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Review Article

The Role of Ceramide and Sphingolipid Metabolism in Cancer Therapeutics

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Abstract

Sphingolipids are a class of bioactive lipids which are highly involved in cellular functions such as signaling, membrane composition, and determining cell fate. The metabolism of these lipids plays important roles in the development and progression of many diseases such as cancer. The role of sphingolipid metabolism in cancer overall is not yet fully understood. However, key sphingolipids such as sphingosine-1-phosphate (S1P) and ceramide have been shown to be influential on the death or survival of cancer cells. S1P is known to exert pro-survival signaling effects when expressed at higher levels in cells. Ceramide, on the other hand, has been established as a pro-death lipid, regulating apoptosis, cell cycle arrest, autophagy, and mitophagy. Cancer cells are typically characterized by an increased ratio in S1P to ceramide, thus granting the survival of the cancer. This ceramide/S1P biostat is the target of many new therapeutics which aim to increase ceramide levels in cancer cells. Additionally, many previously established drugs have been rediscovered for their unexpected ability to perturb sphingolipid metabolism. This review serves to summarize the current use of ceramide and sphingolipid metabolism-related therapies for the treatment of many cancers.

Sphingolipid Metabolism

Many cellular functions such as signaling, membrane composition, and cell fate determination are highly influenced by classes of bioactive lipids known as sphingolipids. Sphingolipids are characterized by alterations to a common sphingoid backbone. Uniquely, the sphingoid backbone includes an amine at the 2-carbon position. The metabolism of these lipids has been determined to play important roles in the development and progression of many diseases such as cancer. An N-acetylated sphingosine molecule known as ceramide forms a hypothetical center of sphingolipid metabolism. As these lipids are metabolized, their structures, functions, and cellular localizations are altered. Synthesis of sphingolipids begins at the endoplasmic reticulum and progresses to the Golgi apparatus and plasma membrane. The catabolism of these lipids occurs predominately at lysosomes within the cell, as well as at the plasma membrane. Many lipid transport proteins (LTPs) such as ceramide-transfer protein (CERT), FAPP2, and ceramide-1-phosphate transfer protein (CPTP) shuttle sphingolipids throughout the cell and regulate their localization and metabolism [1].

The de novo synthesis of ceramide originates at the endoplasmic reticulum as the enzyme serine palmitoyltransferase (SPT) catalyzes the condensation of serine and palmitoyl-CoA to yield 3-ketodihydrosphingosine, also known as 3-ketosphinganine [2]. The enzyme 3- ketodihydrosphingosine reductase (KDSR) then catalyzes reduction to dihydrosphingosine, also known as sphinganine. Subsequent acylation of dihydrosphingosine at the amide position by one of the ceramide synthases (CERS1-5)

yields dihydroceramide. The final product, ceramide, is formed by dihydroceramide desaturase (DEGS1-2), which inserts a 4,5 trans double bond [3]. Various ceramide species, with varying chain lengths or degrees of unsaturation, can be generated by differential addition of acyl chains during synthesis. Specifically, variation in the acylation of dihydrosphingosine by different members of the CERS family can ultimately yield a wide variety of ceramides with varying fatty acid chain length [4].

After its synthesis, ceramide serves at a hypothetical center of sphingolipid metabolism. Over 40 different enzymes have been associated with sphingolipid metabolism in mammals [4]. Ceramide can be modified in many ways to form other complex sphingolipids in this metabolic web. Sphingomyelin synthase (SMS1-2) enzymes can transfer a phosphocholine head group onto ceramide using phosphatidylcholine as a donor to produce sphingomyelin. The enzyme ceramide kinase (CERK) is another enzyme which further processes ceramide via phosphorylation to produce ceramide-1-phosphate (C1P) [4]. In addition, ceramide can be glycosylated to generate glucosylceramide via the enzyme glucosylceramide synthase (GCS). Glucosylceramide then has the potential to be further converted into other glycosphingolipids, including gangliosides [5]. Alternatively, galactosylceramide can be generated and can likewise serve as a building block for more complex glycosphingolipids. Collectively, glucosylceramide and galactosylceramide are known as the cerebrosides. Recently, 1-O-acylation of ceramide by diacylglycerol O- acyltransferase 2 (DGAT2) has also been reported [6]. Enzymes of the ceramidase (CDase) family catalyze the production of sphingosine from ceramide, which can be further processed by sphingosine kinase (SPHK1-2) enzymes to produce sphingosine- 1-phosphate (S1P). Alternatively, the SPHKs can also phosphorylate dihydrosphingosine to generate dihydrosphingosine-1-phosphate (dhS1P). It is important to note that modifications to ceramide and subsequent lipid products are often reversible by opposing enzymes. This dynamic interplay of enzymes demonstrates the fluidity of sphingolipid metabolism and highlights the ways in which this balanced process can be manipulated in diseases related to sphingolipid imbalance.

Immoderate phosphorylation of sphingolipids by lipid kinases, such as the SPHKs, has been identified as an especially important cause of sphingolipid metabolic dysregulation. This is largely because sphingolipids such as S1P are noted for their involvement in cellular survival, proliferation, and migration [4]. The upregulation of SPHKs in cancer has been well established. In this way, the expression of lipid phosphatases, which remove the phosphate from lipids such as S1P, can also be downregulated in cancer.

Ceramide/S1P Biostat and Cancer

The relationship between the phosphosphingolipid, S1P, and the central sphingolipid, ceramide, has been studied extensively. This relationship has been coined the ceramide/S1P biostat. It is known that S1P plays a role in oncogenic activities such as pro-survival pathways, autophagy, metastasis, cytoskeletal rearrangements, angiogenesis, and inflammation [4]. S1P can mediate these effects through a process known as inside-out signaling. After being synthesized inside the cell, S1P can be transported across the plasma membrane and into the microenvironment. From outside the cell, S1P can signal through a group of five GPCRs known as S1P receptors (S1PR1-5) [7]. Different cell types, such as endothelial cells or inflammatory/immune cells, can express different combinations of S1PRs on their surfaces. The collection of S1PRs on the cell surface, as well as the different G proteins they may be coupled with, influence the ultimate effect that is transduced due to S1P binding. For example, S1PR4 has been shown to be expressed on hematopoietic cells, while S1PR5 expression has been observed across a diversity of cells including natural killer cells, dendritic cells, as well as endothelial cells [8, 9].

Ceramide, on the other hand, has been established as a pro-death lipid, regulating apoptosis, cell stress, cell cycle arrest, as well as autophagy/mitophagy. Ceramide can exert its pro-apoptotic effects extrinsically by influencing different signaling pathways that originate at the plasma membrane, such as those mediated by CD95, TNF?, TNF?-related apoptosis-inducing ligand (TRAIL). Alternatively, ceramide has been shown to exert its cellular effects intrinsically by influencing signals that culminate at the mitochondria to initiate the apoptotic cascade. Ceramide-mediated mitochondrial outer membrane permeabilization (MOMP) is an important step involved in allowing the leakage of cytochrome c from the mitochondria to induce apoptosis.

Chemotherapeutics	
doxorubicin (adriamycin)	etoposide
daunorubicin	camptothecin
fluorouracil (5-FU)	irinotecan
cytarabine (Ara-C)	fenretinide
fludarabine	tamoxifen
gemcitabine (Gemzar)	vorinostat
vincristine	sorafenib
vinblastine	paclitaxel (taxol)
cisplatin	oxaliplatin
mitoxantrone (novantrone)	
Other Therapies	**Immunosuppressants**
radiation therapy	cyclosporine A
photodynamic therapy	SDZ PSC-833
Natural products	
resveratrol	Medicinal plant extracts (Devil's Club, Northern Labrador/Tundra Tea, Bog Blueberries)

Table 1: Ceramide-Generating Cancer Therapeutics.

