ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, No. 1, pp. 1-18 © Pleiades Publishing, Ltd., 2025.
Russian Text © The Author(s), 2025, published in Biokhimiya, 2025, Vol. 90, No. 1, pp. 3-21.

REVIEW

4-Methylumbelliferone, an Inhibitor of Hyaluronan Synthase, Prevents the Development of Oncological, Inflammatory, Degenerative, and Autoimmune Diseases

Viktoriya V. Fedorova[1], Alexandra Tsitrina[2], Noreen Halimani[1], and Yuri V. Kotelevtsev[1,a]*

[1]*Skolkovo Institute of Science and Technology, 121205 Moscow, Russia*
[2]*Ben-Gurion University of the Negev, 8410501 Be'er Sheva, Israel*
[a]*e-mail: y.kotelevtsev@skoltech.ru*

Received September 24, 2024
Revised November 24, 2024
Accepted December 8, 2024

Abstract—Hyaluronic acid (HA) is the main structure-forming polymer of the extracellular matrix. HA metabolism plays an important role in intercellular interaction in healthy organism and in various pathologies. HA is synthesized by hyaluronan synthase (HAS); mammals have three highly homologous isoforms of this enzyme: HAS1, HAS2, and HAS3. No highly specific competitive inhibitors of HASs have been described so far. 4-Methylumbelliferone (4-MU), a natural coumarin compound, is commonly used to inhibit HA synthesis *in vivo* and in cell cultures. The review is focused on the molecular mechanisms underlying the therapeutic effects of 4-MU and discusses results of 4-MU application in tissue cultures and animal disease models, as well as in first clinical trials of this compound. It was found that along with receptors and transcription factors, one of the pharmacological targets of 4-MU is HAS2, which is most common isoform of HAS. Moreover, it is inhibition of HA synthesis that underlies the pharmacological effects of 4-MU in oncological, autoimmune, degenerative, and hypercompensated regenerative processes (fibrosis, scar formation). New clinical drugs based on specific HAS2 inhibitors will be the first-in-class compounds to treat a wide range of diseases.

DOI: 10.1134/S0006297924603459

Keywords: 4-methylumbelliferone, Odeston, Hymecromone, hyaluronan synthase inhibition, hyaluronan synthase

"The most fruitful basis of the discovery of a new drug is to start with an old one"
Sir James Black, Nobel Prize Laureate 1988

INTRODUCTION

Development of new drugs in the post-genomic era is based on detailed knowledge of signaling pathways and key effectors or pharmacological targets (enzymes, receptors, and transcription factors). At the same time, physiologically active substances still play an important role in the identification and validation of pharmacological targets. Numerous experimental data have confirmed the therapeutic effect of the natural coumarin compound 4-methylumbelliferone (4-MU) in animal models of oncological, autoimmune, degenerative, and hyperproliferative diseases. This review is focused on the studies on the validation of hyaluronan synthase (HAS) as the main 4-MU pharmacological target, which is an essential step in the development first-in-class drugs, namely, inhibitors of hyaluronic acid (HA) synthesis.

Abbreviations: HA, hyaluronic acid; ECM, extracellular matrix; 4-MU, 4-methylumbelliferone; 4-MUG, 4-methylumbelliferone beta-D-glucuronide; HAS, hyaluronan synthase.

* To whom correspondence should be addressed.

THE ROLE OF EXTRACELLULAR MATRIX IN NORMAL AND PATHOLOGICAL STATES

Extracellular matrix (ECM), which forms the basis of connective tissue, is a highly organized interstitial structure that ensures mechanical integrity and cell–cell interaction.

ECM consists of polymeric carbohydrates glycosaminoglycans (GAGs), proteins (mainly, fibrillar), and proteoglycans (PGs). ECM is a barrier and, at the same time, a depot for peptide hormones and cytokines. It also directly generates chemical and mechanical signals essential for the maintenance of tissue homeostasis. Pathological processes typical of many systemic diseases lead to the ECM rearrangement and changes in its structure, which eventually contributes to changes in the tissue architecture and results in the development of diseases, such as fibrosis, osteoarthritis, and cancer [1, 2].

HA is a linear polymer consisting of D-glucuronic acid and D-N-acetylglucosamine residues connected by alternating β-1,4- and β-1,3-glycosidic bonds; it is the main ECM component by weight. HA homeostasis is maintained through the synthetic activity of HAS enzymes, decomposition by hyaluronidases, and chemical degradation mainly via the action of reactive oxygen species. There are three HAS isoforms. HAS1 is active during embryogenesis. HAS2 is the main isoform both in embryogenesis and in most tissues during the postnatal period; it synthesizes high-molecular-weight (HMW) HA fraction with a weight of 1000-6000 kDa; HAS3 synthesizes low-molecular-weight (LMW) forms of HA weighing less than 250 kDa. HMW HA usually has the anti-inflammatory, antiangiogenic, and anti-cancer properties. On the contrary, LMW fractions of HA exhibit proinflammatory and proangiogenic effects and promote cell adhesion. Although these properties of HA have been well established, the mechanisms underlying them are poorly understood and need further exploration [3].

Human body also has various types of hyaluronidases (HYALs) that cleave HA. The most thoroughly characterized of them are HYAL1 and HYAL2. HYAL2 degrades HA into the fragments approximately 50 monomers in length (~20 kDa), while HYAL1 hydrolyzes HA into tetrasaccharides (~1600 Da), which undergo further degradation in the lysosomes [4]. Pathological processes, such as disruption of HA metabolism, cancer, tissue damage, and inflammation, can change this balance, thus increasing the concentration of LMW HA. There is a large body of evidence indicating HA involvement in the chronic inflammation characteristic of type 2 diabetes, liver cirrhosis, asthma, and cancer progression and metastasis. Thus, HA promotes adhesion and motility of metastatic melanoma cells [5], enhances motility of pancreatic [6] and prostate cancer cells [7], hinders drug delivery to tumors [8-10], promotes drug resistance [11], stimulates cell division [12], and acts as an immune regulatory factor [13]. Upregulated HA synthesis in the tumor stroma is a negative prognostic factor [14-18].

The blood level of HA is a marker of liver fibrosis. In a fibrotic liver, HA is synthesized by fibroblasts originated from activated stellate cells. Normally, stellate cells do not express HAS2 (the main enzyme that produces HA in adult tissues) and do not synthesize HA. Liver damage leads to the production of TGF-β, which triggers transdifferentiation of stellate cells into myofibroblasts and dramatically increases *HAS2* expression in them [19]. HA accumulation in the parenchyma results in the activation of Notch1 signaling pathway in stellate cells, leading to their activation, increased synthesis of the ECM, and development of fibrosis [20]. Therefore, HAS2 and HAS3 are important pharmacological targets in the treatment of diseases associated with pathological activation of HA synthesis, in particular, liver fibrosis.

Elucidation of molecular mechanisms of HA synthesis by mammalian HASs has become important in the context of the targeted search for their specific inhibitors that can be used as drugs. These mechanisms have been discussed in most detail in the review by DeAngelis and Zimmer [21]. Within a few years after the discovery of bacterial HAS in *Streptococcus pyogenes* (SpHAS), three isoforms of vertebrate enzyme (HAS1-3) and viral HAS (CvHAS of *Paramecium bursaria Chlorella* virus-1, PBCV-1) have been identified. CvHAS is similar to the vertebrate enzymes in the overall architecture of the cytoplasmic domain containing the active site and transmembrane (TM) domains, with two TM helices at the N-terminus and four at the C-terminus. All these enzymes belong to class I glycosyltransferases, but vertebrate HASs and viral CvHAS add sugars to the nonreducing end of the growing HA chain (Fig. 1), whereas SpHAS adds sugars to the reducing end.

Vertebrate HASs, CvHAS, and SpHAS have the glycosyltransferase domain of the second type (GT-2), which allows to incorporate both uridine 5′-diphosphoglucuronic acid (UDP-GlcA) and uridine 5′-disphosphate N-acetylglucosamine (UDP-GlcNAc). The three-dimensional structure of CvHAS has been determined by electron cryomicroscopy [22]. 4-MU, which significantly reduces expression of HAS2/HAS3 [1], is a widely used and the only well-characterized inhibitor of HA synthesis that is known under commercial names of Hymecromone and Odeston. It has been approved for the clinical application in Europe and Asia and is routinely used as a hepatoprotector, antispasmodic, and choleretic in biliary dyskinesia. In Italy, this drug has been approved by the Italian Medicines Agency (AIC no. 02130002) and is sold under the name Cantabiline.

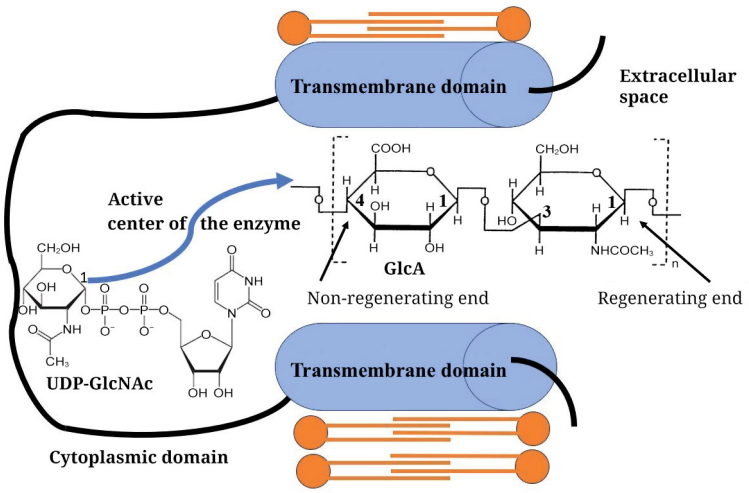

Fig. 1. HA synthesis by HAS enzymes. UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; GlcA, glucuronic acid.

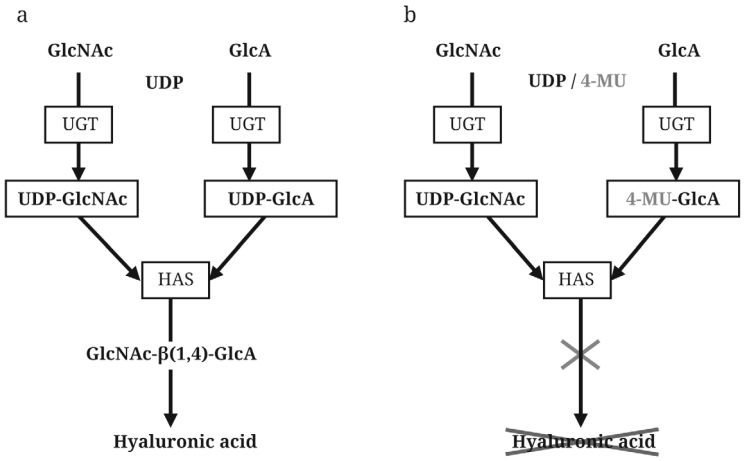

Fig. 2. Proposed mechanism of 4-MU effect on HA synthesis. a) Normal pathway of HA synthesis. b) UDP substitution by 4-MU resulting in the suppression of HA synthesis by HAS (from Nagy et al. [26]).

THE MECHANISM OF 4-MU ACTION ON HYALURONIC ACID SYNTHESIS

There is no evidence of competitive inhibition or even direct interaction of 4-MU with HAS. 4-MU does not affect the enzymatic activity of the solubilized HAS [23]. The most common hypothesis is that 4-MU acts a competitive substrate for uridine 5′-diphosphate-glucuronosyltransferase (UGT), thus depleting the cellular pool of uridine 5′-UDP-GlcA utilized in HA synthesis [24-26] (Fig. 2).

