Sphingosine kinase-1 is a downstream regulator of imatinib-induced apoptosis in chronic myeloid leukemia cells

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We examined the involvement of sphingosine kinase-1 (SphK1), which governs the ceramide/sphingosine-1-phosphate balance, in susceptibility to imatinib of either sensitive or resistant chronic myeloid leukemia cells. Imatinib-sensitive LAMA84-s displayed marked SphK1 inhibition coupled with increased content of ceramide and decreased pro-survival sphingosine-1-phosphate. Conversely, no changes in the sphingolipid metabolism were observed in LAMA84-r treated with imatinib. Overcoming imatinib resistance in LAMA84-r with farnesyltransferase or MEK/ERK inhibitors as well as with cytosine arabinoside led to SphK1 inhibition. Overexpression of SphK1 in LAMA84-s cells impaired apoptosis and inhibited the effects of imatinib on caspase-3 activation, cytochrome c and Smac release from mitochondria through modulation of Bim, Bcl-xL and Mcl-1 expression. Pharmacological inhibition of SphK1 with F-12509a or its silencing by siRNA induced apoptosis of both imatinib-sensitive and -resistant cells, suggesting that SphK1 inhibition was critical for apoptosis signaling. We also show that imatinib-sensitive and -resistant primary cells from chronic myeloid leukemia patients can be successfully killed in vitro by the F-12509a inhibitor. These results uncover the involvement of SphK1 in regulating imatinib-induced apoptosis and establish that SphK1 is a downstream effector of the Bcr-Abl/Ras/ERK pathway inhibited by imatinib but upstream regulator of Bcl-2 family members. Leukemia (2008) 22, 971–979; doi:10.1038/leu.2008.95; published online 10 April 2008

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Introduction

Bcr-Abl is a constitutively activated tyrosine kinase that is necessary and sufficient for the transformation of cells in vitro and induction of a chronic myeloid leukemia (CML)-like disease in mice.1 Imatinib (Glivec or Gleevec, STI571) inhibits Bcr-Abl tyrosine kinase activity and induces apoptosis of Bcr-Abl-positive leukemic cells. Although imatinib is highly effective in the treatment of CML, its continuous administration is associated with development of resistance, particularly in the advanced phase or blast crisis. Cells expressing high amounts of Bcr-Abl, as in blast crisis, are much less sensitive to imatinib and, more significantly, take a substantially shorter time for yielding a mutant subclone resistant to the inhibitor than cells with low expression levels, as in chronic phase.2 In vitro, a similar overexpression of the Bcr-Abl protein has been observed in CML cell lines after exposure to gradually increased doses of imatinib.3–5

The broadened knowledge of the various mechanisms of imatinib resistance has led to the development of novel strategies to address this problem. An attractive strategy relies on targeting Bcr-Abl downstream pathways for overcoming resistance. For example, farnesyltransferase inhibitors (FTIs) antagonize the oncogenicity of Ras, a protein that plays a central role in leukemogenic transformation by Bcr-Abl.6,7 Blocking Ras with FTI has been shown to successfully circumvent imatinib resistance in CML8 or models overexpressing Bcr-Abl,9 and phase I/II studies are under way with such compounds. The Raf kinase is a downstream target of Ras and a point of entry into the MEK/ERK pathway, which appears to be constitutively activated by Bcr-Abl.10–12 Imatinib-induced apoptosis is associated with ERK dephosphorylation in K562 cells13–15 and interference with the MEK/ERK activation with pharmacological inhibitors triggers apoptosis in Bcr-Abl-positive cells.16 Lastly, it has also been established that resistance to imatinib was not associated with resistance to cytosine arabinoside (Ara-C), a well-known antileukemic drug, suggesting that agents like Ara-C may be clinically useful in the management of imatinib-resistant patients.18

