

## Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase II study

Inhye E. Ahn,<sup>1</sup> Mohammed Z.H. Farooqui,<sup>1</sup> Xin Tian,<sup>2</sup> Janet Valdez,<sup>1</sup> Clare Sun,<sup>1</sup> Susan Soto,<sup>1</sup> Jennifer Lotter,<sup>1</sup> Stephanie Housel,<sup>1,3</sup> Maryalice Stetler-Stevenson,<sup>4</sup> Constance M. Yuan,<sup>4</sup> Irina Maric,<sup>5</sup> Katherine R. Calvo,<sup>5</sup> Pia Nierman,<sup>1</sup> Thomas E. Hughes,<sup>6</sup> Nakhle S. Saba,<sup>1,7</sup> Gerald E. Marti,<sup>1</sup> Stefania Pittaluga,<sup>4</sup> Sarah E. M. Herman,<sup>1</sup> Carsten U. Niemann,<sup>1,8</sup> Lone B. Pedersen,<sup>8</sup> Christian H. Geisler,<sup>8</sup> Richard Childs,<sup>1</sup> Georg Aue,<sup>1</sup> and Adrian Wiestner<sup>1</sup>

<sup>1</sup> Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; <sup>2</sup> Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; <sup>3</sup> Leidos Biomedical Research, Inc.; <sup>4</sup> Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD; <sup>5</sup> Department of Laboratory Medicine, Clinical Research Center, National Institutes of Health, Bethesda, MD; <sup>6</sup> Department of Pharmacy, Clinical Research Center, National Institutes of Health, Bethesda, MD; <sup>7</sup> Section of Hematology and Medical Oncology, Department of Medicine, Tulane University, New Orleans, LA; <sup>8</sup> Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Denmark.

### Corresponding author:

Adrian Wiestner

Hematology Branch, NHLBI, NIH

Bldg. 10, CRC 3-5140

10 Center Drive, 20892-1202 Bethesda, MD

phone: +1 (301) 496 5093, fax: +1(301) 496 8396

wiestnera@mail.nih.gov

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## KEYPOINTS

- With 5 years median follow-up, continuous single-agent ibrutinib therapy was well tolerated with deepening of response.
- Previously untreated patients, even those with *TP53* aberration, achieved durable responses.

## ABSTRACT

Safety and efficacy of ibrutinib (420mg) in chronic lymphocytic leukemia (CLL) were evaluated in a phase II study; 51 patients had *TP53* aberration (TP53 cohort) and 35 were enrolled for age  $\geq 65$  years (elderly cohort). Both cohorts included patients with treatment-naïve (TN) and relapsed/refractory (RR) CLL. With the median follow-up of 4.8 years, 49 (57.0%) of 86 patients remain on study. Treatment was discontinued for progressive disease in 20 (23.3%) patients and for adverse events in 5 (5.8%). Atrial fibrillation occurred in 18 (20.9%) patients for a rate of 6.4 per 100 patient-years. No serious bleeding occurred. The overall response rate at 6 months, the primary study endpoint, was 95.8% for the TP53 cohort (95% CI, 85.7-99.5) and 93.9% in the elderly cohort (95% CI, 79.8-99.3). Depth of response improved with time; at best response, 14 (29.2%) of 48 patients in the TP53 cohort and 9 (27.3%) of 33 in the elderly cohort achieved a complete response. Median minimal residual disease (MRD) in peripheral blood was  $3.8 \times 10^{-2}$  at 4 years, with MRD negative ( $<10^{-4}$ ) remissions in 5 (8.3%) patients. In the TP53 cohort, the estimated 5-year progression-free survival (PFS) was 74.4% in TN-CLL compared to 19.4% in RR-CLL ( $P=.0002$ ), and overall survival (OS) was 85.3% versus 53.7%, respectively ( $P=.023$ ). In the elderly cohort, the estimated 5-year PFS and OS in RR-CLL were 64.8% and 71.6%, respectively, while no event occurred in TN-CLL. Long-term administration of ibrutinib was well tolerated and provided durable disease control for most patients. ClinicalTrials.gov #NCT01500733.

## INTRODUCTION

The combination of chemotherapy with an anti-CD20 monoclonal antibody, referred to as chemoimmunotherapy, has been the mainstay of therapy for chronic lymphocytic leukemia (CLL). While a subset of patients achieve durable remissions, most relapse within a few years.<sup>1,2</sup> Clonal evolution leads to the expansion of genetically altered cells that dominate at the time of relapse, limiting efficacy of repeat chemotherapy.<sup>3</sup> In particular, patients with *TP53* aberration, either due to deletion of chromosome 17p (del(17p)) or *TP53* mutation, relapse early after frontline chemoimmunotherapy with a median progression-free survival (PFS) of less than 2 years.<sup>4,5</sup> Toxicity of chemoimmunotherapy can be limiting especially for the majority of patients with CLL who are older than 65. While reduced-intensity regimens are less toxic, durability of response is inferior.<sup>6-8</sup>

Targeted therapy with kinase inhibitors is emerging as an alternative to conventional therapy for CLL.<sup>9-13</sup> Activation of B-cell receptor (BCR) signaling through self-association or interactions with autoantigens is a key driver of CLL pathogenesis.<sup>14-17</sup> Ibrutinib covalently binds Bruton's tyrosine kinase (BTK) leading to sustained inhibition of BCR and NF- $\kappa$ B signaling and tumor proliferation.<sup>18,19</sup> In randomized trials, ibrutinib induced higher overall response rates (ORR) and extended survival compared to the comparator treatment for both relapsed and/or refractory CLL (RR-CLL) and treatment-naïve CLL (TN-CLL).<sup>9,10</sup> In contrast to the experience with chemoimmunotherapy, response to ibrutinib appears to be independent of *TP53* aberration.<sup>11,20,21</sup> However, in RR-CLL with del(17p), one study reported 2-year PFS of 63%.<sup>11</sup> In contrast, we previously reported 2-year PFS of 82% in 51 patients with *TP53* aberration (del(17p) and/or *TP53* mutation), the majority of whom received ibrutinib as first-line therapy.<sup>21</sup> At the time of progression, mutations in *PLCG2* and *BTK* that reduce the inhibitory effects of ibrutinib are often present.<sup>22-27</sup> Computational models that incorporate estimates of mutation frequency and growth kinetics, suggest that subclones carrying these mutations likely exist before initiation of ibrutinib therapy.<sup>28</sup>

Ibrutinib has generally been found to have a favorable safety profile. However, one center reported an estimated cumulative incidence of non-relapse discontinuation of ibrutinib in 15.6% at 18 months.<sup>29</sup> Further, the rate of atrial fibrillation is higher in ibrutinib-treated patients compared to control arms in randomized trials.<sup>30</sup> Finally, the impact of chronic inhibition of BTK on infection risk remains to be better defined. While, the frequency of infections, in particular respiratory infections, has been reported to decrease on continuous therapy,<sup>20</sup> and increases in serum IgA on ibrutinib are associated with a lower risk of infections,<sup>31</sup> others report an apparent increase in some opportunistic infections during ibrutinib therapy, including *Pneumocystis jirovecii* pneumonia and invasive fungal infections.<sup>32,33</sup>

Additional information on safety and efficacy of long-term therapy with ibrutinib will help estimate risks and expected benefit for different patient groups. Here we report the results with single-agent ibrutinib in patients with *TP53* aberration or age 65 years or older enrolled in an investigator-initiated phase II study. Our data suggest that for a large proportion of CLL patients, ibrutinib monotherapy provides durable disease control.