Ceramide can also induce apoptosis by means of activation of protein kinase C d (PKCd) to release cytochrome c for activation of caspase 9. It can also inhibit the effects of AKT signaling on cell survival by recruiting PP2A, PKC?, and p38 [10, 11]. The improper balance of the ceramide/S1P biostat is becoming a distinguished marker of cancer. Cancer cells are typically characterized by an increased ratio in S1P to ceramide. This biostat is the target of many new cancer therapeutics which aim to increase ceramide levels in cancer cells. As this concept is being explored, many previously established drugs have been rediscovered for their unexpected ability to affect sphingolipid metabolism, aside from their other more well-understood mechanisms of action. Additionally, research has found that the collection of ceramides with different chain lengths is important, as various ceramide species are upregulated during different stages of cancer progression. Solar et al. identified accumulation of C18, C22, C24, and C26 ceramides in apoptotic colon cancer cells versus non- apoptotic colon cancer cells [12]. It was also shown by the Ogretman group that C18 ceramides were reduced, while C16, C24, and C24:1 ceramides were increased in head and neck cancers [13]. Many new therapeutics that are being developed today target aspects of ceramide metabolism, or more specifically the ceramide/S1P biostat. These therapeutic strategies include monoclonal antibodies directed against S1P, ceramide analogs, nano-therapies for the delivery of exogenous ceramide, S1PR regulators, and activators/inhibitors of the vital enzymes which control the metabolism of these lipids.

Ceramide-Generating Anticancer Theraputics

Therapeutic agents have been discovered with ceramide metabolism in mind. These therapeutics act in one way or another to either increase endogenous cellular ceramide levels or to deliver exogenous ceramide to cancer cells (Table 1). Either of these strategies ultimately relies on the pro-death properties of ceramide to exert anticancer efficacy (Figure 1). Generation of ceramide has been observed following treatment with anthracyclines, including doxorubicin (Adriamycin), daunorubicin, and mitoxantrone (novantrone), as well as with nucleoside analogs fluorouracil (5-fU), cytarabine (Ara-C), fludarabine, and gemcitabine (Gemzar) [14-20]. In one specific example, Bettaieb et al. showed that mitoxantrone (novantrone) caused ceramide generation by sphingomyelin hydrolysis while inducing cell death of monocytic leukemia cells [16]. This study further helped to distinguish the ceramide-mediated pro-death effect from pro-survival pathways mediated by other lipids such as diacylglycerol. Similarly, ceramide generation has been observed following treatment with DNA cross- linkers, including with cisplatin and oxaliplatin, anti-microtubule Vinca alkaloids vincristine and vinblastine in addition to the anti-microtubule taxane paclitaxel (Taxol), the estrogen receptor antagonist tamoxifen, as well as with the topoisomerase inhibitors etoposide, camptothecin, and irinotecan [14,15, 21-25]. Not surprisingly, ceramide generation occurs following treatment with small molecule CDase inhibitors that block this specific metabolic route of ceramide elimination, including with DM102 and ceranib- 2 [26-29]. In addition to other noteworthy mechanisms, ceramide generation has been reported for the sulfonated naphthylamine derivative suramin as well as for the flavonoids hesperidin and naringenin [14, 30-32]. Further, the synthetic retinoid fenretinide (4-HPR), the histone deacetylase inhibitor vorinostat, the Raf protein tyrosine kinase inhibitor sorafenib], and immune-suppressants cyclosporine A and SDZ PSC- 833 (valspodar), have all also been shown to induce ceramide generation [14, 33-37]. Aside from small molecules, both photodynamic and radiation therapy have also been shown to trigger anticancer ceramide generation [14].

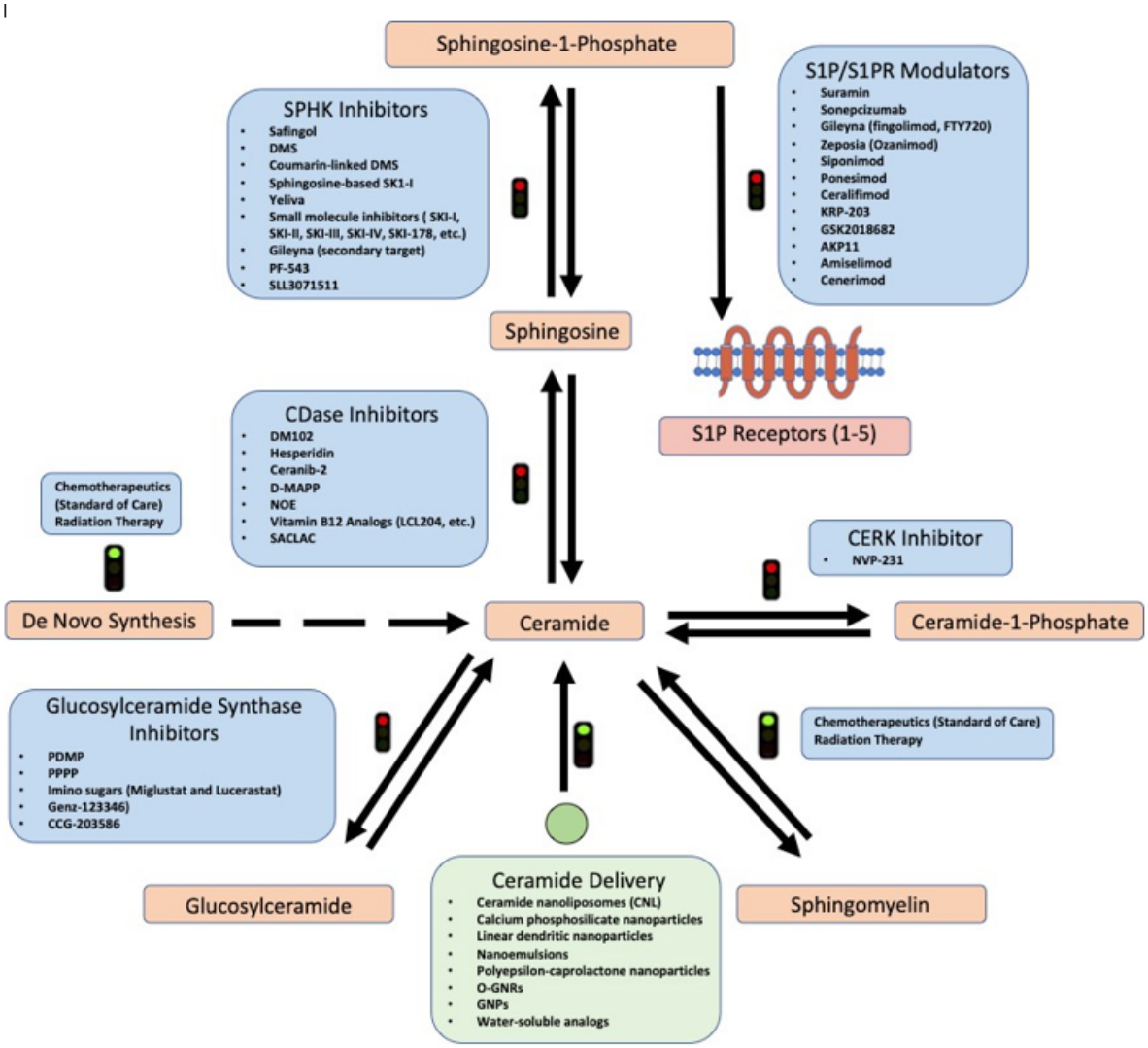

Figure 1: Therapeutics targeting ceramide-centric sphingolipid metabolism. Ceramide lies at a crossroads of sphingolipid metabolism. It can be generated through de novo synthesis as well as through the catabolism of various other sphingolipids. Anticancer therapeutics generally promote ceramide generation (green stoplight), prevent ceramide metabolism (red stoplight), or deliver exogenous forms of ceramide (green stoplight). In addition, ceramide can be metabolized to sphingosine and then onward to sphingosine-1-phosphate (S1P). Various therapeutics also antagonize S1P receptor (S1PR) signaling either by binding S1P or by binding various S1PRs (red stoplight). S1PR-directed therapeutics have been approved to target various rheumatoid conditions, but links between inflammation and cancer, as well as preclinical studies, highlight their potential anticancer utility. For each point of ceramide/S1P-targeting, various experimental and approved therapeutics have been explored (light blue boxes). Ceramide-delivering strategies have been evaluated in preclinical studies, with the ceramide nanoliposome also recently having been studied in a clinical trial (light green box).