However, this hypothesis has not been confirmed experimentally. As an evidence against it, it was shown that 4-MU does not affect the synthesis of other glycosaminoglycans, which utilizes the same monomers as the synthesis of HA. In addition, coumarins with alkylated 7-hydroxy group, which cannot be the substrates for UGT, were still found to inhibit HA synthesis with a high efficiency *in vitro* [27]. 4-MU has been shown to reduce the expression level of *HAS2* mRNA [25, 28, 29] and simultaneously upregulate expression of *Hyal1* gene [30]. It also reduced the levels of phosphorylase and uridine 5′-diphosphate glucose dehydrogenase [31]. It still remains unknown how HA synthesis is regulated at the transcriptional level and

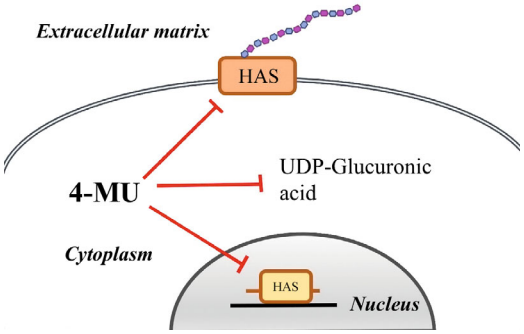

Fig. 3. Mechanisms of HA synthesis inhibition by 4-MU: competition with HA precursor UDP-GlcA; inhibition of *HAS2* gene expression; indirect inhibition of HASs (from Vitale et al. [1]).

whether involved mechanisms are selective for these particular mRNAs (Fig. 3).

As has been shown by our group and other researchers [32, 33], 4-MU has multiple targets not directly related to HA metabolism. It is possible that a decreased HA accumulation observed *in vitro* studies is the cumulative effect of several parallel processes, including possible substrate depletion, downregulation of *HAS2* expression (as experimentally demonstrated), and activation of *Hyal1* expression [30]. *HAS2* expression is also known to be regulated by nuclear receptors, in particular, glucocorticoid receptor. Thus, expression of *HAS2* was almost completely suppressed by dexamethasone [34]. Cells exposed to 4-MU demonstrated alterations in the cell cycle and p53 signaling cascade [35, 36].

EFFECT OF 4-MU ON VARIOUS TYPES OF CANCER AND AUTOIMMUNE AND INFLAMMATORY PROCESSES

4-MU multiple processes associated with tumor progression, such as migration, proliferation, and invasion of cancer cell and angiogenesis, as well as influences cells of tumor microenvironment (fibroblasts, endothelial and immune cells). Cancer development involves rapid changes in the structure and composition of the ECM (whose main component is HA), which requires creation of new drugs capable of changing the properties of the ECM. This approach is promising in the treatment of various types of cancer, and 4-MU has already been approved for the use in this new capacity.

Using a mouse model of carbon tetrachloride-induced liver fibrosis, we demonstrated that formation of collagen fibers is preceded by HA synthesis along the boundaries of liver lobules. 4-MU inhibited HA synthesis and significantly decreased formation of collagen fibers around hepatic lobules [30]. In our recent study, siRNA-mediated knockdown of the *HAS2* gene reproduced the effect of 4-MU on several signaling pathways and transcription of some key genes, resulting in suppression of liver fibrosis [37].

The use 4-MU in the treatment of brain cancer is especially interesting. The ECM of malignant gliomas and glioblastomas is characterized by an increased HA content; HA stimulates adhesive and invasive processes of tumor cells [38]. 4-MU is a small molecule capable of passing through the blood-brain barrier and inhibiting the synthesis of HA, which has promoted studies on its possible application in the treatment for gliomas and glioblastomas. Thus, in mouse models, high doses of 4-MU reduced HA synthesis, reduced proliferation and migration of glioblastoma cells, and stimulated their apoptosis [39-41]. As shown in *in vitro* and *in vivo* experiments, 4-MU reduced proliferation of glioma cells by regulating autophagy [42]. Chistyakov et al. [43] demonstrated that 4-MU inhibited the inflammatory response of astrocytes. Oral administration of 4-MU in mice caused a significant decrease in the HA content in the spinal cord and brain, reduction in synaptic stability, and reactivation of neuroplasticity, which resulted in improved memory [44].

The data on preclinical studies on 4-MU application for the treatment of various diseases are given in Table 1.

THE PROSPECTS OF COMBINED THERAPY WITH 4-MU. THE EFFECT OF 4-MU ON THE TUMOR PHYSICAL BARRIER. THE USE OF 4-MU AS A HYALURONIC ACID SYNTHESIS INHIBITOR

HA-rich ECM forms a biological barrier of the tumor microenvironment. This barrier regulates the activity of immune effectors [13, 98], prevents drug diffusion [99], hinders the adsorption of transgenic vectors in gene therapy [100], and plays an important role in the acquisition of resistance to anticancer drugs [1, 11, 101].

The possibility of changing the properties of tumor microenvironment in order to improve the result of antitumor therapy has been actively investigated. The pathological tumor microenvironment is characterized by hypoxia and high interstitial fluid pressure, leading to tumor progression and resistance to treatment [102]. Increased interstitial pressure is considered to be the most important barrier for efficient drug distribution within the tumor. The reasons for the increased interstitial pressure in the tumor are numerous and include extensive intratumor vascular network, insufficient development of lymphatic vessels, changes in the ECM components, and pressure created by constantly dividing tumor cells [103, 104]. An increased HA content in tissues surrounding the tumor contributes to the increase in the ECM volume and, as a result, increases in the pressure inside the tumor [105, 106]. Such high HA content in the tumor microenvironment forms a physical barrier that restricts the access of monoclonal antibodies and immune cells to the tumor tissue, which is one of the mechanisms of tumor resistance to immunotherapy [107].

Due to the ability to inhibit the synthesis of HA, 4-MU was suggested for the adjuvant therapy in combination with the primary anticancer therapy. Using various models, it has been shown that the use of 4-MU in a combined therapy for various types of cancer increased the treatment efficacy, reduced the toxicity of antitumor drugs, and helped to overcome emerging chemoresistance (Table 2).

Table 1. Preclinical studies on the application of 4-MU in the treatment of various diseases

Organ/system	Studied disease	Year	Type of investigation	Reference
Inflammation	acute respiratory distress syndrome (ARDS)	2013	*in vitro*	[45]
		2015	*in vitro*	[46]
	allergic inflammation	2022	*in vitro*	[47]
	allergic rhinitis	2022	*in vitro*/*in vivo*	[48]
	inflammation	2022	*in vitro*	[49]
Head and neck	oral squamous cell carcinomas	2022	*in vitro*	[50]
Bile ducts	biliary dyskinesia	1984	*in vivo*	[51]
	biliary colic	1995	*in vivo*	[52]
Immune response	Graves' orbitopathy	2020	*in vitro*	[53]
	transplant rejection	2021	*in vitro*/*in vivo*	[54]
	autoimmune response to transplanted islets of Langerhans	2020	*in vitro*/*in vivo*	[55]
	acute lung allograft rejection	2021	*in vitro*/*in vivo*	[56]
Bone marrow	chronic myeloid leukemia	2013	*in vitro*	[57]
		2016	*in vitro*	[58]
		2017	*in vitro*	[59]
Lungs	pleural mesothelioma	2017	*in vitro*/*in vivo*	[60]
	pulmonary fibrosis, pulmonary hypertension	2017	*in vivo*	[61]
Mammary glands	breast cancer	2019	*in vitro*	[62]
		2022	*in vitro*	[63]
Bladder	bladder cancer	2017	*in vitro*/*in vivo*	[64]
Peripheral nervous system	malignant peripheral nerve sheath tumor	2017	*in vitro*/*in vivo*	[65]
Liver	hepatocellular carcinoma	2012	*in vitro*/*in vivo*	[66]
		2015	*in vitro*/*in vivo*	[67]
		2019	*in vitro*/*in vivo*	[29]
		2021	*in vitro*/*in vivo*	[68]
		2022	*in vitro*/*in vivo*	[69]
	liver metastasis of malignant melanoma	2005	*in vitro*/*in vivo*	[70]
	liver fibrosis	2019	*in vivo*	[30]
	steatohepatitis	2021	*in vitro*/*in vivo*	[71]

Table 1 (*cont.*)

Organ/system	Studied disease	Year	Type of investigation	Reference
Pancreas	pancreatic cancer	2006	*in vitro*/*in vivo*	[72]
		2016	*in vitro*/*in vivo*	[73]
		2017	*in vitro*/*in vivo*	[74]
		2018	*in vitro*/*in vivo*	[75, 76]
	pancreatic ductal adenocarcinoma	2019	*in vitro*	[77, 78]
Kidneys	renal cell carcinoma	2013	*in vitro*	[79]
	kidney ischemia-reperfusion injury	2013	*in vivo*	[80]
	metastatic renal cell carcinoma	2020	*in vitro*	[81]
	diabetic nephropathy	2021	*in vivo*	[82]
	advanced renal cell carcinoma	2022	*in vitro*/*in vivo*	[83]
Prostate	prostate cancer	2010	*in vitro*	[84]
		2015	*in vitro*/*in vivo*	[85]
Connective tissue	fibrosarcoma	2017	*in vitro*	[86]
		2019	*in vitro*	[87]
		2020	*in vitro*	[88]
		2021	*in vitro*	[89]
Large intestine	colorectal carcinoma	2015	*in vitro*/*in vivo*	[90]
Central nervous system	glioblastoma	2021	*in vitro*	[39, 40]
		2022	*in vitro*/*in vivo*	[91]
Endometrium	endometriosis	2016	*in vitro*/*in vivo*	[92]
		2020	*in vitro*	[93]
		2023	*in vivo*	[94]
Ovaries	ovarian cancer	2014	*in vitro*	[95]
		2019	*in vitro*/*in vivo*	[96]
		2020	*in vitro*	[97]

Table 2. Preclinical studies on the treatment of various types of cancer using combinations of anticancer drugs and 4-MU

Type of cancer	Main treatment	Type of study	Year	Reference
Hepatocellular carcinoma	immunotherapy: IL-12-encoding adenovirus (AdIL-12)	*in vitro*	2018	[111]
Glioblastoma	temozolomide	*in vitro*	2023	[41]

Table 2 (*cont.*)

Type of cancer	Main treatment	Type of study	Year	Reference
Malignant pleural mesothelioma	trametinib	*in vitro*/*in vivo*	2017	[60]
Colorectal carcinoma	cyclophosphamide with immunotherapy (AdIL-12)	*in vitro*/*in vivo*	2015	[90]
Melanoma	vemurafenib	*in vitro*	2021	[109]
Esophageal squamous cell carcinoma	dichloroacetic acid	*in vitro*/*in vivo*	2019	[108]
Oral squamous cell carcinoma	radiotherapy	*in vitro*	2022	[50]
Renal cell carcinoma	sorafenib	*in vitro*	2013	[79]
Advanced renal cell carcinoma	sorafenib	*in vitro*/*in vivo*	2022	[83]
Pancreatic cancer	5-fluorouracil	*in vitro*/*in vivo*	2018	[76]
Pancreatic cancer	gemcitabine	*in vitro*/*in vivo*	2006	[72]
Ovarian cancer	carboplatin	*in vitro*/*in vivo*	2019	[96]
Bladder urothelial carcinoma	cisplatin or doxorubicin	*in vivo*	2019	[110]
Fibrosarcoma	radiotherapy	*in vitro*	2021	[89]
		in vitro	2019	[87]
		in vitro	2017	[86]
Chronic myeloid leukemia	imatinib	*in vitro*	2017	[59]
Chronic myeloid leukemia	doxorubicin	*in vitro*	2016	[58]

