



A carrier free delivery system of a MAGL inhibitor is effective on ovarian cancer

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ABSTRACT

Monoacylglycerol lipase (MAGL) is a promising target for cancer therapy due to its involvement in lipid metabolism and its impact on cancer hallmarks like cell proliferation, migration, and tumor progression. A potent reversible MAGL inhibitor, MAGL23, has been recently developed by our group, demonstrating promising anticancer activities. To enhance its pharmacological properties, a nanoformulation using nanocrystals coated with albumin was prepared (MAGL23AF). In a previous work, the formulated inhibitor showed potency in ovarian and colon cancer cell lines in terms of IC₅₀, and was tested on mice in order to assess its biocompatibility, organs biodistribution and toxicity. In the present work, we expanded the investigation to assess the potential in vivo application of MAGL23AF. Stability assays in serum and in human derived microsomes showed a good structural stability in physiological conditions of MAGL23AF. The antitumor efficacy tested on mice bearing ovarian cancer tumor xenografts demonstrated that MAGL23AF is more potent than the non-formulated drug, leading to necrosis-driven cancer cell death. In vivo studies revealed that albumin-complexed nanocrystals improved the therapeutic window of MAGL23, exhibiting a favorable biodistribution with slightly increased accumulation in the tumor. In conclusion, the MAGL23AF showed increased in vitro stability in conditions mirroring the bloodstream environment and hepatic metabolism coupled with an optimal antitumor efficacy in vivo. These results not only validates the efficacy of our formulation but also positions it as a promising strategy for addressing challenges related to the solubility of drugs in body fluids.

1. Introduction

Monoacylglycerol lipase (MAGL) is one of the main enzymes of the endocannabinoid system. The serine hydrolase MAGL is responsible for the degradation of the most abundant endocannabinoid 2-arachidonoylglycerol (2-AG) to glycerol and arachidonic acid (AA) in the central nervous system. Therefore MAGL, blocking the 2-AG signalling on cannabinoid receptors CB1 and CB2, interferes with the regulation of many physiological processes, including inflammation, pain,

neuroprotection, food intake and addiction [1]. Moreover, MAGL is also implicated in the metabolism of monoacylglycerols in peripheral tissues, thus enriching the cellular free fatty acid pool. This event leads to the activation of oncogenic signaling, and for this reason MAGL was shown to support cancer cell growth [2]. The multifaceted role of MAGL makes this enzyme an appealing therapeutic target: 2-AG accumulation after MAGL inhibition not only amplifies CB1 and CB2 activation and endocannabinoid signaling, but also reduces the AA-mediated formation of pro-inflammatory prostaglandins in many tissues [3]. Additionally,

Abbreviations: MAGL, Monoacylglycerol lipase; 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; MAGL23-AF, MAGL23 solubilized with Pluronic F127 formulated with albumin; PBS, Phosphate-buffered saline; I.P., intraperitoneal.

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MAGL inhibition in cancer cells diminishes free fatty acid production, reducing proliferation and invasiveness of malignant cells [4]. For these reasons, several MAGL inhibitors were developed and patented [5–7] and most of the recently discovered MAGL inhibitors act by blocking MAGL reversibly, since the transient arrest of MAGL catalytic function is devoid of some undesired effects *in vivo*, which on the contrary are typical of irreversible inhibitors (CB1 desensitization and loss of CB1-mediated biological effects caused by chronic 2-AG increase [8]). Among the most important reversible MAGL inhibitors, benzo[d][1,3]dioxol-5-ylmethyl 6-phenylhexanoate derivative discovered by Hernández-Torres *et al.* is one of the first potent and selective MAGL inhibitors and it proved to reduce the clinical progression of a multiple sclerosis mouse model [9]. Furthermore, azetidine diamide-based derivatives developed at Janssen Research & Development, L.L.C. demonstrated good ADME properties after oral administration, inducing an anti-hyperalgesic efficacy in a rat complete Freund's adjuvant (CFA)-induced inflammatory pain model [10,11]. In the last years, our group developed the class of benzoylpiperidine-based MAGL inhibitors [12–15]; among these derivatives, we developed a MAGL inhibitor (MAGL23, Fig. 1), able to bind the target enzyme with an inhibition potency in the low nanomolar range ($K_i = 39$ nM) [13]. This compound can be considered as a promising compound that could be further developed because: a) it is obtained by a few steps' synthetic preparation; b) it shows a reversible binding mechanism that should render it free of the typical side effects of irreversible MAGL inhibitors observed in *in vivo* studies [8]; c) it shows selectivity for MAGL inhibition over the other main components of the endocannabinoid system. Furthermore, MAGL23 was also able to inhibit the viability of breast, colon and ovarian cancer cell lines with IC_{50} values in the low micromolar range; however, the inhibitor suffered of problems related to poor solubility. To overcome this issue, our group elaborated a new formulation based on nanocrystals and albumin coating [16]. Nanocrystallization is a relative new strategy applied to drug in order to overcome low solubility without the use of a carrier material. In 2017, Park *et al.* introduced this new approach for enhancing the solubility of poorly soluble drugs in water obtaining an aqueous media dispersion of drug nanocrystals [17]. It was utilized Pluronic F-127 and CTAB as surfactants to help the formation of nanocrystals and to stabilize these particles, subsequently exchanging them with human serum albumin (HSA). The presence of surfactants during crystallization helps in regulating nanocrystal size and prevents non-specific protein adsorption, potentially reducing uptake by the mononuclear phagocyte system (MPS). Albumin, being a natural carrier of native ligands and hydrophobic molecules, is stable, digestible, and facilitates cellular internalization of nanocrystals at tumor sites through specific receptor interactions. The nanocrystals, formulated with albumin, offer a safe delivery method, particularly for targeting tumoral sites via specific receptors like GP60 and SPARC. Albumin not only directs the therapeutic compound but also stabilizes the nanocrystals, limiting unwanted protein adsorption during circulation. Toxicity studies in mice indicate that MAGL23-AF is well tolerated and avoids spleen damage observed with MAGL23 [16].