The synthetic retinoid fenretinide (4-HPR) has been employed in numerous cancer clinical trials in recent decades. In these trials, administration of fenretinide alone demonstrated low bioavailability [38]. Recent developments have incorporated fenretinide with other agents to stimulate a synergistic increase in efficacy against malignancies. One clinical trial combined fenretinide with ketoconazole to interfere with the breakdown of fenretinide by the body before it reached the tumor microenvironment [ClinicalTrials.gov Identifier: NCT01535157]. According to the Clinical Trials Registry this trial was terminated due to the lack of efficacy using the combination of fenretinide with ketoconazole. However, it did note that the data gained from that trial may be used for a subsequent trial. Interestingly, fenretinide has also been investigated in combination with the acid CDase (A-CDase) inhibitor DM102 [26]. A-CDase breaks down ceramide to sphingosine and is a rate-limiting step in the biosynthesis of pro- survival and pro-mitogenic S1P. In this study, the combination of DM102 and fenretinide enhanced ceramide generation, produced ROS, blocked metastasis, and induced apoptosis in prostate cancer [26]. In contrast, either compound alone was not able to achieve the same outcomes. Additionally, there has been a phase I trial of fenretinide in combination with intravenous administration of the SPHK1 inhibitor safingol [ClinicalTrials.gov Identifier: NCT01553071] [39]. This study ended earlier than originally planned due to drug supply issues related to the COVID-19 pandemic. However, enough data was generated for some meaningful results. Nearly 90% of the enrolled patients experienced hypertriglyceridemia as an adverse event with over two thirds of those experiencing it as grade 3 or greater. Notably, hypertriglyceridemia as an adverse event in this trial was attributed to the intralipid infusion vehicle for the fenretinide. Unfortunately, one patient experienced dose-limiting toxicities at higher safingol dosage including grade 3 troponinemia and grade 4 myocarditis. Stable disease in two patients (~18%) was the only evidence of minimal activity for this combinatorial therapy although this was a small phase I trial. It should be noted that while ceramide generation has been attributed to fenretinide, it actually stimulates increases in dihydroceramide and other dihydro-sphingolipid species due to blockade of dihydroceramide desaturase. In the context of this clinical trial with safingol, a SPHK1 inhibitor, this may be particularly important as this inhibitory effect would mostly limit the generation of dhS1P than S1P given the fenrettide-induced increase in dihydro-sphingolipid species including dihydrosphingosine. While S1P is a pro-survival and pro-oncogenic sphingolipid, the same may not be the case for dhS1P. In preclinical in vivo studies, dhS1P and a nano-therapeutic approach that stimulates dhS1P were shown to exert antitumor efficacy in part by promoting an antitumor immune response [40]. Therefore, it would be interesting to see safingol be evaluated in a clinical trial in combination with a therapeutic that delivers ceramide so that the safingol effect would be directed more appropriately towards S1P generation.

Flavonoids such as hesperidin and naringenin, are found prominently in citrus fruits. Hesperidin has been shown to elevate intracellular ceramide levels by decreasing A-CDase enzymatic activity in melanoma and renal cell carcinoma cells [31]. Interestingly, hesperidin has also been identified as a HER2 tyrosine kinase inhibitor in breast cancer [32]. The compound called ceranib-2 is another A-CDase inhibitor which has demonstrated efficacy in inducing both early and late apoptosis in prostate, breast, and non-small-cell lung cancers [26-29]. This compound was found to be more effective than two older A-CDase inhibitors, D-MAPP and NOE (N-oleoylethanolamine) [28]. Like other A-CDase inhibitors, this compound is being examined for synergistic enhancing qualities with other chemotherapeutics such as paclitaxel and carboplatin [28].

Suramin is an older medication that is used in treating sleeping sickness due to trypanosome infection. Interestingly, it also is an inhibitor of S1PR3 and S1PR5 [14, 30]. Therefore, the sphingolipid-directed aspect of this compound mainly reduces pro- survival signaling associated with S1P, although it has also been shown to induce ceramide generation. Suramin has completed several clinical trials for the treatment of different cancers, including a phase I trial for the treatment of superficial transitional cell carcinoma of the bladder [ClinicalTrials.gov Identifier: NCT00001381], phase I/II trials of combinatorial treatment with paclitaxel for breast cancer [ClinicalTrials.gov Identifier: NCT00054028] and suramin with carboplatin and paclitaxel for treating non-small cell lung cancer [ClinicalTrials.gov Identifier: NCT00006929], and a phase III trial determining the effects of three different doses of suramin for the treatment of prostate cancer [ClinicalTrials.gov Identifier: NCT00002723] [41-44]. Clinical trials using suramin for cancer have generally found it to be well tolerated as a standalone agent as evidenced by rare and low-grade adverse events. Antitumor efficacy for suramin alone may be minimal. However, it may have palliative efficacy at low dose as it decreased depression and improved the quality of life in patients with metastatic prostate cancer [44]. This effect of decreasing depression is of note given the presence and roles of S1PRs in the brain. In contrast, trials evaluating suramin in combination with other therapeutics have had mixed results. Rates of adverse events are generally higher with some studies reporting somewhat frequent grade 3 and 4 events including anemia and neutropenia, as well as some patients discontinuing treatment due to these toxicities [42, 43]. Generally, combination therapy did not yield efficacy in these trials with patients had already developed chemoresistance. However, combinatorial therapy with suramin seems to have some efficacy in chemotherapy-naive patients [43]. Therefore, suramin appears to have some promise as an anticancer

agent if in the right therapeutic combination. Additional studies continue to evaluate suramin, but the intent continues to be of an old drug repurposing nature and not because of suramin's ability to inhibit S1PR3 and S1PR5 signaling. However, there are yet to be any studies specifically uniting suramin with ceramide-based therapeutics. Perhaps better responses to S1PR modulation would be seen in the context of other ceramide/sphingolipid-directed therapies.

The world of natural products-based drug discovery has also leveraged ceramide metabolism for the identification of therapeutics. Natural products are often sourced from plants or fungi for investigation into their intrinsic anticancer properties. The antioxidant resveratrol is considered a chemo preventative natural product which can increase ceramide generation in cancer cells [45, 46]. Our lab has explored the ability of multiple Alaskan ethnobotanicals such as Devil's Club to decrease the viability of many cancers including acute myeloid leukemia (AML), pancreatic, and colorectal carcinoma. The Alaskan Bog Blueberry (Vaccinium uliginosum) is famous in the world of natural products-based drug discovery for its history of medicinal uses and applications. Bioactive compounds such as the pentacyclic triterpene ursolic acid are found in Alaskan Bog Blueberries and other medicinal plants such as Northern Labrador Tea (also known as Tundra Tea), Devil's Club, plantain leaves, thyme, and coffee [47-49]. The mechanism of action of ursolic acid in cancer cells is still under investigation, but it is generally known for its pro-apoptotic and protective effects. It is hypothesized that the bioactive compounds in these plants may act through various mechanisms which alter sphingolipid metabolism to maintain heightened ceramide levels in cancer cells. Such mechanisms include increasing the activity of enzymes involved in ceramide generation while decreasing the activity of those involved in its elimination or neutralization.

Sphingolipid Metabolism and Regulating Anticancer Theraputics

An established target for the regulation of sphingolipid metabolism in cancer are the SPHKs, which generate S1P. As previously noted, S1P is involved in the survival pathways that are commonly manipulated by cancers, thus deeming SPHKs as targets for the development of therapeutics. For instance, as S1P is a regulator of tumor angiogenesis, SPHK and S1PR inhibitors are being used to enhance the efficacy of anti-angiogenic therapies [13, 15, 50]. These SPHK and S1PR inhibitors include small molecules, lipid mimetics, and immunomodulators. Initial SPHK inhibitors included sphingosine analogs such as dimethylsphingosine (DMS), coumarin-linked DMS, safingol as mentioned above, and sphingosine-based SK1-I [51-54] (Table 2) (Figure 1). The immunomodulator FTY720 (Gilenya, fingolimod) has been approved by the Federal Drug Administration (FDA) for the treatment of multiple sclerosis. It has been additionally investigated for its ability to act as a SPHK1 antagonist. This compound is a structural analog of sphingosine, which has been shown to bind SPHK1 competitively. Other targets of this drug include protein phosphatase 2A (PP2A), the PI3K/AKT pathway, and inhibition of mitochondrial permeability transition pore (MPTP) formation [33]. This drug has also been found to sensitize cancer cells to other chemotherapies including 5-fluorouracil, oxaliplatin, cetuximab, and sunitinib (Sutent) [55, 56]. Despite these findings, hope for FTY720 as a standalone chemotherapeutic is low, as this compound has exhibited high levels of toxicity and immunosuppressive effects on regulatory T cells [33]. Although, a new clinical trial evaluating FTY720 (Gilenya, fingolimod) is planned for non-small cell lung cancer and small cell lung cancer patients whose disease has progressed during chemo-immunotherapy [ClinicalTrials.gov Identifier: NCT06424067]. There have recently been several new drug approvals for compounds that selectively target S1PRs for the treatment rheumatoid conditions such as ulcerative colitis, including ozanimod (Zeposia) [57, 58] (Figure 1). Given links between chronic inflammation and the development and progression of cancer, it may not be surprising if these compounds are further investigated for anticancer efficacy.

