THERAPEUTIC STRATEGY FOR LEUKEMIA BASED ON THE DISRUPTION OF LYSOSOMES

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1. Abstract of the project

Acute myeloid leukemia (AML) is a neoplasia characterized by the rapid expansion of immature myeloid blasts in the bone marrow. The course of the disease is marked by poor prognosis and frequent relapse. Thus, new therapeutic approaches are required for remission induction and prevention of relapse. To search for drugs that may revert early transformation events in AML, an in silico screening was performed to identify small bioactive molecules that may revert this transformation pattern. A group of structural-related antihistamines, previously described as cationic amphiphilic drugs (CADs), was identified and validated in a primary in vitro screening. These CADs exerted a potent anti-leukemic effect on AML cells lines and primary AML patient samples ex vivo. Interestingly, little effect was detected when healthy blood cells were treated. Xenotransplantation assays showed that CADs also behaved as antineoplastic agents in vivo, sparing normal hematopoiesis. Based on their physico-chemical properties, CADs commonly dysregulate lysosome integrity and functionality. Preliminary data for the laboratory suggest that the differential anti-leukemic effect is exerted via lysosome disruption. This proposal aims to study the potentiality of lysosomes as a new organelle therapeutic target for acute leukemia. Two new chemical entities were nominated as lead compounds EDK-87 and EDK-88, based on their highly specific and potent anti-leukemic effect, mimicking the mechanism of action previously described. The non-regulatory preclinical study supports the further development of both compounds in clinical settings.

2. Results

Activity 1. Identification of a family of chemical compounds with anti-leukemic activity mediated by disruption of lysosomal activity.

An in silico screening was performed resulting in the identification of 114 novel compounds with anti-leukemic potential, based on preliminary results obtained in the laboratory (Cornet-Masana, 2017). Nine of these compounds were initially validated, while 2 compounds were selected based on their specific mechanism of action against lysosomal function. These two compounds were named EDK-87 and EDK-88, constituting the lead compounds. Both compounds increase the accumulation of free radicals (ROS) in mitochondria, the activation of effector caspases 3/7, the increase of

positivity for the CytoID probe and the presence of LC3 isoform b. The results obtained in this activity indicate that the lead compounds induce both cell-death programs: apoptosis and autophagy, as desired. Treatment with these compounds compromises the integrity of lysosomes by permeabilizing them (increased galactin-3 spike formation), increasing their size (total lysosomal mass) and increasing lysosomal degradative activity (lentiviral probe Lysosomal-METRIQ). Lysosomal activity is also severely affected with treatment by increasing the activity of the transcription factor TFEB, decreasing c-Myc and inducing terminal differentiation of leukemic cells.

Any potential leukemia treatment has to be specific for leukemic cells, while being harmless for healthy cells. Both EDK-87 and EDK-88 do not reduce cell viability of healthy cells in a biologically meaningful way at the pharmacologically interesting concentrations. In fact, the calculated therapeutic window indicates that the range of this treatment would be safe in future clinical developments.

Pharmacology and pharmacodynamic studies to assess the feasibility of using these naked compounds ensure their viability for use in humans, with subcutaneous administration being optimal. Therefore, clinical development of these compounds is warranted.

Activity 2. The anti-leukemic activity of the series headers is synergistic with conventional treatment.

In clinical practice, monotherapy treatments are the least recommended option due to the potential induction of resistance and reduced effectiveness. Both EDK-87 and EDK-88 show a significant synergistic effect with cytarabine (Ara-C), the backbone of AML treatment. Since EDK-87 and EDK-88, the so-called lead compounds, simultaneously affect lysosomes and mitochondria, drugs approved for hematological diseases were identified as having a mechanism of action that results in damage to either or both organelles. In fact, arsenic trioxide (ATO) has been shown to synergize with EDK-87 and EDK-88 in a statistically significant manner. This synergism is observed both at the level of cytotoxicity and clonogenic capacity.

Activity 3. Study of LAPTM4B as a therapeutic target

LAPTM4B is a membrane protein preferentially localized in the lysosome, whose expression at mRNA level is related to treatment response and survival in AML. In fact,

LAPTM4B expression is increased in relapse episodes, compared to the debut at diagnosis. Overexpression of LAPTM4B induces the acquisition of a cytarabine chemoresistance (Ara-C) phenotype, associated with the activation of genes related to intracellular inactivation of cytarabine, and the expression of the TFEB factor. At the intracellular signaling level, LAPTM4B signals in a leukemic setting through the SER response element, belonging to the MAP-Erk pathway. Overexpression of LAPTM4B increases the regenerative capacity of leukemia in animal models of xenotransplantation, demonstrating its potential as a therapeutic target.

Activity 4. Mutational and gene expression study of the AML subgroup without molecular definition.

In order to determine the mutational profile associated with the lysosomal compartment, global sequencing (RNA-Seq) and whole exome sequencing (WES) were performed in samples from AML patients without molecular definition. Eleven pathogenic variants are found in these samples: 6 affecting genes related to cancer predisposition syndromes (*ATM, DDX41, CHEK2*) and 5 related to bone marrow failure syndromes (*FANCA, FANCM, SBDS, DNAJC21, CSF3R*). In addition, fusion genes not detected by conventional cytogenetic methods are observed (NUP98 rearrangement, *ETV6, NRIP1*).

3. Relevance of the future potential implications

The results obtained have made it possible:

1. To identify a chemical family with physicochemical properties compatible with drug development processes and with specific anti-leukemic activity in in vivo models relevant for clinical use.
2. To nominate EDK-87 and EDK-88 as lead compounds suitable for clinical development.
3. To validate LAPTM4B as a therapeutic target in acute myeloid leukemia.
4. To describe novel germline mutations with high prevalence in adult AML patients, constituting new mutations with value as novel markers of predisposition to AML in the germline.

