

Review

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Lysosome-mediated chemoresistance in acute myeloid leukemia

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How to cite this article: Cuesta-Casanovas L, Delgado-Martínez J, Cornet-Masana JM, Carbó JM, Clément-Demange L, Risueño RM. Lysosome-mediated chemoresistance in acute myeloid leukemia. *Cancer Drug Resist* 2022;5:233-44. <https://dx.doi.org/10.20517/cdr.2021.122>

Received: 18 Nov 2021 **First Decision:** 24 Jan 2022 **Revised:** 27 Jan 2022 **Accepted:** 14 Feb 2022 **Published:** 14 Mar 2022

Academic Editors: Godefridus J. Peters, Claudio Cerchione **Copy Editor:** Xi-Jun Chen **Production Editor:** Xi-Jun Chen

Abstract

Despite the outstanding advances in understanding the biology underlying the pathophysiology of acute myeloid leukemia (AML) and the promising preclinical data published lastly, AML treatment still relies on a classic chemotherapy regimen largely unchanged for the past five decades. Recently, new drugs have been approved for AML, but the real clinical benefit is still under evaluation. Nevertheless, primary refractory and relapse AML continue to represent the main clinical challenge, as the majority of AML patients will succumb to the disease despite achieving a complete remission during the induction phase. As such, treatments for chemoresistant AML represent an unmet need in this disease. Although great efforts have been made to decipher the biological basis for leukemogenesis, the mechanism by which AML cells become resistant to chemotherapy is largely unknown. The identification of the signaling pathways involved in resistance may lead to new combinatory therapies or new therapeutic approaches suitable for this subset of patients. Several mechanisms of chemoresistance have been identified, including drug transporters, key secondary messengers, and metabolic regulators. However, no therapeutic approach targeting chemoresistance has succeeded in clinical trials, especially due to broad secondary effects in healthy cells. Recent research has highlighted the importance of lysosomes in this phenomenon. Lysosomes' key role in resistance to chemotherapy includes the potential to sequester drugs, central metabolic signaling role, and gene expression regulation. These results provide further evidence to support the development



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of new therapeutic approaches that target lysosomes in AML.

Keywords: Lysosome, chemoresistance, AML, lysosomotropic drug, lysosomal sequestration, refractory AML

INTRODUCTION

Relapse and refractory diseases are major clinical challenges during the management of acute myeloid leukemia (AML) patients, and they prevent an optimized response to current treatments. As relapse refers to reappearance of the disease, relapse episodes are strongly related to refractoriness, both of them leading to poor prognosis^[1]. For the last five decades, the standard AML therapy consisted of a combination of cytarabine and an anthracycline^[2], and improvements in the survival rate are mainly due to optimization of the supportive care and hematopoietic cell transplantation protocols. Recently, new targeted drugs have been approved, incorporating the notion of personalised treatments for AML. Continuous assessment of newly approved drugs over time will provide valuable complete efficacy and safety data that will result in an optimal drug regime, as current clinical benefit is controversial^[3,4]. Thus, acquisition of new biological insight in AML pathophysiology represents an unmet need necessary to expand the targetable therapeutic mechanism repertoire to overcome chemoresistance and, then, significantly improve clinical outcomes for AML patients.

Primary and secondary chemoresistance have been widely explored using conventional approaches, searching for gene mutations, chromosomal aberrations, and dysregulated signaling pathways^[5-8]. Changes in the multidrug resistance gene family affect the intracellular concentration of drugs by either reducing the active transport into the tumor cells or increasing the efflux out to the extracellular space. Other mechanisms of action that affect the response to chemotherapy include: modifications in the chemotherapy molecular targets, preventing the pharmacologic action, increased ability to repair tumor DNA damage, defective response to proapoptotic stimuli, and changes in the tumor microenvironment^[8]. Although several inhibitors targeting drug-resistance mechanisms have been reported, their clinical development is still under evaluation.

Oxidative phosphorylation function, metabolic plasticity, and mitochondrial adaptation contribute to chemoresistance in AML, especially towards cytarabine, as resistant AML cells rely more on mitochondrial oxidative phosphorylation and less on glycolysis^[9,10]. Increased mitochondrial mass, mitochondrial membrane potential, reactive oxygen species production, and a characteristic gene signature associated with oxidative phosphorylation are hallmarks of chemoresistance AML cells^[9,11]. Indeed, inhibition of oxidative phosphorylation induces chemosensitivity^[9,11-13]; and mitochondrial oxidative phosphorylation and respiratory capacity correlate with a better response to cytarabine treatment in AML cells^[11]. This metabolic reprogramming might have an important therapeutic implication and metabolic vulnerabilities might be exploited pharmacologically.

Closely related to mitochondria, lysosomes have attracted special interest in oncology due to their growing importance in transformation processes. The traditional view of lysosomes has been challenged by the recognition that lysosomes are not only “degradative organelles”, but also metabolic sensors and regulators, becoming legitimated as intracellular signaling hubs^[14]. Additionally, recent findings highlight the physical and functional interaction of mitochondria and lysosomes, suggesting that this crosstalk plays a major role in metabolic regulation, based on the transfer of Ca²⁺ between organelles^[15,16], affecting the cellular response to treatment. In this review, the role of lysosomes in chemoresistance in AML is discussed and an overview of the potential therapeutic approaches for overcoming refractoriness in leukemia is provided.

LYSOSOMES

Lysosomes were first described in the 1950s as organelles responsible for the degradation of biological macromolecules from extra- and intra-cellular origins^[17]. Their central role in cellular recycling and homeostasis was revealed later when autophagy was discovered^[18,19]. Recent discoveries confirm lysosomes as crucial modulators of cell homeostasis, regulating both cellular metabolism and clearance (reviewed in Ref.^[20]).

Structurally, lysosomes are acidic organelles surrounded by a phospholipid bilayer. The acidic lumen is maintained by vacuolar-type H⁺ ATPase (V-ATPase) on the lysosomal membrane^[21]. Other key proteins on lysosomal membrane are: lysosome-associated membrane protein, soluble N-ethylmaleimide-sensitive factor activating protein receptors, toll-like receptors, and mammalian target of rapamycin (mTOR)^[22]. Luminal hydrolytic enzymes of lysosomes include proteases, sulfatases, nucleases, lipases, phosphatases, and nucleases, which degrade macromolecules.