Inhibitor	Target	Inhibitor	Target
novel sphingosine analogs	sphingosine kinase	cationic analogs of B-13	acid ceramidase
dimethylsphingosine	sphingosine kinase	aminoethanol amides	acid ceramidase
coumarin-linked dimethylsphingosine	sphingosine kinase	P-series (PPMP, PDMP)	Glucosylceramide synthase
safingol	sphingosine kinase	DM102	acid ceramidase
SK1-I (and derivatives)	sphingosine kinase	LCL521	acid ceramidase
small molecule inhibitors (Apogee Biotechnology)	sphingosine kinase	flavonoids (hesperidin, naringenin)	acid ceramidase
sonepcizumab	Sphingosine-1-phosphate	ceranib-2	acid ceramidase
B-13	acid ceramidase	suramin	S1P receptors
D-MAPP	acid ceramidase	ABC294640 (Yeliva)	Sphingosine kinase
N-oleoylethanolamine (NOE)	acid ceramidase	FTY720 (Gileyna; a.k.a. fingolimod) and numerous next- generation compounds	S1P receptors (primarily) and sphingosine kinase (FTY720 off-target effect)

Table 2: Specific Sphingolipid-Regulating Therapeutics.

The humanized S1P monoclonal antibody sonepcizumab (ASONEP) began phase II clinical trials for the treatment of renal cell carcinoma (ClinicalTrials.gov Identifier: NCT01762033). The trial was terminated due to the drug's failure to meet the primary progression-free survival endpoint, although notable overall survival results (21.7 months) in patients with metastatic renal cell carcinoma may retain hope for this compound as a cancer therapeutic with continued research [59].

The SPHK inhibitor ABC294640 (Yeliva, opaganib) has demonstrated efficacy in multiple clinical trials. This compound is believed to possess notable efficacy due to its specificity for inhibiting SPHK2 [60, 61]. Completion of phase I trials in patients with advanced solid tumors saw ABC294640 meet primary and secondary endpoints. This trial demonstrated that it was well tolerated, with a notable decrease in S1P levels as well as disease stabilization in many cases [ClinicalTrials.gov Identifier: NCT01488513] [60]. There was a single patient in the lowest dosage (250 mg qd) cohort that experienced a dose-limiting grade 4 instance of hyperglycemia but that was attributed to pancreatic failure due to their rapidly progressing pancreatic cancer. Otherwise, only low-grade adverse events were observed for patients during low and mid dosages (250 mg qd, 250 mg bid, and 500 mg bid), mostly including nausea, vomiting, and fatigue, alongside rare low-grade nervous system and neuropsychiatric adverse events. This contrasted with the highest dosage (750 mg bid) evaluated where there was an instance of dose-limiting grade 3 nausea and vomiting as well as two other instances of patients unable to complete treatment due to multiple grade 2 and 3 adverse events to include chest discomfort, choking sensation, dysarthria, acute kidney injury, nausea, and agitation. Ultimately, the highest dosage of ABC294640 evaluated was determined to not be tolerable. However, at tolerable lower dosage there were some indicators of efficacy with 40% patients having stable disease and one heavily pretreated cholangiocarcinoma patient experiencing an initial partial response. Unsurprisingly, there are multiple follow-on clinical trials evaluating ABC294640 including for cholangiocarcinoma [ClinicalTrials.gov Identifiers: NCT03377179 and NCT03414489], as well as metastatic castration-resistant prostate cancer [ClinicalTrials.gov Identifier: NCT04207255], and relapsed/refractory multiple myeloma [ClinicalTrials.gov Identifier: NCT02757326]. In the later study, performed with patients that had previously been exposed to proteasome inhibition and immunomodulatory therapies, ABC294640 was well-tolerated even at the higher dosage (750 mg bid) that was limiting in the earlier solid tumor trial. The most common adverse event was low-grade neutropenia. Notably, as a phase I study, one patient had a

partial response and another stable disease indicative of minimal efficacy in this difficult to treat relapsed/refractory multiple myeloma patient population [62]. Altogether, it is not surprising that the FDA has given Yeliva (ABC294640, opaganib) orphan drug designation for the treatment of cholangiocarcinoma as well as recently for neuroblastoma.

Ceramide Analogs for Cancer Therapy

The delivery of exogenous ceramide has existed as a cancer therapeutic strategy for many years now (Figure 1). This strategy is hindered by the intrinsic insolubility of ceramide. To overcome this hurdle, ceramide analogs have been developed which offer greatly increased solubility levels in the human body (Table 3). Short chain ceramides were created for this purpose. Shortening the length of the ceramide molecule modestly increases its solubility, while maintaining relatively similar interactions inside cells [50, 63-66]. Despite the improvement, these ceramide analogs simply are not soluble enough to enable proper efficacy alone. However, with the help of novel delivery systems, including various nanotechnologies, these short chain ceramides can be utilized. Other ceramide analogues include uracil-linked ceramides, ceramines, serinamides, serinols, N-substituted sphingosines, and 4,6-diene-ceramide [67-76]. These ceramide analogs often serve as inhibitors of ceramide neutralization (elimination) enzymes. CDases, which are inherently enzymes that can neutralize ceramide, are important lipid hydrolases that metabolize ceramide into sphingosine. CDases lower cellular ceramide levels while also catalyzing a rate-limiting step in the formation of pro-survival S1P. Multiple classes of CDases exist to function at varying pH optimums. A-CDase is highly upregulated in many cancers including in AML [77]. Neutral CDase and three different alkaline CDases also exist. The ability to break down ceramide into other lipids with varying cellular effects has flagged A-CDase as an important target for the development of many cancer therapeutics.

short-chain ceramide analogs
pyridinium-ceramides (water soluble)
uracil-linked ceramide
ceramines and their derivatives
serinols
novel N-substituted sphingosine analogs
serinamide
4,6-Diene-ceramide
sphingadienes and sphingatrienes
ceramide methylaminoethylphosphonates (CMAEPh)
LCL521, LCL204, and LCL385

Table 3: Ceramide Analogs for Cancer Therapy.

A series of improved versions of the ceramide analog B-13 have been developed. These compounds, including LCL521, LCL204, and LCL385, boast improved structures based on B-13, with the enhanced ability of improved access to the lysosome where A-CDase is mainly located. LCL521 has demonstrated the ability to cause myeloid derived suppressor cell death by means of cathepsin B and D activated by lysosomes [78, 79]. Combinatorial applications of this compound with ionizing radiation or tamoxifen have shown enhanced efficacy versus either treatment independently [79]. The compound LCL204 has been shown to inhibit A-CDase activity and increase ceramide levels in AML. When murine AML cells and human AML patient samples were treated with LCL204, C16 and C24 ceramide levels were increased while the anti-apoptotic MCL-1 protein was decreased. Collectively, this led to a marked rise in apoptosis of the leukemia cells. Survival in a murine AML model treated with LCL204 also was significantly increased [77].