4. Scientific literature generated

This project has generated the following publications:

- Bataller A, Garrido A, Guijarro F, Oñate G, Diaz-Beyá M, Arnan M, Tormo M, Vives S, de Llano MPQ, Coll R, Gallardo D, Vall-Llovera F, Escoda L, Garcia-Guiñon A, Salamero O, Sampol A, Merchan BM, Bargay J, Castaño-Díez S, Esteban D, Oliver-Caldés A, Rivero A, Mozas P, López-Guerra M, Pratcorona M, Zamora L, Costa D, Rozman M, Nomdedéu JF, Colomer D, Brunet S, Sierra J, **Esteve J**. *European LeukemiaNet 2017 risk stratification for acute myeloid leukemia: validation in a risk- adapted protocol*. Blood Adv. 2022 Feb 22;6(4):1193-1206.

- Carbó JM, Cornet-Masana JM, Cuesta-Casanovas L, Delgado-Martínez J, Banús-Mulet A, Clément- Demange L, Serra C, Catena J, Llebaria A, **Esteve J**, **Risueño RM**. *A Novel Family of Lysosomotropic Tetracyclic Compounds for Treating Leukemia*. Cancers (Basel). 2023 Mar 22;15(6):1912.

- Cuesta-Casanovas L, Delgado-Martínez J, Cornet-Masana JM, Carbó JM, Banús-Mulet A, Guijarro F, **Esteve J**, **Risueño RM**. *Prolactin receptor signaling induces acquisition of chemoresistance and reduces clonogenicity in acute myeloid leukemia*. Cancer Cell Int. 2023 May 19;23(1):97.

- Guijarro F, López-Guerra M, Morata J, Bataller A, Paz S, Cornet-Masana JM, Banús-Mulet A, Cuesta-Casanovas L, Carbó JM, Castaño-Díez S, Jiménez-Vicente C, Cortés-Bullich A, Triguero A, Martínez-Roca A, Esteban D, Gómez-Hernando M, Álamo Moreno JR, López-Oreja I, Garrote M, **Risueño RM**, Tonda R, Gut I, Colomer D, Díaz-Beya M, **Esteve J**. *Germ line variants in patients with acute myeloid leukemia without a suspicion of hereditary hematologic malignancy syndrome*. Blood Adv. 2023 Oct 10;7(19):5799-5811.

- Calvente O, Mestre J, **Risueño RM**, Manzanares A, Acha P, Xicoy B, Sole F. *Two-Time Multiplexed Targeted Next-Generation Sequencing Might Help the Implementation of Germline Screening Tools for Myelodysplastic Syndromes/Hematologic Neoplasms*. Biomedicines, 2023 Dec 5;11(12):3222.

- Castaño-Díez S, Pomares H, Esteban D, Guijarro F, Jiménez-Vicente C, Zugasti I, Álamo JR, Mayayo VT, López-Guerra M, de la Fuente C, Charry P, Cortés-Bullich A, Bataller Á, Maluquer C, Colomer D, Rozman M, Arnan M, Xicoy B, **Esteve J**, Díaz-Beyá M. *Characteristics and long-term outcome in a large series of chronic myelomonocytic leukaemia patients including 104 formerly referred to as oligomonocytic*. Br J Haematol. 2024 Mar;204(3):892-897.

The results have been presented at the following congresses:

- ASH 2020 Meeting (virtual):
  - Alex Bataller, Ana Garrido, Francesca Guijarro, Marina Diaz-Beyá, Guadalupe Oñate, Montserrat Hoyos, Montserrat Arnan, Mar Tormo, Susana Vives, Maria Paz Queipo De Llano, Olga Salamero, Rosa Coll, David Gallardo, Ferran Vall-Llovera, Lourdes Escoda, Antonia Sampol, Antonio Garcia-Guiñon, Brayan Merchan, Joan Bargay, Monica Lopez-Guerra, Marta Pratcorona, Lurdes Zamora, Salut Brunet, Josep F Nomdedeu, Jorge Sierra, **Jordi Esteve**. Validation of the European Leukemianet 2017 Prognostic Classification for Patients with De Novo Acute Myeloid Leukemia Treated with a Risk-Adapted Protocol (CETLAM 2012)
  - Sandra Castaño-Díez, Monica Lopez-Guerra, PhD, Francesca Guijarro, MD, Alex Bataller Torralba, MD, Daniel Esteban, Carlos Castillo, MD, Carlos Jiménez-Vicente, MD, Paola Charry, MD, Colomer Dolors, María Rozman, MD, **Jordi Esteve**, Marina Diaz-Beyá, MD. Emergence of NPM1Wild-Type Myeloid Neoplasms after Chemotherapy for Acute Leukemia with NPM1Mutation: Proposed Mechanisms of Clonal Evolution.
  - Jiménez-Vicente C, Charry P, Suárez-Lledó M, et al. Validation of ELN 2022 Risk Classification in Patients Diagnosed with AML Undergoing Allogeneic Hematopoietic Cell Transplantation.
- EHA 2022 Congress (Vienna, Austria):
  - Jennifer Delgado-Martínez, Josep M. Cornet-Masana, Laia Cuesta-Casanovas, José M. Carbó, Lise Clément-Demange, **Ruth M. Risueño**. Role of lysosomal-associated gene LAPTM4B in leukemia. EHA2022 Congress. June 2022. European Hematology Association.

A doctoral thesis related to this project has been submitted:

- Jennifer Delgado Martínez (thesis director: Ruth Muñoz Risueño). *Papel de la proteína lisosomal LAPTM4B en Leucemia Mieloide Aguda*. 6 July 2023. Universidad de Barcelona.