that can be used are good noninvasive biomarkers detected in liquid biopsy samples, such as liquid-based cervical cytology (LBC) samples. However, a few studies have been carried out in these kind of samples to detect the expression profile of miRNAs in cervical cancer precursor lesions. Furthermore, the most of the studies used a small samples or evaluated a few groups of miRNAs. The analysis of miRNAs profile would allow to open diagnostic testing arsenal for the screening of Cervical Intraepithelial Neoplasia (CIN). In view of the above, there is a need for technical standardisation for the analysis of miRNA expression in LBC samples considering the poor evidence in the literature that used LBC samples for the analysis of miRNA expression. Nevertheless, the aimed of this study was to identify housekeeping’s for analysis of miRNA expression in LBC samples.

**Material and methods** Expression of U6, hsa-miR-16 RNU-44, 47, 48 and 49 was measured by Reverse Transcription Quantitative PCR (RT-qPCR). Reference genes expression values were normalised to the reference using the comparative CT method ($2^{ΔΔCt}$). We used one common software, namely NormFinder, to analyse expression stability of the six selected genes. This software candidate reference genes by calculating their stability values, with lower values indicating more stable genes.

**Results and discussions** The stability values calculated using NormFinder to six housekeepings was (U6=0,787; miR-16=9,728; RNU-44=7,912; RNU-47=3,234; RNU-48=5,132; RNU-49=1,716) and the standard error (U6=2,127; miR-16=1,990; RNU-44=1,684; RNU-47=1,084; RNU-48=1,263; RNU-49=1,226). Thereby, we can verify that U6 was the gene that demonstrated to be the best housekeeping, this because it presented smaller variation in the expression in relation to the other genes tested. Similar data were demonstrated in the literature. However, it was the first time that it was performed with liquid-based cervical cytology samples.

**Conclusion** We conclude that the small nucleolar RNA transcript U6 was the best reference gene.

**PO-394 DNA METHYLATION OF ADAM23 IS NEGATIVELY ASSOCIATED WITH HAEMATOGENOUS SPREAD IN BREAST CANCER**

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**Introduction** Circulating tumour cells (CTCs) are associated with metastatic potential in a variety of human cancers. Although DNA methylation of cancer-related genes is a promising diagnostic and prognostic tool, it was rarely studied in relation to the presence of CTCs. ADAM23 gene, member of ADAM (a disintegrin and metalloproteinase domain) family, is involved in various biological events such as cell adhesion, fusion, migration, membrane protein shedding and proteolysis. The aberrant DNA methylation of ADAM23 was associated with expression silencing in breast, gastric, brain and other tumours. In our study we investigated the role of ADAM23 methylation in hematogenous spread of breast cancer (BC).

**Material and methods** To detect CTCs, we analysed expression levels of KRT19, SLUG and TWIST genes in the CD45-enriched peripheral blood samples of 203 primary BC patients. The methylation levels of ADAM23 were quantified by pyrosequencing in formalin-fixed, paraffin-embedded tumour tissues. The hypermethylation cut-off was defined as mean ±2 standard deviation of DNA methylation in healthy breast tissues. Protein expression was evaluated in the same material by ImmunoReactive Score (German IRS system), based on the proportion of positive cells and the staining intensity of the nuclei or cytoplasm. A logistic regression was used to determine the effect of studied variables on presence of CTCs in peripheral blood.

**Results and discussions** CTC positivity (epithelial or mesenchymal markers) was identified in 23% of patients (16% mesenchymal, 9% epithelial and 9% both). ADAM23 hypermethylation (DNA methylation ≥10%) did not occur in mesenchymal CTC positive patients, while it was present in 28.8% of mesenchymal CTC negative patients (p=0.001). Among studied variables, the multivariate analysis identified low ADAM23 methylation level (p=0.004) and high ki67 proliferation index (p=0.005) as two most significant predictors of mesenchymal CTCs. It was shown recently that although ADAM23 inhibition enhanced invasion in breast cancer, it was not sufficient to trigger metastasis (Costa et al., 2015).

**Conclusion** Our data indicate possible involvement of ADAM23 in hematogenous spread of BC cells. Better understanding of reversible epigenetic regulations hold promise for development of novel therapeutic approaches.