Lysosomes belong to the endolysosomal system, a dynamic network of organelles consisting of early, late, and recycling endosomes and lysosomes. Primary lysosomes originate from the Golgi apparatus. Early endosomes formed from the plasma membrane might also progress to late endosomes and lysosomes, as a result of a maturation process. Alternatively, a contact site between lysosomes and late endosomes can be formed, followed by cargo transfer and dissociation (kiss-and-run model), or late endosomes can fuse with lysosomes, creating a hybrid organelle that subsequently evolves in lysosomes (fusion and fission process)^[23].

During malignant transformation, cancer cells adapt their physiological processes to sustain their intrinsic anabolic and catabolic needs. Both lysosomal mass and subcellular localization are widely changed to enable the acquisition of cancer cells' idiosyncratic feature of uncontrolled growth. Recycling of exogenous material provides energy and key molecular components, while autophagy enhances catabolism and, consequently, energy and metabolite precursors are supplied. Nutrient sensing is tightly regulated by lysosomes, based on the activation and translocation of the mTORC1 complex to the lysosome membrane, enhancing lipid catabolism under starving condition in transformed cells (reviewed in Ref.^[24]).

Lysosomes in AML

During leukemogenesis, AML cells increase their lysosomal mass, although their number is not significantly affected^[25]. As AML relies on fatty acids for energy supply, lysosome-dependent fatty acid oxidation rate is higher, inducing an augmented lysosomal mass to support this process^[26]. The gene network that regulates the lysosomal biogenesis is also upregulated, similarly to the expression of key lysosomal enzymes^[27-37]. Indeed, the lysosomal matrix enzyme activity is enhanced in AML, as compared to healthy myeloid cells, probably due to an increase in the quantity of enzymes and the influx rate^[38]. As a consequence of these lysosomal changes, AML cells contain fragile lysosomes due to destabilization of the lysosomal limiting membrane and lower pH.

V-ATPases play both direct and indirect roles in the control of cellular signaling. Growth, survival, and differentiation signaling pathways frequently rely on these ATP-dependent proton pumps. Control of vesicular pH by V-ATPase is essential for proper signaling by many plasma membrane receptors that traffic through the recycling networks, including Notch and Wnt^[39]. Canonical Wnt is required for the development and maintenance of AML^[40-42]. Inhibition of V-ATPase prevents the activating phosphorylation of the Wnt receptor upon ligand binding and dysregulates ligand-mediated internalization of the receptor^[43,44]. Although the importance of Notch as a therapeutic target in AML is still controversial,

this signaling pathway regulates proliferation and cell survival^[45-47]. Activation of the Notch receptor induces the intracellular domain to be cleaved and translocated to the nucleus, a process requiring V-ATPase function^[48-50]. Similarly, PI3K/Akt/mTOR signaling pathway is crucial to many physiological processes, such as proliferation, gene expression regulation, differentiation, cell death, metabolism, cell survival, and migration, and it is frequently hyperactivated in AML^[51-55]. While the precise mechanism involved in the V-ATPase-mediated modulation of mTOR remains widely unknown, inhibition of V-ATPase represses mTOR activation^[56], and mTOR inhibition leads to AML cell death^[57,58].

Lysosomal sequestration of drugs

Lysosomal sequestration or lysosomal trapping is an important mechanism responsible for chemoresistance acquisition^[59]. Many chemotherapeutics used in clinics (i.e., anthracyclines, taxanes, platinum-based drugs) are lipophilic, weak-base drugs and can therefore diffuse freely across lipid membranes, including the plasma membrane and lysosomal membrane. Alternatively, lysosomotropic drugs can be actively transported by inward turned multidrug efflux transporters of the ATP-binding cassette superfamily, embedded in the lysosomal membrane (originally expressed in the plasma membrane and endocytosed into lysosomes), particularly ABCB1^[60-63] and ABCA3^[38]. The acidity of the lysosomal lumen facilitates the rapid protonation of weak-base drugs, impairing their ability to cross back across lipid bilayers, resulting in their marked lysosomal accumulation and compartmentalization^[64,65]. Chemotherapeutics sequestered in lysosomes and associated to drug resistance phenomena include tyrosine kinase inhibitors^[66-68], topoisomerase inhibitors^[69-71], antimetabolites^[72], alkylating agents^[73,74], and microtubule-targeting agents^[75,76]. Lysosomal sequestration severely affects drug subcellular distribution, significantly reducing efficiency, since lysosomes are seldom the target sites for these chemotherapeutics, and the sequestered drugs will not reach their targets^[77]. Therefore, higher concentrations are required to achieve therapeutically relevant concentrations, increasing side effects in patients, and promoting secondary chemotherapy resistance. Treatment with these types of drugs induces expansion of the lysosomal compartment, thereby enhancing their lysosomal sequestration capacity and further increasing chemoresistance, constituting a feedback loop^[78-80].

The transcription factor EB (TFEB) is the master regulator of lysosomal biogenesis, by modulating the expression of genes bearing the coordinated lysosomal expression and regulation motif. In resting conditions, phosphorylated TFEB is retained inactivated in the cytoplasm by the 14-3-3 protein. Calcineurin dephosphorylates and activates TFEB, leading to its dissociation from 14-3-3 and subsequent translocation to the nucleus. mTOR phosphorylates (and inactivates) TFEB, enabling its binding to 14-3-3 in the cytoplasm. The activity of calcineurin is modulated by the release of Ca²⁺ from the lysosomes through the lysosomal Ca²⁺ transporter mucopolin (MCOLN1)^[81]. mTOR can be inhibited by raising the pH, as lysosomotropic drugs do^[82]. Activation of TFEB induces lysosomal biogenesis which increases lysosomal sequestration capacity and exerts a feedback loop.

Clearance of chemotherapeutics sequestered in lysosomes might also provide an additional chemoresistance mechanism. Exocytosis has been proposed as the preferred process^[83], as drug accumulation induces an increase of pH, leading to an activation of exocytosis^[84,85]. Moreover, drug sequestration-induced TFEB activation partially results in the induction of lysosomal exocytosis and clearance of lysosomal content outside the cell^[86,87]. However, once drugs have been released to the extracellular space, they can rediffuse back into the cells, making the exocytosis-mediated chemoresistance a controversial process that requires further clarification.