The sphingolipids ceramide and sphingosine-1-phosphate (S1P) are critical regulators of cell death and survival. Ceramide, the central molecule in sphingolipid metabolism, mediates apoptosis in response to a wide array of anticancer treatments. Conversely, S1P promotes cell survival in response to apoptotic stresses.17 Thus, a ‘sphingolipid biostat’ has been hypothesized where the dynamic balance between ceramide and S1P contents, and subsequent regulation of opposing signaling pathways, may be an important factor determining cell fate.18 A key regulator of this ceramide/S1P balance is sphingosine kinase-1 (SphK1)—an oncogenic enzyme that phosphorylates sphingosine (the catabolite of ceramide) to form S1P—because it reduces pro-apoptotic ceramide levels by driving sphingolipid metabolism toward the pro-survival S1P. Enforced expression of SphK1 results in inhibition of apoptosis induced by stress agents that increase ceramide levels.18–21 In contrast, inhibition of SphK1—which results in reduced levels of pro-survival S1P—has been associated with apoptosis induced by chemotherapy20–22 and radiotherapy.23 Interestingly, SphK1 inhibition is not seen in cancer cells resistant to either radiotherapy23 or chemotherapy,21 hence suggesting a role for SphK1 as an indicator of tumor cell sensitivity or resistance. Importantly, pharmacological or RNA interference (RNAi) inhibition of
SphK1 are sufficient to elicit apoptosis even in chemo- or radioresistant cancer cell lines, demonstrating the crucial role of SphK1 in cell survival. To further substantiate a role for SphK1 as an indicator of sensitivity or resistance in cancer cells, we have investigated its involvement in susceptibility to imatinib of either sensitive or resistant CML LAMA84 cells.

Materials and methods

Cell lines

LAMA84-s cells, established from patients with CML in blast crisis, were cultured in RPMI 1640 containing 10% FBS (fetal bovine serum) and LAMA84-r were grown in the same medium supplemented with 1 μM imatinib. FLAG epitope-tagged wild-type human SphK1 cDNA (complementary DNA) was used for stable transfection of LAMA84-s cells (gift of Dr Wattenberg). Experiments were conducted in the presence of FBS.

Patient samples

Peripheral blood specimens were obtained, after receiving informed consent, from newly diagnosed imatinib-naive patients (chronic phase) and patients who had developed resistance to imatinib (blast crisis). Mononuclear cells were separated on Lymphoprep (Axis-Shield PoC, Oslo, Norway) and cryopreserved prior to use. Imatinib-resistant samples were selected in which Bcr-Abl kinase domain mutations had been detected by pyrosequencing. These mutations were as follows: patient 5 (G250E), patient 6 (M244V), patient 7 (G250R) and patient 8 (T315I and F311L).

Materials

Culture media, serum and antibiotics were obtained from Invitrogen (Cergy-Pontoise, France). Imatinib was a gift of Dr Buchdunger. C2-ceramide (Biornol, Plymouth Meeting, PA, USA), Ac-DEVD-CMK (Bachem, Weil am Rhein, Germany) and F-12509a were prepared in DMSO. Alkaline phosphatase and Ara-C were from Sigma (Saint-Quentin Fallavier, France). Escherichia coli diacylglycerol kinase, octyl-α-glucopyranoside, manumycin and U0126 were from Calbiochem (Nottingham, UK). γ-[32P]-ATP (3000 mCi/mmol) was purchased from Perkin-Elmer (Courtaboeuf, France), and silica gel 60 high-performance TLC (thin layer chromatography) plates were from VWR (Fontenay sous Bois, France).

Cell line viability assay and staining of apoptotic nuclei

Viability of the cell lines was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay. Apoptosis was assessed by staining cells with 8 μg/ml DAPI (4,6-diamidino-2-phenylindole).

Viability assays of primary CML cells

Washed cells from each patient sample were seeded in triplicate in RPMI 1640 containing 10% FBS at a density of 10^6 cells/well in 24-well tissue culture plates. Doses of 0, 1 and 10 μM F-12509a or imatinib were then added to the cells. Aliquots were taken from each well at days 1, 5 and 7 after adding drugs, and cell viability was determined by trypan blue exclusion. Viabilities were normalized to those of vehicle controls for each drug (taken as being 100%) that were included for each sample.

RNA interference experiments

Transient interference was efficiently achieved by double-stranded SphK1-specific small interfering RNA (siRNA) as previously described. siRNA transfection (300 nmol) was performed according to the manufacturer’s instructions with Nucleofactor Solution V (Amaxa, Cologne, Germany). Cells were immediately cultured for 2 days in RPMI with FBS before cell viability experiments or SphK1 activity.