The nanocrystals allowed to obtain a drug content in aqueous media 1000 times higher than its solubility and were then stabilized by human serum albumin. Experimental studies confirmed the maintained potency of the formulated inhibitor in ovarian and colon cancer cell lines. The

formulation process was characterized with different techniques, and preliminary *in vivo* studies indicated a favorable low toxicity profile for MAGL23AF (albumin formulated). That study represents the first-ever nanoformulation of a MAGL inhibitor and its application *in vitro* and *in vivo*. In this study, we conducted a thorough investigation into the stability of MAGL23AF in physiological environments such as serum and microsomes. Additionally, we assessed the *in vivo* antitumor efficacy and pharmacokinetics properties.

2. Materials and methods

2.1. Albumin formulation of MAGL23AF

The nanocrystallization process of MAGL23 is made following a method from a published study [17]. Initially, 6 mg of MAGL23 and 24 mg of Pluronic F-127 (F-127) are mixed in chloroform, then evaporated to obtain an amorphous layer. Nanocrystals are formed using sonication. In the second step, human serum albumin (HSA) is added to the nanocrystal solution, followed by 24 h of rotation to enhance interaction. After washing and centrifugation, the resulting pellet is lyophilized and resuspended physiological solution. Physico-chemical characterizations are reported in [supplementary materials](#) (figure S1-S4).

2.2. *In vitro* stability

Stability MAGL23AF vs MAGL23 was assessed in 50% fetal bovine serum (FBS) in PBS at 37°C. One ml of samples concentrated at 100 µg/ml were incubated and collected at 10 different time points (20, 40 min and 1, 6, 10, 24, 48, 72, 96 and 168 h) and analyzed by liquid chromatography–mass spectrometry (LC MS/MS).

2.3. Microsomes stability

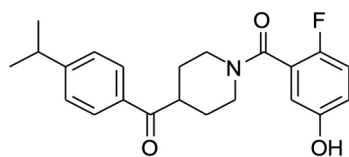
Stability of MAGL23AF vs MAGL23 was assessed in human microsomes purchased by Thermofisher and used following their protocol (HMMCPL, Human Microsomes, 50 Donors, Thermofisher). Samples were collected at 3 different time points (0-40-60 min) and analyzed by LC MS/MS.

2.4. Mouse xenograft

5x10⁶ OVCAR5 cells (Gently Provided by Dr. Gustavo Baldassarre; mycoplasma free) were mixed with 30% of Matrigel HC (BD Bioscience, CA, USA) and implanted subcutaneously into 6 weeks-old female nude mice (Hsd:ATHymicNude-Foxn1nu, Envigo, Italy). When tumors reached a measurable size (>50 mm³), mice were treated intraperitoneally with 20 mg/kg MAGL23AF, MAGL23, or vehicle (physiological solution) one time per week, for 4 treatments. Tumor volumes were measured with a caliper instrument and calculated using the formula: (length × width²)/2. Animal experiments (EU directive (2010/63/EU) were conducted under the authorization of the National Ethical Committee and the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection.