Novel Delivery Systems for Ceramide-Based Anticancer Theraputics

Sphingolipid-based therapeutics face the common obstacle of limited solubility. This hinders the efficacy of these commonly hydrophobic molecules, as they cannot diffuse into cells independently. Novel delivery systems have been developed to circumvent this challenge, and to provide increased selective delivery to tumor microenvironments. Nanoscale delivery systems include nanoliposomes, calcium phosphosilicate nanoparticles (CPSNPs), thermoresponsive and biodegradable linear dendritic nanoparticles, nanoemulsions, polyepsilon-caprolactone nanoparticles, C6 ceramides-oxidized graphene nanoribbons (O-GNRs), and C6 ceramide graphene nanoplatelets (GNPs) [80-96] (Table 4) (Figure 1).

nanoliposomes
calcium phosphosilicate nanoparticles (CPSNPs)
thermoresponsive & biodegradable linear dendritic nanoparticles
nanoemulsions
polyethylene oxide-modified polyepsilon-caprolactate (PEO-PCL) nanoparticles
C6 ceramides-oxidized graphene nanoribbons (O-GNRs)
C6 ceramide graphene nanoplatelets (GNPs)

Table 4: Ceramide-Delivering Nanoscale Systems.

The CPSNP technology presents an effective drug delivery system by means of a stable, non-aggregating calcium phosphate particle loaded with ceramide. These particles are 20 nm in diameter, and they can function as methods for bioimaging or as nano delivery systems for ceramide and other insoluble chemotherapies. This

resorbable system takes advantage of pH changes in the cellular endosomal-lysosomal pathway for the selective delivery and release of the particle's contents. Lower intracellular pH levels promote dissolution of the CPSNP structure, resulting in controlled release of the contained payload [89].

Suhrland et al. created a method to load short chain C6 ceramides onto oxidized graphene nanoribbons (O-GNRs) and graphene nanoplatelets (GNPs) [96]. Oxidation of the graphene nanoribbons allows for increased solubility, while retaining hydrophobic regions for the binding of ceramides. They tested both C6 ceramide-loaded graphene nanoparticles for the ability to decrease HeLa cell viability. It was found that 100 µg/mL of C6 ceramide-loaded O-GNRs reduced cell viability by approximately 93%, while C6 ceramide-loaded GNPs reduced cell viability by approximately 76% [96].

Most notably, advances in nanoliposomal formulations for the delivery of sphingolipid chemotherapeutics have been established in the last decade. Kester et. al began navigating the clinical development of the C6 ceramide nanoliposome (CNL) [81]. CNL is composed of various lipids, including a "drug-like" C6 ceramide analog along with other lipids including a polyethylene glycol (PEG) modified C8 ceramide analog. This CNL therapy is administered intravenously, and thereafter C6 ceramide is delivered to cancer cells through intrabilayer exchange. Selective delivery of the CNL to solid tumor microenvironments is due primarily to the enhanced permeation and retention effect caused by the characteristic 'leakiness' of tumor vasculature. Treatment of multiple cancer models demonstrated the ability of CNL treatment to induce apoptosis and limit vascularization by inhibiting VEGF production, ERK and AKT phosphorylation, as well as expression of CD31 and CD105 [86]. Interestingly, one mechanism of action by which CNL treatment induces apoptosis in cancer cells is by interfering with glycolysis. This results in cell death as cancer cells often rely heavily on glycolysis to allow for increased growth rates, a phenomenon known as the Warburg effect [87].

The CNL therapy has been extensively studied by Kester et al. to determine necessary characteristics of the therapy such as pharmacokinetics and pharmacodynamics for the translation of this drug from the bench to the clinic. In collaboration with the Nanotechnology Characterization Laboratory of National Cancer Institute, it was determined that the CNL therapy has a greatly improved half-life (11-15 hours) in comparison to non-liposomal C6 ceramide (<3 minutes). Non-liposomal delivery of ceramide in DMSO has an LD50 of 10 mg/kg, while the CNL demonstrated no lethality at doses up to 100 mg/kg [81]. The inclusion of PEGylated ceramide in the formulation allows for added stability and enables evasion from immune destruction. The CNL also demonstrated very limited toxicity. The minor toxicity which does present is associated with the overall lipid composition of the nanoliposome and can be prevented by pretreatment with antihistamines [81]. The demonstrated efficacy of this chemotherapeutic, paired with its minimal toxicity level, highlights CNL as a promising and exciting example of sphingolipid-based therapeutics.

Notably, CNL has recently completed a phase I clinical trial with patients impacted by various advanced-stage solid tumor malignancies [ClinicalTrials.gov Identifier: NCT02834611] [97]. The patients in this trial all had metastatic disease, had prior surgery, and had been heavily treated with chemotherapy and/or radiation therapy. For the trial, pre-medication was given prior to liposomal infusion to prevent hypersensitivity reactions typical with lipid formulations. The trial evaluated multiple dosages including 36 mg/m2, 54 mg/m2, 81 mg/m2, 122 mg/m2, 183 mg/m2, 215 mg/m2, and 323 mg/m2, given twice per week with four weeks equating to one cycle of therapy. The only adverse events related to treatment were grade 1 or 2 and occurred in 53% of the patients. These adverse events mostly included headache, constipation, nausea, fatigue, and transaminitis. They were minor and manageable, and so there was no dosage reduction or discontinuation of the study. Importantly, there were no dose-limiting toxicities, and a maximum tolerated dose was not reached. Notably, the highest dosage evaluated represented the maximum allowable infusion volume. Most notably, 38% of patients had stable disease after 8 weeks of treatment and one patient with pancreatic cancer continued to have stable disease until tumor progression at 24 weeks. Overall, CNL is quite promising as a therapeutic given these indicators of efficacy alongside it being very well-tolerated with no reported dose-limiting toxicities [97]. Importantly, CNL therapy may be most promising in combination with other therapeutics as several preclinical studies indicated [81].

The success of the CNL modality has encouraged subsequent investigations with this therapy including synergistic experiments with other standard of care cancer drugs. The CNL treatment has shown synergy with chemotherapeutics such as sorafenib, PPMP, FTY720, gemcitabine, and tamoxifen [61]. Our lab recently published our findings employing combinatorial CNL with vinblastine to treat AML and AML with myelodysplastic syndrome-related changes (AML-MRC). Vinblastine is a microtubule destabilizing agent which interferes with transport of vesicles. This study demonstrated that AML-MRC possesses increased sensitivity to CNL versus De Novo AML, and that the combinatorial therapy of CNL and vinblastine allows for the treatment of both diseases with increased efficacy [82]. Additionally, CNL was recently shown to have combinatorial anti-AML efficacy in preclinical models alongside the standard care therapeutics venetoclax and cytarabine [98]. Importantly, another clinical trial with CNL is planned and this time for patients with relapsed/refractory AML [ClinicalTrials.gov Identifier:

NCT04716452]. This upcoming trial is well-positioned to advance the clinical knowledge of CNL, given the recent preclinical studies highlighting the efficacy of CNL alone or in combination with other chemotherapeutics for AML [82, 98].

Biomarkers and Sphingolipid-Based Therapy

In cancer, sphingolipid metabolism can be dysregulated but not because of mutations to the genes encoding sphingolipid metabolic enzymes or signaling proteins. Rather, the dysfunction that does occur is secondary to molecular alterations typically used to diagnose, risk stratify patients and guide them to therapy. Indeed, many of the sphingolipid-directed therapeutics discussed in this review would likely be more effective if patient populations were identified and targeted for therapy using specific sphingolipid-signatures. One could envision screening to assess the prevalence or upregulation of targets, such as SPHK2 or S1PR1 to guide therapies such as ABC294640 (Yeliva, opaganib) or FTY720 (fingolimod, Gilenya), respectively. Another approach would be to study the sphingolipid metabolic profile of tumors or plasma. This type of "sphingolipidomic" evaluation is commonly done in research laboratories and can be adapted to clinical settings. This was done for the initial clinical trial studying CNL to enable pharmacological studies [97]. Sphingolipidomic profiling could reveal a metabolic signature that can both help select an appropriate sphingolipid-targeted therapeutic as well as follow the treatment for indication of the development of resistance, such as the upregulation of alternative metabolic pathways. Notably, sphingolipidomic approaches have been used in risk prediction for atherosclerosis and cardiovascular disease [99]. More specifically, ratios of different acyl chain lengths and degrees of saturation for various species of ceramide has been leveraged as a ceramide score to evaluate atherosclerosis and cardiovascular disease risk. This demonstrates that this methodology is beginning to be clinically available and could similarly be adapted for cancer.