The anthracyclin daunorubicin (DNR), a backbone chemotherapeutic agent in first-line treatment of AML, displays physicochemical features compatible with lysosomal trapping. Early studies demonstrated that DNR intracellular distribution depends on drug treatment response. Sensitive AML cells preferentially accumulate DNR in the nucleus, where the pharmacology effect is exerted. In contrast, DNR-resistant AML cells tend to sequester DNR in lysosomes^[88]. Indeed, expanded lysosomes are observed in response to DNR treatment, as well as a diminished nuclear drug uptake, exhibiting a 2.5/3-fold less DNR concentration in nucleus in resistant versus sensitive AML cells^[69]. In AML, the relevance of ABCB1 in the active transportation of DNR into lysosomes is limited^[60,69], in contrast to other solid tumor cells^[62,63,89]. Instead, in AML, DNR is actively influxed by lysosomal ABCA3, a transporter upregulated in chemoresistant patients and correlated with poor prognosis^[37,38].

LYSOSOME-BASED THERAPEUTIC APPROACHES

The key contribution of lysosomes to chemoresistance raises increasing interest in lysosome-targeting therapies to either sensitize tumor cells to current approved chemotherapy or as new pharmacologic approaches. The transformation process itself affects the integrity and size of lysosomes, increasing their fragility. Sphingolipid metabolism alterations are also often found in cancer cells, leading to changes in the lysosomal membrane function and structure^[90]. Due to an increased metabolic demand, cancer cells upregulate their lysosomal function, resulting in an augmented lysosomal mass^[91]. Accumulation of lysosomotropic drugs in cancer cells destabilizes lysosomes, causing their failure and eventually activating cell death program. However, healthy lysosomes are fully functional and display compensatory mechanisms that prevent fatal damage. Consequently, a wide therapeutic window is found due to these differences in fragility of lysosomes in cancer cells *vs.* healthy cells. Several strategies have been explored, including lysosomal destabilization. Lysosomotropic compounds can accumulate in lysosomes, causing lysosomal membrane-cell permeabilization, release of cathepsins, and consequently, activation of the cell death program^[92]. Using different screening approaches, the anti-malaria drug mefloquine^[25], cationic amphiphilic antihistamines^[93], and σ_2 receptor agonist siramesine^[94] were described as lysosomal disruptors in AML cells and sensitizers to approved chemotherapeutics. Mefloquine disturbs the lysosomal membrane of AML cells, allowing the release of cathepsins to the cytoplasm and inducing apoptosis^[25]. Cationic amphiphilic antihistamines simultaneously disrupt both lysosomes and mitochondria, based on their physico-chemical properties, inducing both apoptosis and autophagy^[93]. Both the specific cationic amphiphilic antihistamines and mefloquine spare healthy blood cells, confirming the differential effect of lysosomal disruptors in AML and the existence of a preclinically-validated therapeutic window. However, reprofiling of these drugs to AML is difficult due to their pharmacological profile, and no clinical trials have been successfully completed. Medicinal chemistry programs are expected to be necessary to achieve clinically suitable new compounds. Nevertheless, to date, targeting lysosomal integrity is the most promising therapeutic approach to overcome lysosome-mediated chemoresistance in AML [Figure 1].

Accumulation of chemotherapeutics in lysosomes heavily depends on lysosomal lumen pH. Moreover, in resistant AML cells, the pH gradient between the lysosome and cytosol is higher^[95]. Treatment with V-ATPase inhibitor archazolid A induces cell death in leukemic cells, both T-cell acute leukemia and acute myeloid leukemia^[96]. Similar results were obtained with bafilomycin A, another V-ATPase inhibitor, although the mechanism of action responsible for this pharmacological effect is still controversial^[97], as bafilomycin A is unable to resensitize cytarabine-resistant cells^[98]. However, the preclinical data suggest that the therapeutic window was narrow, and their clinical significance might be limited.

As for further lysosome regulators, rapamycin and other mTORC1 modulators, have shown promising results in preclinical assays, specially in combination therapies^[52]. However, mTORC1 regulates key

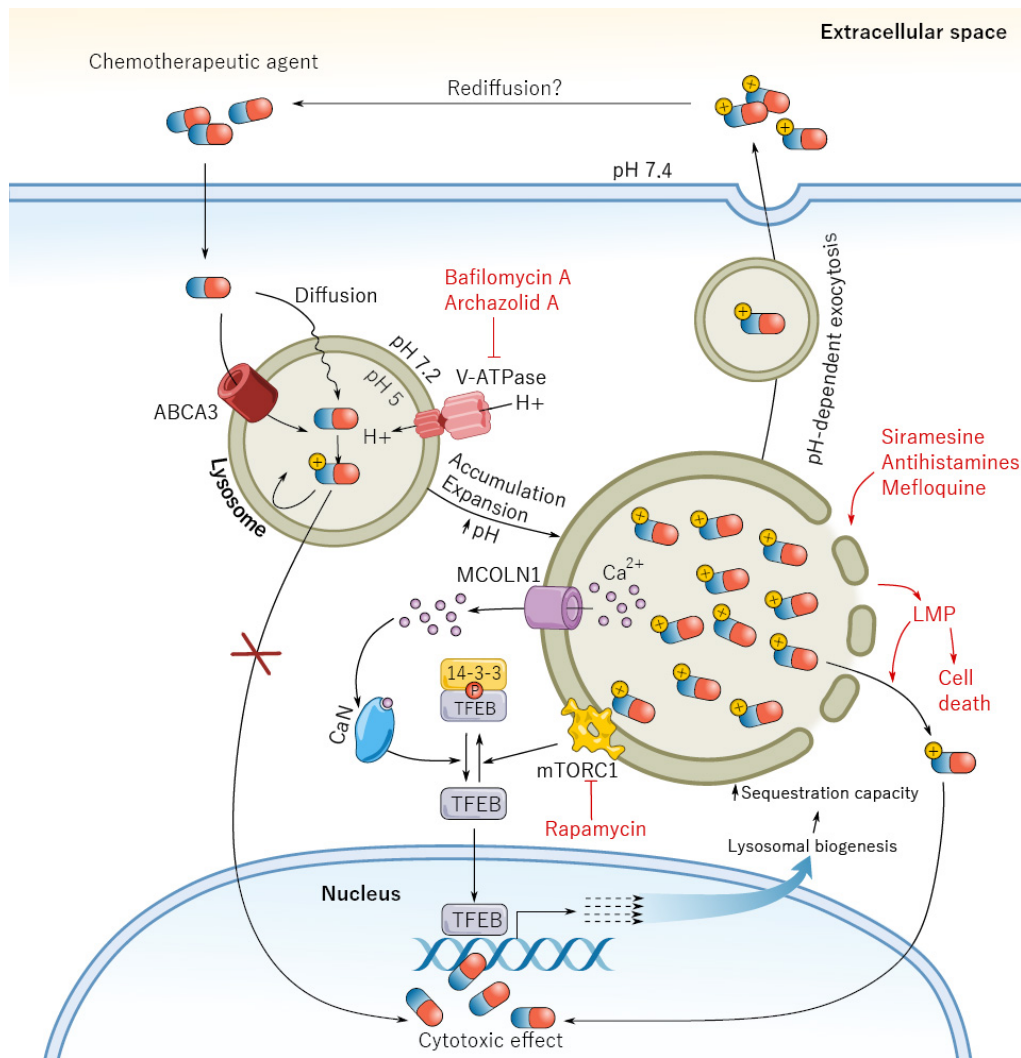


Figure 1. Mechanisms of lysosomal-mediated chemoresistance in acute myeloid leukemia (AML) at a glance. Most chemotherapeutic agents get readily sequestered in lysosomes upon entry in AML cells, causing a remarkable expansion of the lysosomal compartment. Lysosomal expansion is accompanied by an increase in pH, inducing exocytosis and, consequently, clearance of chemotherapy from cells. Both mechanisms prevent chemotherapeutic agents from directly interacting with their molecular targets, commonly located in the nucleus. To revert the undesirable sequestration, two main strategies have been proposed, namely, increasing lysosomal pH by inhibiting V-ATPase or pharmacologically inducing lysosomal membrane leakiness, thus releasing chemotherapeutics and additionally eliciting lysosomal-dependent cell death. Conversely, mTORC1 inhibition contributes to lysosomal biogenesis and sequestration capacity, a mechanism that has been traditionally overlooked in translation of mTORC1 inhibitors and that could partly explain their clinical failure. ABCA3: ATP binding cassette subfamily A member 3; CaN: calcineurin; LMP: lysosomal membrane permeabilization; MCOLN1: mucolipin TRP cation channel 1; mTORC1: mammalian target of rapamycin complex 1; TFEB: transcription factor EB; V-ATPase: vacuolar ATPase.

processes implicated in cellular metabolism and growth. The complexity and broadness of the mTOR signaling networks increase the risk of toxicity due to off-tumor on-target effects, as the therapeutic window is narrow, if existent^[99]. Besides, accumulating evidence suggest that mTOR is not the only specific molecular target for rapamycin. A quantitative chemical proteomics approach has revealed that the rapamycin targetome is extensive, including Stat3, an ubiquitous secondary messenger^[100]. In consequence, the efficacy of mTORC1 modulators in clinical trials is limited^[101-104], probably due to its conserved function of mTOR complex in homeostasis mechanisms in all cell types, preventing their further clinical development [Table 1].

Table 1. Summary of the main lysosome-associated chemoresistance mechanisms and therapeutic approaches

CHEMORESISTANCE MECHANISMS RELATED TO LYOSOMES			
Mechanism	Cause	Effect	Solution
Drug sequestration ^[59]	Drug protonation due to lysosomal acidic lumen ^[64,65]	Changes in subcellular distribution ^[77]	Increase drug concentration ^[78-80]
Exocytosis ^[83]	Drug accumulation due to increased pH ^[84,85] and TFEB activation ^[86,87]	Clearance of lysosomal drug content ^[86,87]	Drugs can rediffuse back into the cells
LYSOSOME-BASED THERAPEUTIC APPROACHES			
Type	Actions	Examples	
Lysosomal destabilizers	Lysosomal membrane permeabilization, cathepsins release, and cell death program activation ^[92]	Mefloquine (anti-malaria drug) ^[25] , cationic amphiphilic antihistamines ^[93] , and siramesine ($\sigma 2$ receptor agonist) ^[94]	
V-ATPase inhibitors	Activation of cell death program ^[96]	Archazolid A ^[96] and bafilomycin A ^[98,96]	
mTOR modulators	Effect on combinatory therapies ^[52]	Rapamycin ^[52]	
Antibody-drug conjugates	Release of the therapeutics coupled to the antibody ^[105]	Gemtuzumab ozogamicin ^[106]	

Gemtuzumab ozogamicin (Mylotarg) is an anti-CD33 monoclonal antibody conjugated to the small molecule chemotherapy drug calicheamicin, recently reapproved for AML. Upon surface CD33 recognition, this antibody-drug conjugate is internalized and translocated to lysosomes. The acidic-labile linker is hydrolyzed in the acidic environment of the lysosome, releasing the cytotoxic drug that is exported to the nucleus, where the pharmacological effect occurs^[105]. Thus, the effectiveness of gemtuzumab ozogamicin greatly depends on lysosome functionality. This targeted therapy was expected to represent a new paradigm in AML therapy; however, the clinical benefit is limited and severe adverse effects are found in a considerable rate^[106]. Discrepancies between expectations and clinical efficacy may be explained based on the lysosomal impairment in AML cells, partially due to the hyperactivation of PI3K/Akt signaling pathway^[51,53,54]. A direct correlation between lysosome function and gemtuzumab ozogamicin-induced cytotoxicity was observed and forced activation of lysosomes led to a synergistic effect with gemtuzumab ozogamicin^[106], demonstrating that these lysosomal-dependent conjugate approaches used as monotherapy may be of limited interest in AML.