2.5. Pharmacokinetics analysis

Pharmacokinetic of MAGL23 was performed on mice (FVB/N) treated with 100 mg/Kg (i.p.) of MAGL23 and MAGL23AF. Blood was collected in EDTA at different time points (0, 0.5, 2, 5, 9, 24 h) from 3 mice each. Plasma was obtained by centrifugation at 1000xg for 5 min, 4°C and stored at –80°C until analysis. The concentrations of drug in plasma and in tumor homogenate were measured with LC-MS/MS as described by Adeel *et al.* [16]. Briefly: samples were precipitated with two volumes of cold acetonitrile containing 100 ng/ml of MAGL24 n-butyl analog working as the internal standard (IS) (Granchi *et al.*, 2019). After vortexing, the samples were kept for 30 min at –80°C than spun at



MAGL 23 structure

Fig. 1. Molecular structure of MAGL23 inhibitor.

13,000 rpm for 15 min at 4°C. The supernatant was diluted with 3 volumes of acetonitrile–water (1:1) solution and 2 µL injected in a Nexera X2 liquid chromatograph coupled with Shimadzu LCMS-8050 tandem mass spectrometer (Shimadzu, Kyoto, Japan) equipped with the electrospray ionization source. Chromatographic separation was performed on Zorbax eclipse 100 × 2.1 mm 1.8 µm C18 column (Agilent, CA USA), equilibrated with a 0.45 mL/min of 0.1% formic/acetonitrile (70:30) and maintained at 40°C. An elution gradient B from 30 to 80% of acetonitrile over 5 min was applied followed by 3 min of equilibration. The retention times were 2.85 min and 2.98 min for MAGL23 and the MAGL24-IS, respectively. Both analytes were monitored by multiple reaction monitoring (MRM) in positive-ion mode using the following m/z transitions: 370.1 → 139.0 m/z and 384.1 → 139 m/z with collision energy voltages set at – 33 V and – 22 V, for MAGL23 and MAGL24-IS, respectively. The spray voltage was set at 4500 V and the source block temperature at 350°C. The results are expressed as ng/mg of tissues.

2.6. Hematoxylin and eosin (H&E) staining and analysis

Tissues were fixed in formalin 10% for 24 h and embedded in paraffin. Tissues were cut at 3 µm thick, counterstained with hematoxylin/eosin and analyzed by the pathologist at 10× and 20× of magnification.

2.7. Statistical analysis

The statistical significance was determined using a two-tailed *t*-test. In vivo tumor growth was analyzed by Wilcoxon matched paired signed rank test. A *p* value less than 0.05 was considered significant for all comparisons done. Bars represent standard errors for tumor volume and body weight. All other bars are standard deviations. Statistical analyses were performed using Microsoft Excel or GraphPad Prism.

3. Results and discussion

3.1. In vitro stability

The in vitro stability was tested under physiological conditions, specifically in 50% FBS in phosphate-buffered saline (PBS) at 37°C, to emulate the environment of the bloodstream. This experimental setup was designed to closely mimic the conditions, which the drug would encounter within the human body, providing insights into its behavior in a complex biological system. MAGL23 AF, the focus of our investigation, exhibited a long-term stability (Fig. 2A). At long-time exposure (168 h), MAGL23AF has a better stability than MAGL23 although not statistically significant. This discovery implies a promising potential for our formulation in vivo, underscoring its effectiveness in enhancing the stability of hydrophobic or less soluble drugs within physiological conditions.

3.2. Microsomes stability

The evaluation of a drug's stability in microsomes stands as a pivotal stage in the process of drug testing and development. Microsomes, integral subcellular constituents housing membrane-bound drug-metabolizing enzymes, including cytochrome P450 (CYP), play a central role in this assessment. Serving as indispensable tools in pharmacology, they enable the meticulous examination of a compound's intrinsic clearance in vitro. In this specific investigation, human liver-derived microsomes are utilized to emulate the intricate metabolic processes that occur within the body [18].