According to the data on the use of 4-MU as an addition to the main therapy, 4-MU enhanced the radiosensitivity of radiation-resistant cells in oral squamous cell carcinoma [50] and fibrosarcoma [86-89]. In renal cell carcinoma, sorafenib in combination with 4-MU inhibited more efficiently proliferation and invasion of cancer cells, suppressed capillary formation, and induces apoptosis of tumor and endothelial cells [79, 83]. 4-MU increased the efficacy of 5-fluorouracil [68] and gemcitabine [72] against pancreatic cancer, inhibited cell proliferation, and decreased the size of primary tumors and metastases, as well as promoted survival of affected animals. 4-MU increased the sensitivity of glioblastoma cells to temozolomide by enhancing the cytotoxic effect of the drug [41]. 4-MU exacerbated the cytotoxic effect of carboplatin on chemoresistant ovarian cancer cells [96]. A combined use of dichloroacetate and 4-MU in a model of esophageal squamous cell carcinoma promoted apoptosis of cancer cells and inhibited tumor growth [108]. 4-MU increased the sensitivity of myeloid leukemia cells to doxorubicin [58] and promoted their senescence [59]. A combination of vemurafenib with 4-MU reduced the survival of melanoma cells more efficiently compared to vemurafenib monotherapy [109]. 4-MU enhanced the chemosensitivity of bladder urothelial carcinoma cells to doxorubicin and cisplatin [110]. 4-MU significantly reduced the interstitial tumor pressure and improved perfusion, thus ensuring more efficient expression of the adenovirus transgene in the IL-12 (AdIL-12) immunotherapy of colorectal cancer [90]. In a liver cancer model, a combination of 4-MU with AdIL-12

led to a more pronounced inhibition of tumor growth and increased survival of mice compared to AdIL-12 monotherapy [111].

THE USE OF 4-MU AS A HEPATOPROTECTOR AND CHOLESTATIC AGENT TO REDUCE THE HEPATOTOXICITY OF PRIMARY THERAPY

Immune checkpoint inhibitors, cytokines, and antibodies against these proteins are used as immunomodulators to enhance the body immune response to tumors and chronic inflammation foci in rheumatoid, autoimmune, and inflammatory diseases [112, 113]. These drugs have successfully passed clinical trials and have been approved for the use in clinical practice by the European and American drug agencies [114]. However, up to 17% patients receiving such immunotherapy suffer from complications associated with the damage of liver and bile duct [115-119].

Depending on the severity of complications, the treatment for hepatotoxicity might include cessation of therapy with immune checkpoint inhibitors. Corticosteroids and immunosuppression (in more severe cases) can be recommended as well [120-123]. Ursodeoxycholic acid (UDCA) is used to improve the liver function in the case of cholestatic hepatotoxicity, when corticosteroids are ineffective [124-127]. UDCA has the hepatoprotective and choleretic effects and is considered as the treatment standard for cholestatic liver diseases with the autoimmune component (primary biliary cholangitis, primary sclerosing cholangitis) [128-130]. To our knowledge, there are no reports on the effect of 4-MU on the risk of hepatotoxicity development in response to immunotherapy. However, its established cholestatic and hepatoprotective properties make 4-MU a promising agent for such studies.

The data accumulated strongly suggest the need for the clinical trials of 4-MU as an agent for the adjuvant/additional antitumor therapy that would reduce the HA content, modify the ECM and tumor microenvironment, decrease interstitial pressure, improve tumor perfusion, facilitate drug access, and produce hepatoprotective and cholestatic effects, thus decreasing the risks of hepatotoxicity during immunotherapy.

TOPICAL APPLICATION OF 4-MU TO PREVENT FORMATION OF STRETCH MARKS, SCARS, KELOID SCARS, SUNBURNS, AND HYPOPIGMENTATION FOCI

Topical application of 4-MU leads to efficient inhibition of HA synthesis in the skin [131]. 4-MU has been shown to prevent keratinocyte activation and to reduce epidermal hyperproliferation [132] and migration rate of keloid keratinocytes, thus decreasing the likelihood of keloid scar formation [133].

4-MU enhances the processes of melanogenesis, which makes it a promising agent in the treatment of skin conditions associated with hypopigmentation, as well as a cosmetic product to provide natural tan [134].

METABOLISM OF 4-MU. TOXICITY AND SAFETY FOR HUMANS

Like all coumarins, 4-MU is poorly soluble in water. It is a nonpolar molecule and therefore, can easily pass through the lipid barrier in the intestine. It is almost completely absorbed upon oral administration and is excreted in urine and bile [26]. The methyl group at position 4 ensures low toxicity of 4-MU by preventing its metabolism to coumarin 3,4-epoxide by cytochrome P450, as well as weak anticoagulation properties compared to other coumarins, such as dicoumarol and warfarin [135].

When ingested, 4-MU is very rapidly and almost completely metabolized to 4-methylumbelliferone beta-D-glucuronide (4-MUG) in the liver and small intestine, which until recently, has limited its use in the treatment of bile ducts only [1, 136-138]. Less than 3% of orally administered 4-MU remains unchanged at the systemic level, while intravenous administration of 4-MU provides 10-30 times higher concentration of this compound in the blood [26, 139]. The half-life of orally administered 4-MU is only 28 min for humans and 3 min for mice [140, 141]. At the same time, the median concentration of 4-MUG in the plasma is more than 3000 times higher than the concentration of 4-MU [26, 141], i.e., most of 4-MU is present as 4-MUG in a body. However, despite its low bioavailability and short half-life, orally taken 4-MU efficiently inhibits HA synthesis. 4-MUG was proven to be as efficient as 4-MU in inhibiting HA synthesis; moreover, it is hydrolyzed back to 4-MU inside the cells [137]. Therefore, to evaluate the pharmacodynamics of 4-MU, it is necessary to take into account the effect of its metabolite 4-MUG. These data suggest that 4-MU can be used for the treatment of diseases beyond the biliary tract. For example, as a small nonpolar molecule, 4-MU is able to cross the blood-brain barrier and inhibit proliferation of glioma cell [42].

A typical regimen of 4-MU administration for an adult is 900-2400 mg/day [26]. No mutagenic or genotoxic effects of 4-MU have been found [1, 142, 143]. Clinical trials in patients with chronic hepatitis B and C (NCT00225537), healthy individuals, and patients with respiratory diseases (NCT02780752) [144] have proven the safety of 4-MU (see Table 3).

Table 3. Clinical trials on the use of 4-MU in the treatment of various diseases

Disease (study)	Status	Year	Reference/ Identifier clinicaltrials.gov
Interstitial lung diseases (SOLID Study)	phase II; recruitment of participants has not started	2024	NCT06325696
Primary sclerosing cholangitis	phase II; participants are being recruited	2022	NCT05295680
COVID-19	no information available	2022	NCT05386420
Pulmonary hypertension, including interstitial lung diseases (SATURN Study)	phase II; completed	2021	NCT05128929
Healthy participants; study of 4-MU effect on HA synthesis	phase I; completed	2016	[144]/NCT02780752
Biliary sludge stage 2	no information available	2016	[145]
Chronic hepatitis C virus and hepatitis B virus	no information available	2005	NCT00225537
Biliary dyskinesia	no information available	2005	[146]
Biliary dyskinesia	no information available	2001	[147]
Biliary dyskinesia	no information available	1995	[52]
Study of 4-MU bioavailability	no information available	1993	[141]
Symptoms after bile duct surgery	no information available	1988	[148]
Biliary dyskinesia after cholecystectomy	no information available	1984	[51]

CONCLUSION

Despite numerous experimental studies demonstrating the efficacy of 4-MU in various animal models of oncological, immune, and degenerative diseases, the molecular mechanisms of its action remain hypothetical. It was demonstrated (at least in the model of liver fibrosis) that the knockdown of the gene encoding HAS2 led not only to the suppression of fibrosis, but also to changes in the transcriptome that were similar to those observed upon oral administration of 4-MU [37]. At the same time, it cannot be excluded that some of effects of 4-MU may be independent of the HA synthesis inhibition [62, 149]. 4-MU may also act through different mechanisms depending on the type of cancer. However, taken together, the data on the effectiveness of 4-MU prove the need for a detailed study of its pharmacokinetic and pharmacodynamic properties to develop the treatment regimen (administration route, doses affecting 4-MU bioavailability, intervals between doses, and administration schedule). The first toxicology studies have already been conducted in phase I clinical trials [144], which allowed to proceed to clinical studies of the drug effectiveness (phase IIa). This is a worldwide trend. Thus, it is currently planned to conduct clinical trials on the use of 4-MU in the treatment of interstitial lung diseases and cholangitis (see Table 3).

The clinical trials of 4-MU include selection of appropriate doses for particular pathologies and investigation of metabolite excretion rates and drug bioavailability. Another important factor is development of new dosage forms (for example, 4-MU-containing nanoparticles) that will not only increase 4-MU bioavailability, but will also lead to the patent protection of a new drug.

Finally, if HAS is indeed the main pharmacological target of 4-MU, development of new chemical compounds using 3D models of HAS2/HAS3 and docking of potential ligands with the help of artificial intelligence will inevitably result in the creation of original, first-in-class targeted drugs based on HAS inhibitors.

Contributions. Yu.V.K. participated in the development of review concept and selection of articles and supervised experimental work of the authors presented in this review; A.C. and N.Kh. provided key conclusions from their experimental articles, edited the manuscript, and participated in its discussion; V.V.F. conducted literature search and analysis and wrote the original version of the manuscript.

Ethics approval and consent to participate. This work does not contain any studies involving human and animal subjects.