## **PATIENTS, MATERIALS, AND METHODS**

### **Study design and participants**

This phase 2, open-label, single-center, study was approved by the Institutional Review Board (clinicaltrials.gov; NCT01500733). All patients provided written informed consent. Eligibility criteria included active CLL or small lymphocytic lymphoma requiring therapy,<sup>34</sup> and del(17p) by fluorescence in situ hybridization (FISH) in  $\geq 10\%$  of nuclei or *TP53* mutation for the *TP53* cohort; or age  $\geq 65$  years for the elderly cohort, ECOG performance status  $\leq 2$ , neutrophil count  $\geq 500/\mu\text{l}$ , platelet count  $\geq 30,000/\mu\text{l}$ . Exclusion criteria included: any histologic transformation of CLL (Richter's syndrome or prolymphocytic leukemia), autoimmune cytopenia requiring steroids, impaired organ function (total bilirubin  $\geq 1.5$  or AST/ALT  $\geq 2.5$  x upper limit of normal; creatinine  $\geq 2.0$

mg/dL or GFR  $\leq$ 50ml/minute), active Hepatitis B infection, HIV infection, concomitant prednisone  $>$ 20mg/day, and/or anticoagulation with warfarin. A four-week interval from prior therapy was required. Pre-treatment evaluation included history, physical examination, laboratory evaluations, bone marrow aspiration and biopsy, peripheral blood and bone marrow flow cytometry, and computed tomography (CT). FISH and sequencing of the immunoglobulin heavy chain variable gene (IGHV) was performed as previously described.<sup>21</sup>

## Procedures

Ibrutinib was administered orally at the dose of 420 mg once daily until disease progression or the development of unacceptable toxicity. Clinical safety monitoring was performed every other week for the first month, then every 4 weeks until 24 weeks, and every 3 months thereafter. Ibrutinib was held for grade 4 neutropenia lasting  $>$ 7 days or grade 4 thrombocytopenia. For the first occurrence of grade 3 or higher adverse events (AEs), ibrutinib was held until the toxicity resolved to grade  $\leq$ 1 or baseline, and restarted at the original dose of 420mg. For subsequent occurrences of grade 3 or higher AEs, ibrutinib was dose-reduced by the increment of 140 mg. Patients experiencing a first occurrence of grade 3 diarrhea, constitutional symptoms, or infection were restarted at the same dose. For new onset or recurrent grade  $\geq$ 2 atrial fibrillation, ibrutinib was held and cardiac evaluation was completed prior to restarting therapy. After a first occurrence of grade  $\geq$ 2 atrial fibrillation, ibrutinib could be restarted at the same dose after toxicity resolved to grade  $\leq$ 1 or baseline. Subsequent occurrences of atrial fibrillation required dose reduction by 140 mg increments. In March 2015, dose modifications for non-hematologic AEs were harmonised with the ibrutinib prescriber information (USPI, Reference ID 3694103). Laboratory testing included complete blood counts, basic metabolic, hepatic, and mineral panels, lactate dehydrogenase, and uric acid. The trial is fully enrolled.

## Outcomes

The primary endpoint was response after 6 cycles of therapy. Secondary endpoints included safety, tolerability, overall survival (OS), PFS, and best response. Data cut-off for this report was December 31, 2017. Non-hematologic AEs were graded by using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Hematologic AEs and responses were called based on the International Workshop on CLL (iwCLL) 2008 criteria, incorporating recent updates (Table S1).<sup>34,35</sup> At 2, 6, 12 months, and yearly thereafter, CT, bone marrow biopsies, and flow cytometry were performed. Spleen volume was calculated from CT using the Vitrea Core Workstation Server, version 6.6 (Vital Images, Minnetonka, MN, USA). Normal spleen volume was considered less than 315mL. We previously reported the activity and safety data of the TP53 cohort (51 patients with *TP53* aberration) at a median follow-up of 24 months.<sup>21</sup>

### **Flow cytometry**

Flow cytometry to quantify residual disease used methods recommended by European Research Initiative in CLL.<sup>36</sup> Minimal residual disease (MRD) negativity was defined as < 1 CLL cell in 10,000 (<10<sup>-4</sup>) leukocytes assessed.

### **Statistical analysis**

We used a Simon's minimax two-stage design to test the null hypothesis that the ORR is ≤15% versus the one-sided alternative. If 3 or more responses were observed among 16 patients of the first stage, an additional 11 would enter the second stage. With 8 or more responses, the null hypothesis would be rejected. For each cohort, 27 patients provide 90% power at the 0.05 significance level when the true response rate is 40%. Eight additional patients could be enrolled to account for non-treatment related discontinuations before 6 months. In September 2012, the study was amended to allow enrollment of up to 35 patients with TN-CLL in the TP53 cohort, adding to 16 patients with RR-CLL. The total for the TP53 cohort was 51 patients. 35 patients were enrolled in the elderly cohort.

We used descriptive statistics to summarize findings. Duration of follow-up was calculated for surviving patients. OS and PFS were estimated by the Kaplan-Meier

method and compared between subgroups by the log-rank test. Response rates were estimated by the proportions for all patients and subgroups, their 95% confidence intervals (CIs) were computed, and compared between subgroups by Fisher's exact test. Wilcoxon signed-rank test was used to assess the change in the quantification of MRD. Spearman's analysis was used to assess the correlation between blood and bone marrow MRD. Statistical analyses were conducted using R version 3.4.0 (R Foundation for Statistical Computing).