Finally, a recent study used sphingolipidomic and genomic (transcriptomic) approaches to stratify AML exclusively based on a sphingolipid profile [100]. The study defined two distinct subtypes based on the prevalence of sphingomyelins (SM) and hexosylceramides (Hex) (glucosylceramide and galactosylceramide). Moreover, a classifier was developed that could leverage genomic data alone and was used to validate the subtypes using both the TCGA-AML and BEAT AML datasets. The HexlowSMhigh subtype had a significantly worse clinical outlook also was associated with a stem cell-like profile. The HexhighSMlow subtype still had a dismal clinical outlook but was instead associated with an inflammatory profile. One could envision many roles for this AML sphingolipid classifier including patient risk stratification but also therapy guidance. For instance, AML patients in the HexlowSMhigh subtype could be directed to therapies that elevate or deliver ceramide in combination with inhibition of the pathways leading to sphingomyelin generation. It may be possible that the approach used to develop this AML sphingolipid classifier could be adapted for other cancers. Similarly, the sphingolipidomic and genomic (transcriptomic) approaches could be used to identify unique sphingolipid signatures that can guide sphingolipid-targeted treatment selection.

Conclusions

Summarizing the current repertoire of sphingolipid metabolism-modulating cancer therapeutics highlights the potential that lies in both established and new drugs. The goal of these therapeutics is commonly to shift the balance of the ceramide/S1P biostat such that higher ratios of the pro-apoptotic lipid ceramide will induce cell death. This goal can be reached in many ways, such as by impacting the enzymes which metabolize ceramide, blocking S1P production, or by administering ceramide or ceramide analogs via nanoliposomal or other delivery systems. The limits to how useful these therapies can be for fighting cancer is set by the limits of our current understanding of sphingolipid biology. As we uncover more about the actions of these lipids, we can review previously established drugs for their potential to modulate sphingolipid metabolism in a helpful way or investigate how sphingolipid metabolism may explain the resistance that develops to current therapies. Additionally, further research into how cancer cells modulate sphingolipid metabolism for their survival will allow us to better leverage such signatures for the development of more targeted cancer therapies.

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References

1. Yamaji T, Hanada K (2014) Sphingolipid Metabolism and Interorganellar Transport: Localization of Sphingolipid Enzymes and Lipid Transfer Proteins. Traffic 16:101-122.
2. Boojar M M A, Boojar, M M A, Golmohammad S (2018) Ceramide pathway: A novel approach to cancer chemotherapy. Egyptian Journal of Basic and Applied Sciences 5:237-244.
3. Kurek K, Miklosz A, Lukaszuk B, Chabowski A, Gorski J, et al. (2015) Inhibition of Ceramide De Novo Synthesis Ameliorates Diet Induced Skeletal Muscles Insulin Resistance. J Diabetes Res 2015:154762.

4. Hannun Y A, Obeid L M (2017) Sphingolipids and their metabolism in physiology and disease. Nat Rev Cell Biol 19:175-191.

5. Ogretmen B (2017) Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 18:33-50.

6. Senkal C E, Salama M F, Snider A J, Allopenna J J, Rana N A, et al. (2017) Ceramide Is Metabolized to Acylceramide and Stored in Lipid Droplets. Cell Metab 25:686-697.

7. Nagahashi M, Takabe K, Terracina K P, Soma D, Hirose Y, et al. (2014) Sphingosine-1-phosphate transporters as targets for cancer therapy. Biomed Res Int 2014:651727.

8. Xiao S, Yang J (2019) Preclinical study of the antitumor effect of sphingosine-1- phosphate receptor 1 antibody (S1PR1-antibody) against human breast cancer cells. Invest New Drugs 37:57-64.

9. Olesch C, Ringel C, Brüne B, Weigert A (2017) Beyond Immune Cell Migration: The Emerging Role of the Sphingosine-1-phosphate Receptor S1PR4 as a Modulator of Innate Immune Cell Activation. Mediators Inflamm 2017:6059203.

10. Truman J P, Barros M G, Obeid L M (2015) Evolving concepts in cancer therapy through targeting sphingolipid metabolism. Biochim Biophys Acta 1841:1174- 1188.

11. Morad S A, Cabot M C (2013) Ceramide-orchestrated signaling in cancer cells. Nat Rev Cancer 13:51-65.

12. del Solar V, Lizardo D Y, Li N, Hurst J J, Brais C J, et al. (2015) Differential Regulation of Specific Sphingolipids in Colon Cancer Cells during Staurosporine-Induced Apoptosis. Chem Biol 22:1662-1670.

13. Ponnusamy S, Meyers-Needham M, Senkal C E, Saddoughi S A, Sentelle D, et al. (2010) Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. Future Oncol 6:1603-1624.

14. Senchenkov A, Litvak D A, Cabot M C (2001) Targeting Ceramide Metabolism - a Strategy for Overcoming Drug Resistance. J Natl Cancer Inst 93:347-357.

15. Saddoughi S A, Song P, Ogretmen B (2004) Roles of bioactive sphingolipids in cancer biology and theraputics. Subcell Biochem 49:413-440.

16. Bettaieb A, Plo I, Mansat-De Mas V, Quillet-Mary A, Levade T, et al. (1999) Daunorubicin- and mitoxantrone-triggered phosphatidylcholine hydrolysis: implication in drug-induced ceramide generation and apoptosis. Mol Pharmacol 55:118-125.

17. Dumitru C A, Weller M, Gulbins E (2009) Ceramide metabolism determines glioma cell resistance to chemotherapy. J Cell Physiol 221:688-695.

18. Dumitru C A, Sandalcioglu I E, Wagner M, Weller M, Gulbins E (2009) Lysosomal ceramide mediates gemcitabine-induced death of glioma cells. J Mol Med 87:1123-1132.

19. Modrak D E, Leon E, Goldenberg D M, Gold D V (2009) Ceramide regulates gemcitabine-induced senescence and apoptosis in human pancreatic cancer cell lines. Mol Cancer Res 7: 890-896.

20. Wu B X, Zeidan Y H, Hannun Y A (2009) Downregulation of neutral ceramidase by gemcitabine: Implications for cell cycle regulation. Biochim Biophys 1791:730-739.

21. Nemoto S, Nakamura M, Osawa Y, Kono S, Itoh Y, et al. (2009) Sphingosine kinase isoforms regulate oxaliplatin sensitivity of human colon cancer cells through ceramide accumulation and Akt activation. J Biol Chem 284:10422-10432.

22. Zeidan Y H, Jenkins R W, Hannun Y A (2008) Remodeling of cellular cytoskeleton by the acid sphingomyelinase/ceramide pathway. J Cell Biol 181:335-350.

23. Saad A F, Meacham W D, Bai A, Anelli V, Elojeimy S, et al. (2007) The functional effects of acid ceramidase overexpression in prostate cancer progression and resistance to chemotherapy. Cancer Biol Ther 6:1455-1460.

24. Rebillard A, Tekpli X, Meurette O, Sergent O, LeMoigneMuller G, et al. (2007) Cisplatin-induced apoptosis involves membrane fluidification via inhibition of NHE1 in human colon cancer cells. Cancer Res 67:7865-7874.

25. Sridevi P, Alexander H, Laviad E L, Min J, Mesika A, et al. (2010) Stress-induced ER to Golgi translocation of ceramide synthase 1 is dependent on proteasomal processing. Exp Cell Res 316:78-91.

26. Gouazé-Andersson V, Flowers M, Karimi R, Fabriás G, Delgado A (2011) Inhibition of acid ceramidase by a 2-substituted aminoethanol amide synergistically sensitizes prostate cancer cells to N-(4-hydroxyphenyl) retinamide. Prostate 2011 1:1064- 1073.

27. Kus G, Kabadere S, Uyar R, Kutlu H M (2015) Induction of apoptosis in prostate cancer cells by the novel ceramidase inhibitor ceranib-2. In Vitro Cell Dev Biol Anim 51:1056-1063.

28. Draper J M, Xia Z, Zhuang Y, Wang W, Smith C D (2011) Discovery and Evaluation of Inhibitors of Human Ceramidase. Mol Cancer Ther 10:2052-2061.

29. Vethakanraj H S, Sesurajan B P, Padmanaban V P, Jayaprakasam M, Murali S et al. (2018) Anticancer effect of acid ceramidase inhibitor ceranib-2 in human breast cancer cell lines MCF-7, MDA MB-231 by the activation of SAPK/JNK, p38 MAPK apoptotic pathways, inhibition of the Akt pathway, downregulation of ERa. Anticancer Drugs 29:50-60.

