LIST ALL AUTHORS and AFFILIATIONS – underline presenting author
Daphné Dupéré-Richer¹, Teresa Ezponda², Teresa Ezponda², Christine Will², Eliza Small², Nobish Varghese³, Sayanta Maji¹, Tej Patel², Behnam Nabet², Jon Oyer², Yupeng Zheng⁴, Xiaoxiao Huang², Manuela Occhionorelli⁵, Giovanni Tonon⁵, Neil Kelleher⁴, Jonathan Keats⁶, Jona than D. Licht¹
¹Division of Hematology/oncology, Genetics Research Complex, The University of Florida Health Cancer Center, ²Division of Hematology/Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, ³Division of Hematology/Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL, ⁴Department of Chemistry, Department of Molecular Biosciences, and the Chemistry of Life Processes Institute, Northwestern University, Evanston, IL, ⁵Functional Genomics of Cancer Unit, Division of Molecular Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) San Raffaele Scientific Institute, Milan, Italy

TITLE
UTX/KDM6A loss enhances the malignant phenotype of multiple myeloma and sensitize cells to EZH2 inhibitor.

HYPOTHESIS:
Loss of KDM6A promotes tumorigenesis of multiple myeloma (MM) by affecting chromatin structure and thus, altering the transcriptome of MM cells.

BACKGROUND/AIMS:
In multiple myeloma (MM), inactivating mutations and deletions encompassing the histone demethylase KDM6A locus is found in up to 10% of newly diagnosed patients and associated with poor prognosis. KMD6A (also name UTX, Ubiquitously transcribed Tetratricopeptide repeat, X chromosome) belongs to a family of Jumonji-C (Jmj-C)-containing demethylases working as a scaffold for a multiprotein complex containing H3K4 specific methyltransferases KMT2D and/or KMT2C (MLL2/3), the histone acetyltransferase CBP/p300 and members of the SWI/SNF chromatin-remodeling complex. In a concerted manner this complex appears to remove the gene repression associated methylation of lysine 27 on histone H3 (H3K27me) a mark placed by EZH2. Hence loss of function mutations of KDM6A may affect the function of this complex, which is common in B-cell malignancies.

METHODS:
We modeled the loss of KDM6A in MM in vitro, using a pair of cell lines, ARP-1 (KDM6A wild type) and ARD (homozygous KDM6A deletion), derived from the same MM patient. As well, we used CRISPR-Cas9 mediated genome editing to disrupt KDM6A gene in cell lines.

RESULTS & CONCLUSIONS
Disruptive mutations in KDM6A locus provide a growth advantage in MM cell in vitro and in vivo. Mass spectrometry analysis of these cell lines and the add-back system showed no difference in global H3K27me3 levels, suggesting that tumor suppressive role of KDM6A does not involve alteration of H3K27 methylation or that the changes in this histone mark following KDM6A loss are loci-specific. We examined gene expression profiles in ARP-1 vs ARD cells, and vs ARD cells upon re-expression of KDM6A, in a doxycycline-inducible manner, by whole transcriptome (RNA sequencing). Many genes induced upon KDM6A add-back are found upregulated in KDM6A wild-type ARP-1 cells. Furthermore, we found that cells null for KDM6A have increased sensitivity to EZH2 inhibitors. Exposure to EZH2 inhibitors alter expression of many genes found deregulated by KDM6A loss. Notably, many of these genes are involve in differentiation of B cells. Loss of KDM6A alters the transcriptome of MM cells possibly affecting the differentiation ability of MM cells.