Conflict of interest. The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Vitale, D. L., Icardi, A., Rosales, P., Spinelli, F. M., Sevic, I., and Alaniz, L. D. (2021) Targeting the tumor extracellular matrix by the natural molecule 4-methylumbelliferone: a complementary and alternative cancer therapeutic strategy, *Front. Oncol.*, **11**, 710061, https://doi.org/10.3389/fonc.2021.710061.
2. Rosales, P., Vitale, D., Icardi, A., Sevic, I., and Alaniz, L. (2024) Role of Hyaluronic acid and its chemical derivatives in immunity during homeostasis, cancer and tissue regeneration, *Semin. Immunopathol.*, **46**, 15, https://doi.org/10.1007/s00281-024-01024-7.
3. Tavianatou, A. G., Caon, I., Franchi, M., Piperigkou, Z., Galesso, D., and Karamanos, N. K. (2019) Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer, *FEBS J.*, **286**, 2883-2908, https://doi.org/10.1111/febs.14777.
4. Jung, H. (2020) Hyaluronidase: an overview of its properties, applications, and side effects, *Arch. Plast. Surg.*, **47**, 297-300, https://doi.org/10.5999/aps.2020.00752.
5. Kudo, D., Kon, A., Yoshihara, S., Kakizaki, I., Sasaki, M., Endo, M., and Takagaki, K. (2004) Effect of a hyaluronan synthase suppressor, 4-methylumbelliferone, on B16F-10 melanoma cell adhesion and locomotion, *Biochem. Biophys. Res. Commun.*, **321**, 783-787, https://doi.org/10.1016/j.bbrc.2004.07.041.
6. Cheng, X. B., Kohi, S., Koga, A., Hirata, K., and Sato, N. (2016) Hyaluronan stimulates pancreatic cancer cell motility, *Oncotarget*, **7**, 4829-4840, https://doi.org/10.18632/oncotarget.6617.
7. Ricciardelli, C., Russell, D. L., Ween, M. P., Mayne, K., Suwiwat, S., Byers, S., Marshall, V. R., Tilley, W. D., and Horsfall, D. J. (2007) Formation of hyaluronan- and versican-rich pericellular matrix by prostate cancer cells promotes cell motility, *J. Biol. Chem.*, **282**, 10814-10825, https://doi.org/10.1074/jbc.M606991200.
8. Jacobetz, M. A., Chan, D. S., Neesse, A., Bapiro, T. E., Cook, N., Frese, K. K., Feig, C., Nakagawa, T., Caldwell, M. E., Zecchini, H. I., Lolkema, M. P., Jiang, P., Kultti, A., Thompson, C. B., Maneval, D. C., Jodrell, D. I., Frost, G. I., Shepard, H. M., Skepper, J. N., and Tuveson, D. A. (2013) Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer, *Gut*, **62**, 112-120, https://doi.org/10.1136/gutjnl-2012-302529.
9. Misra, S., Ghatak, S., Zoltan-Jones, A., and Toole, B. P. (2003) Regulation of multidrug resistance in cancer cells by hyaluronan, *J. Biol. Chem.*, **278**, 25285-25288, https://doi.org/10.1074/jbc.C300173200.
10. Toole, B. P., and Slomiany, M. G. (2008) Hyaluronan: a constitutive regulator of chemoresistance and malignancy in cancer cells, *Semin. Cancer Biol.*, **18**, 244-250, https://doi.org/10.1016/j.semcancer.2008.03.009.
11. Vitale, D. L., Spinelli, F. M., Del Dago, D., Icardi, A., Demarchi, G., Caon, I., García, M., Bolontrade, M. F., Passi, A., Cristina, C., and Alaniz, L. (2018) Co-treatment of tumor cells with hyaluronan plus doxorubicin affects endothelial cell behavior independently of VEGF expression, *Oncotarget*, **9**, 36585-36602, https://doi.org/10.18632/oncotarget.26379.
12. Kultti, A., Zhao, C., Singha, N. C., Zimmerman, S., Osgood, R. J., Symons, R., Jiang, P., Li, X., Thompson, C. B., Infante, J. R., Jacobetz, M. A., Tuveson, D. A., Frost, G. I., Shepard, H. M., and Huang, Z. (2014) Accumulation of extracellular hyaluronan by hyaluronan synthase 3 promotes tumor growth and modulates the pancreatic cancer microenvironment, *Biomed. Res. Int.*, **2014**, 817613, https://doi.org/10.1155/2014/817613.
13. McBride, W. H., and Bard, J. B. (1979) Hyaluronidase-sensitive halos around adherent cells. Their role in blocking lymphocyte-mediated cytolysis, *J. Exp. Med.*, **149**, 507-515, https://doi.org/10.1084/jem.149.2.507.
14. Lipponen, P., Aaltomaa, S., Tammi, R., Tammi, M., Agren, U., and Kosma, V. M. (2001) High stromal hyaluronan level is associated with poor differentiation and metastasis in prostate cancer, *Eur. J. Cancer.*, **37**, 849-856, https://doi.org/10.1016/s0959-8049(00)00448-2.
15. Setälä, L. P., Tammi, M. I., Tammi, R. H., Eskelinen, M. J., Lipponen, P. K., Agren, U. M., Parkkinen, J., Alhava, E. M., and Kosma, V. M. (1999) Hyaluronan expression in gastric cancer cells is associated with local and nodal spread and reduced survival rate, *Br. J. Cancer.*, **79**, 1133-1138, https://doi.org/10.1038/sj.bjc.6690180.
16. Anttila, M. A., Tammi, R. H., Tammi, MI., Syrjänen, K. J., Saarikoski, S. V., and Kosma, V. M. (2000) High levels of stromal hyaluronan predict poor disease outcome in epithelial ovarian cancer, *Cancer Res.*, **60**, 150-155.
17. Bharadwaj, A. G., Kovar, J. L., Loughman, E., Elowsky, C., Oakley, G. G., and Simpson, M. A. (2009) Spontaneous metastasis of prostate cancer is promoted by excess hyaluronan synthesis and processing, *Am. J. Pathol.*, **174**, 1027-1036, https://doi.org/10.2353/ajpath.2009.080501.

18. Sironen, R. K., Tammi, M., Tammi, R., Auvinen, P. K., Anttila, M., and Kosma, V. M. (2011) Hyaluronan in human malignancies, *Exp. Cell Res.*, **317**, 383-391, https://doi.org/10.1016/j.yexcr.2010.11.017.
19. Kim, J., and Seki, E. (2023) Hyaluronan in liver fibrosis: basic mechanisms, clinical implications, and therapeutic targets, *Hepatol. Commun.*, **7**, e0083, https://doi.org/10.1097/HC9.0000000000000083.
20. Yang, Y. M., Noureddin, M., Liu, C., Ohashi, K., Kim, S. Y., Ramnath, D., Powell, E. E., Sweet, M. J., Roh, Y. S., Hsin, I. F., Deng, N., Liu, Z., Liang, J., Mena, E., Shouhed, D., Schwabe, R. F., Jiang, D., Lu, S. C., Noble, P. W., and Seki, E. (2019) Hyaluronan synthase 2-mediated hyaluronan production mediates Notch1 activation and liver fibrosis, *Sci. Transl. Med.*, **11**, eaat9284, https://doi.org/10.1126/scitranslmed.aat9284.
21. DeAngelis, P. L., and Zimmer, J. (2023) Hyaluronan synthases; mechanisms, myths, and mysteries of three types of unique bifunctional glycosyltransferases, *Glycobiology*, **33**, 1117-1127, https://doi.org/10.1093/glycob/cwad075.
22. Maloney, F. P., Kuklewicz, J., Corey, R. A., Bi, Y., Ho, R., Mateusiak, L., Pardon, E., Steyaert, J., Stansfeld, P. J., and Zimmer, J. (2022) Structure, substrate recognition and initiation of hyaluronan synthase, *Nature*, **604**, 195-201, https://doi.org/10.1038/s41586-022-04534-2.
23. Nakamura, T., Funahashi, M., Takagaki, K., Munakata, H., Tanaka, K., Saito, Y., and Endo, M. (1997) Effect of 4-methylumbelliferone on cell-free synthesis of hyaluronic acid, *Biochem. Mol. Biol. Int.*, **43**, 263-268, https://doi.org/10.1080/15216549700204041.
24. Kakizaki, I., Kojima, K., Takagaki, K., Endo, M., Kannagi, R., Ito, M., Maruo, Y., Sato, H., Yasuda, T., Mita, S., Kimata, K., and Itano, N. (2004) A novel mechanism for the inhibition of hyaluronan biosynthesis by 4-methylumbelliferone, *J. Biol. Chem.*, **279**, 33281-33289, https://doi.org/10.1074/jbc.M405918200.
25. Kultti, A., Pasonen-Seppänen, S., Jauhiainen, M., Rilla, KJ., Kärnä, R., Pyöriä, E., Tammi, R. H., and Tammi, M. I. (2009) 4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP-glucuronic acid and downregulation of hyaluronan synthase 2 and 3, *Exp. Cell Res.*, **315**, 1914-1923, https://doi.org/10.1016/j.yexcr.2009.03.002.
26. Nagy, N., Kuipers, H. F., Frymoyer, A. R., Ishak, H. D., Bollyky, J. B., Wight, T. N., and Bollyky, P. L. (2015) 4-methylumbelliferone treatment and hyaluronan inhibition as a therapeutic strategy in inflammation, autoimmunity, and cancer, *Front. Immunol.*, **6**, 123, https://doi.org/10.3389/fimmu.2015.00123.
27. Tsitrina, A. A., Krasylov, I. V., Maltsev, D. I., Andreichenko, I. N., Moskvina, V. S., Ivankov, D. N., Bulgakova, E. V., Nesterchuk, M., Shashkovskaya, V., Dashenkova, N. O., Khilya, V. P., Mikaelyan, A., and Kotelevtsev, Y. (2021) Inhibition of hyaluronan secretion by novel coumarin compounds and chitin synthesis inhibitors, *Glycobiology*, **31**, 959-974, https://doi.org/10.1093/glycob/cwab038.
28. Saito, T., Dai, T., and Asano, R. (2013) The hyaluronan synthesis inhibitor 4-methylumbelliferone exhibits antitumor effects against mesenchymal-like canine mammary tumor cells, *Oncol. Lett.*, **5**, 1068-1074, https://doi.org/10.3892/ol.2013.1124.
29. Sukowati, C. H. C., Anfuso, B., Fiore, E., Ie, S. I., Raseni, A., Vascotto, F., Avellini, C., Mazzolini, G., and Tiribelli, C. (2019) Hyaluronic acid inhibition by 4-methylumbelliferone reduces the expression of cancer stem cells markers during hepatocarcinogenesis, *Sci. Rep.*, **9**, 4026, https://doi.org/10.1038/s41598-019-40436-6.
30. Andreichenko, I. N., Tsitrina, A. A., Fokin, A. V., Gabdulkhakova, A. I., Maltsev, D. I., Perelman, G. S., Bulgakova, E. V., Kulikov, A. M., Mikaelyan, A. S., and Kotelevtsev, Y. V. (2019) 4-methylumbelliferone prevents liver fibrosis by affecting hyaluronan deposition, FSTL1 expression and cell localization, *Int. J. Mol. Sci.*, **20**, 6301, https://doi.org/10.3390/ijms20246301.
31. Vigetti, D., Rizzi, M., Viola, M., Karousou, E., Genasetti, A., Clerici, M., Bartolini, B., Hascall, V. C., De Luca, G., and Passi, A. (2009) The effects of 4-methylumbelliferone on hyaluronan synthesis, MMP2 activity, proliferation, and motility of human aortic smooth muscle cells, *Glycobiology*, **19**, 537-546, https://doi.org/10.1093/glycob/cwp022.
32. Tsitrina, A. A., Halimani, N., Andreichenko, I. N., Sabirov, M., Nesterchuk, M., Dashenkova, N. O., Romanov, R., Bulgakova, E. V., Mikaelyan, A., and Kotelevtsev, Y. (2023) 4-methylumbelliferone targets revealed by public data analysis and liver transcriptome sequencing, *Int. J. Mol. Sci.*, **24**, 2129, https://doi.org/10.3390/ijms24032129.
33. Díaz, M., Pibuel, M., Paglilla, N., Poodts, D., Álvarez, E., Papademetrio, D. L., Hajos, S. E., and Lompardía, S. L. (2021) 4-Methylumbelliferone induces antitumor effects independently of hyaluronan synthesis inhibition in human acute leukemia cell lines, *Life Sci.*, **287**, 120065, https://doi.org/10.1016/j.lfs.2021.120065.
34. Zhang, W., Watson, C. E., Liu, C., Williams, K. J., and Werth, V. P. (2000) Glucocorticoids induce a near-total suppression of hyaluronan synthase mRNA in dermal fibroblasts and in osteoblasts: a molecular mechanism contributing to organ atrophy, *Biochem. J.*, **349**, 91-97, https://doi.org/10.1042/0264-6021:3490091.
35. Saito, T., Tamura, D., Nakamura, T., Makita, Y., Ariyama, H., Komiyama, K., Yoshihara, T., and Asano, R. (2013) 4-methylumbelliferone leads to growth arrest and apoptosis in canine mammary tumor cells, *Oncol. Rep.*, **29**, 335-342, https://doi.org/10.3892/or.2012.2100.
36. Ban, H., Uchakina, O., and McKallip, R. J. (2015) Hyaluronic acid inhibitor 4-methylumbelliferone activates

the intrinsic apoptosis pathway in K562 chronic myelogenous leukemia cells, *Anticancer Res.*, **35**, 5231-5240.

