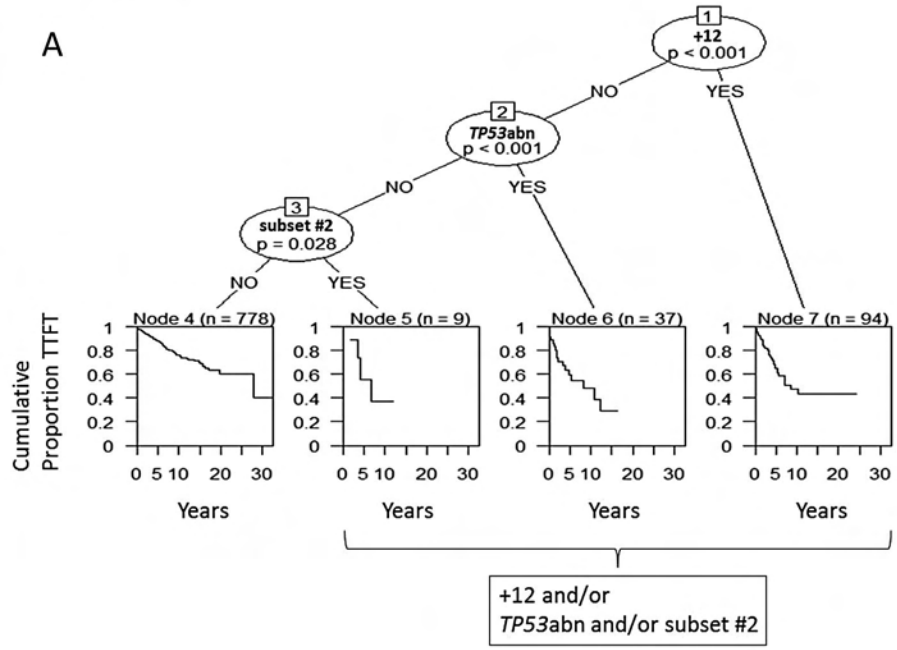

A
+12
n=96
n=3
TP53abs
n=41
#2
n=13
M-CLL
B
100%
75%
50%
25%
0%
% Untreated
Subset #2, n=13
TP53abn, n=41
+12, n=96
p=0.19
0
5
10
15
20
25
Time from diagnosis (years)
C
TP53abs
n=24
n=4
n=6
n=2
del(11q)
n=74
n=13
SF3B1
n=23
U-CLL
D
100%
75%
50%
25%
0%
% Untreated
TP53abn, n=24
SF3B1, n=23
del(11q), n=74
p=0.47
0
2
4
6
8
10
12
Time from diagnosis (years)

Figure 3


A
1
+12
p < 0.001
NO
YES
2
TP53abn
p < 0.001
NO
YES
3
subset #2
p = 0.028
NO
YES
Node 4 (n = 778)
Node 5 (n = 9)
Node 6 (n = 37)
Node 7 (n = 94)
Cumulative Proportion TTFT
Years
+12 and/or
TP53abn and/or subset #2

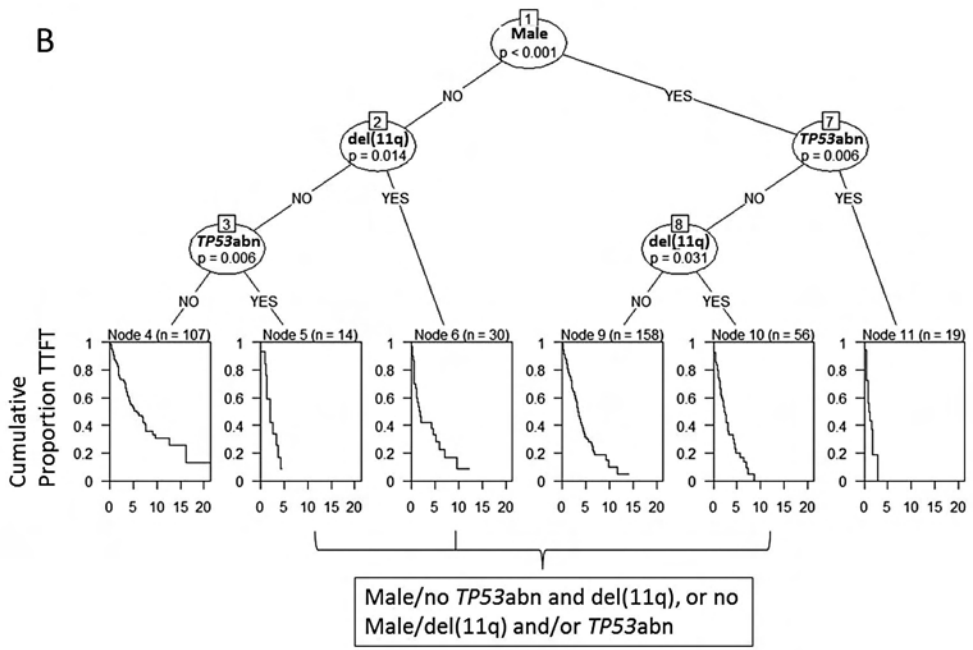
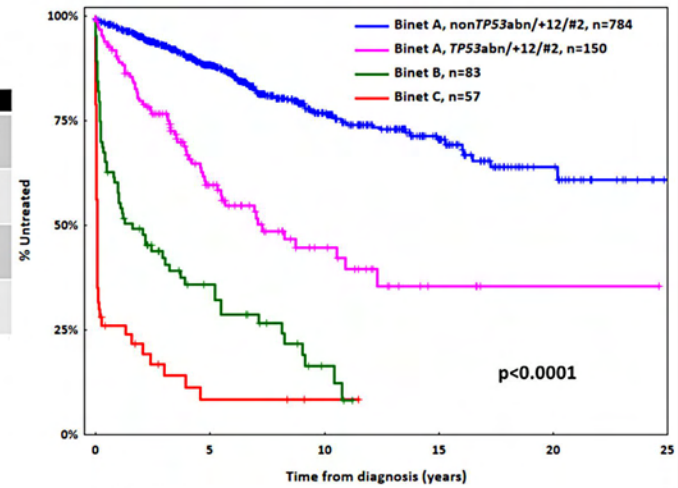

B
1
Male
p < 0.001
NO
YES
2
del(11q)
p = 0.014
7
TP53abn
p = 0.006
3
TP53abn
p = 0.006
8
del(11q)
p = 0.031
Node 4 (n = 107)
Node 5 (n = 14)
Node 6 (n = 30)
Node 9 (n = 158)
Node 10 (n = 56)
Node 11 (n = 19)
Cumulative Proportion TTFT
Years
Male/no TP53abn and del(11q), or no
Male/del(11q) and/or TP53abn

Figure 4

A

Risk group	5-yTP	10-y TP
Very high risk 5% of M-CLL mTTF: 0.1 years	92%	92%
High risk 8% of M-CLL mTTFT: 1.5 years	64%	84%
Intermediate risk 14% of M-CLL mTTFT: 8 years	40%	55%
Low risk 73% of M-CLL mTTFT: nr	12%	25%

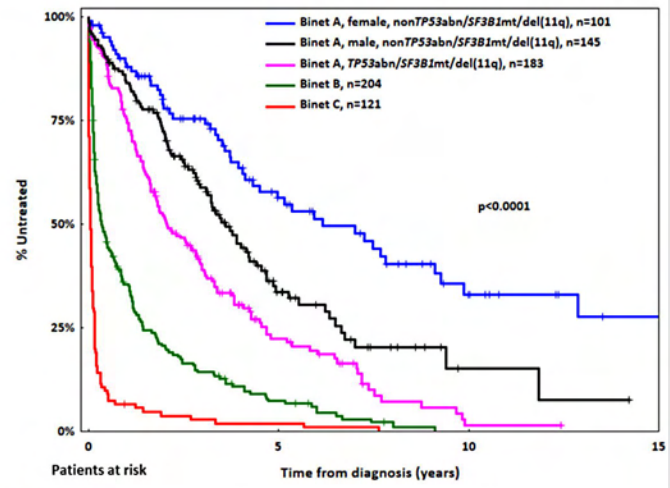


Patients at risk

Very high risk	57	3	1	0	0	0
High risk	83	16	1	0	0	0
Intermediate risk	150	40	9	1	1	1
Low risk	784	417	150	47	10	3

B

Risk group	5-yTP	10-y TP
Very high risk 16% of U-CLL mTTF: 0.1years	100%	100%
High risk 27% of U-CLL mTTFT: 0.4 years	90%	100%
Intermediate risk 24% of U-CLL mTTFT: 2.1 years	78%	98%
Low risk 19% of U-CLL mTTFT: 3.6 years	65%	85%
Very low risk 13% of U-CLL mTTFT: 6.1 years	45%	65%