30. Watters R J, Wang H G, Sung S S, Loughran T P Jr, Liu X (2011) Targeting Sphingosine-1-Phosphate Receptors in Cancer. Anticancer Agents Med Chem 11:810-817.

31. Boojar M M A, Boojar M M A, Colmohammad S, Behrehbar I (2018) Data on cell survival, apoptosis, ceramide metabolism and oxidative stress in A-494 renal cell carcinoma cell line treated with hesperetin and hesperetin-7-O-acetate. Data Brief 20:596-601.

32. Chandrikaa B B, Steephan M, Kumarc T R S, Sabua A, Haridasa M (2016) Hesperetin and Naringenin sensitize HER2 positive cancer cells to death by serving as HER2 Tyrosine Kinase inhibitors. Life Sci 160:47-56.

33. White C, Alshaker H, Cooper C, Winkler M, Pchejetski D (2016) The emerging role of FTY720 (Fingolimod) in cancer treatment. Oncotarget 7:23106-23127.

34. Senkal C E, Ponnusamy S, Rossi M J, Bialewski J, Sinha D, et al. (2007) Role of human longevity assurance gene 1 and C18-ceramide in chemotherapy-induced cell death in human head and neck squamous cell carcinomas. Mol Cancer Ther 6:712-722.

35. Morjani H, Madoulet C (2010) Immunosuppressors as multidrug resistance reversal agents. Methods Mol Biol 596:433-446.

36. Chapman J V, Gouaze-Andersson V, Messner M C, Flowers M, Karimi R, et al. (2010) Metabolism of short-chain ceramide by human cancer cells--implications for therapeutic approaches. Biochem Pharmacol 80:308-315.

37. Barth B M, Gustafson S J, Young M M, Fox T E, Shanmugavelandy S S, et al. (2010) Inhibition of NADPH oxidase by glucosylceramide confers chemoresistance. Cancer Biol Ther 10:1126-1136.

38. Cooper J P, Reynolds C P, Cho H, Kang M H (2017) Clinical development of fenretinide as an antineoplastic drug: Pharmacology perspectives. Exp Biol Med (Maywood) 242:1178-1184.

39. Boulter A C, Maurer B J, Pogue M, Kang M H, Cho H, et al. (2023) Phase I trial of intravenous fenretinide (4- HPR) plus safingol in advanced malignancies. Cancer Chemother Pharmacol 92:97-105.

40. Barth B M, Shanmugavelandy S S, Kaiser J M, McGovern C, Altinoglu E I, et al. (2013) PhotoImmunoNanoTherapy reveals an anticancer role for sphingosine kinase 2 and dihydrosphingosine-1-phosphate. ACS Nano 7:2132-2144.

41. Streeter E, Jones A, Monnier KL, Cranston D, Crew J, et al. (2005) Phase I trial of intravesical Suramin in recurrent superficial transitional cell bladder carcinoma. Br J Cancer 92:2140-7.

42. Lustberg MB, Pant S, Ruppert AS, Shen T, Wei Y, et al. (2012) Phase I/ II trial of non-cytotoxic suramin in combination with weekly paclitaxel in metastatic breast cancer treated with prior taxanes. Cancer Chemother Pharmacol 70:49-56.

43. Villalona-Calero MA, Otterson GA, Wientjes MG, Weber F, Bekaii-Saab T, et al. (2008) Noncytotoxic suramin as a chemosensitizer in patients with advanced non- small-cell lung cancer: a phase II study. Ann Oncol 19:1903-1909.

44. Ahles TA, Small EJ, Vogelzang NJ, Kornblith AB, Ratain MJ, et al. (2004) Quality of life impact of three different doses of suramin in patients with metastatic hormone-refractory prostate carcinoma: results of Intergroup O159/Cancer and Leukemia Group B 9480. Cancer 101:2202-8.

45. Signorelli P, Munoz-Olaya JM, Gagliostro V, Casas J, Ghidoni R, et al. (2009) Dihydroceramide intracellular increase in response to resveratrol treatment mediates autophagy in gastric cancer cells. Cancer Lett 282:238-43.

46. Gatouillat G, Balasse E, Joseph-Pietras D, Morjani H, Madoulet C (2010) Resveratrol induces cell-cycle disruption and apoptosis in chemoresistant B16 melanoma. J Cell Biochem 110:893-902.

47. McGill CM, Brown TJ, Fisher LN, Gustafson SJ, Dunlap KL, et al. (2018) Combinatorial Efficacy of Quercitin and Nanoliposomal Ceramide for Acute Myeloid Leukemia. Int J Biopharm Sci 1:106.

48. McGill CM, Tomco PL, Ondrasik RM, Belknap KC, Dwyer GK, et al. (2018) Therapeutic effect of Northern Labrador tea extracts for acute myeloid leukemia. Phytother Res 32:1636-1641.

49. McGill CM, Alba-Rodriguez EJ, Li S, Benson CJ, Ondrasik RM, et al. (2014) Extracts of Devil's Club (Oplopanax horridus) exert therapeutic efficacy in experimental models of acute myeloid leukemia. Phytother Res 28:1308-14.

50. Fox TE, Finnegan CM, Blumenthal R, Kester M (2006) The clinical potential of sphingolipid-based therapeutics. Cell Mol Life Sci 63:1017-1023.

51. Khodadadian M, LerouxC ME, Auzenne E, Ghosh SC, Farquhar D, et al. (2009) MRP- and BCL-2-mediated drug resistance in human SCLC: effects of apoptotic sphingolipids in vitro. Lung Cancer 66:48-57.

52. Ghosh S C, Auzenne E, Farquhar D, Klostergaard J N (2007) Ndimethylsphingosine- coumarin: synthesis, chemical characterization, and biological evaluation. Bioconjug Chem 18:731-735.

53. Dickson MA, Carvajal RD, Merrill Jr AH, Gonen M, Cane LM, et al. (2011) A Phase I Clinical Trial of Safingol in Combination with Cisplatin in Advanced Solid Tumors. Clin Cancer Res 17:2484-92.

54. Paugh SW, Paugh BS, Rahmani M, Kapitonov D, Almenara JA, et al. (2008) A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia. Blood 112:1382-91.

55. Rosa R, Marciano R, Malapelle U, Formisano L, Nappi L, et al. (2013) Sphingosine kinase 1 overexpression contributes to cetuximab resistance in human colorectal cancer models. Clin Cancer Res 19:138-47.

56. Mousseau Y, Mollard S, Faucher-Durand K, Richard L, Nizou A, et al. (2012) Fingolimod potentiates the effects of sunitinib malate in a rat breast cancer model. Breast Cancer Res Treat 134:31-40.

57. Burg N, Salmon NE, Hla T (2022) Sphingosine 1-phosphate receptor-targeted therapeutics in rheumatic diseases. Nat Rev Rheumatol 18:335-351.

58. Paik J (2022) Ozanimod: A Review in Ulcerative Colitis. Drugs 82:1303-1313.

59. Pal SK, Drabkin HA, Reeves JA, Hainsworth JD, Hazel SE, et al. (2017) A phase 2 study of thesphingosine-1-phosphate antibody sonepcizumab in patients with metastatic renal cell carcinoma. Cancer 123:576-582.

60. Britten CD, Garrett-Mayer E, Chin SH, Shirai K, Ogretmen B, et al. (2017) A Phase I Study of ABC294640, a First-in-Class Sphingosine Kinase-2 Inhibitor, in Patients with Advanced Solid Tumors. Clin Cancer Res 23:4642-4650.

61. Shaw J, Costa-Pinheiro P, Patterson L, Drews K, Spiegel S, et al. (2018) Novel Sphingolipid-Based Cancer Therapeutics in the Personalized Medicine Era. Adv Cancer Res 140:327-366.

62. Kang Y, Sundaramoorthy P, Gasparetto C, Feinberg D, Fan S, et al. (2023) Phase I study of opaganib, an oral sphingosine kinase 2-specific inhibitor, in relapsed and/or refractory multiple myeloma. Ann Hematol 102:369-383.

63. Ryland LK, Fox TE, Liu X, Loughran TP, Kester M (2011) Dysregulation of sphingolipid metabolism in cancer. Cancer Biol Ther 11:138-49.

