Background: Mutations in the BCR-ABL1 kinase domain (KD) are frequently detected in Philadelphia-positive (Ph+) Acute Lymphoblastic Leukemia (ALL) patients (pts) who are refractory or resistant to tyrosine kinase inhibitor (TKI) therapy. Emergence of mutant clones as early as during induction therapy supports the hypothesis that, at least in some cases, TKI therapy may already be present at diagnosis. Routinely, Sanger sequencing (SS) is used for mutation screening but Next Generation Sequencing (NGS) may have considerable advantages.

Aims: We aimed to assess the feasibility and informativity of NGS as compared to routine BCR-ABL1 KD mutation screening of a prospective series of de novo and TKI-resistant Ph+ ALL leukemia patients (MutationALL study).

Methods: Between May 2015 and February 2018, we used NGS in parallel to analyze a consecutive series of 160 Ph+ ALL pts who were either newly diagnosed (n = 44) or had relapsed/refractory disease on TKI therapy (n = 116). NGS of ≥400 bp amplicons generated by nested RT-PCR was performed on a Roche GS Junior (until April 2017) or on an Illumina MiSeq (from May 2017 on). Read alignment and variant calling was done using the AmpSuite software (Smartseq srl), with a lower detection limit set to 3%.

Results: De novo pts positive for mutations were 0/44 by SS and 3/44 (7%) by NGS. All the 3 pts received TKIs effective against the low level mutations they had and achieved remission. Relapsed/refractory pts positive for mutations were 32/40 (80%) by SS and 29/40 (73%) by NGS. NGS identified low level mutations in 18 pts who were negative for mutations by SS. All of them had minimal residual disease (MRD)-positivity to 1st- (n = 10) or 2nd-line (n = 2) therapy or after transplant (n = 6). Most importantly, NGS provided a more accurate picture of BCR-ABL1 mutations status in 37/71 (52%) pts who turned out to have one or more low burden mutations in addition to the dominant mutation(s) detectable by SS. Each low burden mutation detected by NGS could be recognized as poorly sensitive either to the TKI the pt was receiving at the time of testing, or to the previous TKI(s). Out of 37 pts, 28 had hematological relapse and 9 had MRD-positivity. Overall, patients with multiple mutations were 25/71 (35%; up to 13 mutations) by NGS. Mutation complexity correlated with the number of lines of therapy received. T315I was the most frequent mutation; it was detected in 35 (50%) of the 71 mutated pts by SS and in 24 additional pts by NGS (66% of mutated pts). NGS could resolve the clonal complexity of 5 pts who had 2 base substitutions in the same codon so that the actual amino-acid change(s) were impossible to infer, and of 44/51 who had multiple mutations at different codons. Among these 44 pts, 36 were found to carry one or more (up to 4) CMs. CMs included most frequently T315I or F317L.

Summary/Conclusion: In TKI-resistant Ph+ ALL, the underlying mutation landscape may be much more complex than it appears when SS is used for screening. Of note, approximately half of the pts positive for mutations by SS are found to harbor additional TKI-resistant low level mutations. Moreover, NGS may help resolve the subclonal complexity of Ph+ ALL cells. At diagnosis, NGS may identify low level TKI-resistant mutations in some pts, but the 3% lower detection limit might be a limit. More sensitive strategies like digital PCR should be explored in this setting.
non-haematological AE rate was similar in Pathway D to Pathways A-C both for all AE’s and grade 3/4 AE’s. After 18 months 48 patients have relapsed at a median of 11.4 months. To date 68 deaths have been reported; 52 patients died of ALL and 11 from infection. Non intensive Pathway D enabled patients to spend less time in hospital, particularly in Induction I, with an average of 15 inpatient days compared with 28.5 days for Pathway B (p = 0.002).

Summary/Conclusion: Our study reflects the reality that treating ALL in older patients is a difficult balance between efficacy and toxicity. AE rates were high, as expected, but despite this the majority of patients who died did so from ALL. Age but not co-morbidities as measured by Charlson and CRASH appears to be associated with choice of therapy. Patients treated on the non-intensive pathway D, although spending less time in hospital had similar AE. Rates. They had a low CR rate and less than 20% 1 year EFS rate. Patients with Ph+ve disease had a higher CR rate but this did not translate into improved EFS or OS. We noted small numbers of longer-term survivors and anticipate that forthcoming MDR and correlating science data will shed light on the correlates of good outcome in these persons. The UKALL60+ data will be invaluable as a baseline from which to develop more effective regimens including novel agents.