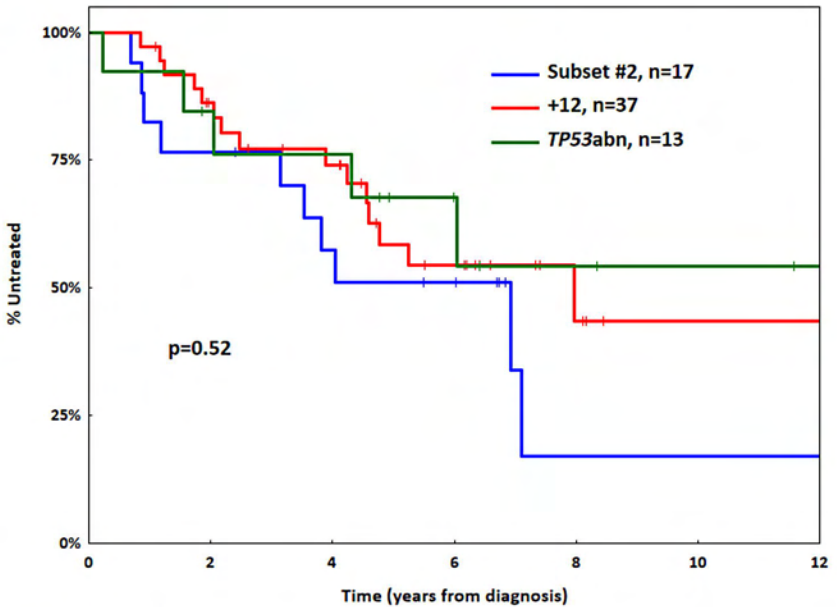


Patients at risk

Very high risk	121	2	0	0	
High risk	204	9	0	0	
Intermediate risk	183	24	1	0	
Low risk	145	11	3	1	
Very low risk	101	38	14	4	

Figure 5

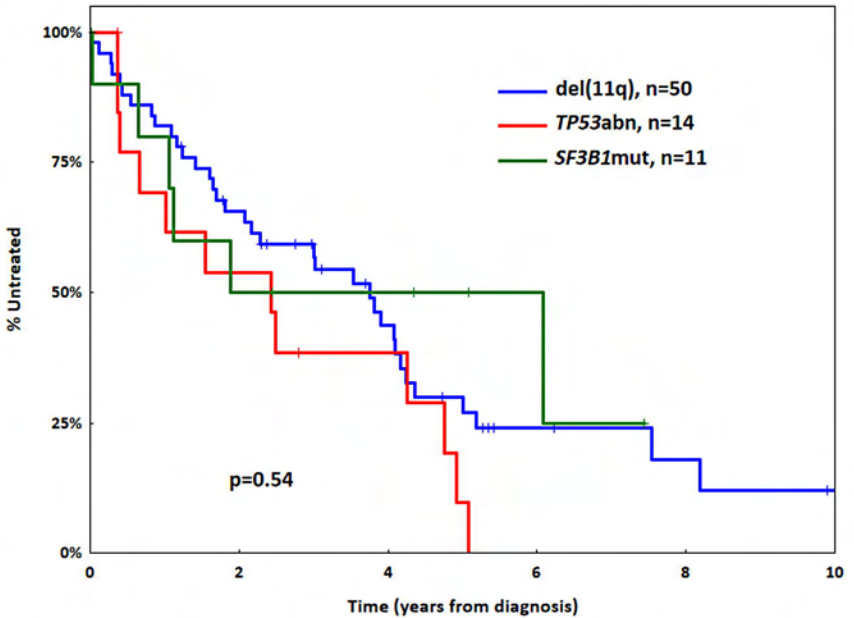
A



Patients at risk

Subset #2	17	14	6	1	1	1
+12	37	24	13	4	1	1
*TP53*abs	13	10	5	3	2	1

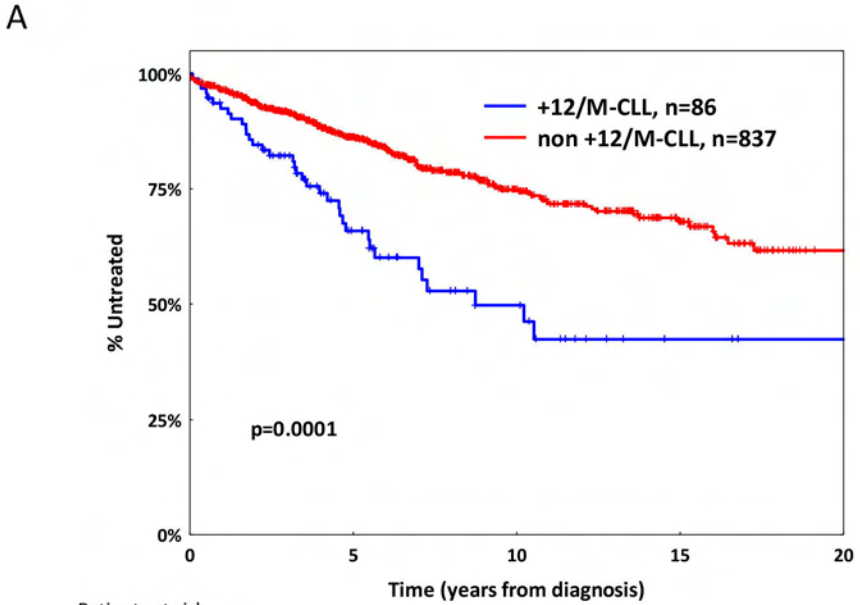
B



Patients at risk

del(11q)	50	31	16	5	3	1
*TP53*abs	14	10	4			
*SF3B1*mut	11	6	4	1		

Figure 6


A
100%
75%
50%
25%
0%
% Untreated
+12/M-CLL, n=86
non +12/M-CLL, n=837
p=0.0001
0
5
10
15
20
Time (years from diagnosis)
Patients at risk
+12
17
14
6
1
Non Trisomy 12
37
24
13
4

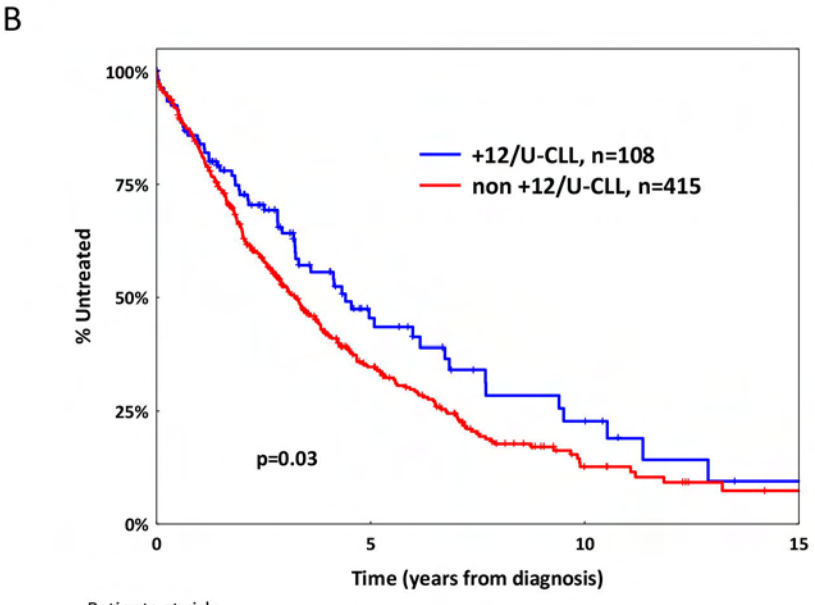
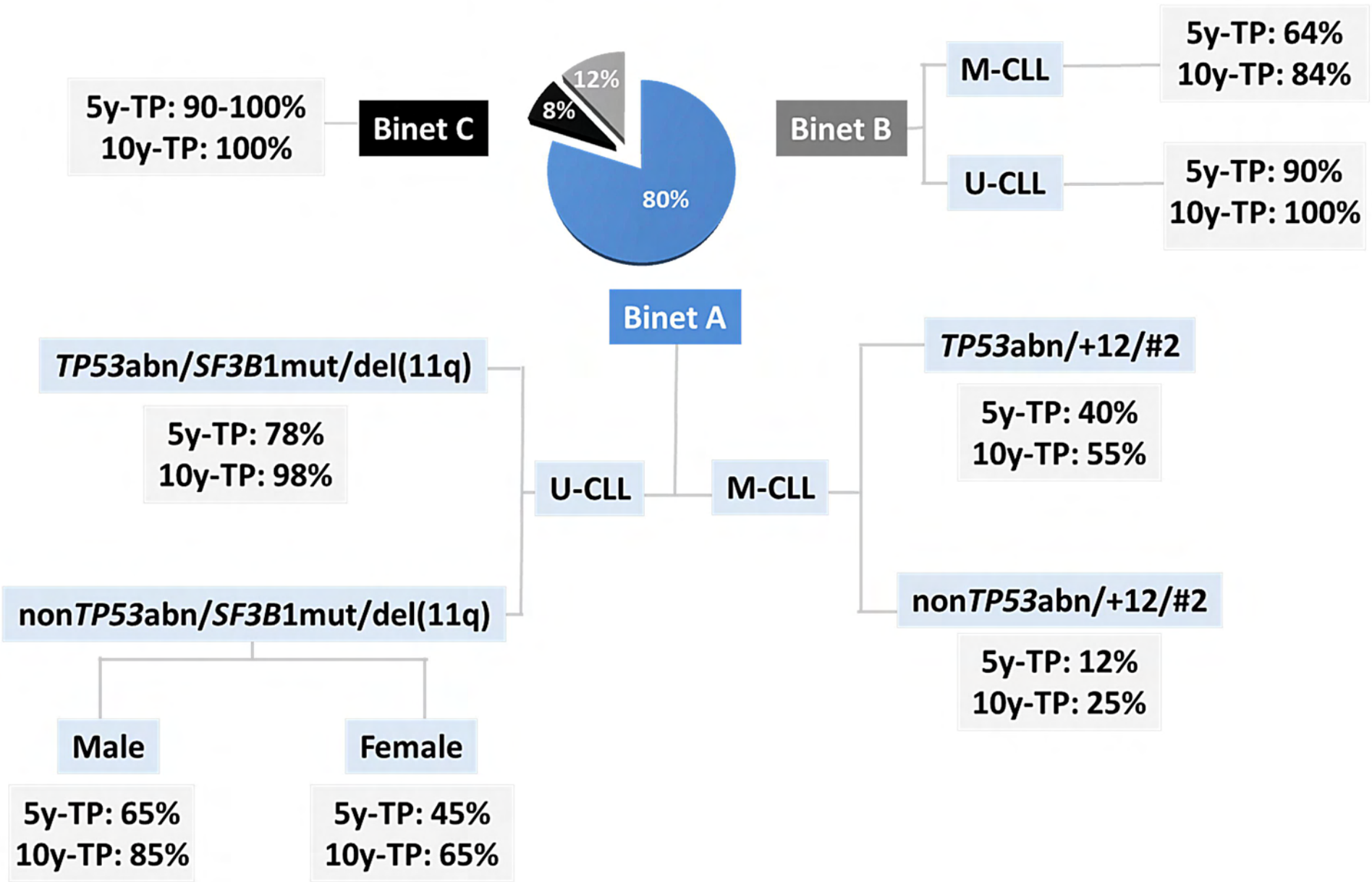