64. Obeid LM, Linardic CM, Karolak LA, Hannun YA (1993) Programmed cell death induced by ceramide. Science 259:1769-71.

65. Gaver RC, Sweeley CC (1966) Chemistry and metabolism of sphingolipids. 3-Oxo derivatives of N-acetylsphingosine and Nacetyldihydrosphingosine. J Am Chem Soc 88:3643-7.

66. Dobrowsky RT, Hannun YA (1992) Ceramide stimulates a cytosolic protein phosphatase. J Biol Chem 267:5048-51.

67. Macchia M, Barontini S, Bertini S, Bussolo VO, Sheets S, et al. (2001) Design, synthesis, and characterization of the antitumor activity of novel ceramide analogues. J Med Chem 44:3994-4000.

68. Dagan A, Wang C, Fibach E, Gatt S (2003) Synthetic, non-natural sphingolipid analogs inhibit the biosynthesis of cellular sphingolipids, elevate ceramide and induce apoptotic cell death. Biochim Biophys Acta 1633:161-9.

69. Granot T, Milhas D, Carpentier S, Dagan A, Ségui B, et al. (2006) Caspase-dependent and -independent cell death of Jurkat human leukemia cells induced by novel synthetic ceramide analogs. Leukemia 20:392-9.

70. Qin JD, Weiss L, Slavin S, Gatt S, Dagan A (2010) Synthetic, nonnatural analogs of ceramide elevate cellular ceramide, inducing apoptotic death to prostate cancer cells and eradicating tumors in mice. Cancer Invest 28:535-43.

71. Antoon JW, Liu J, Ponnapakkam AP, Gestaut MM, Foroozesh M, et al. (2010) Novel D: -erythro N-octanoyl sphingosine analogs as chemo- and endocrine-resistant breast cancer therapeutics. Cancer Chemother Pharmacol 65:1191-5.

72. Liu J, Antoon J W, Ponnapakkam A, Beckman B S, Foroozesh M (2010) Novel anti-viability ceramide analogs: design, synthesis, and structure-

activity relationship studies of substituted (S)- 2-(benzylideneamino)-3-hydroxy-N-tetradecylpropanamides. Bioorg Med Chem 18:5316-5322.

73. Bieberich E, Hu B, Silva J, MacKinnon S, Yu RK, et al. (2002) Fillmore, H.; Broaddus, W. C.; Ottenbrite, R. M. Synthesis and characterization of novel ceramide analogs for induction of apoptosis in human cancer cells. Cancer Lett 181:55-64.

74. Bieberich E, Kawaguchi T, Yu RK (2000) N-acylated serinol is a novel ceramide mimic inducing apoptosis in neuroblastoma cells. J Biol Chem 275:177-81.

75. Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG (2004) Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. J Cell Biol 167:723-34.

76. Struckhoff AP, Bittman R, Burow ME, Clejan S, Elliott S, et al. (2004) Novel ceramide analogs as potential chemotherapeutic agents in breast cancer. J Pharmacol Exp Ther 309:523-32.

77. Tan SF, Liu X, Fox TE, Barth BM, Sharma A, et al. (2016) Acid ceramidase is upregulated in AML and represents a novel therapeutic target. Oncotarget 7:83208-83222.

78. Liu F, Li X, Lu C, Bai A, Bielawski J, et al. (2016) Ceramide activates lysosomal cathepsin B and cathepsin D to attenuate autophagy and induces ER stress to suppress myeloid-derived suppressor cells. Oncotarget 7:83907-83925.

79. Bai A, Mao C, Jenkins RW, Szulc ZM, Bielawska A, et al. (2017) Hannun, Y. A. Anticancer actions of lysosomally targeted inhibitor, LCL521, of acid ceramidase. PLoS One 12:e0177805.

80. Stover T, Kester M (2003) Liposomal delivery enhances short-chain ceramide-induced apoptosis of breast cancer cells. J Pharmacol Exp Ther 307:468-75.

81. Kester M, Bassler J, Fox TE, Carter CJ, Davidson JA, et al. (2015) Preclinical development of a C6-ceramide NanoLiposome, a novel sphingolipid therapeutic. Biol Chem 396:737-47.

82. Barth BM, Wang W, Toran PT, Fox TE, Annageldiyev C, et al. (2019) Sphingolipid metabolism determines the therapeutic efficacy of nanoliposomal ceramide in acute myeloid leukemia. Blood Adv 3:2598-2603.

83. Shabbits JA, Mayer LD (2003) High ceramide content liposomes with in vivo antitumor activity. Anticancer Res 23:3663-9.

84. Shabbits JA, Mayer LD (2003) Intracellular delivery of ceramide lipids via liposomes enhances apoptosis in vitro. Biochim Biophys Acta 1612:98-106.

85. Stover TC, Sharma A, Robertson GP, Kester M (2005) Systemic delivery of liposomal short-chain ceramide limits solid tumor growth in murine models of breast adenocarcinoma. Clin Cancer Res 11:3465-74.

86. Tagaram HRS, Divittore NA, Barth BM, Kaiser JM, Avella D, et al. (2011) Nanoliposomal ceramide prevents in vivo growth of hepatocellular carcinoma. Gut 60:695-701.

87. Liu X, Ryland L, Yang J, Liao A, Aliaga C, et al. (2010) Targeting of survivin by nanoliposomal ceramide induces complete remission in a rat model of NK-LGL leukemia. Blood 116:4192-4201.

88. Tran MA, Gowda R, Sharma A, Park EJ, Adair J, et al. (2008) Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. Cancer Res 68:7638- 7649.

89. Kester M, Heakal Y, Fox T, Sharma A, Robertson GP, et al. (2008) Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells. Nano Lett 8:4116-4121.

90. Morgan TT, Muddana HS, Altinoglu EI, Rouse SM, Tabakovic A, et al. (2008) Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. Nano Lett 8:4108-15.

91. Stover TC, Kim YS, Lowe TL, Kester M (2008) Thermoresponsive and biodegradable linear-dendritic nanoparticles for targeted and sustained release of a pro- apoptotic drug. Biomaterials 29:359-369.

92. Desai A, Vyas T, Amiji M (2008) Cytotoxicity and apoptosis enhancement in brain tumor cells upon coadministration of paclitaxel and ceramide in nanoemulsion formulations. J Pharm Sci 7:2745-56.

93. Devalapally H, Duan Z, Seiden MV, Amiji MM (2008) Modulation of drug resistance in ovarian adenocarcinoma by enhancing intracellular ceramide using tamoxifen-loaded biodegradable polymeric nanoparticles. Clin Cancer Res 14:3193-203.

94. Devalapally H, Duan Z, Seiden MV, Amiji MM (2007) Paclitaxel and ceramide co- administration in biodegradable polymeric nanoparticulate delivery system to overcome drug resistance in ovarian cancer. Int J Cancer 121:1830-8.

95. Vlerken LEV, Duan Z, Seiden MV, Amiji MM (2007) Modulation of intracellular ceramide using polymeric nanoparticles to overcome multidrug resistance in cancer. Cancer Res 67: 4843-4850.

96. Suhrland C, Truman JP, Obeid LM, Sitharaman B (2019) Oxidized graphene nanoparticles as a delivery system for the pro-apoptotic sphingolipid C6 ceramide. J Biomed Mater Res A 107:25-37.

97. Ciner A, Gourdin T, Davidson J, Parette M, Walker SJ, et al. (2024) A phase I study of the ceramide nanoliposome in patients with advanced solid tumors. Cancer Chemother Pharmacol 93:23-29.

98. Khokhlatchev AV, Sharma A, Deering TG, Shaw JJP, Costa-Pinheiro P, et al. (2022) Ceramide nanoliposomes augment the efficacy of venetoclax and cytarabine in models of acute myeloid leukemia. FASEB J 36:e22514.

99. Zelnik ID, Kim JL, Futerman AH (2021) The Complex Tail of Circulating Sphingolipids in Atherosclerosis and Cardiovascular Disease. J Lipid Atheroscler 10:268-281.

100. Paudel BB, Tan SF, Fox TE, Ung J, Golla U, et al. (2024) Acute myeloid leukemia stratifies as 2 clinically relevant sphingolipidomic subtypes. Blood Adv 8:1137-1142.