### **Role of the funding source**

The trial was designed by the investigators, and a draft of the protocol was submitted to Pharmacyclics for comments. The study was funded by the Intramural Research Program of National Heart, Lung and Blood Institute, and National Cancer Institute at the National Institutes of Health (NIH). Pharmacyclics provided the study drug. All data were collected by the investigators and stored at NIH. The investigators analysed the data and wrote the manuscript. A draft of the manuscript was submitted to Pharmacyclics for comments. The corresponding author had full access to all the data in the study and had final responsibility for the content of the report and the decision to submit for publication.

## **RESULTS**

### **Patient characteristics and disposition**

Between December 2011 and January 2014, 86 patients were enrolled; 51 were eligible due to *TP53* aberration (TP53 cohort) and 35 due to age  $\geq 65$  years (elderly cohort; Figure S1; Table 1). Overall, 53 (61.6%) patients had TN-CLL, 58 (67.4%) had advanced Rai stage (III/IV), and 57 (66.3%) had unmutated IGHV. As of December 31, 2017, 49 (57.0%) patients remained on study (median time on study 4.8 years, range 4.0-6.0). Table S2 summarizes the disposition of 37 patients who discontinued the study. Four (4.7%) patients died on study due to reasons other than disease



progression; three from infections, and one due to sudden, presumably cardiac death. Twenty (23.3%) patients discontinued ibrutinib due to progressive disease, and five (5.8%) due to AEs, including newly diagnosed lung and ovarian cancer in one patient each. Six (7.0%) patients withdrew consent for various reasons. Two patients that did not meet enrollment criteria were removed and are included in the safety analysis only.

## Safety

Treatment-related AEs leading to treatment discontinuation were, in one patient each, asymptomatic interstitial pulmonary infiltrates, progressive multifocal leukoencephalopathy on concurrent mycophenolate mofetil, and persistent grade 3 diarrhea with biopsy-proven microscopic colitis. Two (2.3%) patients discontinued due to second malignancies requiring systemic therapy. Nine (10.5%) patients required a dose reduction, six for atrial fibrillation, one for grade 3 rash, one for grade 3 diarrhea, and one patient requested a reduction for grade 2 arthralgia. Treatment-emergent grade 3 or 4 hematologic AEs were neutropenia in 33 (38.4%), thrombocytopenia in 13 (15.1%), and anemia in 6 (7.0%) patients, primarily during the first few months on ibrutinib (Table S3). Most hematologic AEs were attributable to disease and improved with ibrutinib. Most non-hematologic AEs were grade 1 or 2 and consistent with prior experience and USPI. Grade 3 or 4 non-hematologic AEs reported in 2 or more patients were infection (9.3%), atrial fibrillation (5.8%), diarrhea (3.5%), rash (2.3%), and arthritis (2.3%). Infection of any grade occurred in 24 (27.9%) patients. Notably, the overall frequency of infections, irrespective of attribution, decreased with time on therapy, suggesting improvements in immune function.<sup>31</sup> Atrial fibrillation occurred in 18 (20.9%) patients; thirteen grade 1-2 events, and five grade 3 events (Table S4). The rate of atrial fibrillation was 6.4 per 100 patient-years. Three patients underwent electrical cardioversion or ablation. All patients with atrial fibrillation restarted ibrutinib; five patients had dose reduction to 280mg/day, and one patient to 140mg/day. One patient with recurrent atrial fibrillation chose to stop ibrutinib after two years on study. Seven patients received apixaban, and six patients received aspirin. No grade 3 or higher bleeding event occurred on study.

## Response

Eighty-one patients were evaluable for response at 6 months, the protocol defined primary endpoint (Table 2). Three patients were not evaluable; two had died in the first two months, and one patient developed lung cancer. ORR for all patients was 95.1% (95% CI, 87.8-98.6), 95.8% for the TP53 cohort (95% CI, 85.7-99.5), 93.9% for the elderly cohort (95% CI, 79.8-99.3) and was not significantly different between subgroups stratified by treatment history, age, bulky lymphadenopathy, splenomegaly, IGHV subgroups, and baseline  $\beta$ 2-microglobulin (all  $P > .05$ ).

Disease burden progressively decreased in all anatomic compartments (Figure S2) with time on ibrutinib. At 3 years, the median reduction was 97% in absolute lymphocyte count (ALC), 94% in bone marrow, 89% in sum of the product of target lymph nodes, and 89% in spleen volume. The cumulative complete response (CR) rate was 9.9% at 2 years, 21.0% at 3 years, and reached 28.4% at 5 years (Figure 1A). No clinical or laboratory parameter was significantly associated with different CR rates (Figure 1B). Next, we scored each of the iwCLL response criteria in each patient to investigate which criteria were not consistent with CR (Figure 1C). In order of decreasing frequency, the three most common were persistent splenomegaly (volume  $>315\text{mL}$ ) in 33 (40.7%) of 81 patients, residual lymph nodes (diameter  $\geq 1.5\text{cm}$ ) in 27 (33.3%), and persistent lymphocytosis (ALC  $\geq 4,000/\mu\text{L}$ ) in 23 (28.4%). In 36 (44.4%) patients, more than one requirement for CR was not met.

## Minimal residual disease

CLL burden, quantified by flow cytometry at 6 and 12 months and annual time-points thereafter, significantly decreased in both blood and bone marrow (Figure 2AB). Past the first year, the disease burden decreased by 33% in blood and 25% in marrow with each additional year of treatment (Figure S3). The median MRD at 3 years was  $5.3 \times 10^{-2}$  CLL cells/leukocyte in blood and  $7.3 \times 10^{-2}$  in bone marrow. The median MRD at 4 years was  $4.3 \times 10^{-2}$  CLL cells/leukocyte in blood and  $6.2 \times 10^{-2}$  in bone marrow. Measurements

in the two compartments were highly correlated (Spearman's  $\rho=.70$  at 3 years,  $.89$  at 4 years; both  $P<.0001$ ; Figure 2C-F).

Residual disease burden was similar in patients stratified by cohort or prior treatment status. However, patients with mutated IGHV had significantly more residual disease in the blood than patients with unmutated IGHV (Figure 2A). MRD levels in bone marrow were not significantly different between two IGHV subgroups (Figure 2B). MRD negativity ( $<10^{-4}$ ) was achieved in 1 of 64 patients in peripheral blood, and in 2 of 51 in bone marrow aspirate at 3 years, and 5 of 48 patients in peripheral blood, and 2 of 25 patients in bone marrow aspirate at 4 years. Seven patients in CR achieved MRD negativity in either blood or bone marrow. One patient with MRD negativity in blood at 3 and 4 years was not in CR due to persistent splenomegaly (residual spleen volume: 442mL at 4 years).

