

an average value of 1008 putative clones. Of these, only 21, having a frequency higher than 5%, were considered MCL related clones by IMG-T filtering. Each diagnostic sample displayed only one predominant clone, with a median frequency of 98%. Compared to Sanger sequencing data, all the major clones showed the same IGH with a 100% of nucleotide homology in 4 out of 5 cases. Correlation analysis showed a high concordance between ASO qPCR and NGS ($r^2=0.85$). Finally, superimposable performances were observed in single patients MRD monitoring compared to ASO qPCR results (Fig1). HashClone is a new bioinformatics tool for the identification of IGH clonality in MCL patients. It is not affected by biologic biases and is the first tool able to extrapolate the temporal evolution at several time points ("MRD monitoring"). Since this is the first HashClone application, the tool needs to be fine-tuned and validated on large samples series, before being used it in large and prospective clinical trials.

P004

RARE AND UNUSUAL CALR AND MPL GENES MUTATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: SINGLE CENTRE EXPERIENCE

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Driver mutations in Philadelphia-negative myeloproliferative neoplasms (MPNs) are often mutually exclusive and include JAK2, CALR and MPL. JAK2V617F is present in 90-95% patients with Polycythemia vera (PV) and in 50-60% of those with Essential thrombocythemia (ET) or Primary myelofibrosis (PMF). CALR and MPL mutations are absent in PV and their frequencies are 20-25% and 3-4%, respectively, in ET, and 20-25% and 6-7% in PMF. It is known that there are 36 types of somatic mutations in CALR (insertions and deletions) but the most frequent are that of type I (52-bp deletion) and type II (5-bp insertion); about MPL the most common mutation results in an aminoacid substitution (either lysine or leucine) at the 515 position (MPL W515K or MPL515L). The aim of our study was to evaluate CALR and MPL gene mutations in JAK2 negative MPNs patients to find unusual and rare mutations of these two genes. Samples of 156 patients (M/F: 62/94) with documented JAK2 negative MPNs, observed at our center between 2005 and 2016, were re-evaluated for CALR and MPL mutations. Mutations in MPL (exon 10) gene and CALR (exon 9) gene were investigated by sequencing analysis. CALR mutation was found in 51 patients (32.7%): 27 (52.9%) and 19 patients (37.3%) showed type I and type II mutation respectively; 5 patients (9.8%) showed the following patterns: del p.K375 fs*49, delins p.384fs*49 complex, delins p.D384fs*46 complex, Pk377fs*55 complex, p.Glu369fs*38. Six patients (4%) were positive for MPL mutations: 4 patients (66.7%) showed the common mutation W515 (3 patients: W515L, 1 patient: W515K), 2 patients (33.3%) showed rare mutations: W515-P518delinsKT and W515A. Few literature reports are available about the possible prognostic significance of variant mutation of MPL and CALR genes. Our efforts are now oriented to collect samples from a larger cohort of patients to understand the impact of these rare and uncommon patterns of mutations on clinical outcome of patients with MPNs in particular way in the era of new drugs.

P005

THE ASSESSMENT OF SOMATIC MUTATIONS IN ACUTE MYELOID LEUKEMIA IN 2017: A PREDICTIVE MODEL

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After the recent WHO revision, AML patients are now classified as at low, intermediate or high risk on the basis of cytogenetic and molecular integrated parameters. A web available algorithm where clinical, cytogenetic, and molecular features are integrated is now available at <http://cancer.sanger.ac.uk/aml-multistage/>. The NGS techniques today

allow an optimal characterization of each patient; nevertheless, the optimization of NGS is often complicated and it is not available everywhere. Thus, in small laboratories, conventional PCR is still a useful technique for the mutational assessment. In this study, we used the Qiagen AML real-time plates for assessing ASXL1, TET2, IDH1, IDH2, NRAS, WT1, c-KIT, RUNX1, DNMT3A mutations in addition to the conventional diagnostics tests in 38 AML patients at diagnosis. Their median age was 59 years, and 22 were male; in 7 cases AML followed a MDS and in other 7 a previous chemotherapy. Half of patients presented an intermediate, and 1/3 an unfavorable karyotype. After induction, 60% of patients achieved a complete or partial response; the 3-years OS was 31% and PFS 27%. In univariate analysis, the quality of response and karyotype influenced the OS; in addition, the PFS was influenced also by the FLT3 mutation and the allogeneic transplant performance. In multivariate analysis, only the quality of response retained its prognostic value. About somatic mutations, 16% of patients was mutated for NPM1, 13% for FLT3, 24% for N-RAS, 8% for WT1, 10% for IDH1, 16% for IDH2, 50% for c-KIT, 10% for RUNX1, 3% for ASXL1, and 8% for DNMT3A. Thus, 30 out of the 38 enrolled patients presented at least one mutation (30%, 3 mutations); after the adjunctive analysis, 18/38 cases (47%) translated to a poorer risk category, even if no significant impact of the adjunctive mutations on OS and PFS was observed. This was an observational study, thus our therapeutic strategy did not change; nevertheless, we inserted the mutational data in the web site to calculate the probability of survival at 2 years. Then, we compared the output with the real outcome: a discordance of 13% between the prognostic model and the real outcome was observed when we used all the mutational data, but it increased to 23% when only routine data were used (translocations, NPM1, FLT3). In conclusion, the basic molecular assays are fundamental for AML prognostication, but also adjunctive somatic mutational assessments would be useful in the clinical practice.

P006

A NEW IMPROVED DIASORIN Q-LAMP ASSAY FOR THE OPTIMAL AND ULTRA-FAST DETECTION OF BCR-ABL1 COMMON AND RARE ISOFORMS

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Introduction: The molecular detection of BCR-ABL1 transcripts is required to confirm the diagnosis of Philadelphia Positive Leukemias at onset. Although RT-PCR is the most diffuse molecular method so far, a faster and reliable Q-LAMP based assay is nowadays entering in laboratory routine. The Q-LAMP BCR-ABL1 assay detects and discriminates in one hour the most common isoforms of BCR-ABL1 starting directly from RNA in a one-step, close tube format. In this study, we evaluated a new enhanced formulation designed to detect also less frequent isoforms of p190 and p210 (e1a3, e13a3/b2a3, e14a3/b3a3) and the p230 transcripts (e19a2, e19a3). **Methods:** The new Q-LAMP BCR-ABL1 consists in a fluorescent multiplex assay for differential detection of both p190 and p210 BCR-ABL1 (common and rare transcripts) and GUSB endogenous RNA, which acts as internal control. Patient RNA is retro-transcribed and amplified at constant temperature for 60 minutes on the Liaison IAM instrument that displays fluorescent signals in real time and return final elaborated data. The assay has been tested on a total of 148 clinical samples: 52 BCR-ABL1 negative and 96 BCR-ABL1 positive (p190 n=38 and p210 n=56), among which 15 samples presented uncommon isoforms (e1a3 n=5; b2a3 n=8, b3a3 n=2, e19a2 n=2). Results were compared with the ones obtained by conventional RT-PCR method (Biomed Protocol). **Results:** the new enhanced BCR-ABL1 Q-LAMP assay showed 100% concordance with RT-PCR, with average amplification time of 23,41 minutes for common isoforms and 31,3 minutes for rare isoforms. In particular p210 common isoforms average amplification time is 21,16 min respect to 25,03 min of the rarer p210 isoforms and p190 common isoforms average amplification time