B
100%
75%
50%
25%
0%
% Untreated
+12/U-CLL, n=108
non +12/U-CLL, n=415
p=0.03
0
5
10
15
Time (years from diagnosis)
Patients at risk
+12
17
14
6
1
Non Trisomy 12
37
24
13
4

Figure 7

Treatment probability in CLL



Tailored approaches grounded on immunogenetic features for refined prognostication in chronic lymphocytic leukemia

Supplemental Material

Supplemental Material includes detailed information regarding methodology as well as Supplemental Figures 1-6 and Supplemental Tables 1-5**.**

Patients-Methods

Overall, 2366 general practice patients from 10 academic institutions in Europe who were diagnosed with CLL according to the 2008 iwCLL diagnostic criteria and for whom immunogenetic data was available were included in this multicenter retrospective study. Information about the evaluated cohort and biomarkers is provided in Supplemental Table 1. Following the 98% germline identity (GI) cut off, 1364 (58%) patients were classified as mutated (M-CLL) and 1002 (42%) as unmutated (U-CLL). Information regarding gender as well as age and clinical stage at diagnosis were available for the entire cohort. Data on FISH detected abnormalities were available for 1825/2366 (77%) cases with 1162/1260 (92%) of the treated cases being tested before the administration of any treatment. Mutations within the *TP53, NOTCH1, SF3B1, MYD88* and *BIRC3* genes were evaluated in 1544 (65%), 2097 (89%), 1449 (61%), 929 (39%) and 830 (35%) respectively. Regarding time of testing for each mutation, the proportion of treated cases tested before the administration of any treatment ranged from 77-98%. CD38 expression at the time of diagnosis was available in 1649 (70%) patients. A cut-off of 30% was used to indicate CD38 positivity.

Evaluation of biological markers

PCR amplification of IGHV-IGHD-IGHJ rearrangements - Sequence analysis

PCR amplification and sequence analysis of IGHV-IGHD-IGHJ rearrangements were performed on either genomic DNA (gDNA) or complementary DNA (cDNA) as previously reported[1-4]. PCR amplicons were subjected to direct sequencing on both strands. Sequence data were analyzed using the IMGT® databases and the IMGT/V-QUEST tool (http://www.imgt.org). Only productive rearrangements were evaluated. Output data from IMGT/V-QUEST for all productive IGHV-IGHD-IGHJ rearrangements were parsed, reorganized, and exported to a spreadsheet through the use of computer programming. Information was extracted regarding IG gene repertoires, VH CDR3 length and amino acid sequence and SHM; to identify and cluster stereotyped rearrangements, we used an in-house purpose-built bioinformatics method. In brief VH CDR3 sequences were assigned to stereotyped subsets according to the following criteria: (i) Cases were initially clustered

together only if they share at least 50% amino acid identity and 70% similarity within their respective VH CDR3s; (ii) clustered sequences must have identical VH CDR3 lengths and identical locations of shared patterns; (iii) only sequences carrying IGHV genes of the same phylogenetic clan be placed in the same cluster. Iterative clustering ultimately leads to higher levels of hierarchy describing more distant, and thus relaxed, sequence relationships with more widely shared sequence patterns (affecting only the number - and rarely the location - of these patterns, but neither the VH CDR3 length nor the phylogenetic makeup of the cluster) in progressively larger clusters, which eventually form the collection of subsets.

CD38 expression

CD38 expression was assessed with flow-cytometry. The cut-off for positivity was 30%.

FISH analysis

Preparations for FISH analysis were counterstained with 4,6-diamidino-phenyl-indole (DAPI) and a minimum of 200 interphase nuclei were examined using commercially available probes for chromosomal bands 13q14-34, 11q22, 17p13 and chromosome 12.

Analysis of gene mutations

Mutational screening was performed for the following genes: *NOTCH1* (n=1229, 90%): entire exon 34 or targeted analysis for del7544-45/p.P2514Rfs*4; *TP53* (n=743, 54%): exons 4-8 but also exons 9-10 for some centers; *SF3B1* (n=840, 62%): exons 14-16 and, *MYD88* (n=506, 37%): exons 3 and 5 or targeted analysis for p.L265P.

Statistical analysis

Proportional hazard regression

The proportional hazard (PH) assumption was assessed for both the univariable and multivariable case, using the function *cox.zph*(), which correlates for each covariate the corresponding set of scaled Schoenfeld residuals with time, to test for independence between residuals and time. In the multivariable case it also performs a global test for the model as a whole. Within early stage M-CLL patients, from the six variables that were included as predictors in the multivariable model, only Male did not satisfy the PH assumption in both cases. The global test indicated that the PH assumption was satisfied. Within early stage U-CLL patients, all five variables that were included as predictors in the multivariable model satisfied the PH assumption in both cases. The global test also indicated that the PH assumption was satisfied. Harrell's C-index and its standard error were calculated to assess the discriminatory ability of the Cox model within both early stage M-CLL and U-CLL patients for two scenarios. The first is when a multivariable model included as predictors the important factors according to the univariable model and was based on Binet A cases. The second is when a univariable model included as sole predictor the final prognostic index (four and five categories respectively in M-CLL and U-CLL) and was based on all cases.

Internal validation

A bootstrapping procedure was applied separately for M-CLL and U-CLL cases to validate internally the stability of the multivariable Cox model[5]. Initially, 1000 bootstrap samples equal in size to the original CLL population were randomly generated with replacement from the original CLL population. Subsequently, for each bootstrap sample, the multivariable Cox regression model was applied using the same predictors as in the original model. For each predictor, the percentage of cases it was considered statistically significant and included in the model was recorded, as well as the average number of significant predictors per bootstrap sample. A prognostically important predictor would be expected to be included in the multivariable model in the majority of bootstrap samples. In a subsequent step, 1000 additional bootstrap samples were randomly generated following the same procedure. The multivariable Cox model was applied to each bootstrap sample with the same predictors as in the original modeling. The mean of the hazard ratio and the respective 95% confidence interval were recorded for each predictor based on the 1000 bootstrap samples.

The first step of internal validation within early stage M-CLL patients showed that within the 1000 randomly generated bootstrap samples, the average number of predictors included in the multivariable Cox model was 3.2; three variables exhibited selection percentages greater than 60%, i.e. *TP53*abn, +12 and subset #2. Therefore, we argued that these three predictors were the most important according to Cox regression analysis. The first step of internal validation within early stage U-CLL patients, showed that the average number of predictors considered significant in the multivariable Cox model was 3.5. Four variables exhibited selection percentages greater than 60%, i.e. *TP53*abn, *SF3B1*mut, del(11q) and male gender. Therefore, we argued that these four covariates were the most important within this mutational group.