37. Halimani, N., Nesterchuk, M., Tsitrina, A. A., Sabirov, M., Andreichenko, I. N., Dashenkova, N. O., Petrova, E., Kulikov, A. M., Zatsepin, T. S., Romanov, R. A., Mikaelyan, A. S., and Kotelevtsev, Y. V. (2024) Knockdown of Hyaluronan synthase 2 suppresses liver fibrosis in mice via induction of transcriptomic changes similar to 4MU treatment, *Sci. Rep.*, **14**, 2797, https://doi.org/10.1038/s41598-024-53089-x.
38. Kim, Y., and Kumar, S. (2014) CD44-mediated adhesion to hyaluronic acid contributes to mechanosensing and invasive motility, *Mol. Cancer Res.*, **12**, 1416-1429, https://doi.org/10.1158/1541-7786.MCR-13-0629.
39. Pibuel, M. A., Díaz, M., Molinari, Y., Poodts, D., Silvestroff, L., Lompardía, S. L., Franco, P., and Hajos, S. E. (2021) 4-Methylumbelliferone as a potent and selective antitumor drug on a glioblastoma model, *Glycobiology*, **31**, 29-43, https://doi.org/10.1093/glycob/cwaa046.
40. Pibuel, M. A., Poodts, D., Díaz, M., Molinari, Y. A., Franco, P. G., Hajos, S. E., and Lompardía, S. L. (2021) Antitumor effect of 4MU on glioblastoma cells is mediated by senescence induction and CD44, RHAMM and p-ERK modulation, *Cell Death Discov.*, 7, 280, https://doi.org/10.1038/s41420-021-00672-0.
41. Pibuel, M. A., Poodts, D., Sias, S. A., Byrne, A., Hajos, S. E., Franco, P. G., and Lompardía, S. L. (2023) 4-Methylumbelliferone enhances the effects of chemotherapy on both temozolomide-sensitive and resistant glioblastoma cells, *Sci. Rep.*, **13**, 9356, https://doi.org/10.1038/s41598-023-35045-3.
42. Yan, T., Chen, X., Zhan, H., Yao, P., Wang, N., Yang, H., Zhang, C., Wang, K., Hu, H., Li, J., Sun, J., Dong, Y., Lu, E., Zheng, Z., Zhang, R., Wang, X., Ma, J., Gao, M., Ye, J., Wang, X., Teng, L., Liu, H., and Zhao, S. (2021) Interfering with hyaluronic acid metabolism suppresses glioma cell proliferation by regulating autophagy, *Cell Death Dis.*, **12**, 486, https://doi.org/10.1038/s41419-021-03747-z.
43. Chistyakov, D. V., Nikolskaya, A. I., Goriainov, S. V., Astakhova, A. A., and Sergeeva, M. G. (2020) Inhibitor of hyaluronic acid synthesis 4-methylumbelliferone as an anti-inflammatory modulator of LPS-mediated astrocyte responses, *Int. J. Mol. Sci.*, **21**, 8203, https://doi.org/10.3390/ijms21218203.
44. Dubisova, J., Burianova, J. S., Svobodova, L., Makovicky, P., Martinez-Varea, N., Cimpean, A., Fawcett, J. W., Kwok, J. C. F., and Kubinova, S. (2022) Oral treatment of 4-methylumbelliferone reduced perineuronal nets and improved recognition memory in mice, *Brain Res. Bull.*, **181**, 144-156, https://doi.org/10.1016/j.brainresbull.2022.01.011.
45. McKallip, R. J., Hagele, H. F., and Uchakina, O. N. (2013) Treatment with the hyaluronic acid synthesis inhibitor 4-methylumbelliferone suppresses SEB-induced lung inflammation, *Toxins (Basel)*, **5**, 1814-1826, https://doi.org/10.3390/toxins5101814.
46. McKallip, R. J., Ban, H., and Uchakina, O. N. (2015) Treatment with the hyaluronic Acid synthesis inhibitor 4-methylumbelliferone suppresses LPS-induced lung inflammation, *Inflammation*, **38**, 1250-1259, https://doi.org/10.1007/s10753-014-0092-y.
47. Wang, H. N., Xiang, Q. A., Lin, H. H., Chen, J. N., Guo, W. J., Guo, W. M., Yue, X. N., Zhao, Z. F., Ji, K., and Chen, J. J. (2022) Plant-derived molecule 4-methylumbelliferone suppresses FcεRI-mediated mast cell activation and allergic inflammation, *Molecules*, **27**, 1577, https://doi.org/10.3390/molecules27051577.
48. Lee, S. N., Yoon, S. A., Song, J. M., Kim, H. C., Cho, H. J., Choi, A. M. K., and Yoon, J. H. (2022) Cell-type-specific expression of hyaluronan synthases HAS2 and HAS3 promotes goblet cell hyperplasia in allergic airway inflammation, *Am. J. Respir. Cell Mol. Biol.*, **67**, 360-374, https://doi.org/10.1165/rcmb.2021-0527OC.
49. Galkina, S. I., Fedorova, N. V., Ksenofontov, A. L., Golenkina, E. A., Serebryakova, M. V., Stadnichuk, V. I., Baratova, L. A., and Sud'ina, G. F. (2022) Inhibitor of hyaluronic acid synthesis 4-methylumbelliferone suppresses the secretory processes that ensure the invasion of neutrophils into tissues and induce inflammation, *Biomedicines*, **10**, 314, https://doi.org/10.3390/biomedicines10020314.
50. Hasegawa, K., Saga, R., Ohuchi, K., Kuwahara, Y., Tomita, K., Okumura, K., Sato, T., Fukumoto, M., Tsuruga, E., and Hosokawa, Y. (2022) 4-Methylumebelliferone enhances radiosensitizing effects of radioresistant oral squamous cell carcinoma cells via hyaluronan synthase 3 suppression, *Cells*, **11**, 3780, https://doi.org/10.3390/cells11233780.
51. Quaranta, S., Rossetti, S., and Camarri, E. (1984) Double-blind clinical study on hymecromone and placebo in motor disorders of the bile ducts after cholecystectomy, *Clin. Ter.*, **108**, 513-517.
52. Krawzak, H. W., Heistermann, H. P., Andrejewski, K., and Hohlbach, G. (1995) Postprandial bile-duct kinetics under the influence of 4-methylumbelliferone (hymecromone), *Int. J. Clin. Pharmacol. Ther.*, **33**, 569-572.
53. Yoon, Y., Chae, M. K., Lee, E. J., and Yoon, J. S. (2020) 4-Methylumbelliferone suppresses hyaluronan and adipogenesis in primary cultured orbital fibroblasts from Graves' orbitopathy, *Graefes Arch. Clin. Exp. Ophthalmol.*, **258**, 1095-1102, https://doi.org/10.1007/s00417-019-04528-3.
54. Marshall, P. L., Nagy, N., Kaber, G., Barlow, G. L., Ramesh, A., Xie, B. J., Linde, M. H., Haddock, N. L., Lester, C. A., Tran, Q. L., de Vries, C. R., Hargil, A., Malkovskiy, A. V., Gurevich, I., Martinez, H. A., Kuipers, H. F., Yadava, K., Zhang, X., Evanko, S. P., Gebe, J. A., Wang, X., Vernon, R. B., de la Motte, C.,

Wight, T. N., Engleman, E. G., Krams, S. M., Meyer, E. H., and Bollyky, P. L. (2021) Hyaluronan synthesis inhibition impairs antigen presentation and delays transplantation rejection, *Matrix Biol.*, **96**, 69-86, https://doi.org/10.1016/j.matbio.2020.12.001.