We divided patients into two subgroups based on 3-year peripheral blood MRD levels using the cutoff of  $10^{-2}$  as previously described by others: MRD-low ( $<10^{-2}$ ), and MRD-high ( $\geq 10^{-2}$ ).<sup>37</sup> Among 64 patients on ibrutinib at 3 years, 16 (25%) patients were MRD-low, 47 (75%) were MRD-high, and one patient missed MRD assessment. The CR rate was 37.5% in the MRD-low group and 21.3% in the MRD-high group ( $P=.3$ ; Figure S4). Nine (19.1%) of 47 patients in the MRD-high group, and one (6.3%) of 16 patients in the MRD-low group subsequently progressed. PFS was not statistically different between the two groups ( $P=.5$ ).

### **Disease progression and survival**

With the median follow-up of 57 months, the estimated 5-year PFS was 58.2% for the TP53 cohort (95% CI, 44.5-74.5) and 81.2% for the elderly cohort (95% CI, 67.1-98.3) ( $P=.026$ ); and the median OS was 75.7% (95% CI, 64.7-88.7) and 83.8% (95% CI, 70-100), respectively ( $P=.10$ ). In both cohorts, PFS was more favorable for patients receiving ibrutinib as a first-line therapy (Figure 3). In the TP53 cohort, the estimated 5-year PFS was 74.4% (95% CI, 60.2-92.1) for patients with TN-CLL compared to 19.4% (95% CI, 6.3-60) for those with RR-CLL ( $P=.0002$ ), and OS was 85.3% (95% CI, 74.2-

98.1) versus 53.7% (95% CI, 33.4-86.4), respectively ( $P=.023$ ). In the elderly cohort, no progression or death occurred in TN-CLL, and in RR-CLL, the estimated 5-year PFS was 64.8% (95% CI, 43.9-95.7) and OS was 71.6% (95% CI, 51.2-100). There was no statistically significant difference in PFS and OS between subgroups divided by IGHV mutation status (all  $P>.1$ , Figure S5).

Overall, 20 (23.8%) patients progressed. The median time-to-progression was 37.6 months (range, 0.4-54.7). Four (4.8%) patients had Richter's transformation and two (2.4%) had polymphocytic transformation.<sup>21,25</sup> All transformation events occurred within the first 15 months on study. Two patients with Richter's transformation never achieved a response; all others initially responded, three having achieved CR before progressing (Figure S6). Fourteen patients progressed with CLL; 12 (85.7%) of these had *BTK* and/or *PLCG2* mutations at progression.<sup>25</sup> Of nine patients maintained on dose-reduced ibrutinib, only one has progressed to date. Thirteen patients with progressive CLL received salvage therapy with venetoclax or idelalisib plus rituximab. One patient died before initiation of subsequent therapy. The estimated median survival for patients with progressive CLL was 20.5 months from the time of progression with 7 (35.0%) of 20 patients alive at data cutoff.

## DISCUSSION

In most cancers, with the notable exception of chronic myeloid leukemia, single-agent therapy is limited by the rapid emergence of drug resistance. Our data with extended follow-up of patients on ibrutinib suggests that a large proportion of CLL patients achieve durable disease control on single-agent ibrutinib with excellent tolerability. The study population is representative of the clinical spectrum of CLL comprising both previously untreated patients as well as patients relapsing after up to 7 prior regimens, with a majority of patients older than 65. However, due to the preferential inclusion of patients with *TP53* aberration, the genetic risk profile is skewed towards high-risk disease.

We previously reported 2-year PFS of 82% for the TP53 cohort.<sup>21</sup> With the median follow-up of 57 months, the estimated 5-year PFS for these patients decreased to 58.2% (95% CI, 44.5-74.5). The majority of patients progressing had both TP53 aberration and RR-CLL. Notably, the estimated 5-year PFS in the current study was 74.4% (95% CI, 60.2-92.1) for TN-CLL patients with TP53 aberration compared to 19.4% (95% CI, 6.3-60) for those with RR-CLL ( $P=0.0002$ ). These results compare favorably to those achieved with chemoimmunotherapy. In the CLL8 trial, 68% of TN-CLL patients with del(17p) achieved a response to fludarabine, cyclophosphamide, and rituximab with the median PFS of 11.3 months.<sup>4</sup> In the MD Anderson series, 23% of patients with del(17p) were refractory to first-line therapy, the median PFS was 14 months, and 23% developed Richter's transformation.<sup>38</sup> In the current study report, none of the previously untreated patients without TP53 aberration progressed on ibrutinib. Also, patients with RR-CLL without TP53 aberration did well, consistent with data from company-sponsored trials with 3-years of follow-up.<sup>39</sup> Thus, TP53 aberration and prior treatment history are important determinants of PFS on ibrutinib. These observations raise the question whether use of chemoimmunotherapy in first-line could compromise success of subsequent therapy with ibrutinib, or conceivably other targeted agents.<sup>40</sup>

Onset of response was quick, providing symptomatic relief within days, but long-term therapy was required for CRs. The most common reason for not achieving CR was residual lymphadenopathy or splenomegaly on CT scans. We routinely included bone marrow biopsies, which allowed continuous assessment of disease status in this compartment. Only in three patients were bone marrow findings responsible for not meeting CR criteria. MRD negative remissions, defined as less than  $10^{-4}$  CLL cells per leukocyte, remained uncommon. MRD-low remissions ( $<10^{-2}$ ) in the blood were observed in 25% of patients at 3 years. MRD measurements in blood and bone marrow were highly correlated. Thus, bone marrow examination seems to add little to response assessments in patients on ibrutinib.

The safety profile of continuous therapy with ibrutinib for over 5 years was similar to what has been reported with shorter treatment duration.<sup>9,10,21</sup> No new safety signals emerged and most AEs were grade 1 or 2, and transient. The most common treatment-related grade 3 or higher AEs were infection (9.3%) and atrial fibrillation (5.8%). With our extended follow-up, the cumulative incidence of any grade atrial fibrillation at 19.8% is the highest reported.<sup>30</sup> However, the rate of 6.4 per 100 patient-years in our study is consistent with the incidence of 6 per 100 patient-years reported from the pooled analysis of 4 clinical trials using ibrutinib in CLL and MCL.<sup>30</sup> One patient with atrial fibrillation chose to discontinue ibrutinib due to recurrent grade 2 events, all other patients remained on study in consultation with cardiologists. Overall, five (5.8%) patients had to discontinue therapy due to treatment-emergent AEs. The low rate of study discontinuation is notable and, in addition to the good tolerability of the drug, might reflect the high proportion of patients with *TP53* aberration who were determined to continue ibrutinib for as long as possible.

In summary, long-term administration of ibrutinib was well tolerated with deepening of responses over time. Most previously untreated patients, even those with *TP53* aberration, achieved durable responses, making intensification of therapy less urgent and avoidance of unnecessary toxicity more important. Future research should aim to identify patients at risk of early treatment failure who would benefit most from combination therapy.

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## **Author Contributions**

M.F. and A.W. designed the trial. I.A., M.F., and A.W. conducted the clinical trial, analyzed data, and wrote the report. I.A., M.F., J.V., C.S., J.L., G.M., C.S., Y.L., N.S., C.N. and G.A. evaluated patients and collected data. S.S. and P.N. provided research nurse support. S.H. provided regulatory support. M.S. and C.Y. analyzed flow cytometry. I.M. and K.C. evaluated bone marrow biopsies. T.H. provided pharmacy support. C. N., L.P., and C.G. performed and analyzed IGHV sequencing. S.P. reviewed lymph node biopsies. S.H. coordinated laboratory analysis. X.T. performed statistical analysis. R.C. and G.A. provided administrative support. All authors reviewed and approved the report.

## **Conflict of Interest Disclosures**

Pharmacyclics provided study drug and research support for the study. M.F. is employed by Merck, owns stocks and has received travel support from Merck. A.W. received research support from Pharmacyclics. N.S. received research support from Pharmacyclics. C.N. received consultancy fees and/or travel grants outside the current study from Janssen, AbbVie, Novartis, Gilead and Roche. C.G. received consultancy fees from Janssen and Celgene and research support from Novo Nordisk. There are no other conflicts of interest.

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**Table 1. Baseline characteristics**

	<b>All (n=86)</b>	<b>TP53 cohort (n=51)</b>	<b>Elderly cohort (n=35)</b>
<b>Age</b> , median year (range)	66 (33-85)	62 (33-82)	69 (63*-85)
≥ 65, N (%)	55 (64.0)	21 (41.2)	34 (97.1)*
<b>Sex</b> , N (%)			
Female	36 (41.9)	20 (39.2)	16 (45.7)
Male	50 (58.1)	31 (60.8)	19 (54.3)
<b>Prior treatment status</b> , N (%)			
Treatment-naïve	53 (61.6)	35 (68.6)	18 (51.4)
Relapsed/refractory**	33 (38.4)	16 (31.4)	17 (48.6)
<b>Rai stage</b> , N (%)			
I/II	28 (32.6)	19 (37.3)	9 (25.7)
III/IV	58 (67.4)	32 (62.7)	26 (74.3)
<b>Bulky adenopathy</b> (≥ 5 cm), N (%) <sup>#</sup>	31 (36.0)	19 (37.3)	12 (34.3)
<b>Splenomegaly</b> , N (% evaluable) <sup>###</sup>	74 (88.1)	44 (88.0)	30 (88.2)
<b>IGHV unmutated</b> , N (%) <sup>§</sup>	57 (66.3)	34 (66.7)	23 (65.7)
<b>TP53 aberration</b> , N (%)	54 (62.8)	51 (100)	0 (0)
Deletion 17p	50 (58.1)	47 (92.2)	3 (8.6) <sup>§§</sup>
TP53 mutation	4 (4.7)	4 (7.8)	0 (0)
<b>β2-microglobulin</b>			
Median mg/dL (range)	4.0 (1.7-12.9)	3.9 (1.7-12.3)	4.4 (1.9-12.9)
> 4 mg/dL, N (%)	44 (51.2)	24 (47.1)	20 (57.2)

\* One patient not meeting age requirement was removed from study.

\*\* Median number of prior therapies was 3 (range 1-7).

# Target lymph nodes and spleen were assessed with CT scans.

### Two patients had splenectomy. Normal spleen volume is less than 315 mL.

§ Unmutated IGHV indicates <2% change in IGHV sequence compared to germline.

§§ Three patients had 7-9% of nuclei with deletion 17p by FISH; inclusion criteria for the TP53 cohort was >10% of nuclei with deletion 17p.

**Table 2. Response to treatment**

	<b>All</b>	<b>TP53 cohort</b>	<b>Elderly cohort</b>
<b>N*</b>	81	48	33
<b>Response after 6 months, N (%)</b>			
<b>CR</b>	0 (0%)	0 (0%)	0 (0%)
<b>PR</b>	51 (63.0%)	27 (56.3%)	24 (72.7%)
<b>PR-L</b>	26 (32.1%)	19 (39.6%)	7 (21.2%)
<b>SD</b>	1 (1.2%)	0 (0%)	1 (3.0%)
<b>Progression</b>	3 (3.7%)**	2 (4.2%)	1 (3.0%)
<b>Best Response, N (%)</b>			
<b>CR</b>	23 (28.4%)	14 (29.2%)	9 (27.3%)
<b>PR</b>	54 (66.7%)	32 (66.7%)	22 (66.7%)
<b>PR-L</b>	2 (2.5%)	1 (2.1%)	1 (3.0%)
<b>SD</b>	0 (0%)	0 (0%)	0 (0%)
<b>Progression</b>	2 (2.5%)	1 (2.1%)	1 (3.0%)

\* Of 86 enrolled, five patients were not evaluable for response. Two patients did not meet inclusion criteria and were removed from study, two patients died within the first two months, and one patient could not be evaluated due to newly diagnosed lung cancer. Table S2 summarizes patient disposition.

\*\* Two, patients never responded; one patient achieved a partial response at 2 months and progressed (compared to 2-month nadir) at 6 months.

### Abbreviations

CR Complete response

PR Partial response

PR-L Partial response with lymphocytosis

SD Stable disease

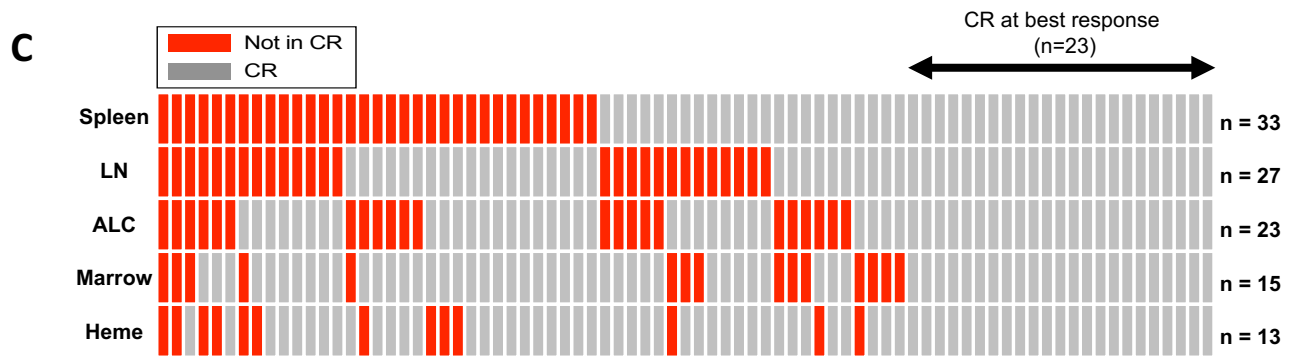
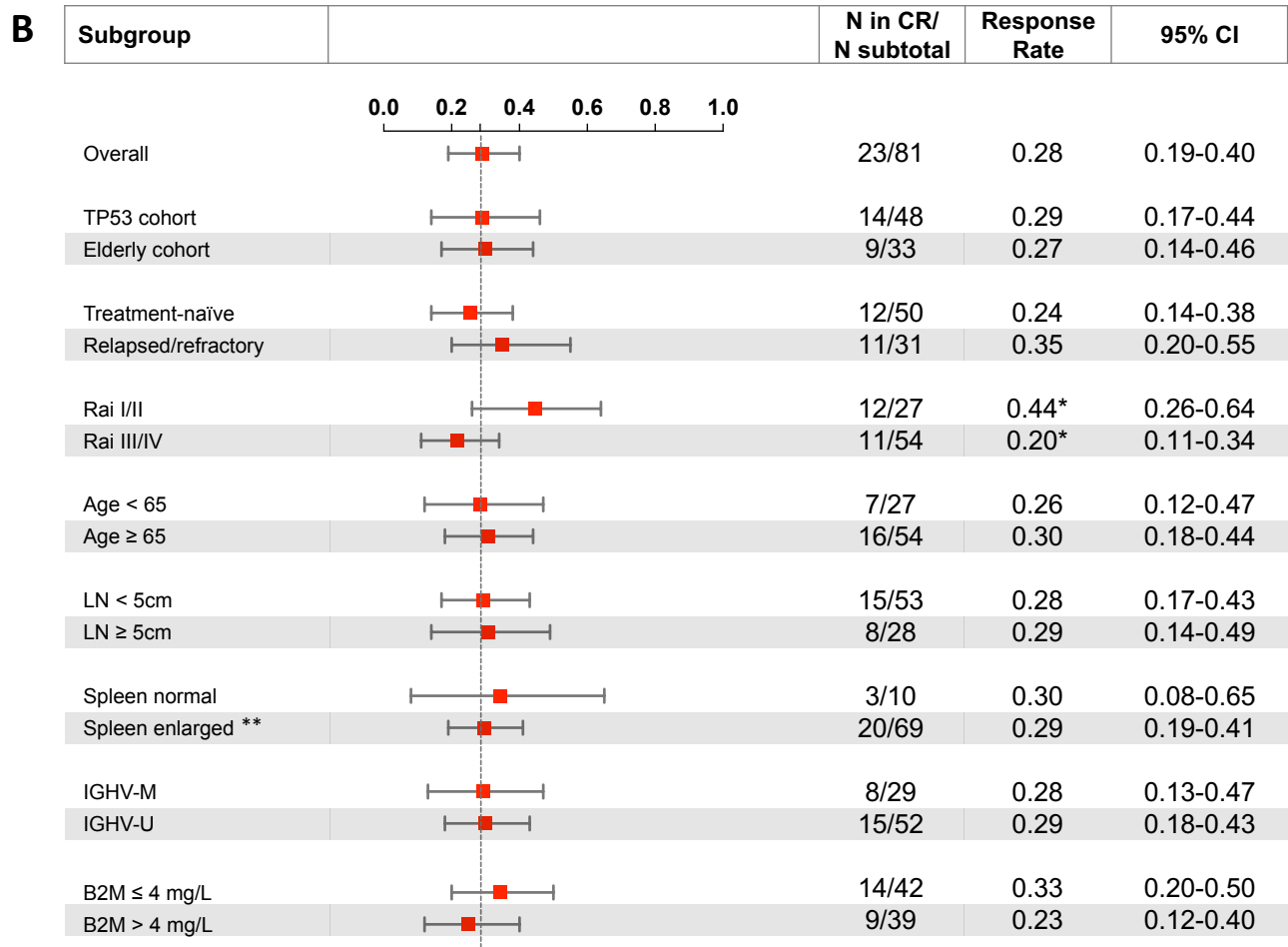
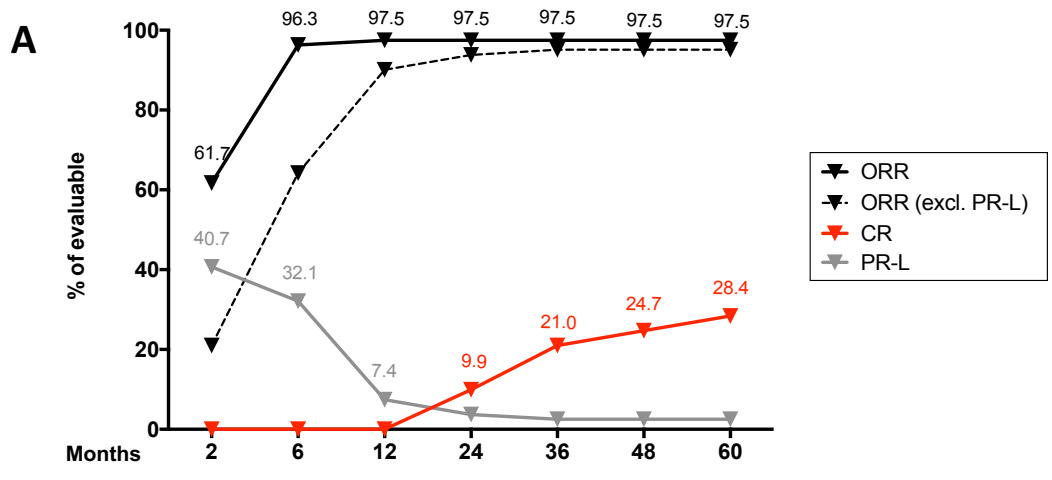
## FIGURE LEGENDS

**Figure 1. Complete response on ibrutinib.** (A) Cumulative best response in 81 evaluable patients; objective response rate (ORR), partial response with lymphocytosis (PR-L), complete response (CR). (B) Forest plot showing CR rates at best response in subgroups divided by clinical and laboratory criteria; the dashed line marks the CR rate in all patients (23.5%). Whiskers indicate 95% confidence interval; \* denotes a trend in CR rate difference between low and advanced Rai stages ( $P=.054$ ). \*\* Spleen size was not evaluated in two patients with history of splenectomy. (C) Status of CR criteria in 79 patients; spleen (volume assessed by CT), target lymph nodes (LN), absolute lymphocyte count (ALC), bone marrow infiltration and cellularity (Marrow), and hematologic recovery (Heme). 23 (28%) patients met all CR criteria (grey blocks), red blocks mark criteria not met. IGHV-M, immunoglobulin heavy-chain variable region gene (IGHV) mutated; IGHV-U, IGHV unmutated; B2M,  $\beta$ 2-microglobulin.