Western blot analysis and antibodies

Western blotting was carried out as previously described. Anti-caspase-3 (CST, Danvers, MA, USA), anti-PARP (CST), anti-c-Abl (Oncogene, Nottingham, UK), anti-FLAG (Sigma), anti-p44/42 MAP kinase (CST), anti-phospho-p44/42 MAP kinase (CST), anti-cytochrome c (BD Pharmingen, Le Pont-de-Clair, France), anti-cytochrome oxidase II (Molecular Probes, Cergy-Pontoise, France), anti-Smac/Diablo (R&D Systems, Lille, France), anti-XIAP (BD Pharmingen), anti-Mcl-1 (BD Pharmingen), anti-Bcl-xL (CST), anti-Bcl-2 (CST) and anti-Bim (Stressgen, Ann Arbor, MI, USA) were used as primary antibodies. Proteins were visualized by ECL detection system using anti-rabbit or anti-mouse HRP-conjugated IgG (Bio-Rad, Marnes la Coquette, France). Equal loading was confirmed by probing the blots with the anti-β-actin antibody (Sigma).

SphK1 assay and mass measurements of ceramide, S1P and phospholipids

SphK1 activity, ceramide and S1P levels normalized to total cellular phospholipids were measured as previously described.

Statistical analysis

The statistical significance of differences between the means of two groups was evaluated using the unpaired Student’s t-test. All statistical tests were two-sided and the level of significance was set at P<0.05. Calculations were performed using Instat (Graphpad, San Diego, CA, USA). Densitometry quantitation was determined using the ImageJ software (NIH, Bethesda, MD, USA).

Results

Imatinib-induced apoptosis is associated with SphK1 inhibition and alteration of the ceramide/S1P rheostat

In contrast to the parental LAMA84-s, the LAMA84-r cell line demonstrated full resistance to imatinib with respect to apoptosis (Supplementary Figure 1). Exposure of parental LAMA84-s cells to imatinib produced a sharp and rapid decrease (within 60 min) in SphK1 activity (Figure 1a). In parallel to SphK1 inhibition, imatinib elicited a rapid increase in ceramide (Figure 1b) and decrease in S1P contents (Figure 1c). The effect on SphK1 activity and S1P content was transient peaking at 60 min, after which SphK1 activity went back to normal (Figure 1a). Such temporary inhibitory effect on SphK1 activity during imatinib treatment might reflect the presence of serum in the medium, which is known to stimulate SphK1 activity. We detected a late and sustained elevation in ceramide starting within 6 h of treatment and increasing by 1.5–2-fold by 24 h (Figure 1b). It should be pointed out that the early peak of ceramide, reduction of S1P and SphK1 inhibition preceded the onset of apoptosis, as no activation of executioner caspase-3 could be found before at least 3–6 h of imatinib

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treatment (data not shown). Remarkably, differing from what was observed in the parental LAMA84-s cell line, we could not detect any significant changes in SphK1 activity, ceramide or S1P content in imatinib-resistant LAMA84-r cells (Figure 1).

SphK1 overexpression inhibits imatinib-induced apoptosis in LAMA84 cells

Transfection with SphK1 resulted in a significantly higher SphK1 expression (Figure 2a) and activity (Figure 2b). As depicted in Figure 2c, SphK1 overexpression rendered LAMA84/SphK1 cells markedly resistant to imatinib-induced cell death. The cytoprotective effect of enforced expression of SphK1 was associated with a strong inhibition of caspase-3 and PARP cleavage as well as degradation of XIAP, an endogenous inhibitor of executioner caspases (Figure 2d). The mechanism of protection induced by SphK1 overexpression was mitochondria-dependent as cytochrome c and Smac/Diablo efflux from mitochondria was strongly reduced in LAMA84/SphK1 as compared with LAMA84/Neo cells (Figure 2e). The expression of Bcl-2 family members—upstream regulators of apoptotic mitochondrial proteins release—was examined. As previously reported in primary CML cells or other Bcr-Abl-overexpressing cell lines,25–28 imatinib treatment was associated with downregulation of both cytoprotective Bcl-xL and Mcl-1 but not Bcl-2 and increased expression of the pro-apoptotic Bcl-2 member Bim in LAMA84/Neo cells. Noteworthy, these effects were remarkably inhibited in LAMA84/SphK1 cells (Figure 2f).