55. Gebe, J. A., Gooden, M. D., Workman, G., Nagy, N., Bollyky, P. L., Wight, T. N., and Vernon, R. B. (2020) Modulation of hyaluronan synthases and involvement of T cell-derived hyaluronan in autoimmune responses to transplanted islets, *Matrix Biol. Plus*, **9**, 100052, https://doi.org/10.1016/j.mbplus.2020.100052.
56. Imani, J., Liu, K., Cui, Y., Assaker, J. P., Han, J., Ghosh, A. J., Ng, J., Shrestha, S., Lamattina, A. M., Louis, P. H., Hentschel, A., Esposito, A. J., Rosas, I. O., Liu, X., Perrella, M. A., Azzi, J., Visner, G., and El-Chemaly, S. (2021) Blocking hyaluronan synthesis alleviates acute lung allograft rejection, *JCI Insight.*, **6**, e142217, https://doi.org/10.1172/jci.insight.142217.
57. Uchakina, O. N., Ban, H., and McKallip, R. J. (2013) Targeting hyaluronic acid production for the treatment of leukemia: treatment with 4-methylumbelliferone leads to induction of MAPK-mediated apoptosis in K562 leukemia, *Leuk. Res.*, **37**, 1294-1301, https://doi.org/10.1016/j.leukres.2013.07.009.
58. Uchakina, O. N., Ban, H., Hostetler, B. J., and McKallip, R. J. (2016) Inhibition of hyaluronic acid formation sensitizes chronic myelogenous leukemia to treatment with doxorubicin, *Glycobiology*, **26**, 1171-1179, https://doi.org/10.1093/glycob/cww064.
59. Lompardía, S. L., Díaz, M., Papademetrio, D. L., Pibuel, M., Álvarez, É., and Hajos, S. E. (2017) 4-methylumbelliferone and imatinib combination enhances senescence induction in chronic myeloid leukemia cell lines, *Invest. New Drugs*, **35**, 1-10, https://doi.org/10.1007/s10637-016-0397-9.
60. Cho, H., Matsumoto, S., Fujita, Y., Kuroda, A., Menju, T., Sonobe, M., Kondo, N., Torii, I., Nakano, T., Lara, P. N., Gandara, D. R., Date, H., and Hasegawa, S. (2017) Trametinib plus 4-methylumbelliferone exhibits antitumor effects by ERK blockade and CD44 downregulation and affects PD-1 and PD-L1 in malignant pleural mesothelioma, *J. Thorac. Oncol.*, **12**, 477-490, https://doi.org/10.1016/j.jtho.2016.10.023.
61. Collum, S. D., Chen, N. Y., Hernandez, A. M., Hanmandlu, A., Sweeney, H., Mertens, T. C. J., Weng, T., Luo, F., Molina, J. G., Davies, J., Horan, I. P., Morrell, N. W., Amione-Guerra, J., Al-Jabbari, O., Youker, K., Sun, W., Rajadas, J., Bollyky, P. L., Akkanti, B. H., Jyothula, S., Sinha, N., Guha, A., and Karmouty-Quintana, H. (2017) Inhibition of hyaluronan synthesis attenuates pulmonary hypertension associated with lung fibrosis, *Br. J. Pharmacol.*, **174**, 3284-3301, https://doi.org/10.1111/bph.13947.
62. Karalis, T. T., Heldin, P., Vynios, D. H., Neill, T., Buraschi, S., Iozzo, R. V., Karamanos, N. K., and Skandalis, S. S. (2019) Tumor-suppressive functions of 4-MU on breast cancer cells of different ER status: regulation of hyaluronan/HAS2/CD44 and specific matrix effectors, *Matrix Biol.*, **78-79**, 118-138, https://doi.org/10.1016/j.matbio.2018.04.007.
63. Choi, B. H., Ryoo, I., Sim, K. H., Ahn, H. J., Lee, Y. J., and Kwak, M. K. (2022) High levels of hyaluronic acid synthase-2 mediate NRF2-driven chemoresistance in breast cancer cells, *Biomol. Ther. (Seoul)*, **30**, 368-379, https://doi.org/10.4062/biomolther.2022.074.
64. Morera, D. S., Hennig, M. S., Talukder, A., Lokeshwar, S. D., Wang, J., Garcia-Roig, M., Ortiz, N., Yates, T. J., Lopez, L. E., Kallifatidis, G., Kramer, M. W., Jordan, A. R., Merseburger, A. S., Manoharan, M., Soloway, M. S., Terris, M. K., and Lokeshwar, V. B. (2017) Hyaluronic acid family in bladder cancer: potential prognostic biomarkers and therapeutic targets, *Br. J. Cancer*, **117**, 1507-1517, https://doi.org/10.1038/bjc.2017.318.
65. Ikuta, K., Ota, T., Zhuo, L., Urakawa, H., Kozawa, E., Hamada, S., Kimata, K., Ishiguro, N., and Nishida, Y. (2017) Antitumor effects of 4-methylumbelliferone, a hyaluronan synthesis inhibitor, on malignant peripheral nerve sheath tumor, *Int. J. Cancer*, **140**, 469-479, https://doi.org/10.1002/ijc.30460.
66. Piccioni, F., Malvicini, M., Garcia, MG., Rodriguez, A., Atorrasagasti, C., Kippes, N., Piedra Buena, I. T., Rizzo, M. M., Bayo, J., Aquino, J., Viola, M., Passi, A., Alaniz, L., and Mazzolini, G. (2012) Antitumor effects of hyaluronic acid inhibitor 4-methylumbelliferone in an orthotopic hepatocellular carcinoma model in mice, *Glycobiology*, **22**, 400-410, https://doi.org/10.1093/glycob/cwr158.
67. Piccioni, F., Fiore, E., Bayo, J., Atorrasagasti, C., Peixoto, E., Rizzo, M., Malvicini, M., Tirado-González, I., García, M. G., Alaniz, L., and Mazzolini, G. (2015) 4-methylumbelliferone inhibits hepatocellular carcinoma growth by decreasing IL-6 production and angiogenesis, *Glycobiology*, **25**, 825-835, https://doi.org/10.1093/glycob/cwv023.
68. Rodríguez, M. M., Onorato, A., Cantero, M. J., Domínguez, L., Bayo, J., Fiore, E., García, M., Atorrasagasti, C., Canbay, A., Malvicini, M., and Mazzolini, G. D. (2021) 4-methylumbelliferone-mediated polarization of M1 macrophages correlate with decreased hepatocellular carcinoma aggressiveness in mice, *Sci. Rep.*, **11**, 6310, https://doi.org/10.1038/s41598-021-85491-0.
69. Weiz, G., Molejon, MI., Malvicini, M., Sukowati, C. H. C., Tiribelli, C., Mazzolini, G., and Breccia, J. D. (2022) Glycosylated 4-methylumbelliferone as a targeted therapy for hepatocellular carcinoma, *Liver Int.*, **42**, 444-457, https://doi.org/10.1111/liv.15084.
70. Yoshihara, S., Kon, A., Kudo, D., Nakazawa, H., Kakizaki, I., Sasaki, M., Endo, M., and Takagaki, K. (2005) A hyaluronan synthase suppressor, 4-methylumbelliferone, inhibits liver metastasis of melanoma cells, *FEBS Lett.*, **579**, 2722-2726, https://doi.org/10.1016/j.febslet.2005.03.079.

71. Yang, Y. M., Wang, Z., Matsuda, M., and Seki, E. (2021) Inhibition of hyaluronan synthesis by 4-methylumbelliferone ameliorates non-alcoholic steatohepatitis in choline-deficient L-amino acid-defined diet-induced murine model, *Arch. Pharm. Res.*, **44**, 230-240, https://doi.org/10.1007/s12272-021-01309-7.
72. Nakazawa, H., Yoshihara, S., Kudo, D., Morohashi, H., Kakizaki, I., Kon, A., Takagaki, K., and Sasaki, M. (2006) 4-methylumbelliferone, a hyaluronan synthase suppressor, enhances the anticancer activity of gemcitabine in human pancreatic cancer cells, *Cancer Chemother. Pharmacol.*, **57**, 165-170, https://doi.org/10.1007/s00280-005-0016-5.
73. Yoshida, E., Kudo, D., Nagase, H., Shimoda, H., Suto, S., Negishi, M., Kakizaki, I., Endo, M., and Hakamada, K. (2016) Antitumor effects of the hyaluronan inhibitor 4-methylumbelliferone on pancreatic cancer, *Oncol. Lett.*, **12**, 2337-2344, https://doi.org/10.3892/ol.2016.4930.
74. Nagase, H., Kudo, D., Suto, A., Yoshida, E., Suto, S., Negishi, M., Kakizaki, I., and Hakamada, K. (2017) 4-methylumbelliferone suppresses hyaluronan synthesis and tumor progression in SCID mice intra-abdominally inoculated with pancreatic cancer cells, *Pancreas*, **46**, 190-197, https://doi.org/10.1097/MPA.0000000000000741.
75. Cheng, X. B., Sato, N., Kohi, S., Koga, A., and Hirata, K. (2018) 4-Methylumbelliferone inhibits enhanced hyaluronan synthesis and cell migration in pancreatic cancer cells in response to tumor-stromal interactions, *Oncol. Lett.*, **15**, 6297-6301, https://doi.org/10.3892/ol.2018.8147.
76. Yoshida, E., Kudo, D., Nagase, H., Suto, A., Shimoda, H., Suto, S., Kakizaki, I., Endo, M., and Hakamada, K. (2018) 4-methylumbelliferone decreases the hyaluronan-rich extracellular matrix and increases the effectiveness of 5-fluorouracil, *Anticancer Res.*, **38**, 5799-5804, https://doi.org/10.21873/anticanres.12919.
77. Kudo, Y., Kohi, S., Hirata, K., Goggins, M., and Sato, N. (2019) Hyaluronan activated-metabolism phenotype (HAMP) in pancreatic ductal adenocarcinoma, *Oncotarget*, **10**, 5592-5604, https://doi.org/10.18632/oncotarget.27172.
78. Suto, A., Kudo, D., Yoshida, E., Nagase, H., Suto, S., Mimura, J., Itoh, K., and Hakamada, K. (2019) Increase of tumor infiltrating γδ T-cells in pancreatic ductal adenocarcinoma through remodeling of the extracellular matrix by a hyaluronan synthesis suppressor, 4-methylumbelliferone, *Pancreas*, **48**, 292-298, https://doi.org/10.1097/MPA.0000000000001211.
79. Benitez, A., Yates, T. J., Shamaldevi, N., Bowen, T., and Lokeshwar, V. B. (2013) Dietary supplement hymecromone and sorafenib: a novel combination for the control of renal cell carcinoma, *J. Urol.*, **190**, 285-290, https://doi.org/10.1016/j.juro.2012.12.011.
80. Colombaro, V., Declèves, A. E., Jadot, I., Voisin, V., Giordano, L., Habsch, I., Nonclercq, D., Flamion, B., and Caron, N. (2013) Inhibition of hyaluronan is protective against renal ischaemia-reperfusion injury, *Nephrol. Dial. Transplant.*, **28**, 2484-2493, https://doi.org/10.1093/ndt/gft314.
81. Jordan, A. R., Wang, J., Yates, T. J., Hasanali, S. L., Lokeshwar, S. D., Morera, D. S., Shamaladevi, N., Li, C. S., Klaassen, Z., Terris, M. K., Thangaraju, M., Singh, A. B., Soloway, M. S., and Lokeshwar, V. B. (2020) Molecular targeting of renal cell carcinoma by an oral combination, *Oncogenesis*, **9**, 52, https://doi.org/10.1038/s41389-020-0233-0.
82. Selman, G., Martinez, L., Lightle, A., Aguilar, A., Woltmann, D., Xiao, Y., Vazquez-Padron, R. I., and Salman, L. H. (2021) A hyaluronan synthesis inhibitor delays the progression of diabetic kidney disease in a mouse experimental model, *Kidney360*, **2**, 809-818, https://doi.org/10.34067/KID.0004642020.
83. Wang, J., Jordan, A. R., Zhu, H., Hasanali, S. L., Thomas, E., Lokeshwar, S. D., Morera, D. S., Alexander, S., McDaniels, J., Sharma, A., Aguilar, K., Sarcan, S., Zhu, T., Soloway, M. S., Terris, M. K., Thangaraju, M., Lopez, L. E., and Lokeshwar, V. B. (2022) Targeting hyaluronic acid synthase-3 (HAS3) for the treatment of advanced renal cell carcinoma, *Cancer Cell Int.*, **22**, 421, https://doi.org/10.1186/s12935-022-02818-1.
84. Lokeshwar, V. B., Lopez, L. E., Munoz, D., Chi, A., Shirodkar, S. P., Lokeshwar, S. D., Escudero, D. O., Dhir, N., and Altman, N. (2010) Antitumor activity of hyaluronic acid synthesis inhibitor 4-methylumbelliferone in prostate cancer cells, *Cancer Res.*, **70**, 2613-2623, https://doi.org/10.1158/0008-5472.CAN-09-3185.
85. Yates, T. J., Lopez, L. E., Lokeshwar, S. D., Ortiz, N., Kallifatidis, G., Jordan, A., Hoye, K., Altman, N., and Lokeshwar, V. B. (2015) Dietary supplement 4-methylumbelliferone: an effective chemopreventive and therapeutic agent for prostate cancer, *J. Natl. Cancer Inst.*, **107**, djv085, https://doi.org/10.1093/jnci/djv085.
86. Saga, R., Monzen, S., Chiba, M., Yoshino, H., Nakamura, T., and Hosokawa, Y. (2017) Anti-tumor and anti-invasion effects of a combination of 4-methylumbelliferone and ionizing radiation in human fibrosarcoma cells, *Oncol. Lett.*, **13**, 410-416, https://doi.org/10.3892/ol.2016.5385.
87. Saga, R., Hasegawa, K., Murata, K., Chiba, M., Nakamura, T., Okumura, K., Tsuruga, E., and Hosokawa, Y. (2019) Regulation of radiosensitivity by 4-methylumbelliferone via the suppression of interleukin-1 in fibrosarcoma cells, *Oncol. Lett.*, **17**, 3555-3561, https://doi.org/10.3892/ol.2019.9990.
88. Hasegawa, K., Saga, R., Takahashi, R., Fukui, R., Chiba, M., Okumura, K., Tsuruga, E., and Hosokawa, Y. (2020) 4-methylumbelliferone inhibits clonogenic potency by suppressing high molecular weight-

hyaluronan in fibrosarcoma cells, *Oncol. Lett.*, **19**, 2801-2808, https://doi.org/10.3892/ol.2020.11370.