CONCLUSION

Refractory and relapse disease are still the main clinical challenges faced in AML. Although new drugs have been approved in the last years, treatment failure and resistance mechanisms remain a major problem in patient management. To date, none of the therapeutic strategies designed to overcome chemoresistance has succeeded in clinics. The identification of lysosomes as key organelles in resistance acquisition opened a new research field and provided new avenues to explore in order to revert the resistant phenotype. The leukemic transformation process results in an augmented metabolic demand, associated with the upregulation of the lysosome function. Consequently, increase in lysosomal mass, pH, and enzymatic activity is induced. Lysosomotropism of several chemotherapeutics enable their sequestration in the lysosomes, becoming “drug-safe house” compartments and reducing their cytotoxic effect in molecular targets. As a consequence of the lysosomal changes induced during leukemogenesis, AML lysosomes are more fragile than those found in healthy cells, with a preclinically demonstrated safe therapeutic window. Developing new drugs that target leukemic lysosome integrity may sensitize AML cells to conventional chemotherapeutics or even constitute a new pharmacological lysosome-centred strategy for relapse and refractory AML patients.

DECLARATIONS

Acknowledgments

The authors acknowledge the support of all Risueño Laboratory members.

Authors' contributions

Preparing and reviewing the manuscript: Cuesta-Casanovas L, Delgado-Martinez J, Cornet-Masana JM, Carbó JM, Clément-Demange L, Risueño RM

Availability of data and materials

Not applicable.

Financial support and sponsorship

This work was funded by the Josep Carreras International Leukaemia Foundation (RMR), l'Obra Social "La Caixa"-Fundació Bancària "La Caixa" (RMR), and CERCA Programme/Generalitat de Catalunya (IJC).

Conflicts of interest

Cornet-Masana JM, Carbó JM, Clément-Demange L, and Risueño RM are inventors in patents related to acute myeloid leukemia treatments. Risueño RM is a shareholder of Leukos Biotech. Cuesta-Casanovas L and Delgado-Martinez J declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med* 2015;373:1136-52. [DOI PubMed](#)
2. Yates JW, Wallace HJ Jr, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep* 1973;57:485-8. [PubMed](#)
3. Estey E, Karp JE, Emadi A, Othus M, Gale RP. Recent drug approvals for newly diagnosed acute myeloid leukemia: gifts or a Trojan horse? *Leukemia* 2020;34:671-81. [DOI PubMed](#)
4. Tran AA, Miljković M, Prasad V. Analysis of estimated clinical benefit of newly approved drugs for US patients with acute myeloid leukemia. *Leuk Res* 2020;96:106420. [DOI PubMed](#)
5. Gurnari C, Pagliuca S, Visconte V. The interactome between metabolism and gene mutations in myeloid malignancies. *Int J Mol Sci* 2021;22:3135. [DOI PubMed PMC](#)
6. Long L, Assaraf YG, Lei ZN, et al. Genetic biomarkers of drug resistance: a compass of prognosis and targeted therapy in acute myeloid leukemia. *Drug Resist Updat* 2020;52:100703. [DOI PubMed](#)
7. Alexa-Stratulat T, Pešić M, Gašparović AČ, Trougakos IP, Riganti C. What sustains the multidrug resistance phenotype beyond ABC efflux transporters? *Drug Resist Updat* 2019;46:100643. [DOI PubMed](#)
8. Fajardo-Orduña GR, Ledesma-Martínez E, Aguiñiga-Sánchez I, Mora-García ML, Weiss-Steider B, Santiago-Osorio E. Inhibitors of chemoresistance pathways in combination with Ara-C to overcome multidrug resistance in AML. A mini review. *Int J Mol Sci* 2021;22:4955. [DOI PubMed PMC](#)
9. Farge T, Saland E, de Toni F, et al. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov* 2017;7:716-35. [DOI](#)
10. Tian Y, Huang Z, Wang Z, et al. Identification of novel molecular markers for prognosis estimation of acute myeloid leukemia: over-expression of PDCD7, FIS1 and Ang2 may indicate poor prognosis in pretreatment patients with acute myeloid leukemia. *PLoS One* 2014;9:e84150. [DOI PubMed PMC](#)
11. Bosc C, Saland E, Bousard A, et al. Mitochondrial inhibitors circumvent adaptive resistance to venetoclax and cytarabine combination therapy in acute myeloid leukemia. *Nat Cancer* 2021;2:1204-23. [DOI PubMed](#)
12. Roca-Portoles A, Rodríguez-Blanco G, Sumpton D, et al. Venetoclax causes metabolic reprogramming independent of BCL-2