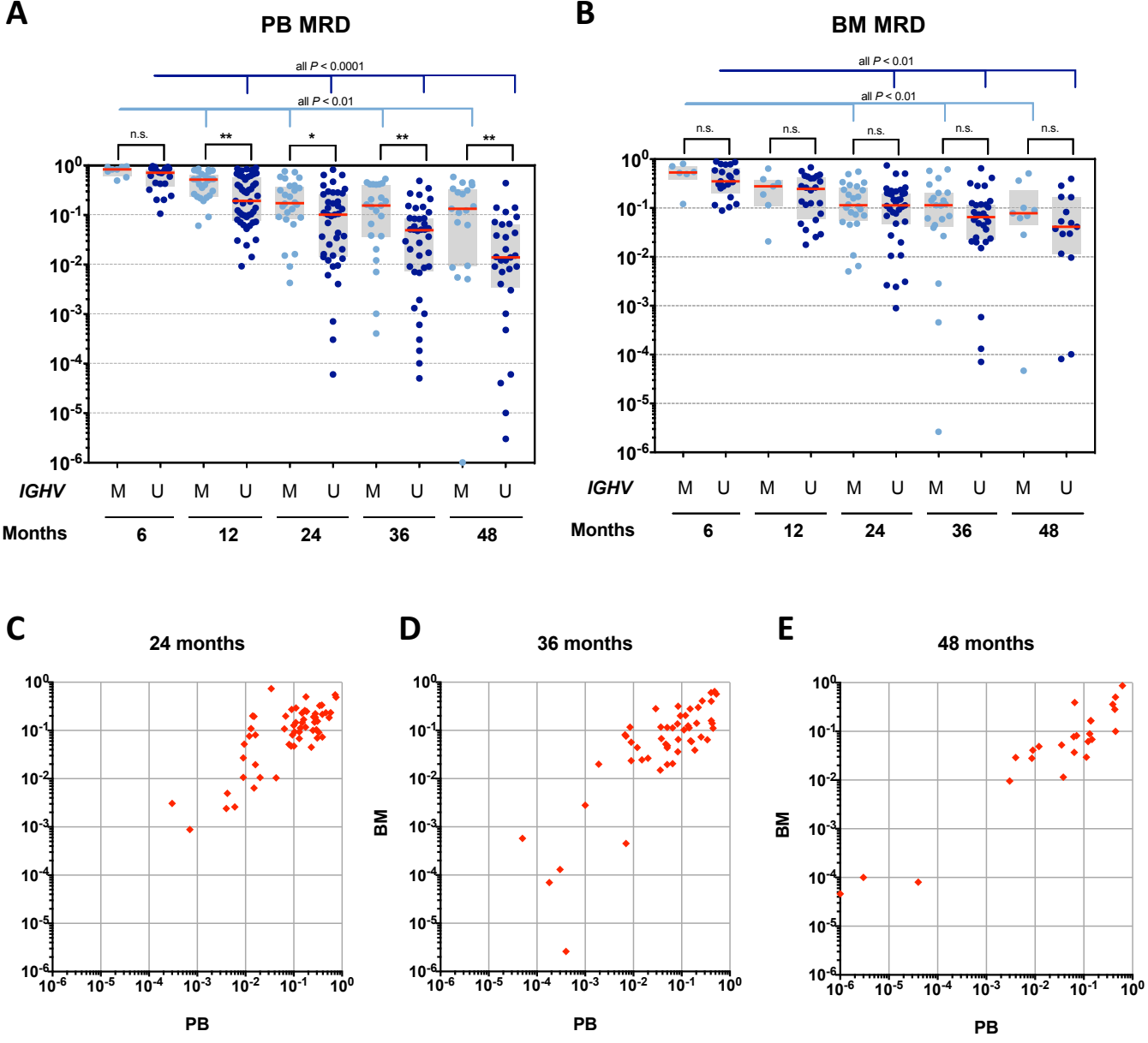
**Figure 2. Minimal residual disease assessment in blood and bone marrow.** (A,B) Minimal residual disease (MRD) was measured by flow cytometry in peripheral blood (PB) and bone marrow (BM). One patient who had undetectable PB MRD at 4 years was plotted at the lowest margin of detection ( $10^{-6}$ ). Patients were stratified by IGHV subgroups; M for mutated, U for unmutated. Red lines indicate median, boxes interquartile ranges. \*  $P<.05$ , \*\*  $P<.01$ , n.s. not significant. (C,D,E) PB and BM MRD in matched samples at 24, 36, and 48 months. (F) Correlation between PB and BM MRD measurements.

**Figure 3. Progression-free survival (PFS) and overall survival (OS).** Kaplan-Meier estimates of PFS and OS of all patients on study (A, B), and by cohort and treatment status (C, D and E, F, respectively). (C, D) for the TP53 cohort and (E, F) for the elderly cohort. B. TN, treatment-naïve CLL; RR, relapsed and/or refractory CLL.