The experiment was conducted strictly following the manufacturer's guidelines, ensuring precision and reliability in the analysis. The stability assessment was conducted at four distinct time points. The compound MAGL23 AF emerged to be more stable when compared to its non-formulated counterpart (Fig. 2B). This observation is particularly

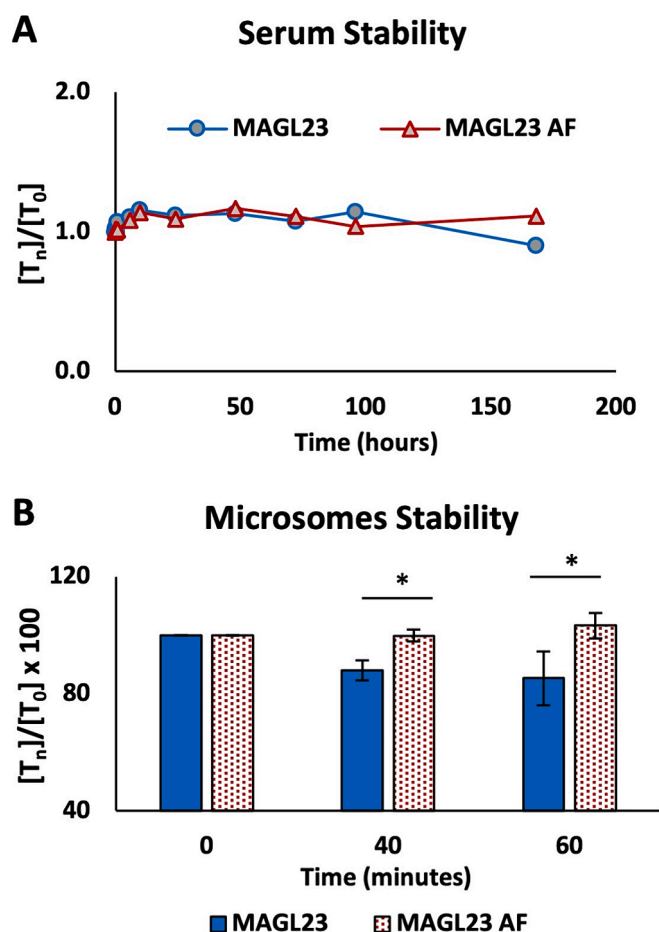


Fig. 2. A) Stability in serum. MAGL23 AF and MAGL23 were incubated in 50% FBS/PBS to assess the stability. MAGL23 AF showed a positive trend in this condition on the long term (168 h). Y-axis is the concentration at T_n divided by the concentration at T_0 used as normalizer. B) Stability test in human derived liver microsomes. Stability of MAGL23 AF was also assessed in human microsomes showing a significant improvement at 40 and 60 min vs MAGL23 (*p* value < 0.05). Y-axis is the concentration at T_n divided by the concentration at T_0 used as normalizer.

significant since suggests that the albumin coating applied to MAGL23 AF imparts a substantial degree of stability within the microsomal system. This stability could potentially be translated into an enhanced bioavailability when the compound is applied in vivo.

The stability of a drug in microsomes, as demonstrated in literature [19], could assume a pivotal role in drug development by predicting the metabolic fate of a drug candidate.

3.3. In vivo antitumor efficacy

The preceding in vitro findings revealed that MAGL23AF demonstrated comparable antitumor effects to MAGL23 in colorectal and ovarian cancer cell lines. Particularly noteworthy was MAGL23AF's enhanced tolerance and reduced toxicity observed in in vivo studies [16]. Consequently, the current investigation is focused on assessing the antitumor efficacy of MAGL23AF. To evaluate this, a model system utilizing nude mice with subcutaneous ovarian cancer tumors was employed to explore the compound's potential.

As depicted in Fig. 3A, B, mice were grafted with OVCAR5 cells and the treatments started once tumors reached a volume of 50 mm³. An intraperitoneal administration of 20 mg/kg of MAGL23AF and MAGL23 was applied weekly, and the outcomes were compared against an untreated control group. After four treatment sessions, a significant

