Thousands of research studies have reported that many kinds of cancer cells and tumors can be killed by treatments that increase the concentration of a simple cellular sphingolipid, ceramide (Cer). While there are many ways to elevate tumor Cer levels, this approach is complicated by the central, complex role of Cer in cell homeostasis: Cer is readily metabolized to form other sphingolipids that increase the tumor’s growth rate, metastasis, and resistance to the patient’s immune system. This review points out the need to prevent this metabolic conversion while simultaneously stimulating the enzymes that increase the formation of Cer. I describe here many of the enzymes that need stimulation or inhibition, and drugs or metabolites or dietary components that modify each of the enzymes. The review also points to the importance of the allylic alcohol group in Cer and in many cancer drugs, suggesting that the hydroxyl group participates in the allylic alcohol group in Cer and in many cancer drugs, suggesting that the hydroxyl group participates in acylation of sphingosine. In addition, Cer concentration can be raised by slowing its conversion to sphingomyelin (SM) which does not seem to be affected. In any given individual, it is important to control several interacting processes. In the example described in this paper, ceramide (the fatty acid amide of sphingosine = Cer) (Figure) is involved in many physiological processes of normal function and disease. Cer has been found by many researchers to block cell growth and proliferation in a large number of cell types, producing apoptosis (suicidal death) in cancer cells if its concentration is high enough. Thus the process needed for cancer treatment is one that forces the patient’s tumor to accumulate Cer. Drugs are known which can (1) accelerate Cer biosynthesis from several precursors, (2) slow its hydrolysis, and (3) slow its conversion to sphingomyelin (SM) which does not seem to affect cell growth. However there is a big complication: Cer also forms products that stimulate cell growth and proliferation, and thus balance growth vs death or differentiation in normal cells. In addition, a group of Cer metabolites secreted by tumors (sialo- and gangliosides) inhibits the immune system’s dendritic cells, which are vital for the complete destruction of cancer cells [1]. These competing, interacting effects make the body’s control of Cer metabolism a vital factor in normal cell function and stability.

If you treat a patient with a drug that speeds Cer synthesis, the resultant accumulation of Cer can be expected to induce more rapid hydrolysis of the Cer by ceramidase or faster conversion to other metabolites. Thus the drug’s beneficial effects will be temporary and the tumor might mutate to a more virulent form in which the extra Cer is converted rapidly to proliferative sphingolipids (sphingosine-1-phosphate, Cer-1-phosphate, glucosyleramide (GicCer), or lactosyleramide). It seems evident that all these processes should be controlled simultaneously, using at least four different drugs in a “Cer cocktail” that act on different Cer enzymes. Since Cer and its metabolites exert many different effects in cells, it may be necessary to control those “side-effects” with additional drugs. The poly-drug proposal has been described [2, 3]. The reader has a wide choice of reviews on sphingolipids [4–9].

Physicians are very familiar with the problem of side-effects, such as the nausea, or diarrhea, or cell damage that results from many antineoplastic drugs, so empirical poly-drug therapy is quite common, using even five drugs simultaneously. Moreover, more and more oncologists are using several different kinds of cancer therapy simultaneously or almost simultaneously, such as radiation together with one or more anti-proliferation drugs. (Radiation causes cells to make Cer faster.) The poly-drug therapy that I am recommending here is the use of drugs to control a single, critical metabolite that will kill the tumor in a “natural” way.
An important factor in this approach is that tumors — at the time of diagnosis — generally consist of several different cell lines that differ in their Cer processing. The dominant cancer cell line might contain a very high activity of a single Cer enzyme, such as ceramidase (which lowers tumor Cer), so a single drug that blocks the enzyme will seem to bring the tumor under control. However this buys the patient only temporary remission. As tumors progress toward higher virulence and metastasis, new mutations appear that repress Cer levels and elevate the proliferative Cer metabolites even more [10]. Thus a wide-acting drug cocktail that controls many aspects of Cer metabolism is essential for (apparently) all cancer patients. This consideration is also important for researchers who test drugs with only one or two cell strains of a given type of cancer instead of a complex mixture of cells.

