Treatment with ibrutinib does not induce a TP53 clonal evolution in chronic lymphocytic leukemia

Ibrutinib is active both in treatment-naïve (TN) and relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) patients, including those with unmutated immunoglobulin heavy chain variable region (IGHV) genes and TP53 disruption.1-3 The acquisition of BTK or PLCγ2 gene mutations conferring resistance, supports the existence of clonal evolution also under ibrutinib treatment.4,5 Whilst it is well described that subclonal TP53 mutations undergo a positive clonal selection following treatment failure, recent studies have suggested that this might not be the case under ibrutinib.6

In order to investigate the dynamics of major and minor TP53 mutations under ibrutinib treatment, we performed longitudinal TP53 monitoring by deep-sequencing in CLL-treated patients. Two cohorts were included: 44 TN and 14 R/R patients. A total of 216 peripheral blood (PB) samples in TN and 52 in R/R were collected at baseline and at subsequent time points during therapy. Among TN patients, 28 were males and 16 females, with a median age of 72 years (range, 54-87); they received ibrutinib plus rituximab (GIMEMA trial LLC1114), with a median ibrutinib exposure of 2.7 years (range, 1.2-3.7) and were evaluated at 6-month intervals for a median number of 5 time points (range, 3-8). R/R patients, nine males and five females, with a median age of 71 years (range, 55-80), received ibrutinib single agent after a median of 1.5 years (range, 1-4) CIT lines; they were evaluated at disease progression (PD) before each line of CIT and after ibrutinib treatment (median: 4 time points; range, 2-6), with 2.5 years ibrutinib exposure (range, 2.1-3.3). Amplicon libraries, covering the entire coding region and splice sites of TP53 gene (exon 2 to 11), were prepared according to the TruSeq Custom Amplicon Low Input Library Prep kit protocol (Illumina, San Diego, CA, USA) and paired-end sequenced on a Miseq Sequencer (Illumina). A mean coverage of 8,956x was obtained; across the target region a coverage >5,000x was obtained in >80% of the sequence in 70% of the samples. Bioinformatic analysis was performed by MiSeq Reporter (Illumina) and an in-house bioinformatics pipeline. A total concordance was observed for the variants with variant allele frequency (VAF) ≥3%; variants with 1%-3% VAF were manually checked on alignment files resulting from MiSeq Reporter analysis of the two DNA strands, using Integrative Genomics Viewer. Variants were manually curated according to the International Agency for Research on Cancer TP53 database (http://p53.iarc.fr/). Validate polymorphism, synonymous variants and variants mapping ≥2 bp outside coding exons were filtered out.

Mutations were defined major if VAF was >10% and minor if ≤10%; the latter were confirmed in an independent deep-sequencing run. VAF was corrected to cancer cell fraction (CCF) by the proportion of CD19+/CD5+ cells in each sample, assessed by flow cytometry. The limit of detection (LOD) was 1% (Online Supplementary Figure S1). A mutation was considered stable when the log fold-change (Log2FC) of CCF values before and after ibrutinib treatment was included between -0.5 and 0.5, decreasing or increasing if the Log2FC was <0.5 or >0.5, respectively.

Among the 44 TN patients at baseline (T0), 27 cases (61%) resulted TP53 wild-type (WT) and 17 (39%) mutated by deep-sequencing analysis. Nine of 43 (21%) carried the del(17p) and 30 (68%) showed unmutated TP53.

Twenty-three TP53 mutations (1.4 mutation/patient; range, 1-5) were identified: 17 (74%) were major (mean VAF 58.8%; range, 18-94.8) and six (26%) minor (mean VAF 5.3%; range, 2.1-9.2). Thirteen patients carried a sole major TP53 mutation; two cases (cases #10025, #10875) showed co-existing major and minor mutations; two cases (#9915, #10671) showed one minor mutation each and 27 none (Online Supplementary Table S1).

According to the CCF, after 2.7 years from ibrutinib treatment, nine of 23 (39%) major mutations decreased, eight of 23 (35%) major mutations persisted stable, four of 23 (17%) (1 major and 3 minor) were undetectable and two of 23 (9%) minor mutations increased (Figure 1A and B; Table 1, Online Supplementary Table S1). Novel TP53 mutations emerged during treatment neither in TP53 mutated nor WT patients.

Thus, TP53 mutated TN patients followed two main patterns: i) major TP53 mutations persisting major from T0 with a stable CCF (6 patients); ii) major TP53 mutations persisting major from T0 with a decreasing CCF (7 patients).

Among TN patients, seven TP53 mutated and 11 WT with a measurable PB residual disease (CD19+/CD5+ >20%) at T20 (n=3), T26 (n=2), T32 (n=7), T38 (n=4) and T44 (n=2), were analyzed by next-generation sequencing for BTK and PLCγ2 mutations and resulted WT for both

| Table 1. Dynamics of TP53 mutations in treatment-naïve under ibrutinib and relapsed/refractory patients under ibrutinib and chemoimmunotherapy. |
|---|
| | Undetectable | Decreasing | Stable | Increasing | Novel |
| TN patients | 4/23 (17.5%) | 9/23 | 8/23 | 2/23 (9%) | 0 |
| R/R patients, ibrutinib phase | 11/31 (35%) | 8/31 | 8/31 | 4/31 (13%) | 2 |
| R/R patients, CIT phase* | 0/29 (10%) | 3/29 | 6/29 | 7/29 (24%) | 13/29 (45%) |

*13 evaluable cases. TN: treatment-naïve; R/R: relapsed/refractory; CIT: chemoimmunotherapy.
Figure 1. Dynamics of TP53 mutations under ibrutinib therapy in treatment-naïve patients. (A) Each line corresponds to the cancer cell fraction (CCF) of each mutation. Dashed lines represent the median CCF. (B) Heatmap with the CCF log2-fold-change (Log2FC) for each mutation. Color code indicates the trend of TP53 mutation over time: decrease (<-0.5), increase (>1), stable (-0.5 and 1). (C) Dynamics of TP53 mutations in the 2 treatment-naïve cases with co-existing major and minor TP53 mutations at time zero (T0).
Among the 14 R/R patients, prior to ibrutinib treatment four patients (29%) resulted TP53 WT and ten (71%) mutated by deep-sequencing analysis. Four of 11 (37%) carried the del(17p) and seven (50%) showed unmutated IGHV. Thirty-one TP53 mutations (31 mutations patients, range, 1-11) were identified: 11 (35.5%) were major (mean VAF 31.9%; range, 10.5-78.8) and 20 (64.5%) minor (mean VAF 2.9%; range 1-6.8). Five patients carried one or two major mutations (#8271, #5708, #3547, #9225, #7458), three cases showed a complex mutational architecture (#5717, #8353 and #3425); two patients showed only minor mutations (3 in #3546 and 11 in #8540, respectively); four patients had no mutations (Online Supplementary Table S2). As expected, before ibrutinib, the TP53 mutational load of R/R patients was higher and more complex than that of TN patients. In the R/R patients, eight of 31 (26%) TP53 mutation (4 major and 4 minor) decreased, eight of 31 (26%) (4 major and 4 minor) persisted stable, 11 of 31 (35%) (2 major and 9 minor) were undetectable, four of 31 (13%) (2 major in #7458 and #8353; 2 minor in #3546) increased in three patients, and two novel minor mutations emerged in two cases already TP53-mutated prior to ibrutinib treatment (cases #5717, #3425) (Table 1; Online Supplementary Table S2). No novel TP53 mutations arose in TP53 WT patients over time under ibrutinib. In R/R patients, because of the more complex mutational profile of each patient we could not identify patient-related patterns, rather we documented different dynamics of different TP53 mutations within the same patient (Online Supplementary Table S2), possibly due to a different sensitivity to the drug, as suggested. Among R/R patients, four TP53-mutated and two WT continued ibrutinib (#8540, #6856, #3547, #9225, #5717, #6123), four discontinued ibrutinib for adverse events (#5708, #8353, #7458, #3380), one shifted to venetoclax (#8425), three died (#3546, #8991, #8271).

No significant changes were observed in the mean CCF of TP53 mutations before and after ibrutinib: 60.7% versus 43.1% and 20.5% versus 20.2%, in TN and R/R patients, respectively. On the contrary, the lymphocytosis count decreased significantly after ibrutinib treatment in TP53-mutated patients from both cohorts: from 40.7x10^9/L (range: 4.9-132.2x10^9/L) to 11.2x10^9/L (range, 1.2-135.7x10^9/L) (P=0.018, Mann-Whitney test) and from 39.7x10^9/L (range, 1.5-99.0x10^9/L) to 7.1x10^9/L (range, 1.4-18.9x10^9/L) (P=0.034), respectively. The decrease in lymphocytosis in the presence of a stable TP53 mutational CCF proves the effectiveness of ibrutinib both on TP53-mutated and WT CLL cells, regardless of previous therapies, at least during the first years of treatment.