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**PO-395 MECHANISTIC INVESTIGATION OF EPIGENETIC MODIFICATIONS INDUCED BY METABOLIC CHANGES IN AML**

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**Introduction** Acute Myeloid Leukaemia is the most common and aggressive adult leukaemia with a high frequency of relapse and mortality. This is likely due to the failure to eliminate the malignant stem cell population (LSCs) composed by low cycling cells that give rise to highly proliferating blast type progenitors. Although the currently available chemotherapeutic agents efficiently eradicate blasts, LSCs can be retained and cause relapse. Therefore, new therapeutic strategies aimed to specifically eradicate them is urgently needed.

These insights led to an increasing interest in understanding the molecular mechanisms governing LSCs biology. Along this line, recent reports (Raffel et al. Nature 2017) highlight the crucial role of Branched Chain Amino Acid (BCAA) catabolism in LSCs. Indeed, BCAA Transaminase 1 (BCAT1) is highly expressed in LSCs and led to reduction of alpha-ketoglutarate (aKG) levels, with consequent impairment of aKG-dependent dioxygenases activity, such as TET1/2, and increase in DNA dioxygenases activity, such as TET1/2, and increase in DNA
Abacavir is also a co-factor of histone-demethylases and it remains unclear whether BCAT1 also affects these enzymes, which regulate chromatin structure and activity. Material and methods  

Genome wide approaches (i.e. ChIPseq for specific histone marks, ATACseq, RNAseq, DNA methylation) are used to mechanistically dissect the connection between BCAT1 mediated metabolic changes and alterations in the chromatin landscape of AML cell lines (HL-60, SKM1-MOLM-13) and LSCs. Results and discussions  

In order to understand if BCAT1 is controlling the chromatin structure, the transcription factor binding and the histone methylation status, we performed H3K27ac-H3K27me3-H3K4me1-H3K4me3 ChIPseq experiments and ATACseq on control and BCAT overexpressing cells. Despite the global DNA methylation changes observed in BCAT1 overexpressing cells, Raffel et al., Nature 2017 ChIPseq and ATACseq analyses did not show a global change in chromatin structure and modifications. Nonetheless, differences in specific loci were identified in BCAT1 overexpression suggesting a more complicated scenario and opening the road for further analyses.  

Conclusion This work will provide rationale for the use of metabolic enzyme inhibitors (alone or in combination with epigenetic inhibitors) as a therapeutic approach in AML and other solid BCAT1 overexpressing tumour entities.

Deep Sequencing

PO-396 ABSTRACT WITHDRAWN

PO-397 DNA DAMAGE TOLERANCE IS ESSENTIAL FOR THE DNA DAMAGE RESPONSE NETWORK AND HEMATOPOIEIC STEM CELL MAINTENANCE

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Introduction Stem cell fitness dictates essential biological processes like tissue homeostasis and ageing. The overall contribution of DNA damage tolerance (DDT) in maintaining stem cell fitness remains unknown. DDT pathways which enable replication in the presence of DNA replication impediments are facilitated by PCNA\(^{K164R}\) ubiquitination and REV1. By intercrossing Pcn\(^a\)\(^{K164R}\) mutant and Rev1 deficient mice, DDT was found to be essential for mammalian life.  

Material and methods We crossed mouse models with Rev1 deletion and Pcn\(^a\)\(^{K164R}\) mutation. We use flow cytometry, pathology, and single cell RNA sequencing in these mouse models to determine the relevance of DDT in mice.  

Results and discussions By intercrossing Pcn\(^a\)\(^{K164R}\) mutant and Rev1 deficient mice, DDT was found to be essential for mammalian life. A compound mutation of Rev1 and Pcn\(^a\)\(^{K164R}\) rendered hematopoietic stem cells (HSCs) and their immediate precursors genetically instable, instigating a pathological process where the associated HSC depletion culminated in a severe embryonic lethal anaemia. Single cell RNA-sequencing of the remaining LSK cells revealed a remarkable stress-induced plasticity of multipotent progenitors, and the existence of a novel CD24\(^a\)\(^{high}\)CD93\(^low\) erythroid-committed progenitor (ECP) compartment. Conclusion DDT is a key activity within the DNA damage response network, where PCNA\(^{K164}\) and REV1 primarily serve non-epistatic DDT pathways and are essential in maintaining HSCs. Furthermore, we reveal a novel CD24\(^a\)\(^{high}\)CD93\(^low\) erythroid-committed progenitor (ECP) within the LSK compartment.