Another factor that is rarely recognized by researchers working with cultured cells is the difference in drug sensitivity between normal cells and cancer cells. Drugs expected to have antineoplastic activity are typically tested in available cancer cells, which are easier to handle. Researchers should recognize the need to promptly move to animal testing to evaluate the true potential of their drug. While the evidence is incomplete, all the reported studies show that cancer cells have an abnormally high rate of sphingolipid synthesis, and that tumors that have progressed to the drug resistant stage make sphingolipids even faster [11]. It is thus likely that realistic poly-drug Cer therapy will work quickly, using below-typical dosages, minimizing toxicity to the patient.

1. Therapy by stimulating Cer synthesis de novo

1a. Cer is made from palmitic acid and serine, which form 3-keto sphinganine and CO₂ (the latter is from the COOH of serine). The amino group of the lipid is then acylated by a fatty acid, typically ranging in size from C₁₄ to C₂₆, forming 3-keto dihydroceramide. The ketone moiety is reduced to an OH, forming dihydroceramide. This lipid seems to have little or no physiological activity, but a dehydrogenase acting between carbons 4 and 5 produces a trans-Δ⁴ double bond that makes true Cer. The vital difference between the two amides is the double bond, which — together with the C₃ hydroxyl — is part of an allylic alcohol group.

Several researchers have shown that elevating tissue precursor levels (palmitic acid and serine) will speed the de novo synthesis of Cer and aid the appearance of apoptosis. Conversely, it is helpful to use an inhibitor (etomoxir) to slow the transport of palmitic acid to mitochondria by carnitine, which leads to the “burning” of the fatty acid. Administration of extra (dietary) carnitine makes true Cer. The vital difference between the two amides is the double bond, which — together with the C₃ hydroxyl — is part of an allylic alcohol group.

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1b. Radiation stimulates the serine–palmitic condensation by inducing faster formation of keto–sphinganine synthase, thus elevates the Cer in the tumor. In addition, the high–energy photons generate reactive oxygen species (ROS), which speed the hydrolysis of sphingomyelin (SM = Cer–phosphocholine) to form additional Cer (see section 3a). Apparently any energetic form of radiation works: UVA, UVB, gamma-rays, and (probably) X-rays. Recent publications have supported the use of simultaneous or near–simultaneous radiation, combined with chemotherapy that elevates tumor Cer. A merit of using chemotherapy first, to shrink the tumor, is that one can reduce the volume of normal tissue exposed to radiation. However the advantage of simultaneous use is that the tumor is killed promptly, before mutated, more resistant cancer cells can appear.

1c. Several drugs with antineoplastic activity have been shown to stimulate the serine–palmitic condensation: 1) tetrahydrocannabinol (the active component of marijuana); 2) PSC 833 (valspodar, which neutralizes the multi–drug insensitivity of advanced tumors); 3) retinoic acid (a metabolite of Vitamin A); 4) campothecin and irinotecan (popular antineoplastic drugs); 5) etoposide (an anticancer drug that inhibits topoisomerase II and breaks DNA strands); and 6) paclitaxel (Taxol, a well–known anticancer drug).

Modification of these structures has yielded superior anticancer drugs, e.g., fenretinide (N–(4–hydroxyphenyl) retinamide) and homocamptothecin. Fenretinide produces Cer and apoptosis and fludarabine, an antineoplastic drug that causes Cer accumulation, probably also acts by stimulating keto–sphinganine synthesis. Modifications of tetrahydrocannabinol that produce less change in behavior appear to be more effective for chemotherapy.

2. Therapy by treatment with ceramide

2a. Cer is very insoluble in water but it can be dissolved in organic solvents, such as ethanol and dimethylsulfoxide, or incorporated into a water/oil cream. Thus it can readily be applied to skin tumors. For injection, Cer can be incorporated into an emulsion with a suitable detergent or incorporated into liposomes. Use of short–chain Cer (acetyl sphingosine or a little longer) greatly enhances the dispersibility. The short–chain ceramides are readily absorbed by cells and metabolized (mainly by hydrolysis and reacylation of the released sphingosine), so they may be excellent therapeutic agents.