89. Saga, R., Matsuya, Y., Takahashi, R., Hasegawa, K., Date, H., and Hosokawa, Y. (2021) 4-Methylumbelliferone administration enhances radiosensitivity of human fibrosarcoma by intercellular communication, *Sci. Rep.*, **11**, 8258, https://doi.org/10.1038/s41598-021-87850-3.
90. Malvicini, M., Fiore, E., Ghiaccio, V., Piccioni, F., Rizzo, M., Olmedo Bonadeo, L., García, M., Rodríguez, M., Bayo, J., Peixoto, E., Atorrasagasti, C., Alaniz, L., Aquino, J., Matar, P., and Mazzolini, G. (2015) Tumor microenvironment remodeling by 4-methylumbelliferone boosts the antitumor effect of combined immunotherapy in murine colorectal carcinoma, *Mol. Ther.*, **23**, 1444-1455, https://doi.org/10.1038/mt.2015.112.
91. Zhan, D., Yalcin, F., Ma, D., Fu, Y., Wei, S., Lal, B., Li, Y., Dzaye, O., Laterra, J., Ying, M., Lopez-Bertoni, H., and Xia, S. (2021) Targeting UDP-α-d-glucose 6-dehydrogenase alters the CNS tumor immune microenvironment and inhibits glioblastoma growth, *Genes Dis.*, **9**, 717-730, https://doi.org/10.1016/j.gendis.2021.08.008.
92. Olivares, C. N., Alaniz, L. D., Menger, M. D., Barañao, R. I., Laschke, M. W., and Meresman, G. F. (2016) Inhibition of hyaluronic acid synthesis suppresses angiogenesis in developing endometriotic lesions, *PLoS One*, **11**, e0152302, https://doi.org/10.1371/journal.pone.0152302.
93. McLaughlin, J. E., Santos, M. T., Binkley, P. A., Sultana, M., Tekmal, R. R., Schenken, R. S., and Knudtson, J. F. (2020) Inhibition of hyaluronic acid synthesis decreases endometrial cell attachment, migration, and invasion, *Reprod. Sci.*, **27**, 1058-1063, https://doi.org/10.1007/s43032-019-00100-w.
94. Olivares, C. N., Ricci, A. G., Bilotas, M. A., Alaniz, L., Barañao, R. I., and Meresman, G. F. (2023) Effects of pharmacological inhibition of hyaluronic acid synthesis on experimental endometriosis, *Eur. J. Clin. Invest.*, **53**, e13899, https://doi.org/10.1111/eci.13899.
95. Tamura, R., Yokoyama, Y., Yoshida, H., Imaizumi, T., and Mizunuma, H. (2014) 4-Methylumbelliferone inhibits ovarian cancer growth by suppressing thymidine phosphorylase expression, *J. Ovarian Res.*, **7**, 94, https://doi.org/10.1186/s13048-014-0094-2.
96. Lokman, N. A., Price, Z. K., Hawkins, E. K., Macpherson, A. M., Oehler, M. K., and Ricciardelli, C. (2019) 4-Methylumbelliferone inhibits cancer stem cell activation and overcomes chemoresistance in ovarian cancer, *Cancers (Basel)*, **11**, 1187, https://doi.org/10.3390/cancers11081187.
97. An, G., Park, S., Lee, M., Lim, W., and Song, G. (2020) Antiproliferative effect of 4-methylumbelliferone in epithelial ovarian cancer cells is mediated by disruption of intracellular homeostasis and regulation of PI3K/AKT and MAPK signaling, *Pharmaceutics*, **12**, 640, https://doi.org/10.3390/pharmaceutics12070640.
98. Spinelli, F. M., Vitale, D. L., Icardi, A., Caon, I., Brandone, A., Giannoni, P., Saturno, V., Passi, A., García, M., Sevic, I., and Alaniz, L. (2019) Hyaluronan preconditioning of monocytes/macrophages affects their angiogenic behavior and regulation of TSG-6 expression in a tumor type-specific manner, *FEBS J.*, **286**, 3433-3449, https://doi.org/10.1111/febs.14871.
99. Höbarth, K., Maier, U., and Marberger, M. (1992) Topical chemoprophylaxis of superficial bladder cancer with mitomycin C and adjuvant hyaluronidase, *Eur. Urol.*, **21**, 206-210, https://doi.org/10.1159/000474839.
100. Ruponen, M., Honkakoski, P., Tammi, M., and Urtti, A. (2004) Cell-surface glycosaminoglycans inhibit cation-mediated gene transfer, *J. Gene Med.*, **6**, 405-414, https://doi.org/10.1002/jgm.522.
101. Miyamoto, H., Murakami, T., Tsuchida, K., Sugino, H., Miyake, H., and Tashiro, S. (2004) Tumor-stroma interaction of human pancreatic cancer: acquired resistance to anticancer drugs and proliferation regulation is dependent on extracellular matrix proteins, *Pancreas*, **28**, 38-44, https://doi.org/10.1097/00006676-200401000-00006.
102. Fang, H., and Declerck, Y. A. (2013) Targeting the tumor microenvironment: from understanding pathways to effective clinical trials, *Cancer Res.*, **73**, 4965-4977, https://doi.org/10.1158/0008-5472.CAN-13-0661.
103. Heldin, C. H., Rubin, K., Pietras, K., and Ostman, A. (2004) High interstitial fluid pressure – an obstacle in cancer therapy, *Nat. Rev. Cancer*, **4**, 806-813, https://doi.org/10.1038/nrc1456.
104. Padera, T. P., Stoll, B. R., Tooredman, J. B., Capen, D., di Tomaso, E., and Jain, R. K. (2004) Pathology: cancer cells compress intratumour vessels, *Nature*, **427**, 695, https://doi.org/10.1038/427695a.
105. Toole, B. P. (2004) Hyaluronan: from extracellular glue to pericellular cue, *Nat. Rev. Cancer*, **4**, 528-539, https://doi.org/10.1038/nrc1391.
106. Friman, T., Gustafsson, R., Stuhr, LB., Chidiac, J., Heldin, N. E., Reed, R. K., Oldberg, A., and Rubin, K. (2012) Increased fibrosis and interstitial fluid pressure in two different types of syngeneic murine carcinoma grown in integrin β3-subunit deficient mice, *PLoS One*, **7**, e34082, https://doi.org/10.1371/journal.pone.0034082.
107. Singha, N. C., Nekoroski, T., Zhao, C., Symons, R., Jiang, P., Frost, G. I., Huang, Z., and Shepard, H. M. (2015) Tumor-associated hyaluronan limits efficacy of monoclonal antibody therapy, *Mol. Cancer Ther.*, **14**, 523-532, https://doi.org/10.1158/1535-7163.MCT-14-0580.
108. Twarock, S., Reichert, C., Bach, K., Reiners, O., Kretschmer, I., Gorski, D. J., Gorges, K., Grandoch, M., and Fischer, J. W. (2019) Inhibition of the hyaluronan matrix enhances metabolic anticancer therapy by dichloroacetate *in vitro* and *in vivo*, *Br. J. Pharmacol.*, **176**, 4474-4490, https://doi.org/10.1111/bph.14808.

109. Abildgaard, C., Rizza, S., Christiansen, H., Schmidt, S., Dahl, C., Abdul-Al, A., Christensen, A., Filomeni, G., and Guldberg, P. (2021) Screening of metabolic modulators identifies new strategies to target metabolic reprogramming in melanoma, *Sci. Rep.*, **11**, 4390, https://doi.org/10.1038/s41598-021-83796-8.
110. Anand, V., Khandelwal, M., Appunni, S., Gupta, N., Seth, A., Singh, P., Mathur, S., and Sharma, A. (2019) CD44 splice variant (CD44v3) promotes progression of urothelial carcinoma of bladder through Akt/ERK/STAT3 pathways: novel therapeutic approach, *J. Cancer Res. Clin. Oncol.*, **145**, 2649-2661, https://doi.org/10.1007/s00432-019-03024-9.
111. Rodríguez, M. M., Fiore, E., Bayo, J., Atorrasagasti, C., García, M., Onorato, A., Domínguez, L., Malvicini, M., and Mazzolini, G. (2018) 4Mu decreases CD47 expression on hepatic cancer stem cells and primes a potent antitumor T cell response induced by interleukin-12, *Mol. Ther.*, **26**, 2738-2750, https://doi.org/10.1016/j.ymthe.2018.09.012.
112. Hountondji, L., Ferreira De Matos, C., Lebossé, F., Quantin, X., Lesage, C., Palassin, P., Rivet, V., Faure, S., Pageaux, G. P., Assenat, É., Alric, L., Zahhaf, A., Larrey, D., Witkowski Durand Viel, P., Riviere, B., Janick, S., Dalle, S., Maria, A. T. J., Comont, T., and Meunier, L. (2023) Clinical pattern of checkpoint inhibitor-induced liver injury in a multicentre cohort, *JHEP Rep.*, **5**, 100719, https://doi.org/10.1016/j.jhepr.2023.100719.
113. Tawbi, H. A., Schadendorf, D., Lipson, E. J., Ascierto, P. A., Matamala, L., Castillo Gutiérrez, E., Rutkowski, P., Gogas, H. J., Lao, C. D., De Menezes, J. J., Dalle, S., Arance, A., Grob, J.-J., Srivastava, S., Abaskharoun, M., Hamilton, M., Keidel, S., Simonsen, K. L., Sobiesk, A. M., Li, B., Hodi, F. S., and Long, G. V. (2022) Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma, *N. Engl. J. Med.*, **386**, 24-34, https://doi.org/10.1056/NEJMoa2109970.
114. Malnick, S. D. H., Abdullah, A., and Neuman, M. G. (2021) Checkpoint Inhibitors and Hepatotoxicity, *Biomedicines*, **9**, 101, https://doi.org/10.3390/biomedicines9020101.
115. De Martin, E., Michot, JM., Rosmorduc, O., Guettier, C., and Samuel, D. (2020) Liver toxicity as a limiting factor to the increasing use of immune checkpoint inhibitors, *JHEP Rep.*, **2**, 100170, https://doi.org/10.1016/j.jhepr.2020.100170.
116. Hernandez, N., and Bessone, F. (2022) Hepatotoxicity induced by biological agents: clinical features and current controversies, *J. Clin. Transl. Hepatol.*, **10**, 486-495, https://doi.org/10.14218/JCTH.2021.00243.
117. Shah, P., Sundaram, V., and Björnsson, E. (2020) Biologic and checkpoint inhibitor-induced liver injury: a systematic literature review, *Hepatol. Commun.*, **4**, 172-184, https://doi.org/10.1002/hep4.1465.
118. Peeraphatdit, T. B., Wang, J., Odenwald, M. A., Hu, S., Hart, J., and Charlton, M. R. (2020) Hepatotoxicity from immune checkpoint inhibitors: a systematic review and management recommendation, *Hepatology*, **72**, 315-329, https://doi.org/10.1002/hep.31227.
119. Delire, B., De Martin, E., Meunier, L., Larrey, D., and Horsmans, Y. (2022) Immunotherapy and gene therapy: new challenges in the diagnosis and management of drug-induced liver injury, *Front. Pharmacol.*, **12**, 786174, https://doi.org/10.3389/fphar.2021.786174.
120. Haanen, J., Obeid, M., Spain, L., Carbonnel, F., Wang, Y., Robert, C., Lyon, A. R., Wick, W., Kostine, M., Peters, S., Jordan, K., and Larkin, J. (2022) Management of toxicities from immunotherapy: ESMO clinical practice guideline for diagnosis, treatment and follow-up, *Ann. Oncol.*, **33**, 1217-1238, https://doi.org/10.1016/j.annonc.2022.10.001.
121. Puzanov, I., Diab, A., Abdallah, K., Bingham, C. O., 3rd, Brogdon, C., Dadu, R., Hamad, L., Kim, S., Lacouture, M. E., LeBoeuf, N. R., Lenihan, D., Onofrei, C., Shannon, V., Sharma, R., Silk, A. W., Skondra, D., Suarez-Almazor, M. E., Wang, Y., Wiley, K., Kaufman, H. L., and Ernstoff, M. S. (2017) Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group, *J. Immunother. Cancer*, **5**, 95, https://doi.org/10.1186/s40425-017-0300-z.
122. Brahmer, J. R., Lacchetti, C., Schneider, B. J., Atkins, M. B., Brassil, K. J., Caterino, J. M., Chau, I., Ernstoff, M. S., Gardner, J. M., Ginex, P., Hallmeyer, S., Holter Chakrabarty, J., Leighl, N. B., Mammen, J. S., McDermott, D. F., Naing, A., Nastoupil, L. J., Phillips, T., Porter, L. D., Puzanov, I., Reichner, C. A., Santomasso, B. D., Seigel, C., Spira, A., Suarez-Almazor, M. E., Wang, Y., Weber, J. S., Wolchok, J. D., and Thompson, J. A. (2018) Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline, *J. Clin. Oncol.*, **36**, 1714-1768, https://doi.org/10.1200/JCO.2017.77.6385.
123. Dougan, M., Wang, Y., Rubio-Tapia, A., and Lim, J. K. (2021) AGA Clinical practice update on diagnosis and management of immune checkpoint inhibitor colitis and hepatitis: expert review, *Gastroenterology*, **160**, 1384-1393, https://doi.org/10.1053/j.gastro.2020.08.063.
124. Doherty, G. J., Duckworth, A. M., Davies, S. E., Mells, G. F., Brais, R., Harden, S. V., Parkinson, C. A., and Corrie, P. G. (2017) Severe steroid-resistant anti-PD1 T-cell checkpoint inhibitor-induced hepatotoxicity driven by biliary injury, *ESMO Open*, **2**, e000268, https://doi.org/10.1136/esmoopen-2017-000268.
125. Onishi, S., Tajika, M., Bando, H., Matsubara, Y., Hosoda, W., Muro, K., and Niwa, Y. (2020) Ursodeoxycholic acid and bezafibrate were useful for steroid-

