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ABSTRACT BOOK

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SEGRETERIA SIES
Via Marconi, 36 - 40122 Bologna
Tel. 051 6390906 - Fax 051 4219534
E-mail: segreteriasies@ercongressi.it
www.siesonline.it

SEGRETERIA ORGANIZZATIVA
Studio ER Congressi
Via Marconi, 36 - 40122 Bologna
Tel. 051 4210559 - Fax 051 4210174
E-mail: ercongressi@ercongressi.it
www.ercongressi.it

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Contributors

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CK2 inactivation, which could account for GATA1 instability. However, GATA-1 and CK2β also colocalized in the nucleus in presence of the β-estradiol suggesting a possible direct mechanism in CK2-mediated control of this transcription factor.

Conclusions: CK2β is essential for erythroid maturation, regulating STAT5, AKT and GATA-1. Further research will clarify if CK2β subunit could influence these factors in an indirect or direct mechanism.

CO045

COMPORTAMENTO DELLE MUTAZIONI DRIVER E NON DRIVER NEI PAZIENTI CON MIELOFIBROSI IN TRATTAMENTO CON RUXOLITINIB

S. Galimberti[1], S. Balducci[1], F. Guerrini[1], S. Grassi[1,2], M.G. Massantini[1], F. Ricci[1], D. Cattaneo[3], A. Iurlo[3], P. Vigneri[4], M. Petrini[1], G.A. Palumbo[5], C. Baratè[1]

[1]UO Ematologia, Dipartimento Medicina Clinica e Sperimentale, Università di Pisa; [2]GENOMEC scuola di dottorato, Università di Siena; [3]UO Ematologia, IRCCS Ca' Granda, Milano; [4]Oncologia, Università di Catania; [5]UO Ematologia, Università di Catania, Italy

Background: Introduction of ruxolitinib changed the outcome of patients with myelofibrosis (MF), offering longer survivals. Nevertheless, 50% of patients loss response; in some cases, this phenomenon has been ascribed to driver and non-driver mutations, but with conflicting results: Patel *et al.* (Blood 2015) reported that having >3 mutations well correlated with shorter time to discontinuation and overall survival, whereas in the COMFORT-II study the MF-associated mutations did not correlate with response, survival or discontinuation probability (Guglielmelli, Blood 2014).

Aims: in order to investigate if ruxolitinib could play any role in changing the mutational landscape in MF, we assessed the 3 driver and 8 non-driver mutations in 36 MF patients; all were assessed a diagnosis, 19 also after 12 months of ruxolitinib, and other 4 after hydroxyurea.

Methods: In addition to assessment of the driver mutations (JAK2, CALR, MPL), a PCR plate with pre-spotted primers able to detect 8 non-driver mutations was designed (Custom qBiomarker Somatic Mutation PCR Array® - Qiagen, Italy). ASXL1, EZH2, DNMT3A, IDH1, IDH2, SRSF2, TET2, TP53, for total 38 hot-spot sites, were assessed.

Results: JAK2 was mutated in 70% of cases, CALR in 20%, whereas 10% of cases were triple-negative. The median OS was significantly longer for primary MF (160 months) vs post-ET (80 months) or post-PV MF (35 months)(p=0.03), and for CALR- vs JAK2-mutated patients. At the last follow-up, 4 patients (11%) progressed to AML, and 12 (33%) died. The non-driver mutations were found at diagnosis in 33% of cases receiving ruxolitinib and in one/4 patients treated with hydroxyurea. Considering both driver and non- driver mutations, 24 cases (67%) were mutated, with 16 cases carrying one, and 10 two mutations. The most frequently detected mutations belonged to the methylation pathway (DNMT3A, IDH, TET2 = 75%), followed by TP53 (17%), SRSF2 (8%), ASXL1 (8%), and EZH2 (8%). During treatment, JAK2 allele burden remained stable, whereas non-driver mutations changed in 13 cases: 9 acquired a new mutation (DNMT3A in 5, IDH2 in one, and TP53 in another one) while other 4 lost mutation. None of the CALR-mutated cases carried non-driver mutations. In the 4 cases treated with hydroxyurea, one acquired the TP53 and another one the DNMT3A mutation. On the other hand, 4 cases in the group of ruxolitinib, and none in the group of hydroxyurea lost mutations present at diagnosis (TP53, IDH2, ASXL1, DNMT3A). Presence/absence of non-driver mutations, their number (>1), the molecular subgroup (methylation, splicing, chromatin) did not significantly condition OS.