Overcoming imatinib resistance by inhibiting the Ras/MAPK signaling pathway requires SphK1 downregulation

As previously reported with another FTI,8 manumycin could overcome resistance to imatinib in LAMA84-r cells in a manner similar to that for the parental LAMA84-s cells (Figure 3a). SphK1 activity was strongly inhibited (within 60 min) by manumycin (Figure 3b). This was paralleled by a downregulation of the ceramide/S1P biostat with a decrease in S1P and increase in ceramide levels (Figure 3c). The role of SphK1 inhibition in manumycin-induced cell death was confirmed by cell viability assays that showed that SphK1-overexpressing LAMA84-s were >50% more resistant than LAMA84-s transfected with empty vector (Figure 3d).

It has been previously reported that imatinib could induce ERK dephosphorylation in K562 cells13–15 as well as in primary CML cells.29 ERK dephosphorylation could be observed in LAMA84-s cells as soon as 20–30 min of treatment with imatinib with a very sharp decline in ERK activity at 60 min (Figure 4a). As anticipated, we could not detect dephosphorylation of ERK in imatinib-resistant LAMA84-r cell extracts (Figure 4a). Interruption of the ERK signaling by the MEK inhibitor UO126 was seen within minutes of incubation both in LAMA84-r (Figure 4b) and LAMA84-s (data not shown). UO126-treated LAMA84-r underwent a rapid decrease in SphK1 activity (Figure 4c). Concomitantly, as a consequence of SphK1 inhibition, ceramide and S1P levels were, respectively, up and down (Figure 4c). Both LAMA84-s and LAMA84-r were more or less equally sensitive to UO126 (Figure 4d). Confirming that SphK1 downregulation was a consequence of ERK inhibition, enforced expression of SphK1 rendered LAMA84 cells significantly more resistant to UO126 (Figure 4e).

Ara-C triggers cell death in both LAMA84-s and LAMA84-r via downregulation of SphK1 activity

As previously reported,15 Ara-C treatment showed a dose-dependent cytotoxic effect on LAMA84 cells regardless of their sensitivity/resistance status to imatinib (Figure 5a). SphK1 activity was rapidly inhibited (<30 min) by Ara-C in imatinib-resistant LAMA84-r (Figure 5b, left) and parental LAMA84-s (data not shown), which was paralleled by a 50% increase in ceramide content and a 35% decrease in S1P (Figure 5b, right). As noted for imatinib (Figure 1b), manumycin (Figure 3c)
and UO126 (Figure 4c), we found a strong ceramide elevation at later times (4-24 h). Ceramide accumulation was not associated with significant changes in S1P levels or SphK1 activity, suggesting a different mechanism of production. As expected, SphK1 overexpression in LAMA84-s cells was clearly associated with an increased resistance of these cells to Ara-C (Figure 5c).

**Figure 2** Sustained sphingosine kinase-1 (SphK1) activity confers resistance to LAMA84. SphK1 expression (a) was analyzed by western blotting and basal SphK1 activity (b) was measured in LAMA84/Neo and LAMA84/SphK1 cells. (c) Cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test after treatment with 1 μM imatinib for the indicated times. Columns, mean of 10 independent experiments; bars, s.d. Two-tailed \( P \)-values: *** \( P < 0.001 \). (d) Caspase-3 activation, PARP cleavage and XIAP cleavage were analyzed by western blotting after treatment of LAMA84/Neo and LAMA84/SphK1 cells with or without 1 μM imatinib for 24 h. Arrowheads, the migration of full-length caspase-3 and XIAP. The proteolytically processed forms of caspase-3 (p20 and p17), PARP (p89) and XIAP (p45) are indicated. (e) Mitochondria-free cell extracts from LAMA84/Neo and LAMA84/SphK1 incubated with or without 1 μM imatinib for 24 h were analyzed by immunoblotting using anti-cytochrome c (Cyt c), anti-Smac/Diablo and anti-COX II (cytochrome oxidase serves as a marker for mitochondrial contamination of cytosolic extracts). A mitochondrial extract from untreated cells (mit. Fr. Con) was used as a positive control for Cyt c, Smac/Diablo and COX II. (f) Expression of Bim, Bcl-xL, Bcl-2 and Mcl-1 in cell extracts obtained as described in (d). For all western blotting experiments, similar results were obtained in three independent experiments.