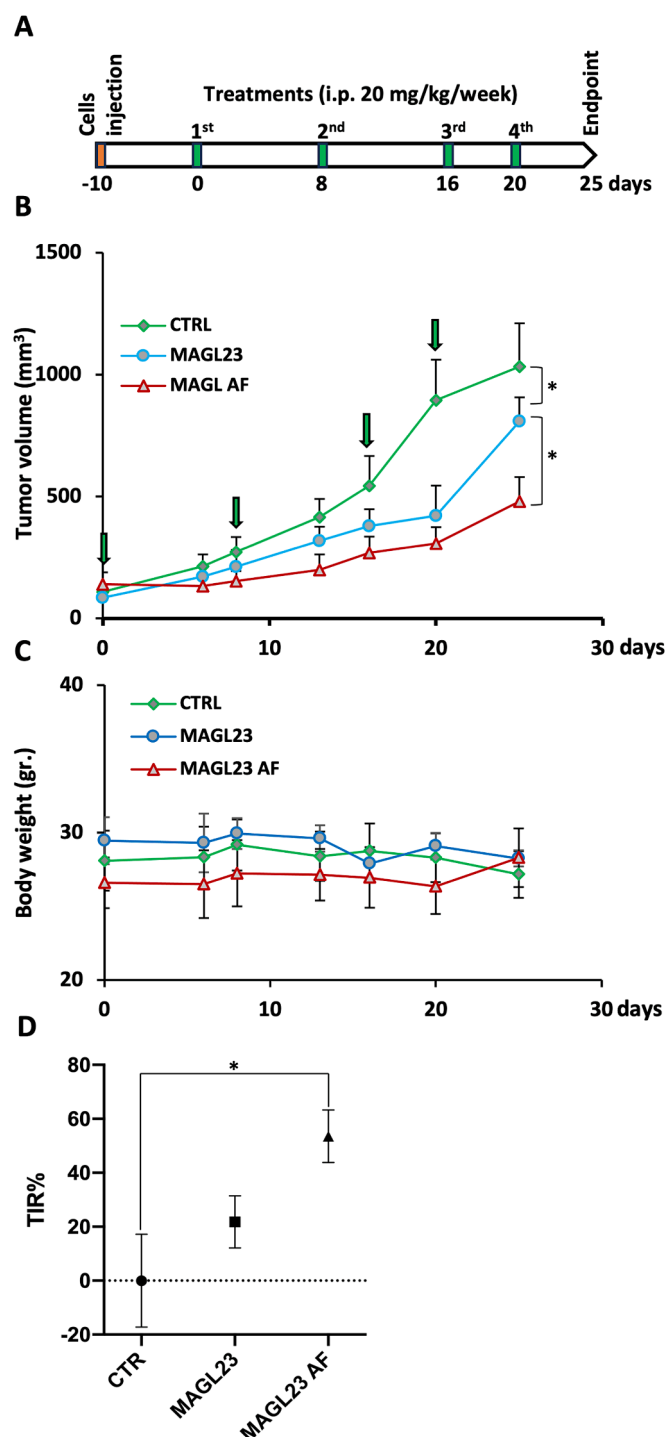


Fig. 3. A) Schematic design of tumor growth study. Mice were injected subcutaneously and after tumors started growing, mice were treated four times (20 mg/kg, once a week) and tumor volumes ($n = 6$) were followed up for 25 days. B) MAGL23 AF antitumor efficacy. After tumors reached a volume of 50 mm³ mice were treated once per week 20 mg/kg, intraperitoneally. Arrows indicates treatment. At the end of the experiment, mice treated with MAGL23 AF showed a slight reduced tumor volume compared to MAGL23 treated mice and a significant tumor volume reduction compared to the control group (p -value < 0.05). C) Mice weight was monitored throughout the experiment, revealing non-significant variations among the three groups. D) Tumor inhibition rate (TIR%). Tumor inhibition rate is calculated as $(1 - (\text{mean volume of treated tumors}) / (\text{mean volume of control tumors})) \times 100\%$ at day 25 (p -value < 0.05).

reduction in tumor volume was observed in mice treated with MAGL23AF, reaching statistical significance when compared to mice treated with MAGL23. Both treated groups exhibited a statistically significant reduction in tumor volume when compared to the untreated control group ($p < 0.05$). Tumor inhibition rate (TIR%) of MAGL23AF is 53% vs 21% of MAGL23 at the end (day 25) of in vivo antitumor efficacy experiment (Fig. 3d) highlighting the efficacy of the MAGL23 AF ($p < 0.05$).

Tumor specimens were histologically evaluated by an experienced pathologist by H&E staining. Mice tumor specimens treated with MAGL23AF and MAGL23 or untreated (CTR) showed areas of intratumoral necrosis quantifiable respectively in around 40%, 20% and < 10% of the whole sections, respectively. MAGL23AF is more effective in inducing necrosis (Fig. 4).

Throughout all the experiment, mice bodyweight did not decrease indicating a good tolerability toward the drug (Fig. 3c).

This observed difference between MAGL23AF and MAGL23 led to a further exploration of the pharmacokinetic (PK) profile of both formulations. The pharmacokinetic data, as depicted in Fig. 5, as area under the curve (AUC), revealed a subtle yet significant alteration in the PK of MAGL23AF, characterized by a substantial increase in the AUC. This observation indicates an augmented amount of circulating drug, leading to an increased exposure of tumor cells to a drug.