Conclusions: In this work, even if on a small series of patients, we showed that during ruxolitinib about the half of cases developed non-driver mutations, a percentage overlapping to that observed in cases receiving hydroxyurea. Interestingly, ruxolitinib and not hydroxyurea allowed disappearance of mutations in one third of cases.

CO046

DEVELOPMENT OF A COMBINATION STRATEGY BASED ON ER AND OXIDATIVE STRESS TO TARGET ACUTE MYELOID LEUKEMIA

S. Masciarelli[1], E. Capuano[1], T. Ottone[2], M. Divona[2], S. Lavorgna[2], A. Picardi[3], M.T. Voso[2], F. Lo Coco[2] F. Fazi[1]

[1]Department of Anatomical, Histological, Forensic & Orthopedic Sciences, Section of Histology & Medical Embryology, Sapienza University of Rome, laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Rome; [2]Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome; [3]Stem Cell Transplant Unit, Rome Transplant Network, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy

Introduction: Acute Myeloid Leukemia (AML) is a heterogeneous disease caused by different molecular genetic aberrations. These result in the expression of fusion or mutant proteins that cause impaired differentiation and enhanced proliferation and survival. We previously showed that APL cell lines and primary blasts induced to differentiate by Retinoic Acid (RA) become highly sensitive to amounts of ER stress not detrimental for the same cells in the absence of RA. Furthermore the same cells resulted sensitive to a combination of ER stress inducers with Arsenic Trioxide (ATO) that generates oxidative stress. Importantly we observed that ER stress caused increased amounts of disulphide-bound high molecular weight aggregates of PML-RARα and PML, exacerbating the alteration of cellular proteostasis already generated by induction of ER stress. This observation provides the rationale to translate the findings we observed in APL to other types of AML characterized by fusion or mutant proteins. The presence of mutant proteins that are easily prone to aggregation or mis-folding, because of their mutant structure or because of mis-localization, could render the cells sensitive to levels of ER and oxidative stress that could be recovered in their absence.

Methods: We treated AML cell lines and AML primary leukemic blasts with RA and ER and oxidative stress inducers, evaluating cell proliferation and death, activation of the ER/oxidative stress responses, localization and possible aggregation of the mutant proteins by confocal microscopy, colony forming capacity.

Results: We first tested a panel of AML cell lines characterized by different oncogenic fusion or mutant proteins and we found that ML-2 cells, bearing the MLL-AF6 fusion protein, and MV-4-11 cells, expressing the fusion protein MLL-AF4 and FLT3-ITD are highly sensitive to the combination of sub-lethal amounts of RA, Tm and ATO. In the cells undergoing ER and oxidative stress in combination, we found prolonged activation of the antioxidant response and of the unfolded protein response (UPR), activated by ER stress, as indicated by the expression of HMOX, CHOP, BiP and sXBP1. The antioxidant agent N-acetyl-cystenine and the inhibitor of the UPR player GADD34 determine resistance of the cells to the treatments. Furthermore, an inhibitor of the PERK branch of the UPR dramatically exacerbates the sensitivity to the combination of ER and oxidative stress pointing to this pathway as a possible new therapeutic molecular target. Importantly, the combination of ER and oxidative stress significantly reduces the colony forming capacity of primary leukemic blasts isolated from the bone marrow of FLT3-ITD positive patients.

Conclusions: Altogether our data suggest that the combination of low levels of ER and oxidative stress leads to apoptosis rather than recovery, achieved instead when the same stresses are induced alone.