- inhibition. *Cell Death Dis* 2020;11:616. DOI PubMed PMC
13. Niu X, Rothe K, Chen M, et al. Targeting AXL kinase sensitizes leukemic stem and progenitor cells to venetoclax treatment in acute myeloid leukemia. *Blood* 2021;137:3641-55. DOI PubMed PMC
 14. Appelqvist H, Wåster P, Kågedal K, Öllinger K. The lysosome: from waste bag to potential therapeutic target. *J Mol Cell Biol* 2013;5:214-26. DOI PubMed
 15. Peng W, Wong YC, Krainc D. Mitochondria-lysosome contacts regulate mitochondrial Ca²⁺ dynamics via lysosomal TRPML1. *Proc Natl Acad Sci U S A* 2020;117:19266-75. DOI PubMed PMC
 16. Wong YC, Kim S, Peng W, Krainc D. Regulation and function of mitochondria-lysosome membrane contact sites in cellular homeostasis. *Trends Cell Biol* 2019;29:500-13. DOI PubMed PMC
 17. Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem J* 1955;60:604-17. DOI PubMed PMC
 18. Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 1992;119:301-11. DOI PubMed PMC
 19. Duve C, Wattiaux R. Functions of lysosomes. *Annu Rev Physiol* 1966;28:435-92. DOI PubMed
 20. Lamming DW, Bar-Peled L. Lysosome: the metabolic signaling hub. *Traffic* 2019;20:27-38. DOI PubMed PMC
 21. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol* 2007;8:917-29. DOI PubMed
 22. Zhang Z, Yue P, Lu T, Wang Y, Wei Y, Wei X. Role of lysosomes in physiological activities, diseases, and therapy. *J Hematol Oncol* 2021;14:79. DOI PubMed PMC
 23. Trivedi PC, Bartlett JJ, Pulinilkunnil T. Lysosomal biology and function: modern view of cellular debris bin. *Cells* 2020;9:1131. DOI PubMed PMC
 24. Tang T, Yang ZY, Wang D, et al. The role of lysosomes in cancer development and progression. *Cell Biosci* 2020;10:131. DOI PubMed PMC
 25. Sukhai MA, Prabha S, Hurren R, et al. Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors. *J Clin Invest* 2013;123:315-28. DOI PubMed PMC
 26. Samudio I, Harmancey R, Fiegl M, et al. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. *J Clin Invest* 2010;120:142-56. DOI PubMed PMC
 27. Eppert K, Takenaka K, Lechman ER, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat Med* 2011;17:1086-93. DOI PubMed
 28. Kikushige Y, Shima T, Takayanagi S, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* 2010;7:708-17. DOI PubMed
 29. Gentles AJ, Plevritis SK, Page P, Majeti R, Alizadeh AA. Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. *JAMA* 2010;304:2706-15. DOI PubMed PMC
 30. Metzeler KH, Hummel M, Bloomfield CD, et al. An 86-probe gene expression signature can predict survival in AML with normal karyotype independently of FLT3 ITD and NPM1 mutation status - a collaborative study from the AMLCG and CALGB study groups. *Blood* 2007;110:596-596. DOI
 31. Rapin N, Bagger FO, Jendholm J, et al. Comparing cancer vs normal gene expression profiles identifies new disease entities and common transcriptional programs in AML patients. *Blood* 2014;123:894-904. DOI PubMed
 32. Metzeler KH, Hummel M, Bloomfield CD, et al; Cancer and Leukemia Group B, German AML Cooperative Group. An 86-probe-set gene-expression signature predicts survival in cytogenetically normal acute myeloid leukemia. *Blood* 2008;112:4193-201. DOI PubMed PMC
 33. Ng SW, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature* 2016;540:433-7. DOI PubMed
 34. Herold T, Jurinovic V, Batcha AMN, et al. A 29-gene and cytogenetic score for the prediction of resistance to induction treatment in acute myeloid leukemia. *Haematologica* 2018;103:456-65. DOI PubMed PMC
 35. Valk PJ, Verhaak RG, Beijnen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med* 2004;350:1617-28. DOI PubMed
 36. Steinbach D, Gillet JP, Sauerbrey A, et al. ABCA3 as a possible cause of drug resistance in childhood acute myeloid leukemia. *Clin Cancer Res* 2006;12:4357-63. DOI PubMed
 37. Li Z, Herold T, He C, et al. Identification of a 24-gene prognostic signature that improves the European LeukemiaNet risk classification of acute myeloid leukemia: an international collaborative study. *J Clin Oncol* 2013;31:1172-81. DOI PubMed PMC
 38. Chapuy B, Koch R, Radunski U, et al. Intracellular ABC transporter A3 confers multidrug resistance in leukemia cells by lysosomal drug sequestration. *Leukemia* 2008;22:1576-86. DOI PubMed
 39. Aasebø E, Bartaula-Brevik S, Hernandez-Valladares M, Bruserud Ø. Vacuolar ATPase as a possible therapeutic target in human acute myeloid leukemia. *Expert Rev Hematol* 2018;11:13-24. DOI PubMed
 40. Majeti R, Becker MW, Tian Q, et al. Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci* 2009;106:3396-401. DOI PubMed PMC
 41. Benoit YD, Mitchell RR, Risueño RM, et al. Sam68 allows selective targeting of human cancer stem cells. *Cell Chem Biol* 2017;24:833-844.e9. DOI PubMed
 42. Wang Y, Krivtsov AV, Sinha AU, et al. The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. *Science* 2010;327:1650-3. DOI PubMed PMC