In 13 of the 14 R/R patients, TP53 mutations were retrospectively evaluated by deep sequencing also before each line of CIT. At the first evaluated time point, three patients were mutated (2 with minor and 1 with one major mutation) and ten resulted WT; of the latter, six acquired major or minor TP53 mutations over time. Overall, among the nine mutated cases, 29 mutations were identified with the following dynamics: 13 (45%) (3 major and 10 minor) novel mutations emerged, seven (24%) minor mutations increased, six (21%) (3 major and 3 minor) persisted stable and three (10%) (1 major and 2 minor) decreased. The decrease in CCF for the latter was from 84.44% to 46%, from 3.3% to 2.01% and from 4.69% to 1.83%, respectively. No mutation was undetectable. While the increased and novel mutations were significantly more common during the CIT phase (20/29 vs. 6/33, during CIT and ibrutinib, respectively; P<0.0001, Fisher’s exact test), the decreased and undetectable mutations were more frequent under ibrutinib treatment (3/29 vs. 19/33, during CIT and ibrutinib, respectively; P<0.0001) (Table 1).

In the present study, in TP53-mutated TN and R/R CLL patients, ibrutinib appears to decrease the major and minor mutations’ numerosity and complexity, since most mutations decreased (39% and 24%) or were undetectable (17% and 34%) and one third of mutations remained stable. On the other hand, a small proportion of TP53 mutations (9%, 2 minor, in TN; 13%, 2 major and 2 minor, in R/R cases) increased in CCF under ibrutinib treatment, although without clear clinical consequences with the current follow-up. We observed no association between the dynamics of TP53 mutations and the type of mutation, or the exon involved, neither the type of karyotype (data not shown) nor the presence of del(17p) (8 TN with vs. 9 without del(17p), decreased/undetectable vs. increased/novel mutations, P=0.46; 4 R/R with vs. 3 without del(17p), P=1 at Fisher’s exact test).

With a prolonged follow-up of more than 2 years, up to 44 months, our data add to the initial findings of a general stability of TP53 subclones over the early treatment period and support the notion that there is no specific positive selection of TP53 mutations under ibrutinib.7,14 Emergence of novel mutations proved exceptional mechanisms of cell fitness control in addition to the BCR pathway,7 that can make the difference over time.

In conclusion, in TP53-mutated CLL patients ibrutinib in any line of therapy decreases the TP53 complexity at least within the first years of treatment and it does not exert a positive selective pressure on pre-existing TP53 mutated clones, unlike CIT. In TP53 WT patients, ibrutinib never induced the emergence of novel TP53 mutations after >2 years of exposure. These findings reinforce a broader use of a BTK inhibitor rather than CIT in the management of CLL, particularly for patients with an unfavorable genetic profile or with R/R disease.

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References

1. O’Brien S, Furman RR, Coutre S, et al. Single-agent ibrutinib in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. Blood. 2018;131(17):1910-1919.
2. Murir T, Brown JR, O’Brien S, et al. Final analysis from RESONATE-2 study. Blood. 2020;134(9):787-796.
3. Woyach JA, Bumpert AS, Guine D, et al. BTK/C48I-mediated resistance to ibrutinib in chronic lymphocytic leukemia. J Clin Oncol. 2017;35(13):1437-1443.
4. Burger JA, Landau DA, Taylor-Weiner A, et al. Clonal evolution in patients with chronic lymphocytic leukemia developing resistance to BTK inhibition. Nat. Commun. 2016;7:11589.
5. Ahn IE, Underbayev C, Albitar A, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood. 2017;129(11):1469-1479.
6. Landau DA, Sun C, Rosebrock D, et al. The evolutionary landscape of chronic lymphocytic leukemia treated with ibrutinib targeted therapy. Nat Commun. 2017;8(1):2185.
7. Kanagal-Shamanna R, Jain P, Patel KP, et al. Targeted multigene deep sequencing of Bruton tyrosine kinase inhibitor-resistant chronic lymphocytic leukemia with disease progression and Richter transformation. Cancer. 2019;125(4):559-574.
8. Rossi D, Khibabian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. Blood. 2014;123(14):2139-2147.
9. Malcikova J, Stano-Kozubik K, Tichy B, et al. Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia. Leukemia. 2015;29(4):877-885.
10. Nadeu F, Ortega J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood. 2016;127(17):2122-2130.
11. Kadi S, Lee J, Fitzpatrick C, et al. Clonal evolution underlying leukemia progression and Richter transformation in patients with ibrutinib-relapsed CLL. Blood Adv. 2017;1(12):715-727.
12. Gängò A, Alpár D, Galik B, et al. Dissection of subclonal evolution by temporal mutation profiling in chronic lymphocytic leukemia patients treated with ibrutinib. Int J Cancer. 2020;146(1):83-93.
13. Guarini A, Peragine N, Messina M, et al. Unravelling the suboptimal response of TP53-mutated chronic lymphocytic leukemia to ibrutinib. Br J Haematol. 2019;184(3):392-396.