**PF174 GERIATRIC ASSESSMENT-BASED TREATMENT OF ELDERLY PHILADELPHIA-NEGATIVE ACUTE LYMPHBLASTIC LEUKEMIA PATIENTS. RESULTS OF THE GIMEMA LAL1104 PROTOCOL**

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Background: The prognosis of acute lymphoblastic leukemia (ALL) in the elderly is poor and many patients (pts) are not included in clinical trials because of concomitant comorbidities/poor performance status (PS). In order to overcome these aspects, the GIMEMA designed a trial (LAL1104) for Ph-negative ALL (Ph- ALL) pts >60 years (ys).

Aims: Aim of the study was to assess the overall survival (OS) of elderly Ph- ALL pts treated front-line according to a geriatric assessment.

Methods: In the LAL1104 front-line phase II multicenter trial elderly Ph- ALL pts were stratified into 3 categories (fit, frail, intermediate), on the basis of a geriatric multidimensional evaluation: they were considered fit if they had normal activities of daily living (ADL), no grade (G) 2 comorbidities and no geriatric syndromes; intermediate if they had a normal ADL, G 2 comorbidities and no geriatric syndromes; intermediate if they had a normal ADL, G 2 comorbidities and 2 or G 3 comorbidities ≥1, ≥1 geriatric syndromes. Treatment was more intensive for fit and intermediate pts: including daunorubicine 25 mg/m2 on days 1–3, 22–24 and vincristine 1.4 mg/m2 on days 1, 8, 15, 22 in induction, and methotrexate (MTX) 1 g/m2 and cytarabine 1.5 g/m2 as consolidation, autologous transplantation (auto-SCT) in fit pts followed by maintenance with MTX, 6-mercaptopurin (6-MP) and vincristine; frail pts received vinblastine 5 mg/m2 on days 1, 8, 15, 22 as induction, and MTX and 6-MP as maintenance. All pts underwent central nervous system prophylaxis.

Results: From October 2007 to December 2016, 102 previously untreated elderly Ph- ALL pts were enrolled. Median age was 69.6 ys (range: 60.5–84.6). 85% were B-lineage ALL; t(4;11) was detected in 7% of pts. According to the geriatric evaluation, 49 pts (51%) were considered fit, 28 (29%) intermediate and 19 (20%) frail. Twenty pts (21%) had a PS ≥2. A response - complete + partial response, CR and PR - was achieved in 63 pts (63.5%), 17 pts (18%) died in induction. With a median follow-up of 32.8 months (range: 0.2–88.8) the 2- and 4-ys OS and disease-free survival (DFS) are 40.5% and 38.3%, and 22.1% and 19.5%, respectively. There were no differences in 2- and 4-ys OS and DFS (fit vs intermediate vs frail: 40% vs 43% vs 41% and 41% vs 32% vs 44%; 26% vs 19% vs 10% and 24% vs 19% vs 0% according to the geriatric assessment; contrariwise, younger pts (60–70 ys) showed significantly better 2- and 4-ys OS than older pts (48% vs 30% and 32% vs 9%, respectively, p = 0.007, Figure). Fifteen pts (14%) underwent an auto-SCT, which was beneficial in terms of OS, with a time dependent cure advantage (p = 0.02). The most common G2 adverse events were infections (41%), in particular sepsis (20%) and pneumonia (15%). Ten pts (10%) developed paralytic ileus during treatment, with intestinal perforation in 1; hepatic and cardiac toxicities were observed in 6 pts (6%). Peripheral neuropathy was reported in 6 pts (6%).

Summary/Conclusion: Despite being less intensive than other trials designed for elderly ALL the GIMEMA 1104 trial designed for Ph- ALL pts compared favorably with literature data. As expected, age represented the most important prognostic factor in terms of OS. Importantly, the geriatric assessment proved effective in reducing early fatal side effects in unfit pts and abolished the differences in terms of OS, with a survival rate of 26% at 4-ys for the fit pts. Enrollment in clinical trials designed specifically for elderly Ph- ALL, including new drugs and a geriatric assessment, will likely improve further the survival rates of these difficult to treat pts.

**PF175 MULTICENTRE STANDARDIZATION OF MINIMAL RESIDUAL DISEASE DETECTION AND QUANTITATION USING THE EUROCLONALITY-NGS ASSAY**


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