Ibrutinib and rituximab versus fludarabine, cyclophosphamide, and rituximab for patients in FLAIR Study with previously untreated chronic lymphocytic leukaemia: Assessment of mutational landscape of patients at baseline and prognostic impact

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Introduction: Recent studies using next generation sequencing (NGS) have revealed recurrent mutated genes in chronic lymphocytic leukaemia (CLL) that have been associated with clinical outcome. In this study we assessed the baseline mutational profile of 771 FLAIR patients treated with either fludarabine, cyclophosphamide and rituximab (FCR) or ibrutinib and rituximab (IR). The prognostic impact of individual gene mutations on disease progression was investigated.

Method: FLAIR is an ongoing, phase III, multicentre, randomised, controlled, open, parallel group trial for previously untreated CLL requiring therapy according to International Workshop on CLL criteria. Patients with >20% chromosome 17p deletion were excluded from recruitment. A total of 771 participants were randomised on a 1:1 basis to receive standard therapy with FCR or IR. Somatic hypermutation (SHM) status was determined by PCR amplification of IGHV-IGHD-IGHJ gene rearrangements using IGH VH leader/FR1 primers. Bidirectional Sanger sequencing was analysed using IMGT V-Quest and the ARResT/AssignSubsets tool. Extracted DNA was sequenced using an Illumina MiSeq and analysed using an in-house pipeline. Amplicon based targeted sequencing of 33 recurrently mutated genes in lymphoid malignancies were performed in parallel. Detected variants were reported down to minimum variant allele fractions of 3-5% and coverage of 100X. Low level variants were confirmed by repeat sequencing.

Results: Of 1924 patients assessed for eligibility, 771 were randomly assigned to receive FCR (385) or IR (386). IGHV gene SHM status was available for 738/771 patients (94.4%) of which 388 (50%) were IGHV unmutated, 294 (38%) IGHV mutated and 46 (6%) Subset 2 (28 were IGHV mutated and 18 IGHV mutated). Gene mutations were assessed in 767/771 (99.5%) patients at baseline. Gene mutations ($\geq$1) were detected in 480/767 (62.6%) patients. Mutation frequencies ranged from 0.1-18.8% with mutations in SF3B1 (18.8%), ATM (14.5%), NOTCH1 (10.0%), MYD88 (6.1%), POT1 (5.7%), BRAF (4.4%) and RPS15 (4.0%) being the most frequent. Patients with no detectable mutations at baseline did not show a significant difference in PFS or OS compared to patients with $\geq$1 mutation.

Of the recurrent gene mutations commonly associated with a poorer prognosis in CLL, a significant shorter PFS and OS was observed in our TP53 mutated patient cohort at baseline compared to wildtype (HR 2.23 [95% CI 1.31-3.79]; p=0.003 and HR 2.43 [95% CI 1.04-5.66]; p=0.039 respectively). When patients were further subdivided by treatment arms, a trend for shorter PFS was observed in TP53 mutated patients in both arms compared to wildtype, but significance was only achieved for FCR (HR 2.48 [95% CI 1.25-4.91]; p=0.009) and not IR (HR 2.27 [95% CI 0.97-5.30]; p=0.059) treated patients. A significantly shorter PFS was also observed for FCR compared to IR treated patients who had ATM (HR 0.35 [95% CI 0.16-0.80]; p=0.012) and RSP15 (HR 0.19 [95% CI 0.04-0.96]; p=0.044) gene mutations at baseline. As previously described in other studies, a trend for shorter PFS was also observed in NOTCH1 mutated FCR treated patients however, this was not found to be significant when compared to wildtype or NOTCH1 mutated IR treated patients.

Assessment of PFS and OS for some of the less frequently mutated genes in our panel revealed that patients with CREBBP mutations at baseline (n=19; 2.5%) were associated with shorter PFS (HR 2.63 [95% CI 1.29-5.35]; p=0.008) and OS (HR 4.37 [95% CI 1.75-10.94]; p=0.002) compared to wild type. When further subdivided by treatment arm, a shorter PFS and OS was also observed for CREBBP mutated patients treated with FCR compared to wildtype (HR 4.37 [95% CI 1.75-10.94]; p=0.002 and HR 10.14 [95% CI 3.83-26.83]; p<0.001). A similar trend was not observed in IR treated patients (PFS HR 0.79 [CI 0.11-5.74]; p=0.819 and OS HR NE [95% CI NE-NE]; p=0.988). A shorter PFS was however observed between the CREBBP mutated FCR and IR treated patients (HR 0.1 [95% CI 0.01-0.86]; p=0.036).

The PFS and OS for IGHV subset 2 was similar to IGHV mutated patients. In FCR treated patients there was no difference between mutated and unmutated subset-2 but with IR there was a trend that IGHV mutated subset 2 patients had better PFS and OS than their IGHV mutated counterparts.

Conclusions: Our study confirms the effect of TP53 mutations on shorter PFS and OS and demonstrates an improved PFS when patients with ATM or RPS15 mutations are treated with IR compared to FCR. Mutations in the CREBBP gene have recently been described as a novel candidate driver in CLL. In this study we report a significant improvement in disease progression in CREBBP mutated

patients treated with IR when compared to those treated with FCR. In this trial IGHV subset 2 patients had a similar outcome to IGHV mutated patients.