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POSTER ABSTRACTS

642.CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND EPIDEMIOLOGICAL

Molecular Analysis at Relapse of Patients Treated on the Ibrutinib and Rituximab Arm of the National Multi-Centre Phase III FLAIR Study in Previously Untreated CLL Patients

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Background

The NCRI FLAIR trial confirmed ibrutinib + rituximab (IR) improved progression free survival (PFS) over FCR in untreated CLL. Durable responses are achieved on continuous Bruton's tyrosine kinase inhibitor (BTKi) therapy, but acquired resistance mutations in *BTK*, and *PLCG2* are reported in up to 80% of relapsed/refractory patients (pts) failing BTKi often detected at low variant allele frequencies (VAF) several months before relapse. Their frequency, evolutionary dynamics, and relevance to progression in the upfront-treatment setting are poorly characterised. We present the observed clonal evolution in standard risk pts with disease progression (DP) on IR, integrating next generation sequencing (NGS) with serial measurable disease monitoring by flow cytometry, and tracking individual *BTK* mutations (*BTK* ^{mt}) by digital droplet PCR (ddPCR) from initial detection to DP.

Methods

771 randomised pts to FCR (n=385) or IR (n=386). Pts treated with IR received rituximab at 375 mg/m ^{2} on D1/C1 and 500 mg/m ^{2} on D1/C 2-6 of every 28-days. I was given orally at 420 mg/day for up to 72 mo. All 61 DP pts on IR were included in the analysis. DNA from baseline and progression peripheral blood (PB) samples were processed for sequencing of 33 genes recurrently mutated in lymphoid malignancies including *BTK* (exon 15) and *PLCG2* (exons 19-20) with an Illumina MiSeq. Variants were reported down to minimum VAF of 3-5% and coverage of 100X. Low VAF (3-6%) *BTK* ^{mt} were validated by ddPCR using the QX200 ddPCR protocol (sensitivity 1-0.1%). ddPCR was used for the individual serial analysis at baseline and 6 monthly in pts with confirmed *BTK*hot spot mutations (p.C481S (c.1441T>A, c.1442G>C)), p.C481R (c.1441T>C), p.T474I (c.1421C>T) and presented with concurrent PB MRD monitoring using highly sensitive flow cytometry with a detection limit of one CLL cell in 100,000leukocytes (0·001%).

Results

61/386 pts progressed after a median follow up of 44 mo. on I with 50/61 pts progressing after >24 mo. No deaths were recorded before DP. 47/61 IR DP samples were sequenced by MiSeq, 10 further samples with low disease burden were only analysed by ddPCR for hotspot *BTK* ^{mt}. 7/47 sequenced at baseline and DP, developed new isolated mutations in *ATM, SF3B1, NOTCH1, BIRC3, BRAF, KLF2, CDKNB1 or TRAF2*. The most frequently mutated gene at DP was *BTK*. From 57/61 sequenced samples at DP, 7 were found to have hotspot *BTK* ^{mt} most with multiple mutations (Fig/Table). No *BTK* ^{mt} detected at DP were detected at baseline by NGS or ddPCR. One pt had a *PLCG2* mutation at DP (VAF 33.3%). The 8 *BTK* ^{mt}/ *PLCG2* ^{mt} IR

progressions were mostly in late DP pts (median 60.3 mo.) compared to a median time to DP of 54.3 mo in *BTK* ^{wt}/ *PLCG2* ^{wt} pts. The majority of *BTK* ^{mt} IR DP (5/7) were reported after stopping therapy at 72 mo. 6 monthly samples during I were studied for *BTK* ^{mt}. Evolution of *BTK* ^{mt} in the context of increasing disease burden on I, shows a steady rise in the VAF preceding frank DP (Fig). 148/386 (38.3%) IR pts were 100% homologous to germline *IGHV* unmutated and 31/148 (20.9%) progressed. 12/15 pts, who acquired additional mutations at DP were *IGHV* unmutated and 9/12 were 100% homologous to germline including 6/8 with acquired *BTK/PLCG2* mutations.

Conclusion

In upfront IR treated standard risk CLL pts 15.8% progressed (61/386), the majority late. Only 13% of the IR DP cohort (8/61) had a *BTK/PLCG2* mutation, suggesting other non- *BTK/PLCG2* mutations or disease related factors as contributors to DP after initial response in the upfront setting. Interestingly, the background mutation profile did not change from baseline in those who acquired *BTK/PLCG2* mutations at DP, but 7/15 acquired non- *BTK/ PLCG2*mutations at DP. The majority of IR DP with *BTK/PLCG2* mutations (6/8), were 100% homologous to germline *IGHV* unmutated, suggesting an inherent higher risk disease profile as a risk factor for acquiring *BTK* ^{mt} with prolonged BTKi therapy. *BTK/PLCG2* mutations were enriched amongst very late progressors with 6/8 pts progressing shortly after stopping therapy at 72 mo., which is later than previously reported for relapsed/refractory CLL pts.