2b. Cer can also be converted to a more dispersible prodrug by attaching a water–soluble polymer — poly(ethylene glycol) — with a connecting spacer, such as succinic acid. Presumably the ester linkages would be hydrolyzed inside the tumor cells, liberating Cer. It might also be useful to make Cer with sphingosine and a water–soluble fatty acid, such as the ω-poly(ethylene glycol) ether of decanoic acid. This might act directly to produce apoptosis.

2c. Cer has been made more dispersible by attachment to glucuronic acid as a glycoside in the C–1 position. Added to the diet, it was found to undergo hydrolysis and reduce carcinogenesis in the colon [13]. This approach deserves more study, especially with polysaccharide substituents, which would allow the use...
of higher doses. Natural sphingolipids in food also undergo some hydrolysis to Cer in the intestine and are easy to prepare from animal spinal cords or brains. While nervous system products might contain pathogenic prions, treatment with alkali would not harm the lipids but would surely denature the prions.

2d. The recent growth of interest in the potential use of Cer in chemotherapy has encouraged chemists to synthesize variations on Cer structure [3, 14, 15]. Since dihydroceramide shows little activity, it would appear that the allylic alcohol group of Cer must play an important role in producing tumor death. This concept finds support in the observation that many antineoplastic drugs also contain an allylic alcohol or generate an allylic alcohol group under metabolic activation [14]. Evidence has been cited to support the possibility that the C–3 OH of Cer is oxidized to a ketone group in mitochondria, by interaction with coenzyme Q or a factor closely related to CoQ [16]. The allylic ketone presumably then condenses in a Michael reaction with glutathione or cysteine thiol groups or other reactive substances, possibly an enzyme acting on CoQ. The apoptogenic consequences of such a condensation are described in section 3a. It is probably significant that an allylic ketone group is seen in many antineoplastic drugs. Readers of this journal may be especially interested to note that landomycin, an allylic ketone (quinone), has potent antitumor and antibiotic activity [17].

Another suggestion [18] is based on the known ability of Cer to stimulate some protein kinases and protein phosphatases [19, 20]. Since the activity of many proteins depends on the presence or absence of phosphate esters at certain sites, Cer catalyzes a wide variety of biological phenomena. An appropriate complex of phosphate changes might result in apoptosis. This catalytic or cofactor activity also requires the high reactivity of an allylic OH, so the hypothesis was that the phosphate anion forms a transient 1,3–cyclic ester with Cer which is then transferred to a protein or — in a phosphatase — anion forms a transient 1,3-cyclic ester with Cer which is then transferred to a protein or — in a phosphatase — is simply hydrolyzed. Conceivably the transient phosphate group is simply on the C–3 hydroxyl.

Variations on the structure of Cer should then include features that enhance the activity of the allylic group. Moving the C–3 OH to the C–5 position, and the ∆4 double bond to the ∆3 position (i.e., inverting the position of the allylic alcohol) yielded an iso–Cer that was much more active in slowing cell proliferation [21]. The OH in this isomer is further from the amide group and thus more exposed to the environment than the C–3 OH. Another approach is to add an OH to position C–6, which would yield Cer with two allylic alcohols instead of one [22]. Adding one or more conjugated double bonds to natural Cer (i.e., at ∆4, ∆6, etc.) may enhance the reactivity of the C–3 OH.

Modifying the fatty acid part of Cer might also be productive. For example, retinoic acid and conjugated linoleic acid have anticancer activity, probably as the result of oxygenase action that inserts an allylic OH into the chain. They could be readily incorporated into Cer. Ceramides containing a 2–OH fatty acid are common in mammals and addition of a ∆3 double bond to the fatty acid (yielding an extra allylic alcohol group) would probably enhance the Cer activity. Many other Cer analogs with promising properties have recently been described [15]. It seems evident that the synthesis of new Cer analogs will yield powerful antineoplastic drugs.

It should be remembered that a test with a new Cer analog might show good interference with cancer cell proliferation simply because it protects endogenous Cer against conversion to a proliferative metabolite. For example, it might simply inhibit ceramidase rather than mimic Cer’s ability to produce apoptosis. Conversely, a new Cer analog might have little effect on cell proliferation simply because it inhibits a Cer enzyme that occurs at a very high level of activity in the cell type chosen for the evaluation. Cer containing a pyrrolidine ring at C–1 instead of an OH failed to inhibit growth of MDCK cells, yet showed high inhibition of GlcCer synthase, thus is potentially very useful in a cancer cocktail (see section 4).

3. Therapy by stimulating sphingomyelin hydrolysis

3a. Several kinds of SMase hydrolyze SM to Cer and phosphocholine. Neutral, Mg2+-stimulated SMase has received the most attention, probably because its activity depends on the oxidoreduction status of the tumor and because the substrate (SM) occurs at a relatively high concentration in cells. Thus SM acts as a reservoir for the rapidly formation of Cer. Glutathione (GSH), the major thiol in tissues, inhibits the enzyme, so any factor that lowers its concentration will speed Cer synthesis [23]. It is significant that many tumors contain high levels of GSH and γ-glutamylcysteine synthase, thus tend to keep Cer levels low and avoid apoptosis.

GSH levels are lowered by oxidative stress. While oxygen is relatively scarce in solid tumors, some parts of the tumors are oxygenated enough to attain the benefits of oxidative stress. The sulfur atom in GSH can be oxidized to a disulfide (as a dimer or reaction with protein–bound cysteine), or an S–O or S=O or S–NO derivative. Agents that produce ROS oxidize GSH, elevate Cer, and kill cancer cells. An important generator of ROS is Cer itself, which produces ROS in mitochondria. This ROS oxidizes GSH and stimulates SM hydrolysis, generating additional Cer. Thus one sees a gradual rise in cellular Cer by what amounts to an autocatalytic growing spiral. GSH can also be oxidized to a mixed disulfide by various disulfide drugs, such as disulfiram. This anti-alcoholism drug was shown to induce apoptosis of mela-noma cells. Betathine (β-alanyl cysteamine disulfide) has shown some promise in cancer patients, possibly by forming a mixed disulfide with GSH.

GSH can also be depleted by inhibiting its biosynthesis with buthionine sulfoximine, which also induces apoptosis. Various detoxification reactions consume GSH, generally forming thio ethers. The common anti-inflammatory drug, acetaminophen, is detoxified in part via this reaction. Glutamic acid, one of the GSH precursors, can be removed from the body in relatively large amounts by letting it react (after conversion to glutamine) with phenylacetic acid. The resultant amide, phenylacetylglutamine, is excreted in urine. 4–Phenylbutyrate
reacts similarly. These simple molecules decreased plasma glutamine levels in patients, as well as GSH [24]. The two arylalkanoic acids have shown some anticancer activity and ability to prevent carcinogenesis, particularly when used with other Cer inducers, such as radiation and doxorubicin. (Incidentally, they are useful for treating patients whose urea cycle is inadequate.)

Because of the many normal functions of GSH, reducing its concentration in tumors should not be complete and the method used should be part of other Cer elevating treatments. Many antineoplastic drugs react with GSH by a Michael condensation or thiol condensation, an effect which must enhance the effectiveness of the drugs.

3b. SMase is stimulated by arachidonic acid, one of the major unsaturated fatty acids of phospholipids [25]. Arachidonate can be elevated by ingestion or by stimulating phospholipase A, which liberates arachidonate, increases Cer, and produces apoptosis. Hydrogen peroxide stimulates the phosphatase, so Cer itself (which produces peroxide in cells) may increase its concentration in an expanding spiral like the one described above. Short-chain Cer — and probably ordinary Cer — can act as an arachidonate acceptor from phospholipids, especially plasmalogen, forming the 1-arachidonoyl-Cer ester. The ester is hydrolyzed readily, offering another way to stimulate SM hydrolysis. In other pathways, arachidonate is attacked by oxygenases, forming several allylic alcohols, which can be expected to produce apoptosis [26]. Arachidonate metabolism is complex and conversion of the acid to prostaglandins should be minimized since they stimulate tumor growth.

3c. Oxidized low density lipoprotein stimulates neutral SMase, alkaline and acid ceramidase, sphingosine kinase, and the formation of lactosylceramide from GlcCer [27]. Cer levels are raised by the SMase. But the other enzymes lower Cer and form proliferative sphingolipids, so the lipoprotein must be used together with inhibitors of the other enzymes. In a study with human macrophages, the Cer increase was also accompanied by increased neutral and acidic SMase. Oxidized LDL may contain an oxidized form of cholesterol, 4-cholesten-3-one (an allylic ketone), as well as allylic alcohol oxidation products of polyunsaturated fatty acids, which should help its apoptogenic activity.

3d. Exogenous SM, as a component of the Cer cocktail, can be considered a prodruk, since it is converted to Cer in the tumor. Some tumors contain little SM, probably because they convert most of their Cer to the proliferative sphingolipids. Use of a short-chain SM might be better than natural SM, since it should penetrate tumors more rapidly. Dietary SM was reported to slow the appearance of colonic tumors [28], and the effectiveness of 5-fluorouracil and irinotecan for treating tumor xenografts in mice was enhanced by the inclusion of SM. (Irinotecan, like camptothecin, is an allylic alcohol that induces faster Cer biosynthesis.) SM resembles lecithin structurally, so it can be incorporated into a liposomal dispersion of anticancer drugs (such as vincristin). It is readily prepared from natural sources.

3e. Cholesterol binds strongly to SM, apparently reducing its sensitivity to SMase. Thus any steps available for reducing tumor levels of cholesterol may prove helpful in cancer therapy. Dietary control of fat (a known stimulator of cancer growth) and cholesterol intake seems like a simple way to enhance the Cer cocktail. Statins, which slow cholesterol biosynthesis, have shown promising anticancer activity and apoptosis in cultured cells, but human studies have not been encouraging, possibly because statins also reduce the cellular level of ubiquinone. One mechanism by which Cer induces apoptosis is by interference with ubiquinone function (section 2d) so a deficiency of ubiquinone in a tumor might be undesirable. Perhaps statins would be effective if used together with supplementary ubiquinone and the Cer cocktail.

3f. Dihydroxy-vitamin D3, the active form of vitamin D, stimulates SM hydrolysis [29], produces apoptosis, and has been found to be helpful in cancer chemotherapy. It is significant that it is an allylic alcohol. High doses upset body Ca metabolism and efforts are currently underway to synthesize improved analogues.

4. Forcing ceramide accumulation by blocking its glucosylation

4a. Glucosylceramide and lactosylCer stimulate cell proliferation and appear to be involved in tumor multidrug resistance, so the enzymes that produce these glucosphinoglycals should be blocked. Cer analogs in which the terminal OH is replaced by an amine are potent inhibitors of Cer glucosylation. Similar analogs in which the last 15 carbon atoms of sphingosine are replaced by a phenyl ring (the “P-drugs”) are also highly effective, in vitro and in vivo [30, 31]. Injection of D-threo-1-(ethylenedioxy)phenyl–2-palmitoylamino–3-pyrrolidino–propanol–1 into mice with Fabry disease for 3 days at 1 mg every 12 h resulted in 50% depletion of renal GlcCer [32]. During the period of treatment, GlcCer synthesis is strongly slowed and the complex glucosphingolipids and GlcCer gradually disappear by hydrolytic turnover. This sort of compound, now under commercialization, has been recommended for use in treating sphingolipidoses [33], cancer, and other diseases of proliferation. In mice bearing Ehrlich ascites cancer cells, administration of a P-drug for only 10 days succeeded in permanently curing 30–40% of the mice and greatly prolonged life in the others [30]. The cured mice were unaffected by reinoculation with more cancer cells, suggesting that the drug also enabled the development of immune resistance.

N—butyldideoxyojirimycin has also been reported to be useful for slowing the conversion of Cer to GlcCer, but the data on its effectiveness are not entirely satisfying.

4b. Tamoxifen is an anti-estrogen with valuable anticancer activity. It also inhibits Cer glucosylation, especially in multi-drug resistant cancer cells [34]. Tamoxifen seems to help prevent the appearance of cancer, a feature in agreement with other data suggesting that chronic elevation of tissue Cer slows or prevents cancer. Raloxifene, a related antineoplastic drug, may also slow GlcCer synthesis.
4c. RU486 (mifepristone), the abortion-inducing drug, is an inhibitor of Cer glucosylation, producing Cer accumulation and apoptosis [35]. It has also shown promise in human chemotherapy. It is a conjugated aliphatic ketone which inactivates a cytochrome c, probably by Michael condensation with a CysH moiety.

4d. Since testosterone stimulates Cer glucosylation and inhibits GlcCer hydrolysis in mouse kidneys, it elevates the synthesis of the proliferative glucosphingolipids [36]. This effect is seen in growing mice, in which the male kidneys grow much faster than female kidneys. Anti-androgen chemotherapy can be expected to slow Cer glucosylation, especially in androgen-sensitive tumors (prostate, kidney, lung, brain). The effects on other sphingolipids need study.

4e. Cellular glucose levels affect the rate of UDP-glucose biosynthesis, thereby affecting Cer glucosylation. High blood glucose levels, as in diabetics, speed the diversion of Cer into glucosphingolipids, and thus should be avoided. Studies have shown the proliferative effects of high glucose availability, as well as high fat availability and the consequent stimulation of tumor growth.

5. Elevating ceramide in tumors by blocking ceramidase

Acid and neutral/alkaline ceramidases, particularly in mitochondria, tend to prevent Cer accumulation and apoptosis. Some tumors have a high ceramidase activity and thus protect themselves from apoptosis [37]. Blocking ceramidase is especially important for cancer therapy since its product, sphingosine, is converted in part to the mitogenic lipid, sphingosine–1–phosphate.

5a. N–oleylethanolamine, a very simple amide that resembles the first two carbons of Cer, inhibits the acid type of ceramidase and tends to produce the typical changes of Cer–induced apoptosis [38, 39]. Some studies have shown that the amide also stimulates neutral SMase and inhibits Cer glucosylation, thus it elevates tumor Cer by modulating three ceramidases. More effort should be invested in searching for a more stable and potent substitute.

5b. An aromatic Cer analog, D–erythro–2–tetrade canoylamino–1–phenyl–1–propanol, inhibits neutral/alkaline ceramidase, elevating Cer and slowing cell growth. Like natural Cer, it has the D–erythro configuration. This analog, as well as a related one (2–N–myristoylamino–1–(4–nitrophenyl)–1,3–propandiol) was active against acid ceramidase, producing Cer accumulation and apoptosis in normal and melanoma cells [40]. As the authors suggest, this approach to cancer chemotherapy looks very promising.

5c. Mitochondrial alkaline Cerase can be efficiently inhibited with a secondary amine version of Cer (stearyl sphingosine that has been reduced to stearyl sphingosine) [41].

6. Blocking the conversion of ceramide to sphingomyelin

A portion of freshly synthesized Cer is diverted by reaction with a phospholipid, particularly phosphatidylcholine (lecithin), forming SM. The Cer is thus unavailable for producing apoptosis (although the hydrolysis of SM can be stimulated — section 3). The reaction is a simple, reversible transesterification, in which the phosphocholine moiety is attached to the Cer C–1 OH, leaving diacyl glycerol. A similar exchange between phosphorylcholamine and Cer yields Cer–phosphoethanol, which then gets methylated to form more SM. An elevated lecithin content in cells, seen in many tumors, minimizes the apoptogenic action of several Cer elevating agents. Conversely, subnormal lecithin content tends to produce apoptosis. The P–drug inhibitors of Cer glucosylation divert the extra Cer into SM synthesis, so such drugs should be used together with agents that lower tumor phospholipid content. Emulsification of anticancer drugs with the aid of lecithin should also be avoided.

Consistent with these observations are the findings that Cer inhibits lecithin synthesis and GlcCer stimulates it. Moreover Cer destroys lecithin and phosphatidylethanolamine by transferring the phospholipid arachidonate to their C–1 position (section 3b). Tumors, by keeping their Cer content low and GlcCer high thus stimulate their own growth.

6a. Phospholipases lower phospholipid levels, helping to keep Cer levels high. Several agents stimulate these enzymes. Cer–1–phosphate is a direct activator of cytosolic phospholipase A2 [42], a factor that needs study. Hexadecylphosphocholine probably acts this way, inducing an increase in ceramide level, lowering lecithin and SM, and producing apoptosis. It is considered too toxic to use in patients as a solitary drug but might well be useful at low dosages in the Cer cocktail. Other lecithin–like drugs might be better.

6b. Tamoxifen (section 4b) stimulates phospholipases C and D, lowering lecithin levels. This effect augments its inhibition of Cer glucosylation.

7. Slowing synthesis of sphingosine phosphate

Sphingosine–1–phosphate, like Cer–1–phosphate and decacylated SM (sphingosyl phosphocholine) is a highly potent stimulator of cell growth and proliferation, also producing a wide variety of physiological effects. Since it is formed by phosphorylation of sphingosine, the product of ceramidase action, it is important to slow Cer hydrolysis (section 5). It is possible to inhibit the kinase that attacks the free base with N,N–dimethyl sphingosine, N,N,N–trimethyl sphingosine, or L–three–sphinganine. The latter, also called safinol, showed some promise in cancer patients when used alone. One of these inhibitors would be useful if the ceramidases are not fully inhibited. No stimulator is yet available for the phosphatases acting on the above phosphate esters or on the lyase that cleaves sphingosine phosphate, but it might be useful to try synthesizing one.

The same possibility applies to the kinase that converts Cer to Cer–1–phosphate.

Unfortunately, sphingosine phosphate and Cer phosphate stimulate mitochondrial ceramidase, an additional reason to prevent their formation [42].

8. Stimulating glucosylceramide hydrolysis

8a. GlcCer stimulates cell growth and proliferation and generates the gangliosides, which prevent the pa-
tient’s immune system from attacking the abnormal components of tumors. Thus its synthesis should not only be slowed (section 4) but its hydrolysis (to yield Cer) should be stimulated. The glucosidase acting on GlcCer is normally activated by acidic phospholipids, and ingesting them could be useful. However, the natural acidic phospholipids are readily attacked by enzymes and might not last long enough to be useful. A search for a stable synthetic phospholipid should be made.

8b. The glucosidase also needs a small, heat-stable protein called saposin C, which stimulates the enzyme and protects it against proteolytic breakdown. Studies of saposin C showed that only a small portion of the peptide is needed for activity, and efforts have been made to synthesize an unnatural but stable mimic of this peptide. A 14-mer peptide analog, Prosaptide TX14(A), looks promising and has shown promise as a neurological deficit drug in human trials. While its ability to stimulate the glucosidase does not seem to have been measured, it produces the expected increase in gangliosides when added to neuroblastoma cells [43].

8c. GlcCer glucosidase catalyzes transglycosidation as well as hydrolysis, allowing the degradation of GlcCer to proceed faster than hydrolysis. Retinol and pentanol accept the glucose moiety, liberating Cer [44]. Individuals whose tumors possess good β-glucosidase activity (a glucosidase unable to attack GlcCer) may be able to destroy the tumor’s GlcCer rapidly. Retinol (Vitamin A) is a known antioxidant, helping to prevent the appearance of cancer. It undergoes oxidation to 4-keto retinol, retinoic acid, and 4-keto retinoic acid. Retinoic acid and the keto metabolites (conjugated allylic ketones) very likely exert anticancer activity [14]. The transglycosidation approach deserves more study.

8d. The human glucosidase that hydrolyzes GlcCer is commercially available for Gaucher disease patients (whose enzyme is too slow). Supplementing a cancer patient’s glucosidase with the medicinal enzyme may increase the tumor’s rate of GlcCer destruction and help force the tumor to accumulate Cer. While the enzyme is very expensive, it would not be needed for a long time.

9. Other Cer-controlling factors

The mechanisms by which Cer exerts its signals for cell function are under intense investigation, and these undoubtedly raise additional potentially useful possibilities for therapy [45].

Chlorpromazine, the anti-psychotic drug, increased the Cer concentration in mouse liver by 25% within 6 h after injection [46] and produced apoptosis in cultured CHO cells. Administered to mice bearing sarcoma 37 cells, chlorpromazine (at a high dosage) almost completely halted the growth of the tumors [47]. The cancer cells in the treated mice revealed formation of fatty granules, stained with Oil Red O, consistent with accumulation of Cer. However, the slow growth could be attributed to the heavy sedation of the mice. A more recent study with rats also showed that chlorpromazine produced apoptosis; in this study, the cells were in the thymus and revealed an increase of 15-fold in the degree of apoptosis [48]. The authors attributed the effect to interference with Ca²⁺ metabolism. Many reports of Cer effects have shown that marked changes in Ca²⁺ distribution can occur. Cytokines affect sphingolipid enzymes and might be useful to control but also may exert too many effects. The Cer content of cultured cells seems to rise rapidly as the cells approach confluency, which can make in vitro experiments difficult to interpret. Age of the patient may be an important variable, since GlcCer synthase decreases rapidly in late life.

Since so many established or proposed anticancer drugs contain an allylic alcohol moiety in their structure, they may work like ceramide, interfering with Coenzyme Q function in mitochondria or by activating protein phosphorylation and dephosphorylation. They differ from each other in the tissue of action or type of tumor that is attacked, so it seems worth using the most suitable one for each patient together with the Cer cocktail. In addition, the many antineoplastic drugs that contain an allylic ketone moiety may mimic my proposed ketone Ceramide and could then also be used together with the Cer-inducing cocktail. It is possible that they undergo reduction to allylic alcohols in the tumor, and mimic Cer more closely. Perhaps their main function is to decrease the tumor’s GSH content (section 3a).

The impact of dietary allylic alcohols and allylic ketones should not be overlooked, since so many foods contain them and so many are available in purified form. Curcumin (in Indian spice), indole–3-carbinol (in broccoli), genistein (in soy tofu), and conjugated linoleic acid (in milk fat) are commercially available and show usefulness in cancer studies. While this last item does not contain an allylic group, it is undoubtedly oxygenated in the body to yield an allylic alcohol. This is true of vitamin A, which is metabolized to allylic ketones (section 8c). Vitamin D3, normally formed in the skin by sunlight and activated by metabolic hydroxylation to form the allylic alcohol, seems to protect against the appearance of cancer by stimulating SM hydrolysis (section 3f). People living in northern latitudes or who work indoors have been shown to resist carcinogenesis by eating extra vitamine D. Another kind of dietary factor to consider is the need to eliminate the contamination of corn by a parasitic mold, which forms an inhibitor of Cer synthesis, fumonisin B1. This widely prevalent contaminant has been found at dangerous levels that chronically slow the acylation of sphingosine and keto sphinganine. The inhibitor, a sphingosine analog, tends to produce cancer.

Recent work with nitric oxide has shown that the chronic application of NO sources to cultured cells results in gradual Cer elevation, then apoptosis [49]. Other work has suggested there is a mutual stimulation relationship that should be considered.

Readers who plan to try the above recommendations in animals or patients should keep in mind the complexity of Cer’s interactions with normal tissues and tumors, as opposed to cultured cancer cells. It is essential to make many measurements of the changes induced by their poly-drug Cer-inducing cocktail. This review definitely does not list all the known (and unknown) relevant interactions.
REFERENCES