SphK1 pharmacological inhibition or knockdown induces apoptosis in imatinib-resistant LAMA84-r cells

A substantial decrease in SphK1 activity was observed in SphK1 RNAi-treated cells compared with scrambled RNAi (Figure 6a). This was further illustrated by decreases in S1P content and SphK1 protein (Figure 6a, inset). The decrease in SphK1 activity was accompanied by a significant loss of cell viability in
imatinib-resistant LAMA84-r cells (Figure 6b). We next examined the impact of SphK1 pharmacological inhibition on cell viability. Under exposure to F-12509a, SphK1 activity was strongly inhibited in imatinib-resistant LAMA84-r cells (Figure 6c). Concomitant with SphK1 inhibition, a rapid drop in S1P and an increase in ceramide levels were observed (Figure 6d). Similar to imatinib treatment, long exposure (>24 h) to F-12509a was correlated with large increase in ceramide levels (Figure 6d), which occurred subsequent to the executioner phase of apoptosis, namely caspase-3 activation (Figure 6f). F-12509a induced a dose- and time-dependent cytotoxicity in imatinib-resistant cells (Figure 6e). The anti-proliferative effect of F-12509a was clearly related to apoptosis as manifested by the strong nuclear fragmentation observed in treated LAMA84-r cells compared with vehicle-treated control (Figure 6f, bottom). Executioner caspase-3 activation was distinctly seen as early as 6 h in LAMA84-r cells after exposure to F-12509a (Figure 6f, up).

F-12509a induces a significant reduction in viability of cells from imatinib-naive and imatinib-resistant patients

Pilot titration experiments indicated that the most effective cytotoxic doses of imatinib and F-12905a for primary CML cells were 1 and 10 μM, respectively, and that the most informative time for a detectable effect in liquid culture was around 1 week. In all four imatinib-naive samples, F-12509a induced a significant decline in viability by day 7, ranging from 24 to 42% of the vehicle control cultures, whereas that induced by imatinib was more varied (2–62% of control; Table 1). In three out of four imatinib-resistant patients the viability of cells after 7 days in culture in 10 μM F-12509a was 6–38% lower than that of imatinib-treated cells (Table 1). For the three imatinib-resistant samples, which were more sensitive to F-12509a than imatinib, viability was reduced by nearly 50% of the control (52%, patient 5) or by more than 50% of the control (27.4%, patient 6; 37.8%, patient 7). These latter reductions in viability are within the range seen for the imatinib-naive samples. In only one of the four imatinib-resistant patients (patient 8), was F-12509a less effective than imatinib at inducing cell death (38.5% vs 73.1% of control, respectively).

Discussion

In this study, we show that SphK1 acts as a sensor during imatinib-induced apoptosis of CML cells. SphK1 overexpression protects LAMA84 cells from imatinib by blocking the mitochondrial release of cytochrome c and Smac/Diablo, both implicated in the activation of executioner caspases. Such findings are consistent with our previous reports suggesting that SphK1 overexpression or addition of S1P inhibit apoptosis by interfering with the efflux of apoptogenic factors from mitochondria. Bcl-2 family members have been reported to affect imatinib sensitivity. On the one hand, imatinib-induced Bcl-xL and Mcl-1 down-regulation was inhibited by SphK1 overexpression. On the other hand, the pro-apoptotic Bcl-2 member Bim upregulation induced by imatinib was inhibited by SphK1 overexpression. SphK1 knockdown by RNAi strategy could induce significant cell viability loss both in imatinib-sensitive (data not shown) and -resistant LAMA84 cells. These data strongly suggest that SphK1 is required for cell survival and that decrease of SphK1 activity can be important for execution of cell death.

In CML cells, the oncoprotein Ras plays a key role in leukemogenic transformation by Bcr-Abl, and Ras inhibition by various FTIs can block the effect of Bcr-Abl reactivation. Furthermore, inhibition of MEK/ERK in Bcr-Abl-overexpressing K562 cells is sufficient to induce apoptosis. Here, we show that targeting Ras or MEK1/2 elicits SphK1 activity downregulation associated with a shift toward ceramide of the ceramide-to-S1P balance. Interestingly, SphK1 activation was demonstrated to be a direct consequence of ERK signaling and a downstream target of Ras. Thus, it is not surprising that selective inhibition of these two proteins leads to SphK1 activity downregulation. Importantly, overexpression of SphK1 in LAMA84 cells could protect from manumycin A- and U0126-induced cell death, clearly suggesting the involvement of SphK1 as a downstream element of the Ras pathway.