Binary Recursive partitioning

Recursive partitioning was performed using tree-structured regression models that describe the conditional distribution of TTFT given the same predictors that were included in the multivariable Cox model.

We followed Hothorn et al. who proposed a recursive binary partitioning approach within a theoretically structured conditional inference framework[6]. The algorithm used for the partitioning is briefly described below:

1. Test the global null hypothesis of independence between TTFT and any of the covariates. Stop if this hypothesis cannot be rejected. Otherwise, select the covariate which exhibits the strongest association to TTFT.
2. Once the best covariate is selected, the optimal binary split is determined.
3. Recursively repeat steps 1 and 2.

This procedure enables the hierarchical classification of the significant covariates, from the most important, which splits the primary node (entire population), to those which extend to the terminal nodes. By separating the covariate selection and following the splitting

procedure algorithm, the two main problems when fitting such models are addressed, i.e. overfitting and selection bias towards covariates with many possible splits or missing values. The *ctree* function from the package *party* in *R* was applied. Notable parameters that control aspects of the tree construction are: (i) the minimum sum of patients in a node in order to be considered for splitting (set to 20); (ii) the minimum sum of patients in a terminal node (set to 9); and (iii) the split criterion according to a log-rank scores-based statistic, which was set as $p<0.05$.

Amalgamation

Any two nodes within the tree that arise from the same parent node exhibit significantly different survival behavior. This is not the case for each pair of the terminal nodes that do not share the same parent. Therefore, an amalgamation algorithm is applied to merge terminal nodes that exhibit similar survival behavior[7]. At first, the log-rank test is applied, for each different pair of terminal nodes, to test the null hypothesis that their survival distributions are the same, against the alternative that they differ. The p-value is recorded for each comparison and the maximum p-value of all possible comparisons is considered. When the latter is greater than 0.05, the corresponding nodes are merged to a new terminal node and the procedure is repeated until the maximum p-value is less than 0.05.

Supplemental Table 1. Main clinicobiological features of the entire cohort (n=2366).

	Entire cohort n, %	M-CLL n, %	U-CLL n, %	X^2 test p-value
Clinical stage				
Binet A	1900/2366, 80%	1224/1364, 90%	676/1002, 68%	<0.001
Binet B	287/2366, 12%	83/1364, 6%	204/1002, 20%	<0.001
Binet C	179/2366, 8%	57/1364, 4%	122/1002, 12%	<0.001
Gender				
Male	1449/2366, 61%	806/1364, 59%	643/1002, 64%	0.233
Age at Diagnosis				
Median age (years)	64.3 (22-92)	63.7 (22-91)	63.5 (26-92)	0.575
CD38 expression				
High	293/1649, 18%	167/1319, 13%	126/330, 38%	<0.001
FISH detected abnormalities				
idel(13q)	671/1373, 49%	503/1013, 50%	168/360, 47%	0.603
Trisomy 12	263/1798, 15%	114/1043, 11%	149/755, 20%	<0.001
del(11q)	220/1813, 12%	35/1047, 3.3%	185/766, 24%	<0.001
del(17p)	114/1825, 6%	34/1057, 3.2%	80/768, 10.5%	<0.001
Recurrent gene mutations				
MYD88	21/929, 2.2%	21/506, 4.1%	0/423, 0%	<0.001
NOTCH1	166/2097, 8%	22/1229, 1.8%	144/868, 16.5%	<0.001

SF3B1	115/1449, 8%	31/840, 3.7%	84/609, 14%	<0.001
TP53	137/1535, 9%	42/743, 5.6%	95/801, 12%	<0.001
BIRC3	24/830, 3%	7/458, 1%	17/372, 5%	0.020
***TP53*abn**	183/2095, 9%	55/1154, 4.8%	128/941, 14%	<0.001
Immunogenetic features				
GI: 97-97.99%	104/2366, 4.4%	104/1364, 7.6%	-	-
GI: 100%	750/2366, 32%	-	750/1002, 75%	-
Stereotyped #1	55/2366, 2.3%	-	55/1002, 5.5%	-
Stereotyped #2	33/2366, 1.5%	27/1364, 2%	6/1002, 0.6%	0.009
Stereotyped #4	35/2366, 1.5%	35/1364, 2.6%	-	

High CD38 expression: positivity >30%, idel(13q): isolated deletion of chromosome 13q, del(11q): deletion of chromosome 11q, del(17p): deletion of chromosome 17p, *TP53*abn: deletion of chromosome 17p (del(17p)) and/or *TP53* mutation, GI: germline identity, Stereotyped #2: assignment to stereotyped subset #2, stereotyped #4: assignment to stereotyped subset #4, M-CLL: patients carrying mutated IGHV genes, U-CLL: cases carrying unmutated IGHV genes.

Supplemental Table 2. Main clinicobiological features of the validation cohort. The p-value stems from the comparison of each biomarker between the main and the validation cohort.

	n, %	p-value (X^2 test)
Gender		
Male	397/649, 62%	1
Age at diagnosis		
Median age	63.6 (29-89) years	0.630
FISH detected abnormalities		
idel(13q)	301/522, 58%	0.061
Trisomy 12	65/598, 11%	0.049
del(11q)	59/598, 10%	0.203
del(17p)	13/598, 2%	<0.001
Recurrent gene mutations		
SF3B1	28/553, 5%	0.046
TP53	27/623, 4%	<0.001
***TP53*abn**	29/632, 5%	0.002
Immunogenetic features		
M-CLL	442/649, 68%	0.020
Stereotyped #2	20/649, 3%	0.008

idel(13q): isolated deletion of chromosome 13q, del(11q): deletion of chromosome 11q, del(17p): deletion of chromosome 17p, *TP53*abn: deletion of chromosome 17p (del(17p)) and/or *TP53* mutation, Stereotyped #2: assignment to stereotyped subset #2, M-CLL: patients carrying mutated IGHV genes.

Supplemental Table 3. Main clinicobiological features of M-CLL cases assigned to different Binet clinical stages. Bonferroni correction was applied and the significance level was set at $p < 0.017$.

	Binet A n=1224	**Binet B n=83**	**Binet C n=57**	**p-value A vs B**	**p-value A vs C**	**p-value B vs C**
Male gender	711/1224, 58%	58/83, 70%	37/57, 65%	0.034	0.31	0.53
CD38 expression	127/1186, 11%	22/78, 28%	18/55, 32.8%	<0.0001	<0.0001	0.57
NOTCH1	15/1104, 1.3%	4/75, 5.3%	3/50, 6%	0.008	0.009	0.87
MYD88	16/436, 3.7%	3/41, 7.3%	2/29, 6.8%	0.25	0.38	0.94
SF3B1	20/750, 2.7%	6/53, 11.3%	5/37, 13.5%	0.0005	0.0002	0.75
idel(13q)	459/906, 51%	27/64, 42%	17/43, 40%	0.2	0.15	0.78
+12	100/972, 10.3%	8/66, 12%	10/44, 22.7%	0.63	0.009	0.14
del(11q)	23/939, 2.4%	7/65, 10.7%	5/43, 11.6%	0.0001	0.0004	0.88
***TP53*abn**	46/1035, 4.4%	5/72, 7%	4/47, 8.5%	0.32	0.19	0.75
GI: 97-97.99%	80/1224, 6.5%	13/83, 15.7%	11/57, 19.2%	0.001	0.0002	0.57
Subset #2	13/1224, 1%	8/83, 9.6%	6/57, 10.5%	<0.0001	<0.0001	0.86

CD38 expression: cut-off for positivity >30%, idel(13q): isolated deletion of chromosome 13q, +12: trisomy 12, del(11q): deletion of chromosome 11q, *TP53*abn: deletion of chromosome 17p [del(17p)] and/or *TP53* mutation, GI: germline identity, subset #2: assignment to stereotyped subset #2.

Supplemental Table 4. Main clinicobiological features of U-CLL cases assigned to different Binet clinical stages. Bonferroni correction was applied and the significance level was set at $p < 0.017$.

	Binet A n=676	Binet B n=204	Binet C n=122	p-value A vs B	p-value A vs C	p-value B vs C
Male gender	406/676, 61%	148/204, 73%	89/122, 73%	0.001	0.006	0.93
CD38 expression	78/213, 37%	36/82, 44%	12/35, 34%	0.24	0.79	0.33
NOTCH1	88/587, 15%	33/174, 19%	23/107, 21%	0.23	0.09	0.61
BIRC3	9/241, 4%	4/71, 6%	4/60, 7%	0.48	0.31	0.81
SF3B1	45/416, 11%	25/118, 21%	14/75, 19%	0.003	0.05	0.67
idel(13q)	125/266, 47%	24/54, 44%	19/40, 48%	0.73	0.95	0.91
+12	108/523, 21%	29/138, 21%	12/94, 13%	0.92	0.075	0.11
del(11q)	118/529, 22%	42/143, 29%	25/94, 27%	0.078	0.36	0.64
***TP53*abn**	73/636, 12%	28/188, 15%	27/117, 23%	0.21	0.0007	0.07
GI: 100%	502/676, 74%	152/204, 75%	96/122, 79%	0.94	0.29	0.39
IGHV1-69	160/676, 24%	54/204, 26%	28/122, 23%	0.41	0.86	0.48
Subset #1	34/555, 6%	12/177, 7%	9/115, 8%	0.75	0.49	0.73

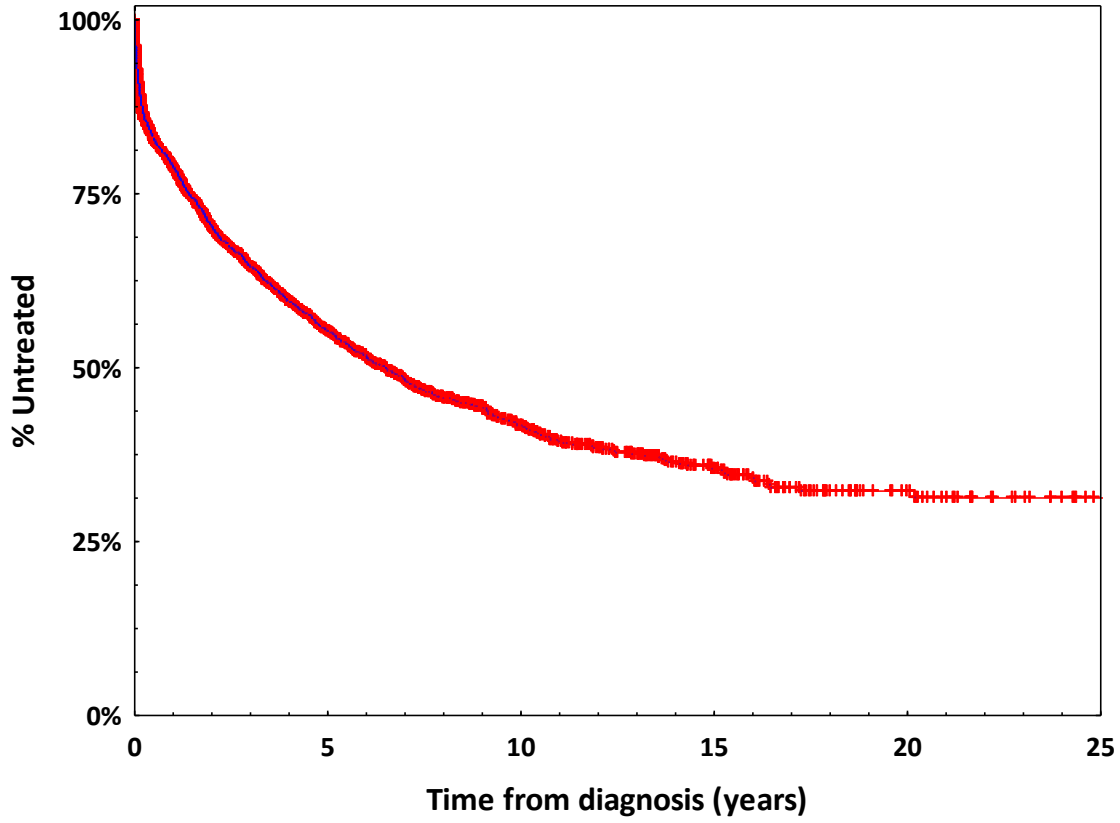
CD38 expression: positivity >30%, idel(13q): isolated deletion of chromosome 13q, +12: trisomy 12, del(11q): deletion of chromosome 11q, *TP53*abn: deletion of chromosome 17p [del(17p)] and/or *TP53* mutation, GI: germline identity, subset #1: assignment to stereotyped subset #1.

Supplemental Table 5. Overview of the entire cohort and the subgroup of cases included in the multivariable analysis. No significant difference is detected regarding the evaluated biomarkers.

Feature	Entire cohort, M-CLL n, %	Cases included in the multivariable - analysis, M-CLL; n, %	p-value
Gender			
Male	711/1224, 58%	530/919, 58	0.84
Age at diagnosis			
Median (years)	64.6	64.5	0.99
CD38 expression			
High	127/1186, 11%	97/864, 11%	0.71
FISH detected abnormalities			
Idel(13q)	432/731, 59%	432/731, 59%	-
Trisomy 12	100/972, 10.3%	119/919, 12.5%	0.07
del(11q)	23/939, 2.4%	34/904, 3.5%	0.1
Recurrent gene mutations			
MYD88	16/436, 3.7%	14/435, 3.2%	0.71
NOTCH1	15/1104, 1.3%	12/902, 1.3%	0.95
SF3B1	20/750, 2.7%	22/686, 3.2%	0.54
***TP53*abn**	46/1035, 4.4%	36/919, 4%	0.56
Immunogenetic features			
GI: 97-97.99%	80/1224, 6.5%	57/919, 6.2%	0.75
Stereotyped #2	13/1224, 1%	12/919, 1.3	0.60
	Entire cohort, M-CLL n, %	**Cases included in the multivariable analysis, U-CLL; n, %**	**p-value**
Gender			
Male	406/676, 61%	233/384, 61%	0.84
Age at diagnosis			0.79
Median (years)	64.4	64.5	
FISH detected abnormalities			
idel(13q)	125/266, 47%	97/194, 50%	0.52
Trisomy 12	108/523, 21%	80/384, 21%	0.94
del(11q)	118/529, 22%	89/384, 23%	0.75
Recurrent gene mutations			
NOTCH1	88/587, 15%	69/380, 18%	0.19
SF3B1	45/416, 11%	43/384, 12%	0.86
BIRC3	9/241, 4%	9/236, 3.8%	0.96
***TP53*abn**	73/636, 12%	36/384, 9.5%	0.29
Immunogenetic features			
GI: 100%	502/676, 74%	284/384, 74%	0.91
Stereotyped #1	34/555, 6%	17/303, 5.5%	0.26

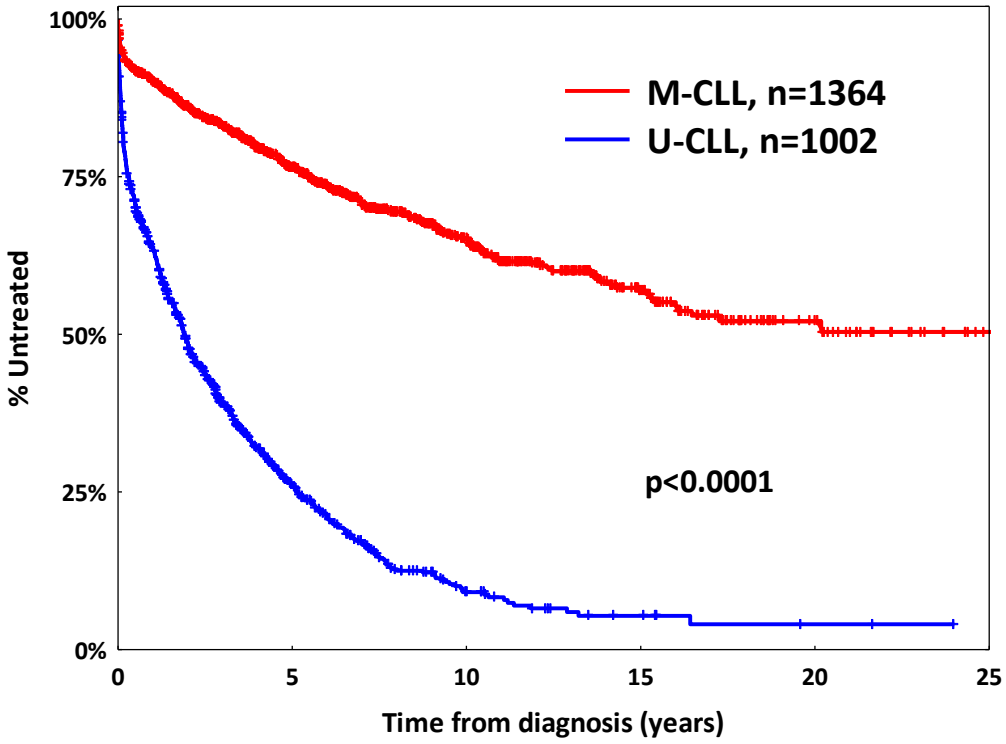
idel(13q): isolated deletion of chromosome 13q, del(11q): deletion of chromosome 11q, del(17p): deletion of chromosome 17p, *TP53*abn: deletion of chromosome 17p (del(17p)) and/or *TP53* mutation, Stereotyped #2: assignment to stereotyped subset #2, M-CLL: patients carrying mutated IGHV genes, U-CLL: patients carrying unmutated IGHV genes, MV-analysis: multivariable analysis.

Supplemental Figure 1. Kaplan Meier curve for time-to-first-treatment (TTFT) for the entire cohort (n=2366).

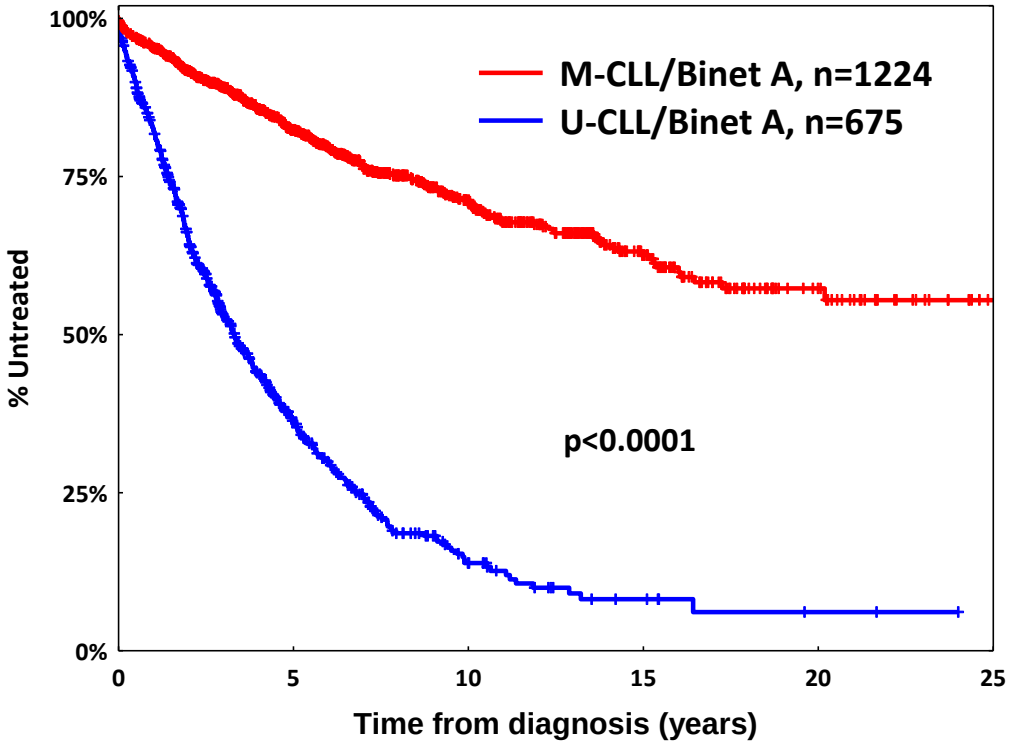


Supplemental Figure 2. (A) Kaplan Meier curves for time-to-first-treatment (TTFT) for M-CLL and U-CLL. (B) Kaplan Meier curves for TTFT for Binet A M-CLL and U-CLL.

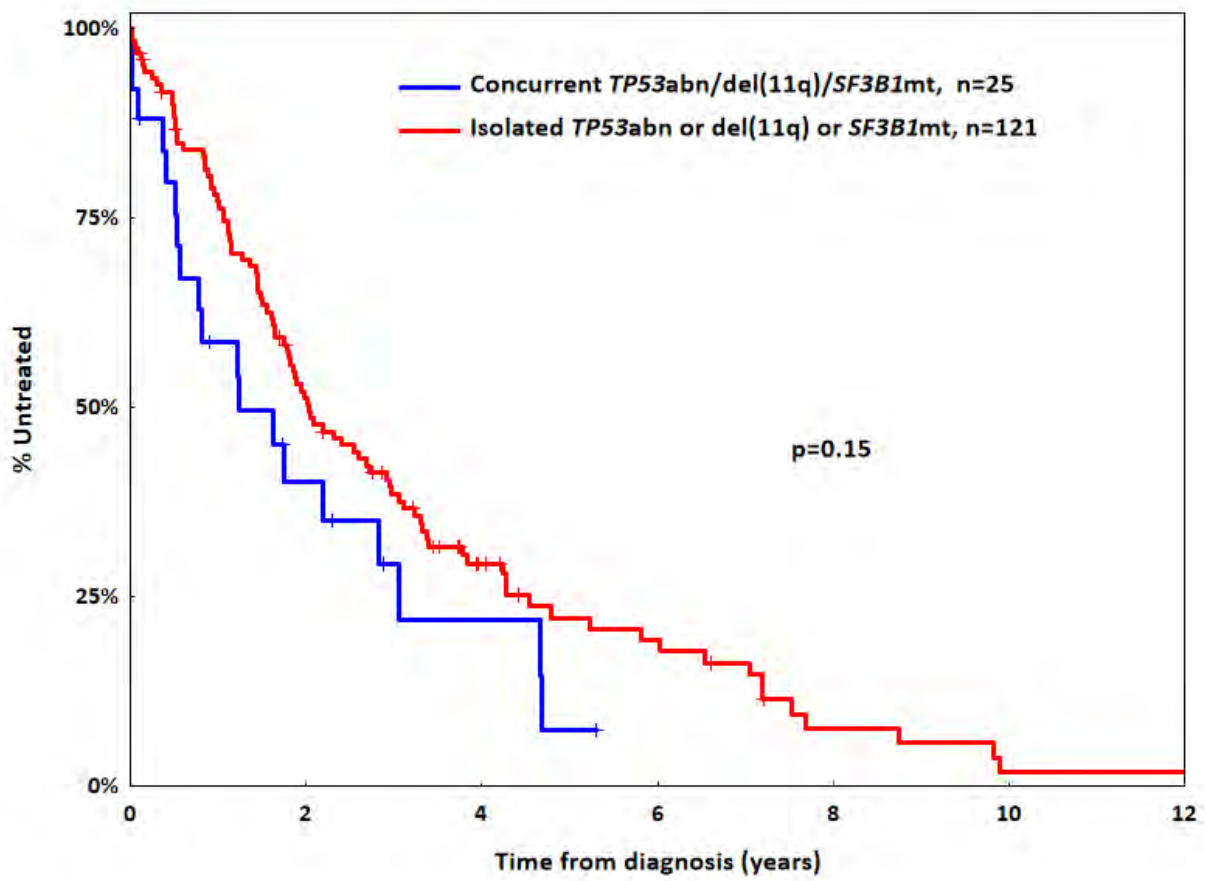
2A



2B

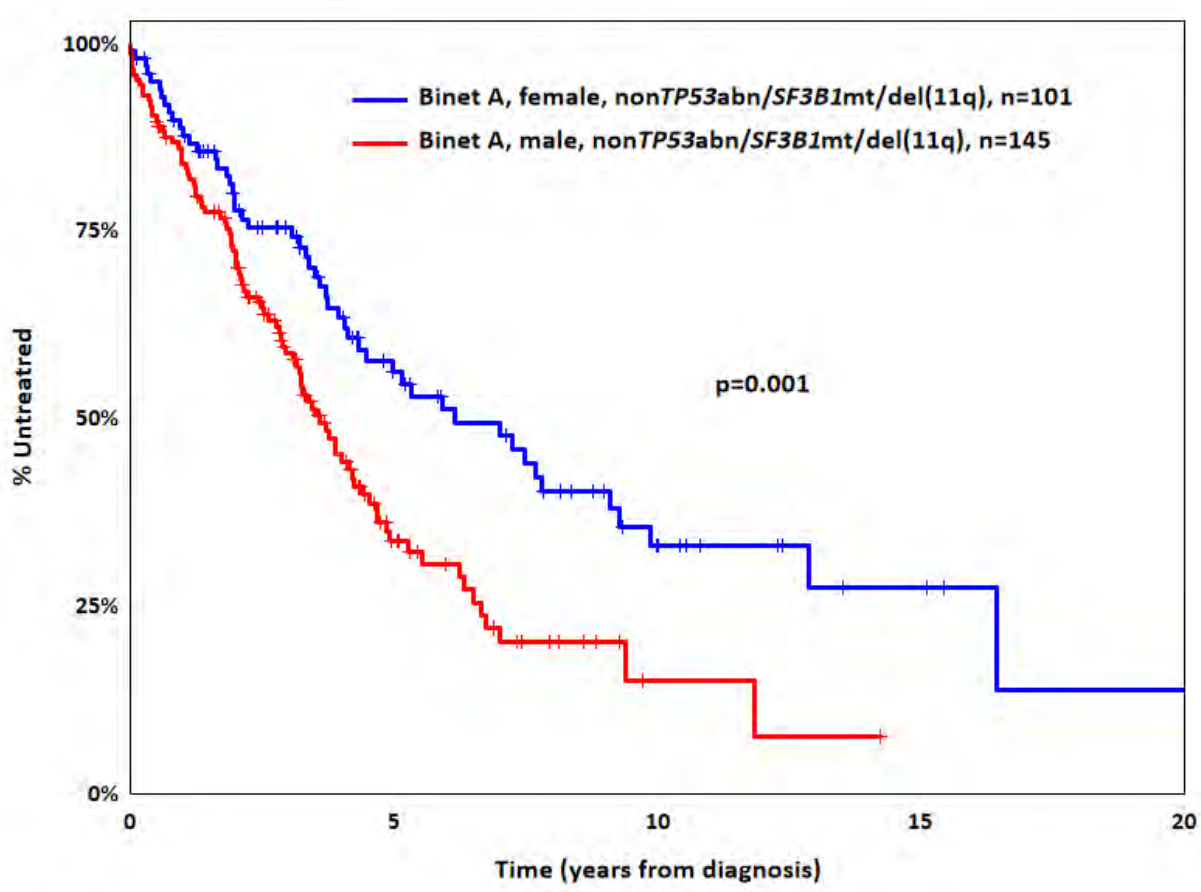

100%
75%
50%
25%
0%
% Untreated
M-CLL/Binet A, n=1224
U-CLL/Binet A, n=675
p<0.0001
0
5
10
15
20
25
Time from diagnosis (years)

Supplemental Figure 3. Kaplan Meier curves for time-to-first-treatment (TTFT) for Binet A U-CLL cases with either isolated *TP53*abn, *SF3B1* mutations and del(11q) vs cases with 2 concurrent of the mentioned aberrations

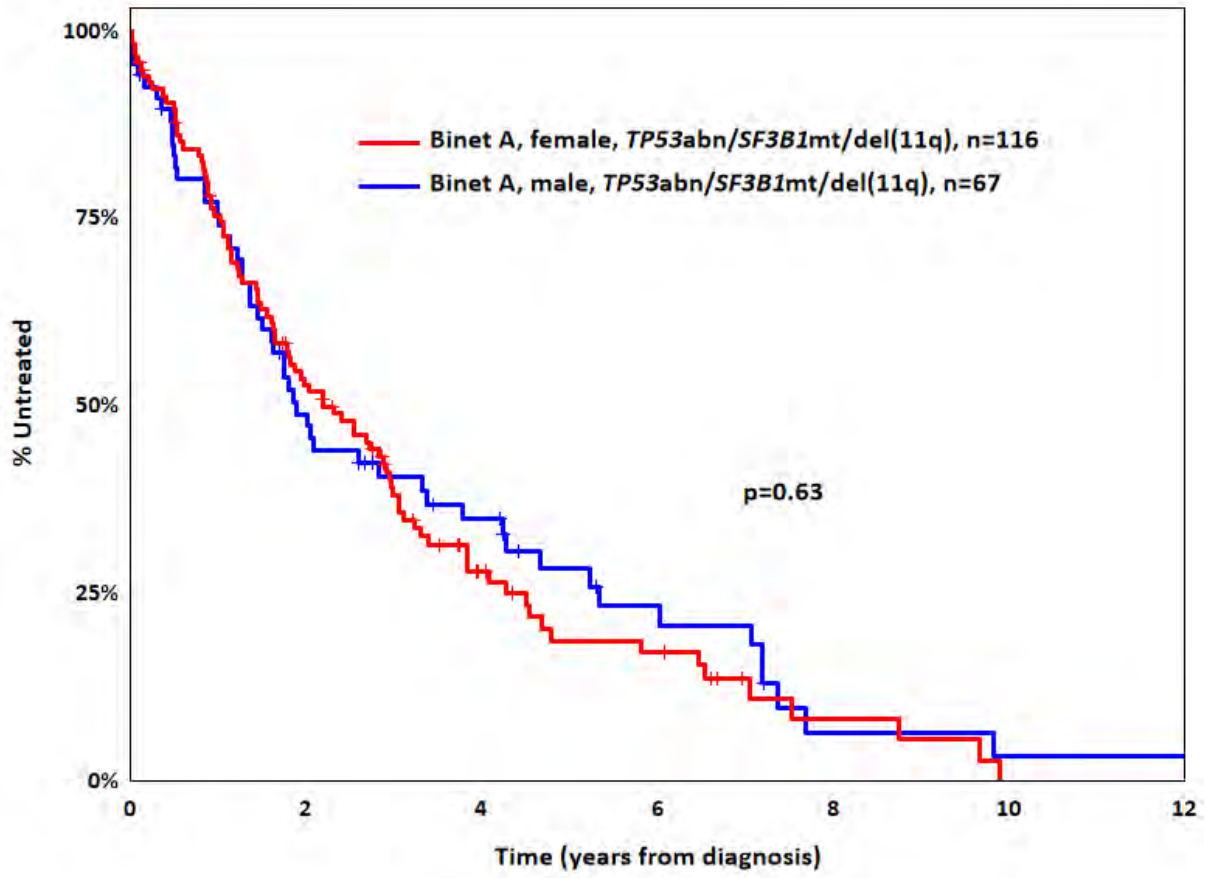


Supplemental Figure 4. Kaplan Meier curves for time-to-first-treatment (TTFT) in Binet A U-CLL. (A): Male gender is correlated with shorter TTFT within the non *TP53*abn/*SF3B1*mut/del(11q) Binet A. (B): No impact of male gender within the *TP53*abn/*SF3B1*mut/del(11q) Binet A cases.

4A

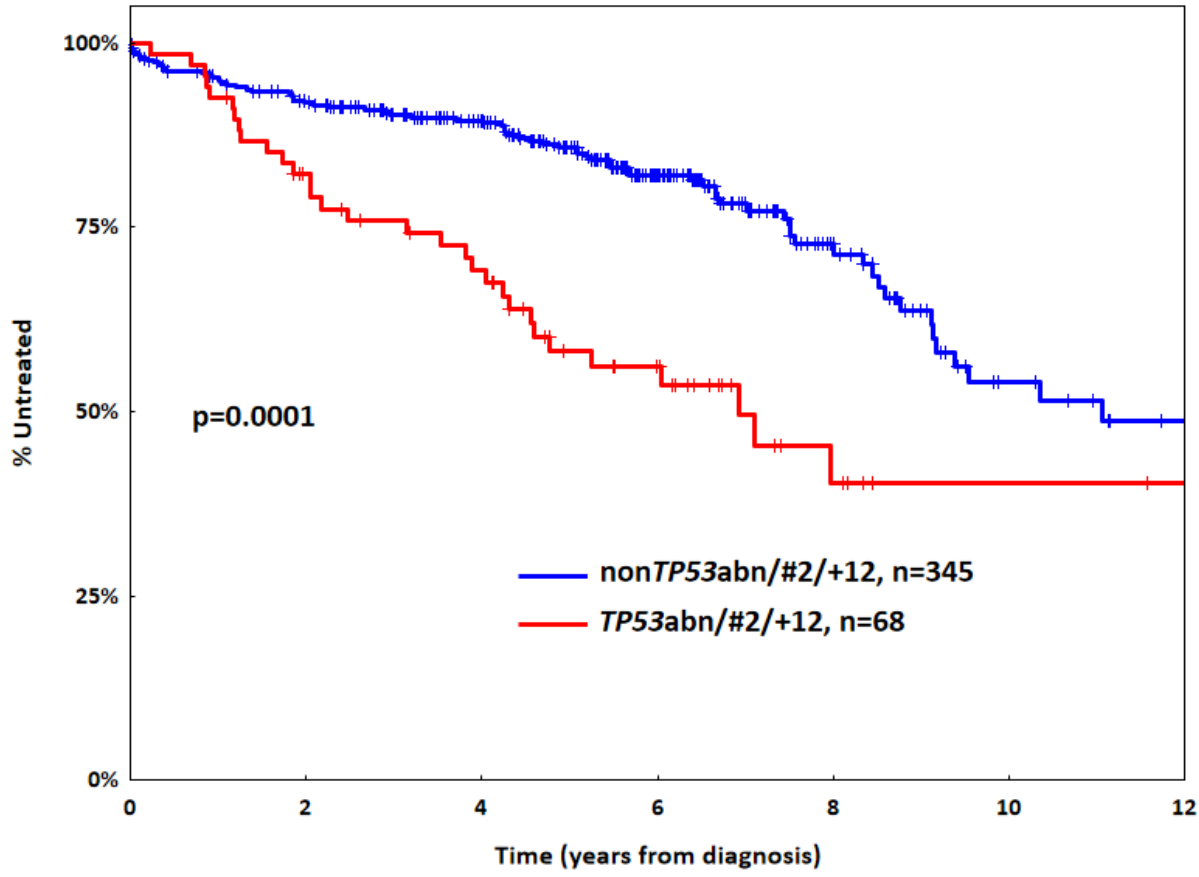


4B

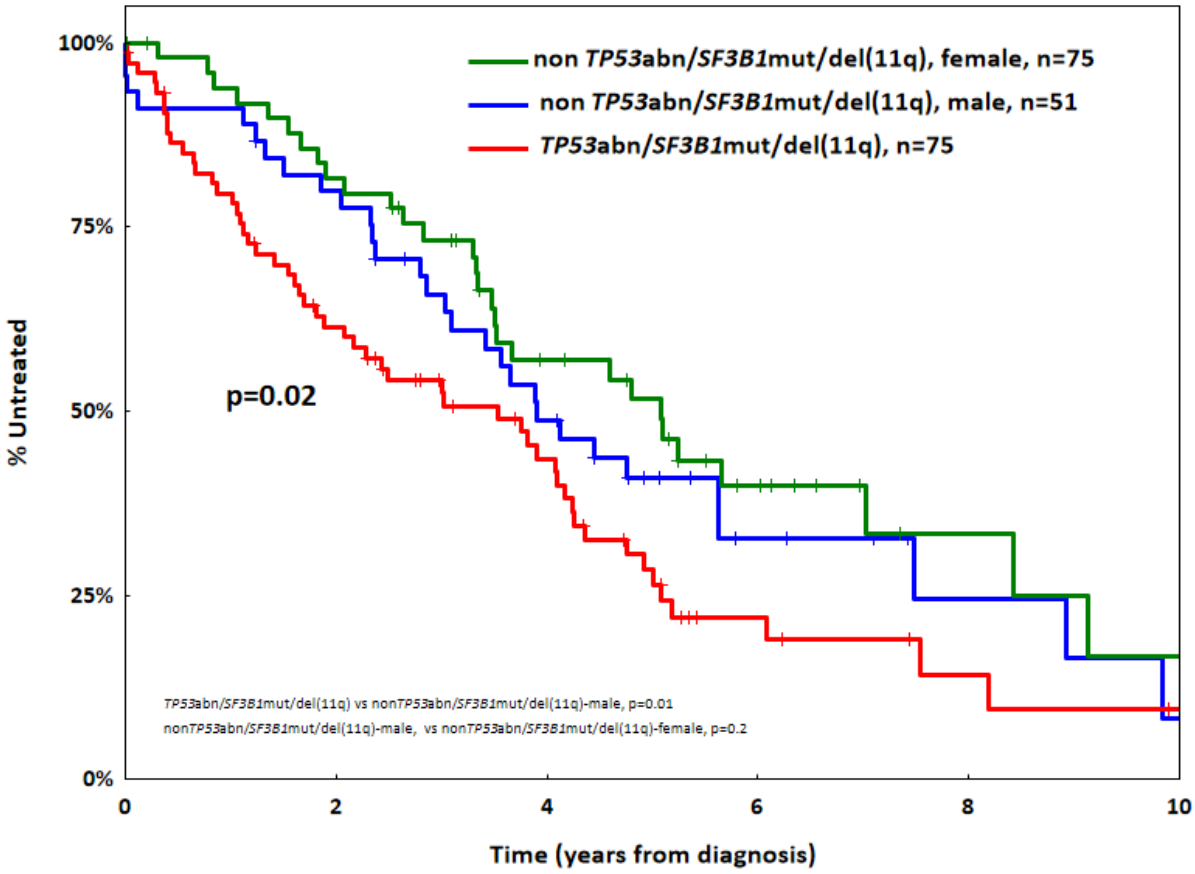

100%
75%
50%
25%
0%
% Untreated
Binet A, female, TP53abn/SF3B1mt/del(11q), n=116
Binet A, male, TP53abn/SF3B1mt/del(11q), n=67
p=0.63
0
2
4
6
8
10
12
Time (years from diagnosis)

Supplemental Figure 5. Kaplan Meier curves for time-to-first-treatment (TTFT) in the validation cohort (n=649). (A) M-CLL cases carrying *TP53*abn, trisomy 12 (+12) or assigned to stereotyped subset #2, display shorter TTFT compared to non *TP53*abn/#2/+12 cases; (B) Within Binet A U-CLL, *TP53*abn/*SF3B1*mut/del(11q) cases exhibit the shortest TTFT. Within the remaining cases the difference between male and female patients does not exhibit statistical significance.

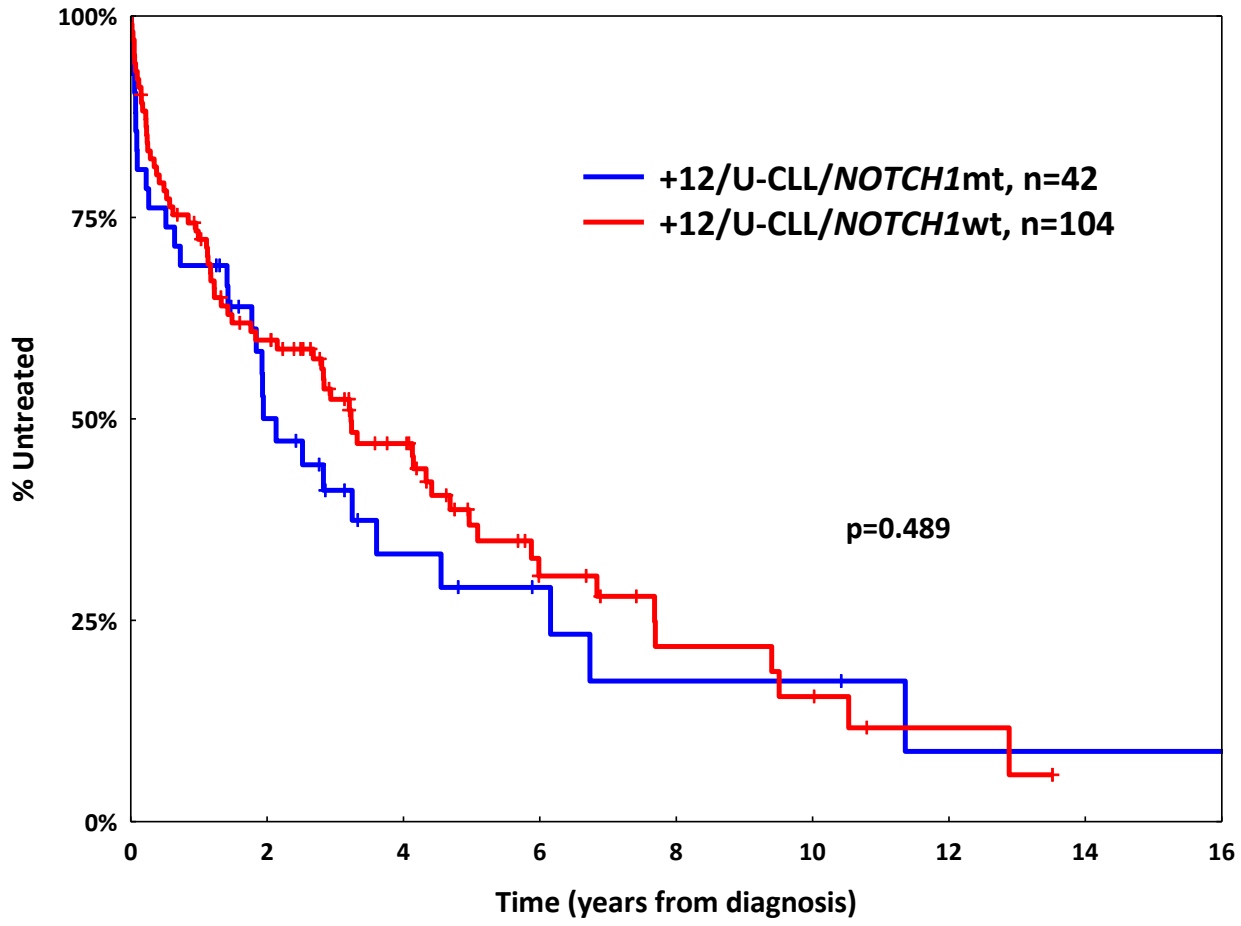
5A



5B


non TP53abn/SF3B1mut/del(11q), female, n=75
non TP53abn/SF3B1mut/del(11q), male, n=51
TP53abn/SF3B1mut/del(11q), n=75
p=0.02
TP53abn/SF3B1mut/del(11q) vs nonTP53abn/SF3B1mut/del(11q)-male, p=0.01
nonTP53abn/SF3B1mut/del(11q)-male, vs nonTP53abn/SF3B1mut/del(11q)-female, p=0.2
% Untreated
100%
75%
50%
25%
0%
0
2
4
6
8
10
Time (years from diagnosis)

Supplemental Figure 6. Kaplan Meier curves for time-to-first-treatment (TTFT) within cases carrying trisomy 12 (+12). No impact of *NOTCH1* mutation in cases with unmutated IGHV genes (U-CLL).



References

1.Agathangelidis A, Darzentas N, Hadzidimitriou A, et al: Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood 119:4467-75, 2012

2.Ghia P, Stamatopoulos K, Belessi C, et al: ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 21:1-3, 2007

3.Langerak AW, Davi F, Ghia P, et al: Immunoglobulin sequence analysis and prognostication in CLL: guidelines from the ERIC review board for reliable interpretation of problematic cases. Leukemia 25:979-984, 2011

4.Murray F, Darzentas N, Hadzidimitriou A, et al: Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. Blood 111:1524-33, 2008

5.Chen CH, George SL: The bootstrap and identification of prognostic factors via Cox's proportional hazards regression model. Stat Med 4:39-46, 1985

6.Hothorn T, Hornik K, Zeileis A: Unbaised Recursive Partitioning: A Conditional Inference Framework. Journal of Computational and Graphical Statistics 15:651-674, 2006

7.Rossi D, Rasi S, Spina V, et al: Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood 121:1403-12, 2013