Material and methods Several myeloid leukemic cell lines as well as patient samples were examined. Effects of drugs on viability were determined by flow cytometry with Annexin-V/Sytox staining. Glucose and mitochondrial metabolism changes were determined using XFe24 Seahorse and by mass spectrometer using glucose- C^{13} or glutamine-C^{13}.
Results and discussions Under normoxic and hypoxic conditions, the constitutive activation of oncogenic tyrosine kinases supports glucose metabolism of leukemic cells. Consequently, inhibition of BCR-Abl or $FLT3^{ITD}$ by TKI leads to severe metabolic changes including drastic reduction in glucose uptake and glycolysis, as well as decrease in mitochondrial metabolism. However, a sub-population of leukemic cells maintains viability even after several days of *in vitro* treatment with TKI and is characterised by higher mitochondrial membrane potential, confirming the primordial role of mitochondrial metabolism in the survival of TKI-treated cells. Interestingly, the association of TKI and L-Asparaginase (l-ase), an enzyme that depletes extracellular asparagine and glutamine, decreases OxPhos resulting in the eradication of TKI-tolerant cells. Moreover, the association of TKI and l-ase kills $BCR\text{-}Abl^{+}$ leukemic cells from patients with acquired resistance to TKI. By a co-culture of mesenchymal (MS-5) and leukemic cells, we have also demonstrated that the presence of MS-5 managed to rescue TKI-treated cells, nevertheless the addition of l-ase eradicates the persistent cells that survived under TKI treatment alone, without affecting MS-5 cells viability.
Conclusion These results might enable the development of new therapeutic strategies based on anti-metabolic cooperativity combining TKI and L-Ase in patients who develop myeloid leukaemia or in patients with acquired resistance to TKI.

Poster Presentation: Cancer Cell Biology

PO-266 METABOLIC PROFILING OF OSTEOSARCOMA CANCER STEM CELLS AS TOOL TO IDENTIFY POTENTIAL TARGET FOR CANCER THERAPY

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Introduction Recently, several studies have highlighted the key role of cancer stem cells (CSC) in tumour initiation, metastasis, and relapses. The CSC pool generally exhibits higher resistance to conventional chemo-radiotherapy and a different cell metabolism. Aim of our study is to investigate the main metabolic differences in the human osteosarcoma stem-like cells (3ABOS) and differentiated osteosarcoma cells (MG63) to unveil new metabolic therapeutic targets.
Material and methods Metabolic analyses were performed with Seahorse Bioanalyzer. Live cell imaging for ROS content, mitochondrial membrane potential and morphology, were performed by confocal microscopy by using DCF-DA, Mito-Tracker Red and NAO as selective probes, respectively. Protein expression was revealed by qPCR and western blot.
Results and discussions Our results showed a significant reduction of the mitochondrial oxygen consumption rate in 3ABOS compared with MG63 cells. Next, we assessed the specific contributions of glucose, fatty acid and glutamine to the respiratory phenotype, unveiling larger reliance on oxidation of these three main fuels with a significant reduction in mitochondrial flexibility in 3ABOS. The lower OXPHOS is compensated by a shift in glucose metabolism demonstrated by increased extracellular acidification rate.These results were further supported by a significant reduction of 3ABOS proliferation in glucose shortage. According to this scenario, confocal microscopy highlighted reduced mitochondrial membrane potential, and increased ROS content in 3ABOS compared to MG63. Additionally, 3ABOS displayed a lower mitochondrial DNA amount associated with more elongated mitochondrial network confirmed by both live cell imaging and Mitofusin expression analysis. Moreover, members of the NADPH oxidases family resulted to be differently expressed in the two cell lines, thus suggesting a potential role of ROS mediated signalling in cancer cell phenotype.
Conclusion Overall our results demonstrated that the oxidative metabolic phenotype hallmarks cancer biology. Further investigations are ongoing to define specific drugs acting on metabolic target and their effectiveness as a therapeutic approach.

PO-267 PHGDH AND PSAT CONFER METABOLIC VULNERABILITY TO IDH2-DRIVEN REPROGRAMMING IN BREAST CANCER

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Introduction Reprogrammed energy metabolism is one among the distinguished cancer hallmarks that unravels the dysregulated bioenergetics of cancer cells. Isocitrate dehydrogenase 2 (IDH2), a mitochondrial metabolic enzyme has been reported to be overexpressed in several cancers, yet the mechanisms of wildtype IDH2-induced transformation are still obscure. We previously identified IDH2, as a novel poor prognosis marker of breast cancer progression. In agreement, transcriptomics (TCGA) and proteomics data of breast cancer clinical samples showed similar expression changes of increased wildtype IDH2 from luminal, to triple-negative and to HER-2 positive, which correlated with tumour aggressiveness.
Material and methods We performed intracellular metabolite tracing with heavy labelled glucose ($^{13}C_6$) and glutamine ($^{13}C_5$) followed by ultra-high performance liquid chromatography separation and mass spectrometric analysis on the Q-Exactive Plus instrument. Genome-scale metabolic modelling analysis was performed to identify the IDH2-dependent metabolic genes using Minimization of Metabolic Adjustment (MOMA) algorithm.
Results and discussions Overexpression of IDH2 increased serine biosynthesis, increased glycolytic rate and oxidative stress resistance. Genome-scale metabolic modelling of breast cancer tumour data identified dependence on serine biosynthesis as one of the metabolic vulnerability of IDH2 overexpressing cells. CRISPR-Cas9 mediated knockout of phosphoglycerate dehydrogenase (PHGDH) and phosphoserine aminotransferase (PSAT) specifically inhibited IDH2 overexpressing cells, even in the presence of exogenous serine and glycine, and reduced