Genomic Profile of Patients with Triple Negative (JAK2, CALR and MPL) Essential Thrombocythemia and Primary Myelofibrosis

Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) are myeloproliferative neoplasms (MPN) with similar driver mutations. The three hallmark molecular alterations in these diseases are JAK2, MPL and CALR mutations. Nevertheless, roughly 10% of these patients do not present mutations in neither of these genes. Recent data suggest that triple negative PMF patients have a more aggressive clinical course. While the molecular alterations present in patients with MPN have been extensively studied, the genomic profile of triple negative TE/PMF/RARS-T has not been extensively characterized. We performed whole exome / genome sequencing of paired granulocytes and skin from 15 triple negative MPN patients [PMF (N=6)/ TE (N=8)/ RARS-T (N=1)] were analyzed. DNA was extracted from CD66b+ magnetic bead selected granulocytes and matched skin biopsies. Whole-exome targeted capture was carried out using Agilent SureSelect Human Exome Kit 51Mb version 4. Somatic variants calls were generated by combining the output of Somatic Sniper (Washington University), Mutect (Broad Institute) and Pindel (Washington University). Tumor coverage was 150x and germline coverage was 60x. The combined output of these 3 softwares was further filtered by in-house criteria in order to reduce false-positive calls. All JAK2 and CALR mutations were validated through Sanger sequencing. Validations of other somatic mutations are under way at this point. First we asked whether other hematopoietic related genes could be responsible for the pathogenesis of the triple negative cases. With that goal we searched for high confidence mutations in genes that are mutated in at least 1% of patients with hematopoietic tumors on COSMIC (catalog of somatic mutations in cancer) database and also genes known to be recurrently mutated in myeloid malignancies. Only 6 out of 15 patients presented mutations in other myeloid related genes. The diagnosis of all these patients was PMF. The hematopoietic related genes mutated in these patients were: ASXL1 (n=4), CUX1 (n=3), NRAS (n=2) and ATM, CBL, CSFR3, CREBBP, DNMT3A, ETV6, EZH2, JARID2, MLL2, PHF6, SRSF2, STAG2, TET2, GNAS, U2AF1 (n=1). Remarkably, the average number of hematopoietic related mutations in these patients was 5, significantly higher than the total number of mutations found in another cohort of patients with either JAK2 (average = 1.7) or CALR mutations (average = 1.9). Although our numbers are small, we may speculate that the high incidence of ASXL1 mutations (28%) [1] associated with a high number of prognostically detrimental mutations [2] can partially explain the worse outcomes associated with triple negative MPN [3]. Regarding the other 9 patients for whom no hematopoietic mutations could be identified, 8 patients had ET and one patient had RARS-T. We have shown that: i- patients with triple negative MPN are molecularly heterogeneous, with one group

presenting a high number of hematopoietic related mutations, ii-the most common mutations present in these patients are ASXL1, CUX1 and NRAS, iii-The majority of these patients do not present mutations in hematopoietic related genes, what suggests that non-described molecular mechanisms are operating in these patients.