LIST ALL AUTHORS and AFFILIATIONS – underline presenting author
Camille Jacques, Richard L. Bennett, Daphné Dupere-Richer, Jianping Li, Sayantan Maji, Aditya Bele, Alberto Riva, Sharon Norton, Crissandra Piper, Jonathan D. Licht

TITLE
Interplay between oncogenic signaling pathways and epigenetic modifiers in the development of Acute Myeloid Leukemia.

HYPOTHESIS
While its etiology is not completely understood, Acute Myeloid Leukemia (AML) is a heterogeneous clonal disorder characterized by an abnormal hematopoiesis and the accumulation of immature/incompletely differentiated myeloid precursors in the bone marrow. We hypothesize that epigenetic events such as inactivating mutations/deletions of the Histone Methyl Transferase KMT2C cooperate with the activation of the Ras pathways to trigger the transformation and spreading of leukemia cells, as well as driving both the malignant features of these cells and their chemoresistance.

BACKGROUND/AIMS:
AML is the most common acute leukemia, with more than 13,700 cases annually in the US. Although routine use of dose-intensive chemotherapy regimens improves outcomes for AML, most patients die of refractory disease and suffer from significant toxicities. In an attempt to better treat this disease, delineating the genetic aberrations and signaling pathways’ deregulations that allow blood cells to transform into leukemia cells are of urgent clinical importance. Recent sequencing studies identified a set of loss of functions mutations in chromatin regulators such as KMT2C, and the complete loss of chromosome 7 or 7q36.1 is also found in AML. Furthermore, a large proportion of AMLs have mutations in N-RAS, K-RAS, or genes that activate Ras signaling, and main Ras effectors are activated in nearly all AML patient. Thus, the aim of this study is to explore how these genetic events could be related and how they could cooperate, leading to the development of AML.

METHODS:
We have created genetically matched cell systems in which the Ras signaling pathway has been activated by lentiviral infection with a constitutively activated mutant of the K-Ras oncogene (K-Ras G12V). These cells have then been modified by using the CRISPR/Cas9 method in order to inactivate the KMT2C gene by introducing mutations in exon 38. Single clones have been isolated by cell sorting and the combined effects of both the activation of the Ras pathway and the inactivation of KMT2C have been examined at both functional and molecular levels. Functional tests have been performed to assess: cytokine and growth factor independence (Alamar Blue staining), apoptotic response after starvation (caspase 3/7 activation assay), clonogenic capabilities in semi-soft media and differentiation potential.

RESULTS & CONCLUSIONS
The K-Ras G12V expressing hematopoietic cells display cytokines/growth factor-independent growth, increased clonogenic abilities and a poor apoptotic response compared with normal cells, either in human or in murine cell lines. Moreover, the activation of the Ras pathway in such cells decreases their cell-differentiation potential. So far, our preliminary data show that loss of KMT2C decreased cell growth and promotes sensitivity to cytokine/growth factor deprivation. In addition, the KMT2C mutant clones isolated in a K-Ras activated background do not display an activation of caspase 3/7 after starvation compared to the KMT2C mutant in a WT background. Taken together, these first results open the road to study more in depth the interplay between the Ras oncogenic signaling and the KMT2C gene in the context of AML.