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# POSTER 6: Ferritinophagy is a Druggable Vulnerability of Quiescent Leukemic Stem Cells

**Clement Larrue**[1,2,3,4,#], Sarah Mouche[1], Paolo Angelino[1,5], Maxime Sajot[2,3,4], Rudy Birsen[1,6,7], François Vergez[8], Christian Recher[8], Véronique Mansat-De Mas[8], Qiong Gu[9], Jun Xu[9], Petros Tsantoulis[1], Jean-Emmanuel Sarry[2,3,4], and Jerome Tamburini[1,#]

[1]Translational Research Centre in Onco-hematology, Faculty of Medicine, University of Geneva, and Swiss Cancer Center Leman, Geneva, Switzerland. [2]Centre de Recherches en Cancérologie de Toulouse, Université de Toulouse, Inserm U1037, CNRS U5077, Toulouse, France. [3]LabEx Toucan, Toulouse, France. [4]Équipe labellisée Ligue Nationale Contre le Cancer 2023, Toulouse, France. [5]Translational Data Science, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland. [6]Université de Paris, Institut Cochin, CNRS U8104, Inserm U1016, Paris, France. [7]Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Centre, Service d'Hématologie Clinique, Paris, France. [8]Centre Hospitalier Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopole, Service d'Hématologie, Toulouse, France. [9]Research Center for Drug Discovery, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China.
[#]: Correspondence to Clement Larrue : clement.larrue@inserm.fr and Jerome Tamburini : jerome.tamburinibonnefoy@unige.ch.

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Acute myeloid leukemia (AML) poses significant challenges in terms of prognosis and treatment options. Leukemic stem cells (LSCs) contribute to therapy resistance, relapse and adverse outcomes. This study aimed to explore one of the major features of LSCs (e.g. quiescence) and its underlying molecular mechanisms, as well as identify potential targets for therapeutic intervention.

Using *in vivo* cell labeling in patient-derived xenograft (PDX) assays, we identified a quiescent cell subpopulation with high LSC potential. Using transcriptomics experiments conducted separately on quiescent and cycling cell subsets as well as single-cell RNA sequencing, we identified genes specifically overexpressed in quiescent cells of AML. Interestingly, quiescent cells clustered very well with gene signatures related to LSCs. Through a machine learning approach, we identified a gene set highly specific of quiescent cells (QS35 gene signature) that emerged as a significant prognostic factor for poor outcomes in three independent cohorts of AML patients.

Using transcriptomics and proteomics, we identified elevated autophagy and reduced expression of TFRC and ferritin in quiescent cells, prompting an exploration into the synchronized regulation of autophagy and iron metabolism in the quiescent LSC subset. Autophagy inhibition selectively induced cytotoxicity in the CD34+CD38- compartment and reduced the frequency of long-term culture-initiating cells (LTC-IC), and these effects were rescued by exogenous iron supply, underscoring the critical role of autophagy in iron bioavailability within the quiescent LSC subpopulation.

To target ferritinophagy, we depleted NCOA4, the main carrier in ferritinophagy responsible for delivering ferritin-bound iron to the autophagosome, using shRNAs in primary AML samples. NCOA4 invalidation abrogated leukemia initiation and self-renewal in serial transplantation experiments *in vivo*, particularly within the quiescent LSC population.

Additionally, in PDX models, treatment with a novel small molecule inhibitor named compound 9a that disrupts the NCOA4-ferritin interaction, decreased tumor burden and induced cell death within the quiescent CD34+CD38- LSC population.

These findings highlight the critical vulnerability of quiescent LSCs to ferritinophagy. Thus, ferritinophagy inhibition represents an innovative therapeutic approach for AML patients.