43. Cruciati CM, Ohkawara B, Acebron SP, et al. Requirement of prorenin receptor and vacuolar H⁺-ATPase-mediated acidification for Wnt signaling. *Science* 2010;327:459-63. [DOI](#) [PubMed](#)
44. Tuttle AM, Hoffman TL, Schilling TF. Rabconnectin-3a regulates vesicle endocytosis and canonical Wnt signaling in zebrafish neural crest migration. *PLoS Biol* 2014;12:e1001852. [DOI](#) [PubMed](#) [PMC](#)
45. Wang Y, Liu Y, Malek SN, Zheng P, Liu Y. Targeting HIF1 α eliminates cancer stem cells in hematological malignancies. *Cell Stem Cell* 2011;8:399-411. [DOI](#) [PubMed](#) [PMC](#)
46. Kannan S, Sutphin RM, Hall MG, et al. Notch activation inhibits AML growth and survival: a potential therapeutic approach. *J Exp Med* 2013;210:321-37. [DOI](#) [PubMed](#) [PMC](#)
47. Lobry C, Ntziachristos P, Ndiaye-Lobry D, et al. Notch pathway activation targets AML-initiating cell homeostasis and differentiation. *J Exp Med* 2013;210:301-19. [DOI](#) [PubMed](#) [PMC](#)
48. Kobia F, Duchi S, Deflorian G, Vaccari T. Pharmacologic inhibition of vacuolar H⁺ ATPase reduces physiologic and oncogenic Notch signaling. *Mol Oncol* 2014;8:207-20. [DOI](#) [PubMed](#) [PMC](#)
49. Vaccari T, Duchi S, Cortese K, Tacchetti C, Bilder D. The vacuolar ATPase is required for physiological as well as pathological activation of the Notch receptor. *Development* 2010;137:1825-32. [DOI](#) [PubMed](#) [PMC](#)
50. Yan Y, Deneff N, Schüpbach T. The vacuolar proton pump, V-ATPase, is required for notch signaling and endosomal trafficking in *Drosophila*. *Dev Cell* 2009;17:387-402. [DOI](#) [PubMed](#) [PMC](#)
51. Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. *Blood* 2003;102:972-80. [DOI](#) [PubMed](#)
52. Récher C, Beyne-Rauzy O, Demur C, et al. Antileukemic activity of rapamycin in acute myeloid leukemia. *Blood* 2005;105:2527-34. [DOI](#) [PubMed](#)
53. Yilmaz OH, Valdez R, Theisen BK, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 2006;441:475-82. [DOI](#) [PubMed](#)
54. Tamburini J, Elie C, Bardet V, et al. Constitutive phosphoinositide 3-kinase/Akt activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. *Blood* 2007;110:1025-8. [DOI](#) [PubMed](#)
55. Récher C, Dos Santos C, Demur C, Payrastra B. mTOR, a new therapeutic target in acute myeloid leukemia. *Cell Cycle* 2005;4:1540-9. [DOI](#) [PubMed](#)
56. Marino ML, Fais S, Djavaheri-Mergny M, et al. Proton pump inhibition induces autophagy as a survival mechanism following oxidative stress in human melanoma cells. *Cell Death Dis* 2010;1:e87. [DOI](#) [PubMed](#) [PMC](#)
57. Carneiro BA, Kaplan JB, Altman JK, Giles FJ, Platanius LC. Targeting mTOR signaling pathways and related negative feedback loops for the treatment of acute myeloid leukemia. *Cancer Biol Ther* 2015;16:648-56. [DOI](#) [PubMed](#) [PMC](#)
58. Chapuis N, Tamburini J, Green AS, et al. Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. *Clin Cancer Res* 2010;16:5424-35. [DOI](#) [PubMed](#)
59. Halaby R. Influence of lysosomal sequestration on multidrug resistance in cancer cells. *Cancer Drug Resist* 2019;2:31-42. [DOI](#)
60. Ferrao P, Sincok P, Cole S, Ashman L. Intracellular P-gp contributes to functional drug efflux and resistance in acute myeloid leukaemia. *Leukemia Research* 2001;25:395-405. [DOI](#) [PubMed](#)
61. Yamagishi T, Sahni S, Sharp DM, Arvind A, Jansson PJ, Richardson DR. P-glycoprotein mediates drug resistance via a novel mechanism involving lysosomal sequestration. *J Biol Chem* 2013;288:31761-71. [DOI](#) [PubMed](#) [PMC](#)
62. Fu D, Roufogalis BD. Actin disruption inhibits endosomal traffic of P-glycoprotein-EGFP and resistance to daunorubicin accumulation. *Am J Physiol Cell Physiol* 2007;292:C1543-52. [DOI](#) [PubMed](#)
63. Shapiro AB, Fox K, Lee P, Yang YD, Ling V. Functional intracellular P-glycoprotein. *Int J Cancer* 1998;76:857-64. [DOI](#) [PubMed](#)
64. MacIntyre AC, Cutler DJ. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos* 1988;9:513-26. [DOI](#) [PubMed](#)
65. Duve C, De Barsey T, Poole B, Trouet A, Tulkens P, Van Hoof F. Lysosomotropic agents. *Biochem Pharmacol* 1974;23:2495-531. [DOI](#) [PubMed](#)
66. Gotink KJ, Broxterman HJ, Labots M, et al. Lysosomal sequestration of sunitinib: a novel mechanism of drug resistance. *Clin Cancer Res* 2011;17:7337-46. [DOI](#) [PubMed](#) [PMC](#)
67. Van Der Steen N, Keller K, Dekker H, et al. Crizotinib sensitizes the erlotinib resistant HCC827GR5 cell line by influencing lysosomal function. *J Cell Physiol* 2020;235:8085-97. [DOI](#) [PubMed](#) [PMC](#)
68. Klerk DJ, Honeywell RJ, Jansen G, Peters GJ. Transporter and lysosomal mediated (multi)drug resistance to tyrosine kinase inhibitors and potential strategies to overcome resistance. *Cancers (Basel)* 2018;10:503. [DOI](#) [PubMed](#) [PMC](#)
69. Hurwitz SJ, Terashima M, Mizunuma N, Slapak CA. Vesicular anthracycline accumulation in doxorubicin-selected U-937 cells: participation of lysosomes. *Blood* 1997;89:3745-54. [PubMed](#)
70. Herlevsen M, Oxford G, Owens CR, Conaway M, Theodorescu D. Depletion of major vault protein increases doxorubicin sensitivity and nuclear accumulation and disrupts its sequestration in lysosomes. *Mol Cancer Ther* 2007;6:1804-13. [DOI](#) [PubMed](#)
71. Smith PJ, Sykes HR, Fox ME, Furlong IJ. Subcellular distribution of the anticancer drug mitoxantrone in human and drug-resistant murine cells analyzed by flow cytometry and confocal microscopy and its relationship to the induction of DNA damage. *Cancer Res* 1992;52:4000-8. [PubMed](#)
72. Marshall LA, Rhee MS, Hofmann L, Khodjakov A, Schneider E. Increased lysosomal uptake of methotrexate-polyglutamates in two methotrexate-resistant cell lines with distinct mechanisms of resistance. *Biochem Pharmacol* 2005;71:203-13. [DOI](#) [PubMed](#)
73. Groth-Pedersen L, Ostenfeld MS, Høyer-Hansen M, Nylandsted J, Jäätelä M. Vincristine induces dramatic lysosomal changes and sensitizes cancer cells to lysosome-destabilizing siramesine. *Cancer Res* 2007;67:2217-25. [DOI](#) [PubMed](#)