Before the advent of imatinib, Ara-C had a prominent role in the treatment of CML. Ara-C exerts antileukemic effect via mechanisms that are largely undetermined. Interestingly, resistance to imatinib in LAMA84 cells is not associated with
Imatinib inhibits ERK in LAMA84-s but not in LAMA84-r cells, and UO126 induces a sphingolipid-mediated cell death that is blocked by sphingosine kinase-1 (SphK1) overexpression. Expression of phosphorylated and basal ERK was analyzed by western blotting after treatment with 1 μM imatinib for the indicated times in LAMA84-s and LAMA84-r (a), or with 10 μM UO126 in LAMA84-r (b). Similar results were obtained in three independent experiments. (c) LAMA84-r cells were incubated for the indicated times with 10 μM UO126, then tested for SphK1 activity, ceramide (Cer) and sphingosine-1-phosphate (S1P) levels. (d) LAMA84-s and LAMA84-r cells were incubated for 72 h with 10 μM UO126 and cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). (e) Cell viability of LAMA84/Neo and LAMA84/SphK1 was assessed by MTT test after treatment for 72 h with 10 μM UO126. Columns, mean of eight independent experiments for MTT assays and four independent experiments for SphK1 activity and sphingolipid dosages; bars, s.d. Two-tailed $P$-values: *$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, not significant.

Cytosine arabinoside (Ara-C) equally induces a sphingolipid-mediated cell death of LAMA84-s and LAMA84-r that is inhibited by sphingosine kinase-1 (SphK1) overexpression. (a) LAMA84-s and LAMA84-r cells were incubated for 72 h with increasing concentrations of Ara-C and cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. (b) LAMA84-r cells were incubated for the indicated times with 10 μM Ara-C, then tested for SphK1 activity, ceramide (Cer) and sphingosine-1-phosphate (S1P) levels. (c) Cell viability of LAMA84/Neo and LAMA84/SphK1 was assessed by MTT test after treatment for 72 h with 10 μM Ara-C. Columns, mean of eight independent experiments for cell viability assays and five independent experiments for SphK1 activity and sphingolipid dosages; bars, s.d. Two-tailed $P$-values: *$P<0.05$; **$P<0.01$; ***$P<0.001$. 

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resistance to near-therapeutic doses of Ara-C, implying that
this drug could be efficient for imatinib-resistant patients.\textsuperscript{16}
Remarkably, we found that Ara-C-induced LAMA84 cell death
relied on SphK1 inhibition. Hence, both Ara-C- and imatinib-
induced dissociated signaling cascades seem to converge at the
SphK1 regulatory checkpoint.

Of note, the rapid and transient increase in ceramide after
treatment with imatinib, FTI, UO126, Ara-C or F-12509a was
followed by a delayed, persistent and robust elevation. Such
biphasic ceramide generation has been documented with other
pro-apoptotic stimuli in various leukemic cells\textsuperscript{36,37}
and is believed to play a role in amplification rather than initiation of
apoptosis.\textsuperscript{36} As a matter of fact, the rapid ceramide increase
triggered by imatinib occurred before executioner caspase
activation. It was not affected by executioner caspase inhibition
in contrast to the late and sustained ceramide elevation,
suggesting that this second ceramide generation occurred
downstream of caspases (data not shown).

Strategies to destroy cancer cells by increasing their ceramide
content or blocking accumulation of pro-survival S1P should
have a favorable therapeutic index. As previously shown in
multidrug-resistant HL-60 AML,\textsuperscript{21} the F-12509a SphK1 inhibitor
concomitantly blocked S1P biosynthesis and caused ceramide
accumulation, efficiently accomplishing this goal in one
single step.

More remarkably in the clinical context, F-12509a was
overall at least as effective as imatinib in killing CML primary
cells, irrespective of them being imatinib sensitive or resistant. It
is interesting to note that patient 8, the only one whose cells were less sensitive to F-12509a than to imatinib, had imatinib-resistant subclones with Abl kinase domain mutations F311L and T315I. The latter mutation is considered the most resistant to imatinib mesylate in chronic myeloid leukemia. Cancer Res 2005; 65: 8912–8919.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu).