BAALC-mediated signalling complexes involved in DNA repair were identified by immunoprecipitation.

Results and discussions BAALC, but not EV, overexpression resulted in elevated DNA fragmentation (comet assay), and sustained gH2AX and ATM activation (pSer1981) post-daunorubicin and etoposide-induced DNA damage, indicative of an inability to repair the damage. Gene expression analyses in BAALC overexpressing cells identified that the expression of components (*RAD51D*, *TP53BP1*, *BTG2*) of the high-fidelity homologous recombination double strand break repair pathway were significantly decreased. Furthermore, BAALC interacts with several proteins (RAD51, CaMKII, dermcidin) implicated in DNA repair. Further supporting a role for BAALC overexpression in inducing defective homologous recombination repair, BAALC overexpressing AML cells were hypersensitive to cisplatin treatment.

Conclusion As perturbations in DNA repair pathways in AML have been associated with increased resistance to a range of chemotherapeutics, and BAALC overexpression is associated with primary refractory AML, our data suggests that BAALC overexpression induces decreased chemosensitivity by perturbing DNA repair pathways.

PO-145 ERK5 PATHWAY INHIBITORS INHIBIT THE MAINTENANCE OF CHRONIC MYELOID LEUKAEMIA STEM CELLS

[1]E Rovida*, [2]I Tusa, [2]G Cheloni, [3]A Gozzini, [4]X Deng, [4]NS Gray, [5]S Li, [6]P Dello Sbarba. *[1]University of Florence, Department of Clinical and Experimental Biomedical Sciences, Florence, Italy; [2]University of Florence, Department of Experimental and Clinical Biomedical Sciences- University of Florence, Florence, Italy; [3]AOU Careggi, Hematology Unit, Florence, Italy; [4]Dana Farber Cancer Institute, Harvard Medical School, Boston, USA; [5]University of Massachusetts Medical School, Department of Medicine, Worcester, USA; [6]University of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy*

10.1136/esmoopen-2018-EACR25.186

Introduction Chronic myeloid leukaemia (CML) is a hematopoietic stem cell (HSC)-driven neoplasia characterised by the expression of the constitutively active tyrosine kinase BCR/ABL. CML therapy based on tyrosine kinase inhibitors (TKi) is highly effective in inducing remission but not in targeting leukaemia stem cells (LSC), which sustain the minimal residual disease and are responsible for CML relapse following discontinuation of treatment. Our aim was to address the effects of the inhibition of the ERK5 pathway on the maintenance of CML LSC.

Material and methods KCL22 and K562 CML cell lines, patient-derived CML cells or CD34 +peripheral blood cells from healthy donors (informed consent) were incubated in normoxic or hypoxic (0.1% O_2) primary cultures (LC1) in the presence or the absence of drugs. At the end of incubation (day 7), cells were analysed on a flow cytometer to determine the expression of stem cell markers or transferred to drug-free normoxic secondary cultures (LC2) to measure LC2 repopulation as a read-out of progenitor/stem cell potential (CRA assay). In the serial Colony Formation Ability (CFA) assay colonies were scored on day 7 of each passage (III passages). In the Long-Term Culture-Initiating Cells (LTC-IC) assay the number of colonies was scored after 14 days. Compounds: XMD8-92 (ERK5 inhibitor) and BIX02189 (MEK5 inhibitor); imatinib and dasatinib (BCR/ABL inhibitors).

Results and discussions In CML patient-derived cells and cell lines, we found that the MEK5/ERK5 pathway is active and necessary for optimal proliferation in low oxygen, a condition typical of normal hematopoietic and leukemic stem cell niches. Treatment of primary CML cells with XMD8-92 or BIX02189, but not with TKi, strikingly reduced Culture Repopulation Ability (CRA), serial Colony Formation Ability and Long-Term Culture-Initiating Cells (LTC-IC). Importantly, inhibition of MEK5/ERK5 was effective on CML cells regardless of the presence or absence of imatinib (IM), and did not reduce CRA or LTC-IC of normal CD34 +cells. Interestingly, in hypoxia, combined treatment XMD8-92/IM decreased the expression of genes relevant for stem cell maintenance such as c-MYC, SOX2 and NANOG and the expression of CD26, a CML LSC marker.

Conclusion We propose ERK5 pathway inhibitors as a novel therapeutic approach to prevent CML relapse and, in combination with TKi, enhance induction of remission.

Metastases and EMT

PO-146 MULTIPLE MYELOMA-DERIVED EXOSOMES CARRY AMPHIREGULIN AND ARE RESPONSIBLE FOR THE UNCOUPLED BONE REMODELLING

[1]R Alessandro*, [1]S Raimondo, [2]L Saieva, [3,4]E Vicario, [4]F Costa, [4]N Giuliani. *[1]University of Palermo, Biopathology and Medical Biotechnologies, Palermo, Italy; [2]University of Palermo, Biopathology and Medical Biotechnology, Palermo, Italy; [3]University of Palermo, Biopathology and Medical Biotechnology, Palermo, Italy; [4]University of Parma, Department of Medicine and Surgery, Parma, Italy*

10.1136/esmoopen-2018-EACR25.187

Introduction Multiple myeloma (MM) is a hematologic malignancy associated with osteolytic bone disease caused by the perturbation of the functional balance between bone resorption and bone formation. Exosomes, nanosize lipoproteic structures, have been recently recognised as a new mechanism of cell to cell communication during tumour growth and progression. We have previously shown that MM-exosomes are involved in osteolytic lesions but the underlying mechanism is still understood. We hypothesise that the epidermal growth factor receptor ligand Amphiregulin (AREG) can be delivered by multiple myeloma-derived exosomes and participate in modulating the response of the bone microenvironment to the tumour.

Material and methods Exosomes were isolated from the conditioned medium of MM1 cell line and from BM plasma samples of patients. In order to test whether MM-exosomes could affect osteoclastogenesis through the activation of the EGFR pathway, primary CD14 +monocytes and a murine cell line (RAW264.7) were used as osteoclast (OC) models. Cells were treated with exosomes from both MM1 and plasma samples, pre-treated or not with anti-AREG neutralising antibodies and OC specific markers were measured. In addition, to further explore whether exosomes were able to promote osteoclastogenesis by affecting mesenchymal stem cells, hTERT-MSC were treated with exosomes; the conditioned medium were collected to measure the secretion of IL8 and to stimulate primary CD14 +monocytes.

Results and discussions We found that AREG was specifically enriched in exosome samples, leading to the activation of EGFR in pre-OC. In addition we showed a significant increase of the expression of the OC markers Cathepsin K, Matrix Metalloproteinases 9 and Tartrate-resistant Acid Phosphatase in