# Figure 1



**Figure 2**

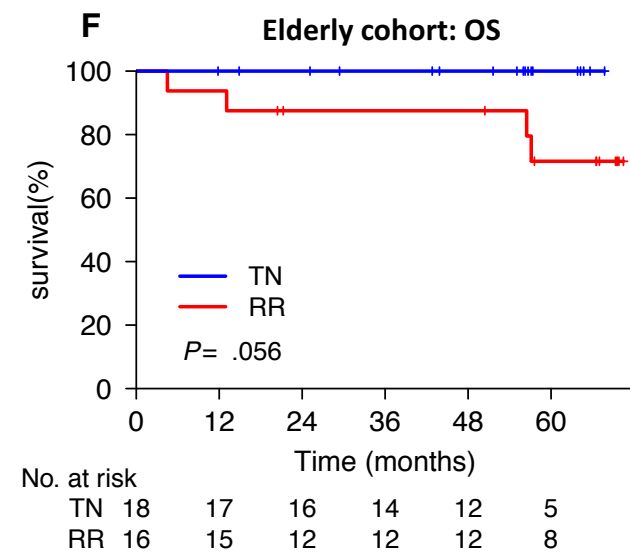
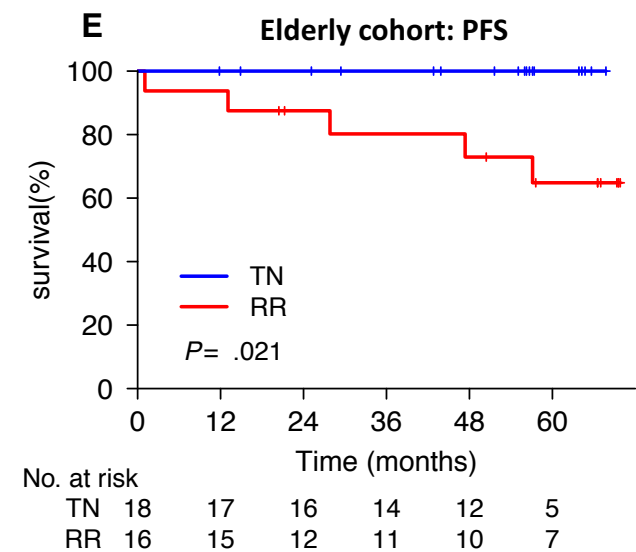
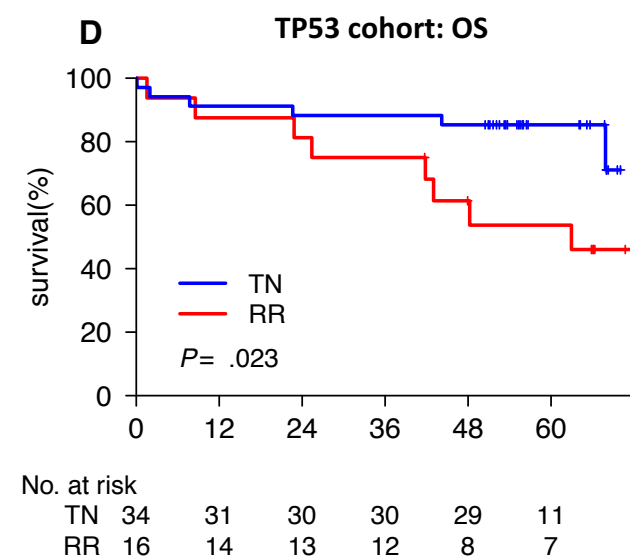
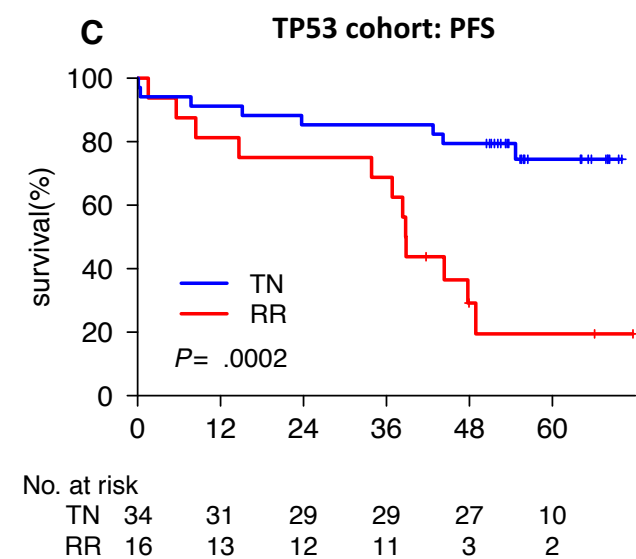
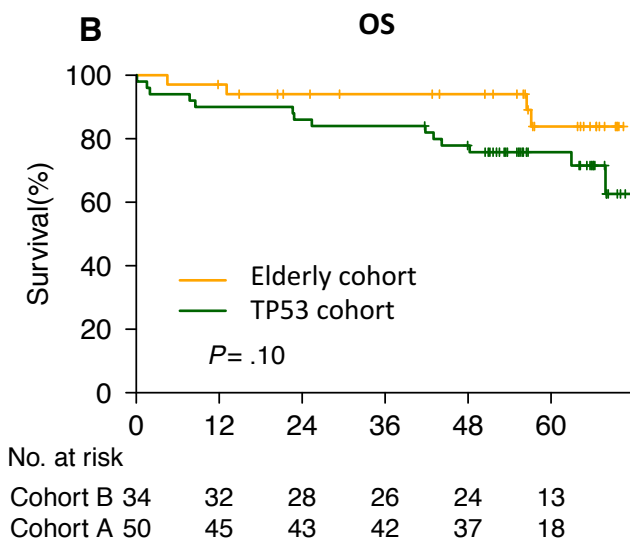
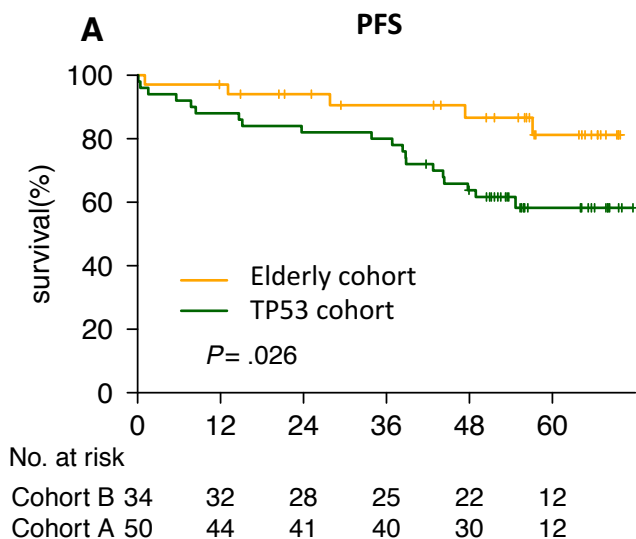


**F**

Months	24	36	48
PB & BM pairs	58	51	26
Spearman R	0.62	0.70	0.89
<i>P</i>	<0.0001	<0.0001	<0.0001



**Figure 3**





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## **Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase II study**

Inhye E. Ahn, Mohammed Z.H. Farooqui, Xin Tian, Janet Valdez, Clare Sun, Susan Soto, Jennifer Lotter, Stephanie Housel, Maryalice Stetler-Stevenson, Constance M. Yuan, Irina Maric, Katherine R. Calvo, Pia Nierman, Thomas E. Hughes, Nakhle S. Saba, Gerald E. Marti, Stefania Pittaluga, Sarah E.M. Herman, Carsten U. Niemann, Lone B. Pedersen, Christian H. Geisler, Richard Childs, Georg Aue and Adrian Wiestner

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