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Table: *BTK*^{mt} patient genetic characteristics at baseline and progression

Patient number	Sex	Variants at baseline	IGHV status	Variants at progression	BTKmt	p.Cys481Ser (c.1441T>A) MiSeq VAF%/ddPCR	p.Cys481Ser (c.1442G>C) MiSeq VAF%/ddPCR	p.Cys481Arg (c.1441T>C) MiSeqVAF%/ddPCR	p.Thr474Ile (c.1421C>T) MiSeq VAF%/ddPCR
1	M	**SF3B1**	Mutated	**BTK**/SF3B1	p.Cys481Ser (c.1441T>A)	31.7	-	-	-
2	M	**NOTCH1**	Unmutated	**BTK**/NOTCH1	p.Cys481Ser (c.1442G>C)	-	6.8	-	-
3	M	**SF3B1**	Mutated	**BTK**/SF3B1	p.Cys481Arg (c.1441T>C)			18.2	
4	M		Unmutated	**BTK x2**	p.Cys481Ser (c.1442G>C) / p.Cys481Ser (c.1441T>A)	3.2/3.47	7.9/6.16	-	-
5	M	**SF3B1**	Unmutated	**BTK x2** /TP53 /SF3B1	p.Cys481Ser x2 (c.1442G>C and c.1441T>A)	49.5	18.7	-	-
6	F	**ATM / ATM / MYD88 / POT1**	Unmutated	**BTK x3**/ATM	p.Cys481Ser (c.1441T>A) / p.Thr474Ile (c.1421C>T) / p.Cys481Arg (c.1441T>C)	11	-	5.1/3.18	6.9/3.49
7	F	**ATM**	Unmutated	**BTK x3** /ATM	p.Cys481Arg (c.1441T>C) / p.Cys481Ser (c.1442G>C and c.1441T>A)	19	4.7	32	-

Figure: BTK kinase domain mutation variants and distribution at disease progression

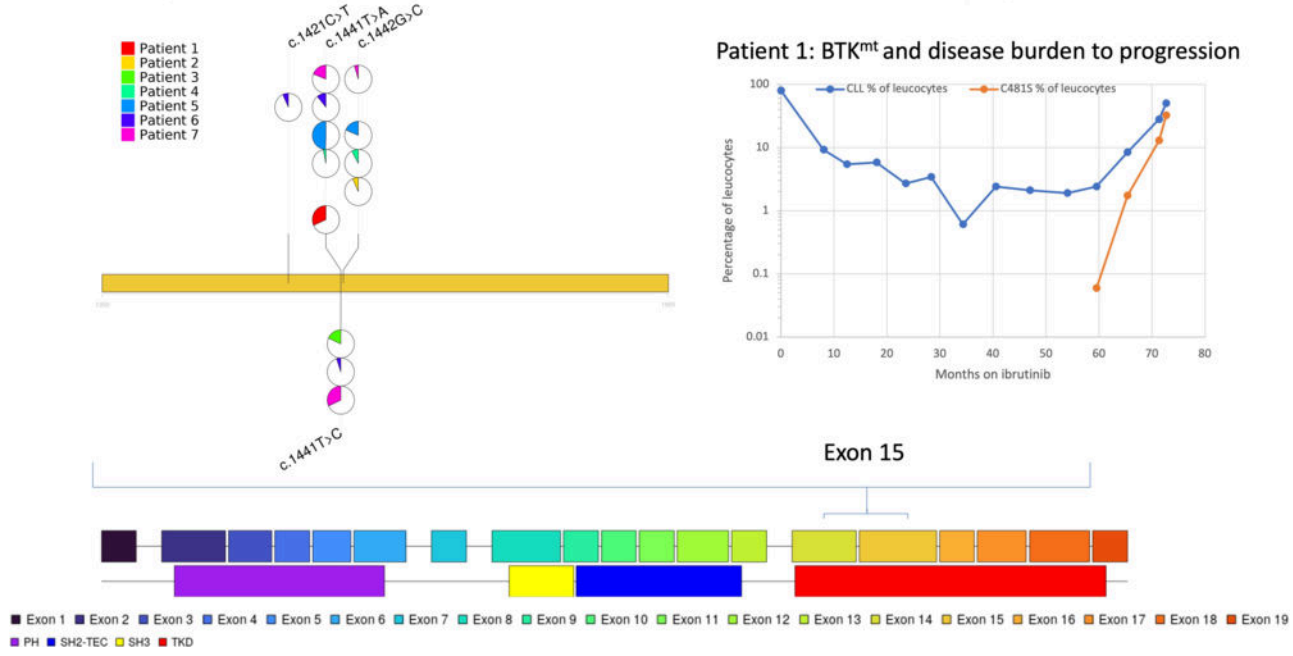

Figure 1

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