refractory, immune-related hepatitis: a case report, *J. Med. Case Rep.*, **14**, 230, https://doi.org/10.1186/s13256-020-02541-3.

126. Sato, K., Hayashi, M., Abe, K., Fujita, M., Takahashi, A., and Ohira, H. (2020) Pembrolizumab-induced sclerosing cholangitis in a lung adenocarcinoma patient with a remarkable response to chemotherapy: a case report, *Clin. J. Gastroenterol.*, **13**, 1310-1314, https://doi.org/10.1007/s12328-020-01178-5.
127. Robles-Díaz, M., Nezic, L., Vujic-Aleksic, V., and Björnsson, E. S. (2021) Role of ursodeoxycholic acid in treating and preventing idiosyncratic drug-induced liver injury. A systematic review, *Front. Pharmacol.*, **12**, 744488, https://doi.org/10.3389/fphar.2021.744488.
128. Cullen, S. N., Rust, C., Fleming, K., Edwards, C., Beuers, U., and Chapman, R. W. (2008) High dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis is safe and effective, *J. Hepatol.*, **48**, 792-800, https://doi.org/10.1016/j.jhep.2007.12.023.
129. Ma, H., Zeng, M., Han, Y., Yan, H., Tang, H., Sheng, J., Hu, H., Cheng, L., Xie, Q., Zhu, Y., Chen, G., Gao, Z., Xie, W., Wang, J., Wu, S., Wang, G., Miao, X., Fu, X., Duan, L., Xu, J., Wei, L., Shi, G., Chen, C., Chen, M., Ning, Q., Yao, C., and Jia, J. (2016) A multicenter, randomized, double-blind trial comparing the efficacy and safety of TUDCA and UDCA in Chinese patients with primary biliary cholangitis, *Medicine (Baltimore)*, **95**, e5391, https://doi.org/10.1097/MD.0000000000005391.
130. Xiang, X., Yang, X., Shen, M., Huang, C., Liu, Y., Fan, X., and Yang, L. (2021) Ursodeoxycholic acid at 18-22 mg/kg/d showed a promising capacity for treating refractory primary biliary cholangitis, *Can. J. Gastroenterol. Hepatol.*, **2021**, 6691425, https://doi.org/10.1155/2021/6691425.
131. Steen, E. H., Short, W. D., Li, H., Parikh, U. M., Blum, A., Templeman, N., Nagy, N., Bollyky, P. L., Keswani, S. G., and Balaji, S. (2021) Skin-specific knockdown of hyaluronan in mice by an optimized topical 4-methylumbelliferone formulation, *Drug Deliv.*, **28**, 422-432, https://doi.org/10.1080/10717544.2021.1886376.
132. Rilla, K., Pasonen-Seppänen, S., Rieppo, J., Tammi, M., and Tammi, R. (2004) The hyaluronan synthesis inhibitor 4-methylumbelliferone prevents keratinocyte activation and epidermal hyperproliferation induced by epidermal growth factor, *J. Invest. Dermatol.*, **123**, 708-714, https://doi.org/10.1111/j.0022-202X.2004.23409.x.
133. Supp, D. M., Hahn, J. M., McFarland, K. L., and Glaser, K. (2014) Inhibition of hyaluronan synthase 2 reduces the abnormal migration rate of keloid keratinocytes, *J. Burn Care Res.*, **35**, 84-92, https://doi.org/10.1097/BCR.0b013e3182a2a9dd.
134. Kim, T., Kim, K. B., and Hyun, C. G. (2023) A 7-hydroxy 4-methylcoumarin enhances melanogenesis in B16-F10 melanoma cells, *Molecules*, **28**, 3039, https://doi.org/10.3390/molecules28073039.
135. Vassallo, J. D., Hicks, S. M., Born, S. L., and Daston, G. P. (2004) Roles for epoxidation and detoxification of coumarin in determining species differences in Clara cell toxicity, *Toxicol. Sci.*, **82**, 26-33, https://doi.org/10.1093/toxsci/kfh237.
136. Egan, D., O'Kennedy, R., Moran, E., Cox, D., Prosser, E., and Thornes, R. D. (1990) The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds, *Drug Metab. Rev.*, **22**, 503-529, https://doi.org/10.3109/03602539008991449.
137. Nagy, N., Gurevich, I., Kuipers, H. F., Ruppert, S. M., Marshall, P. L., Xie, B. J., Sun, W., Malkovskiy, A. V., Rajadas, J., Grandoch, M., Fischer, J. W., Frymoyer, A. R., Kaber, G., and Bollyky, P. L. (2019) 4-Methylumbelliferyl glucuronide contributes to hyaluronan synthesis inhibition, *J. Biol Chem.*, **294**, 7864-7877, https://doi.org/10.1074/jbc.RA118.006166.
138. Okhlobystin, A. V., and Ufimtseva, A. K. (2020) The use of hymecromone in diseases of the biliary tract: opportunities and prospects [in Russian], *Vopr. Detsk. Dietol.*, **18**, 66-74, https://doi.org/10.20953/1727-5784-2020-5-66-74.
139. Garrett, E. R., and Venitz, J. (1994) Comparisons of detections, stabilities, and kinetics of degradation of hymecromone and its glucuronide and sulfate metabolites, *J. Pharm. Sci.*, **83**, 115-116, https://doi.org/10.1002/jps.2600830128.
140. Takeda, S., and Aburada, M. (1981) The choleretic mechanism of coumarin compounds and phenolic compounds, *J. Pharmacobiodyn.*, **4**, 724-734, https://doi.org/10.1248/bpb1978.4.724.
141. Garrett, E. R., Venitz, J., Eberst, K., and Cerda, J. J. (1993) Pharmacokinetics and bioavailabilities of hymecromone in human volunteers, *Biopharm. Drug Dispos.*, **14**, 13-39, https://doi.org/10.1002/bdd.2510140103.
142. Marshall, M. E., Mohler, J. L., Edmonds, K., Williams, B., Butler, K., Ryles, M., Weiss, L., Urban, D., Bueschen, A., and Markiewicz, M. (1994) An updated review of the clinical development of coumarin (1,2-benzopyrone) and 7-hydroxycoumarin, *J. Cancer Res. Clin. Oncol.*, **120**, S39-S42, https://doi.org/10.1007/BF01377124.
143. Lake, B. G. (1999) Coumarin metabolism., toxicity and carcinogenicity: relevance for human risk assessment, *Food Chem. Toxicol.*, **37**, 423-453, https://doi.org/10.1016/s0278-6915(99)00010-1.
144. Rosser, J. I., Nagy, N., Goel, R., Kaber, G., Demirdjian, S., Saxena, J., Bollyky, J. B., Frymoyer, A. R., Pacheco-Navarro, A. E., Burgener, E. B., Rajadas, J., Wang, Z., Arbach, O., Dunn, C. E., Kalinowski, A., Milla, C. E., and Bollyky, P. L. (2022) Oral hymecromone decreases hyaluronan in human study participants, *J. Clin. Invest.*, **132**, e157983, https://doi.org/10.1172/JCI157983.
145. Selezneva, E. Ya., Mechetina, T. A., Orlova, Yu. N., Koricheva, E. S., Voynovan, I. N., Bezaeva, I. V., Dubtsova, E. A., and Bordin, D. S. (2016) Comparative study of the UDCA monotherapy effectiveness

and UDCA-hymecromone combination in patients with stage 2 biliary sludge [in Russian], *Eksp. Klin. Gastroenterol.*, **10**, 94-98.
146. Hoffmann, R. M., Schwarz, G., Pohl, C., Ziegenhagen, D. J., and Kruis, W. (2005) Bile acid-independent effect of hymecromone on bile secretion and common bile duct motility, *Dtsch. Med. Wochenschr.*, **130**, 1938-1943, https://doi.org/10.1055/s-2005-872606.
147. Abate, A., Dimartino, V., Spina, P., Costa, P. L., Lombardo, C., Santini, A., Del Piano, M., and Alimonti, P. (2001) Hymecromone in the treatment of motor disorders of the bile ducts: a multicenter., double-blind, placebo-controlled clinical study, *Drugs Exp. Clin. Res.*, **27**, 223-231.
148. Camarri, E., and Marchettini, G. (1988) Hymecromone in the treatment of symptoms following surgery of the bile ducts, *Recenti Prog. Med.*, **79**, 198-202.
149. Ishizuka, S., Askew, E. B., Ishizuka, N., Knudson, C. B., and Knudson, W. (2016) 4-methylumbelliferone diminishes catabolically activated articular chondrocytes and cartilage explants via a mechanism independent of hyaluronan inhibition, *J. Biol. Chem.*, **291**, 12087-12104, https://doi.org/10.1074/jbc.M115.709683.