4. Conclusions

The MAGL protein is highly expressed in several tumors, promoting cell aggressiveness by stimulating invasion, proliferation, and migration [2]. Due to its involvement in tumors, several MAGL inhibitors have been developed and reported in the literature with positive applications both in vitro and in vivo [4,7]. Many of those are accompanied by a lot of side effects due to their irreversible interaction with the protein target thus limiting their further application and development [8]. In contrast, others reversible MAGL inhibitors have been developed but their application is limited by low solubility in physiological solvent possessing high lipophilicity. MAGL23, a newly introduced reversible inhibitor of monoacylglycerol lipase, showing high selectivity against other endocannabinoid system components [13]. Its development is further streamlined by a straightforward synthetic preparation process. Starting by this compound, a new formulation based on albumin nanocrystals formation has been developed and successfully employed by Adeel et al. [16]. Technological advancements in biomaterials have led to the development of immune-regulating camouflage strategies,

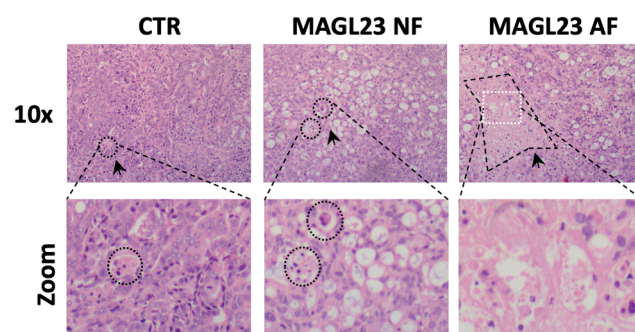


Fig. 4. H&E of tumor treated with MAGL23 AF and MAGL23. H&E-stained sections from tumor treated with MAGL23 AF, MAGL23, and untreated (CTR) specimens revealed quantifiable areas of intratumoral necrosis (black arrowhead and dotted outlines), accounting for approximately 40%, 20%, and < 10% of the entire sections, respectively. Notably, there are differences in necrosis distribution in the different specimens. In detail, necrosis is mainly intraglandular in CTR and MAGL23 treated samples as an effect of "tumor growth", whereas in MAGL23 AF specimens the necrosis is massive as drug anti-tumor effect.

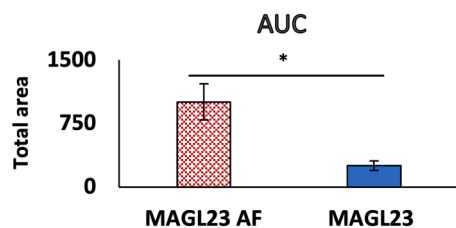


Fig. 5. Area under the curve (AUC). The AUC demonstrates that the formulated drug is capable of being absorbed better than its free form, making it more bioavailable (p -value < 0.05).

which are innovative approaches in cancer therapy. These biomimetic systems act as stealth shields, evading immune surveillance and prolonging circulation in the blood before reaching the tumor site. Upon arrival, the camouflage coating can enhance immune responses against tumors through various mechanisms. These strategies involve coating nanoplateforms with different polymers or cell membranes, including cell-derived exosomes. By leveraging their unique characteristics and functionalities, immune-regulating camouflage enables nanosystems to overcome immune-induced limitations, resulting in prolonged circulation and enhanced anticancer efficacy [20].

The nanocrystals, formulated with albumin, offer a safe delivery method, particularly for targeting tumoral sites via specific receptors like GP60 and SPARC. Albumin not only directs the therapeutic compound but also stabilizes the nanocrystals, preventing unwanted protein adsorption during circulation. Toxicity studies in mice indicate that MAGL23-AF is well tolerated and avoids spleen damage observed with MAGL23.

In the present work the efficacy of MAGL23AF was evaluated in term of stability in physiological environment (serum and microsomes) in order to be applied in vivo. In mice, the antitumor efficacy has been highlighted coupled with good tolerance and low toxicity. An increased induction of necrosis suggests that MAGL23AF exhibits superior efficacy compared to MAGL23.

In conclusion, the transition from in vitro success to in vivo efficacy assessment in ovarian cancer mouse xenografts has yielded encouraging results. The differences in the pharmacokinetics of MAGL23AF, underline its superior performance. These findings not only reinforce the preclinical relevance of MAGL23AF as a new formulation for a biocompatible delivery of an anticancer drug, but also underscore the importance of albumin nanocrystals-based drug formulation to improve pharmacological properties.

5. Data availability

The data supporting the findings of this study are available within the paper and from the corresponding authors upon reasonable request. Source data are provided with this paper.