74. Samimi G, Katano K, Holzer AK, Safaei R, Howell SB. Modulation of the cellular pharmacology of cisplatin and its analogs by the copper exporters ATP7A and ATP7B. *Mol Pharmacol* 2004;66:25-32. DOI PubMed
75. Shimomura M, Yaoi T, Itoh K, et al. Drug resistance to paclitaxel is not only associated with ABCB1 mRNA expression but also with drug accumulation in intracellular compartments in human lung cancer cell lines. *Int J Oncol* 2012;40:995-1004. DOI PubMed PMC
76. Zhang J, Wang J, Wong YK, et al. Docetaxel enhances lysosomal function through TFEB activation. *Cell Death Dis* 2018;9:614. DOI PubMed PMC
77. Duvvuri M, Krise JP. A novel assay reveals that weakly basic model compounds concentrate in lysosomes to an extent greater than pH-partitioning theory would predict. *Mol Pharm* 2005;2:440-8. DOI PubMed
78. Zhitomirsky B, Assaraf YG. Lysosomes as mediators of drug resistance in cancer. *Drug Resist Updat* 2016;24:23-33. DOI PubMed
79. Zhao B, Dierichs L, Gu JN, et al. TFEB-mediated lysosomal biogenesis and lysosomal drug sequestration confer resistance to MEK inhibition in pancreatic cancer. *Cell Death Discov* 2020;6:12. DOI PubMed PMC
80. Medina DL, Di Paola S, Peluso I, et al. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat Cell Biol* 2015;17:288-99. DOI PubMed PMC
81. Li DL, Wang ZV, Ding G, et al. Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. *Circulation* 2016;133:1668-87. DOI PubMed PMC
82. Groth-Pedersen L, Jäättelä M. Combating apoptosis and multidrug resistant cancers by targeting lysosomes. *Cancer Lett* 2013;332:265-74. DOI PubMed
83. Sundler R. Lysosomal and cytosolic pH as regulators of exocytosis in mouse macrophages. *Acta Physiol Scand* 1997;161:553-6. DOI PubMed
84. Kazmi F, Hensley T, Pope C, et al. Lysosomal sequestration (trapping) of lipophilic amine (cationic amphiphilic) drugs in immortalized human hepatocytes (Fa2N-4 cells). *Drug Metab Dispos* 2013;41:897-905. DOI PubMed PMC
85. Medina DL, Fraldi A, Bouche V, et al. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev Cell* 2011;21:421-30. DOI PubMed PMC
86. Zhitomirsky B, Assaraf YG. The role of cytoplasmic-to-lysosomal pH gradient in hydrophobic weak base drug sequestration in lysosomes. *Can Cell Microenviron* 2015. DOI
87. Gervasoni JE, Fields SZ, Krishna S, et al. Subcellular distribution of daunorubicin in P-glycoprotein-positive and -negative drug-resistant cell lines using laser-assisted confocal microscopy. *Cancer Res* 1991;51:4955-63. PubMed
88. Breuninger LM, Paul S, Gaughan K, et al. Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution. *Cancer Res* 1995;55:5342-7. PubMed
89. Petersen NH, Olsen OD, Groth-Pedersen L, et al. Transformation-associated changes in sphingolipid metabolism sensitize cells to lysosomal cell death induced by inhibitors of acid sphingomyelinase. *Cancer Cell* 2013;24:379-93. DOI PubMed
90. Perera RM, Stoykova S, Nicolay BN, et al. Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature* 2015;524:361-5. DOI PubMed PMC
91. Aits S, Jäättelä M. Lysosomal cell death at a glance. *J Cell Sci* 2013;126:1905-12. DOI PubMed
92. Cornet-Masana JM, Banús-Mulet A, Carbó JM, et al. Dual lysosomal-mitochondrial targeting by antihistamines to eradicate leukaemic cells. *EBioMedicine* 2019;47:221-34. DOI PubMed PMC
93. Nielsen IØ, Groth-Pedersen L, Dicroce-Giacobini J, et al. Cationic amphiphilic drugs induce elevation in lysoglycerophospholipid levels and cell death in leukemia cells. *Metabolomics* 2020;16:91. DOI PubMed
94. Duvvuri M, Gong Y, Chatterji D, Krise JP. Weak base permeability characteristics influence the intracellular sequestration site in the multidrug-resistant human leukemic cell line HL-60. *J Biol Chem* 2004;279:32367-72. DOI PubMed
95. Zhang S, Schneider LS, Vick B, et al. Anti-leukemic effects of the V-ATPase inhibitor Archazolid A. *Oncotarget* 2015;6:43508-28. DOI PubMed PMC
96. Dykstra KM, Fay HRS, Massey AC, et al. Inhibiting autophagy targets human leukemic stem cells and hypoxic AML blasts by disrupting mitochondrial homeostasis. *Blood Adv* 2021;5:2087-100. DOI PubMed PMC
97. Visser N, Lourens HJ, Huls G, Bremer E, Wiersma VR. Inhibition of autophagy does not re-sensitize acute myeloid leukemia cells resistant to cytarabine. *Int J Mol Sci* 2021;22:2337. DOI PubMed PMC
98. Bao EL, Nandakumar SK, Liao X, et al; FinnGen, 23andMe Research Team. Inherited myeloproliferative neoplasm risk affects haematopoietic stem cells. *Nature* 2020;586:769-75. DOI PubMed PMC
99. Sun L, Yan Y, Lv H, et al. Rapamycin targets STAT3 and impacts c-Myc to suppress tumor growth. *Cell Chem Biol* 2021. DOI PubMed
100. Liesveld JL, O'Dwyer K, Walker A, et al. A phase I study of decitabine and rapamycin in relapsed/refractory AML. *Leuk Res* 2013;37:1622-7. DOI PubMed
101. Liesveld JL, Baran A, Azadniv M, et al. A phase II study of sequential decitabine and rapamycin in acute myelogenous leukemia. *Leuk Res* 2022;112:106749. DOI PubMed
102. Rizzieri DA, Feldman E, Dipersio JF, et al. A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res* 2008;14:2756-62. DOI PubMed
103. Park S, Chapuis N, Saint Marcoux F, et al; GOELAMS (Groupe Ouest Est d'Etude des Leucémies aiguës et Autres Maladies du Sang). A phase Ib GOELAMS study of the mTOR inhibitor RAD001 in association with chemotherapy for AML patients in first relapse. *Leukemia* 2013;27:1479-86. DOI PubMed

104. Bross PF, Beitz J, Chen G, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin Cancer Res* 2001;7:1490-6. [PubMed](#)
105. Petersdorf SH, Kopecky KJ, Slovak M, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood* 2013;121:4854-60. [DOI](#) [PubMed](#) [PMC](#)
106. Mizutani Y, Inase A, Maimaitili Y, et al. An mTORC1/2 dual inhibitor, AZD2014, acts as a lysosomal function activator and enhances gemtuzumab ozogamicin-induced apoptosis in primary human leukemia cells. *Int J Hematol* 2019;110:490-9. [DOI](#) [PubMed](#)