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Disclaimer

This manuscript has undergone grammar and syntax correction using ChatGPT, an AI language model developed by OpenAI. While every effort has been made to improve the clarity and accuracy of the language in this document, the authors acknowledge that the final content and scientific interpretations remain the sole responsibility of the authors and their collaborators. ChatGPT has been used exclusively to enhance the manuscript's readability and expression, and not to generate any type of data.

CRediT authorship contribution statement

Stefano Palazzolo: Writing – original draft, Investigation. **Gloria Saorin:** Methodology. **Giuseppe Corona:** Data curation. **Carlotta Granchi:** Writing – review & editing, Data curation. **Tiziano Tuccinardi:** Writing – review & editing, Data curation, Conceptualization. **Urska Kamensek:** Writing – review & editing, Formal analysis. **Simona Kranjc Brezar:** Writing – review & editing, Formal analysis. **Maja Cemazar:** Writing – review & editing, Formal analysis. **Vincenzo Canzonieri:** Writing – review & editing, Investigation, Funding acquisition. **Flavio Rizzolio:** Writing – review & editing, Supervision, Data curation, Conceptualization.

Data availability

The data supporting the findings of this study are available within the paper and from the corresponding authors upon reasonable request. Source data are provided with this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejpb.2024.114397>.

References

- [1] G.F. Grabner, R. Zimmermann, R. Schicho, U. Taschler, Monoglyceride lipase as a drug target: at the crossroads of arachidonic acid metabolism and endocannabinoid signaling, *Pharmacol. Ther.* 175 (2017) 35–46, <https://doi.org/10.1016/j.pharmthera.2017.02.033>.
- [2] D.K. Nomura, J.Z. Long, S. Niessen, H.S. Hoover, S.-W. Ng, B.F. Cravatt, Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis, *Cell* 140 (2010) 49–61, <https://doi.org/10.1016/j.cell.2009.11.027>.
- [3] D.K. Nomura, B.E. Morrison, J.L. Blankman, J.Z. Long, S.G. Kinsey, M.C. G. Marcondes, A.M. Ward, Y.K. Hahn, A.H. Lichtman, B. Conti, et al., Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation, *Science* 334 (2011) 809–813, <https://doi.org/10.1126/science.1209200>.
- [4] H. Deng, W. Li, Monoacylglycerol lipase inhibitors: modulators for lipid metabolism in cancer malignancy, neurological and metabolic disorders, *Acta Pharm. Sin. B* 10 (2020) 582–602, <https://doi.org/10.1016/j.apsb.2019.10.006>.
- [5] C. Granchi, I. Caligiuri, E. Bertelli, G. Poli, F. Rizzolio, M. Macchia, A. Martinelli, F. Minutolo, T. Tuccinardi, Development of terphenyl-2-methylloxazol-5(4H)-one derivatives as selective reversible MAGL inhibitors, *J. Enzyme Inhib. Med. Chem.* 32 (2017) 1240–1252, <https://doi.org/10.1080/14756366.2017.1375484>.
- [6] C. Granchi, I. Caligiuri, F. Minutolo, F. Rizzolio, T. Tuccinardi, A patent review of Monoacylglycerol Lipase (MAGL) inhibitors (2013–2017), *Expert Opin. Ther. Pat.* 27 (2017) 1341–1351, <https://doi.org/10.1080/13543776.2018.1389899>.
- [7] G. Bononi, G. Poli, F. Rizzolio, T. Tuccinardi, M. Macchia, F. Minutolo, C. Granchi, An updated patent review of monoacylglycerol lipase (MAGL) inhibitors (2018–present), *Expert Opin. Ther. Pat.* (2020) 1–16, <https://doi.org/10.1080/13543776.2021.1841166>.
- [8] J.E. Schlosburg, J.L. Blankman, J.Z. Long, D.K. Nomura, B. Pan, S.G. Kinsey, P. T. Nguyen, D. Ramesh, L. Booker, J.J. Burston, et al., Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system, *Nat. Neurosci.* 13 (2010) 1113–1119, <https://doi.org/10.1038/nn.2616>.
- [9] G. Hernández-Torres, M. Cipriano, E. Hedén, E. Björklund, A. Canales, D. Zian, A. Feliú, M. Mecha, C. Guaza, C.J. Fowler, et al., A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis, *Angew. Chem. – Int. Ed.* 53 (2014) 13765–13770, <https://doi.org/10.1002/anie.201407807>.
- [10] B. Zhu, P.J. Connolly, S.-P. Zhang, K.M. Chevalier, C.M. Milligan, C.M. Flores, M. J. Macielag, The discovery of diazetidinyl diamides as potent and reversible inhibitors of monoacylglycerol lipase (MAGL), *Bioorg. Med. Chem. Lett.* 30 (2020) 127198, <https://doi.org/10.1016/j.bmcl.2020.127198>.
- [11] B. Zhu, P.J. Connolly, Y.-M. Zhang, M.E. McDonnell, H. Bian, S.-C. Lin, L. Liu, S.-P. Zhang, K.M. Chevalier, M.R. Brandt, et al., The discovery of azetidine-piperazine di-amides as potent, selective and reversible monoacylglycerol lipase (MAGL) inhibitors, *Bioorg. Med. Chem. Lett.* 30 (2020) 127243, <https://doi.org/10.1016/j.bmcl.2020.127243>.
- [12] C. Granchi, F. Rizzolio, S. Palazzolo, S. Carmignani, M. Macchia, G. Saccomanni, C. Manera, A. Martinelli, F. Minutolo, T. Tuccinardi, Structural Optimization of 4-Chlorobenzoylpiperidine Derivatives for the Development of Potent, Reversible,

- and Selective Monoacylglycerol Lipase (MAGL) Inhibitors, *J. Med. Chem.* 59 (2016) 10299–10314, <https://doi.org/10.1021/acs.jmedchem.6b01459>.
- [13] C. Granchi, M. Lapillo, S. Glasmacher, G. Bononi, C. Licari, G. Poli, M. El Boustani, I. Caligiuri, F. Rizzolio, J. Gertsch, et al., Optimization of a Benzoylpiperidine Class Identifies a Highly Potent and Selective Reversible Monoacylglycerol Lipase (MAGL) Inhibitor, *J. Med. Chem.* 62 (2019) 1932–1958, <https://doi.org/10.1021/acs.jmedchem.8b01483>.
- [14] C. Granchi, G. Bononi, R. Ferrisi, E. Gori, G. Mantini, S. Glasmacher, G. Poli, S. Palazzolo, I. Caligiuri, F. Rizzolio, et al., Design, synthesis and biological evaluation of second-generation benzoylpiperidine derivatives as reversible monoacylglycerol lipase (MAGL) inhibitors, *Eur. J. Med. Chem.* 209 (2021) 112857, <https://doi.org/10.1016/j.ejmech.2020.112857>.
- [15] G. Bononi, G. Tonarini, G. Poli, I. Barravecchia, I. Caligiuri, M. Macchia, F. Rizzolio, G.C. Demontis, F. Minutolo, C. Granchi, et al., Monoacylglycerol lipase (MAGL) inhibitors based on a diphenylsulfide-benzoylpiperidine scaffold, *Eur. J. Med. Chem.* 223 (2021) 113679, <https://doi.org/10.1016/j.ejmech.2021.113679>.
- [16] M. Adeel, G. Saorin, G. Boccalon, A.A. Sfriso, S. Parisi, I. Moro, S. Palazzolo, I. Caligiuri, C. Granchi, G. Corona, et al., A carrier free delivery system of a monoacylglycerol lipase hydrophobic inhibitor, *Int. J. Pharm.* 613 (2022) 121374, <https://doi.org/10.1016/j.ijpharm.2021.121374>.
- [17] J. Park, B. Sun, Y. Yeo, Albumin-coated nanocrystals for carrier-free delivery of paclitaxel, *J. Control. Release* 263 (2017) 90–101, <https://doi.org/10.1016/j.jconrel.2016.12.040>.
- [18] V.B. Siramshetty, P. Shah, E. Kerns, K. Nguyen, K.R. Yu, M. Kabir, J. Williams, J. Neyra, N. Southall, Đ.T. Nguyễn, et al., Retrospective assessment of rat liver microsomal stability at NCATS: data and QSAR models, *Sci. Rep.* 10 (2020) 1–14, <https://doi.org/10.1038/s41598-020-77327-0>.
- [19] L. Di, E.H. Kerns, X.J. Ma, Y. Huang, G.T. Carter, Applications of high throughput microsomal stability assay in drug discovery, *Comb. Chem. High Throughput Screen.* 11 (2008) 469–476, <https://doi.org/10.2174/138620708784911429>.
- [20] B.Q. Chen, Y. Zhao, Y. Zhang, Y.J. Pan, H.Y. Xia, R.K. Kankala, W.S. Bin, G. Liu, A. Z. Chen, Immune-regulating camouflaged nanoplateforms: a promising strategy to improve cancer nano-immunotherapy, *Bioact. Mater.* 21 (2023) 1–19, <https://doi.org/10.1016/j.bioactmat.2022.07.023>.