Polyamines are highly regulated essential cations that are elevated in rapidly proliferating tissues, including diverse cancers. Expression analyses in neuroblastomas suggest that up-regulation of polyamine pro-synthetic enzymes and down-regulation of catabolic enzymes is associated with poor prognosis. Polyamine sufficiency may be required for MYCN oncogenicity in MYCN amplified neuroblastomas, and targeting polyamine homeostasis may therefore provide an attractive therapeutic approach. ODC1, an oncogenic MYCN target, is rate-limiting for polyamine synthesis, and is overexpressed in many cancers including neuroblastoma. Inhibition of ODC1 by difluoromethylornithine (DFMO) decreased tumor penetrance in TH-MYCN mice treated pre-emptively, and extended survival and synergized with chemotherapy in treating established tumors in both TH-MYCN and xenograft models. Efforts to augment DFMO activity, or otherwise maximally reduce polyamine levels, are focused on antagonizing polyamine uptake or augmenting polyamine export or catabolism. Since polyamine inhibition appears to be clinically well tolerated, these approaches, particularly when combined with chemotherapy, have great potential for improving neuroblastoma outcome in both MYCN amplified and non-MYCN amplified neuroblastomas.

Keywords: polyamines, MYCN, neuroblastoma, ODC1, DFMO

INTRODUCTION

Neuroblastoma originates from the primitive cells of the sympathetic nervous system and is the most common solid tumor of early childhood. It is an aggressive cancer that often presents with high risk clinical and genetic features. In these cases, despite the use of intense multimodal therapies, long-term survival rates remain below 50% (Maris et al., 2007). Current treatment regimens are also associated with substantial morbidity, so novel therapeutic strategies are urgently needed. MYCN amplification, identified in up to 30% of neuroblastomas, is a powerful and reliable marker of aggressive disease and is strongly prognostic of poor outcome (Cohn and Tweddle, 2004). As a transcription factor, MYCN induces and represses a large number of genes involved in multiple biological processes including cell growth and differentiation. However, the genes necessary or sufficient to initiate neuroblastoma tumorigenesis downstream of MYCN remain to be established.

The polyamine pathway is frequently deregulated in neuroblastoma, and a number of genes involved in polyamine homeostasis are known to be MYCN or c-MYC targets (Bello-Fernandez et al., 1993; Lutz et al., 1996; Fernandez et al., 2003; Li et al., 2003; Forshell et al., 2010), while the expression of others is linked to MYCN status (Hogarty et al., 2008; Roubehler et al., 2009). This suggests a mechanism by which MYCN may contribute to the malignant phenotype of neuroblastoma. Therapeutic approaches targeting the polyamine pathway may therefore provide an effective strategy for the treatment of high risk neuroblastoma, particularly in tumors dependent on deregulated Myc activity, such as those with MYCN amplification.

REGULATION OF THE POLYAMINE PATHWAY

Polyamines are positively charged multifunctional polycations derived from amino acids and found in all living organisms. They are indispensable for cell growth, differentiation, and cell survival and function by forming electrostatic bonds with negatively charged macromolecules to mediate a number of biological processes. These include DNA synthesis and stability, replication, transcription and translation, ribosome biogenesis, modulation of ion channels and receptors, and protein phosphorylation (Pegg, 1988; Panagiotidis et al., 1995; Johnson, 1996; Igarashi and Kashwagi, 2000; Childs et al., 2003; Gerner and Meyers, 2004; Pegg, 2006). Polyamines are also required for covalent activation of eIF5A, a major protein translation factor, via hypusination, a polyamine-dependent modification (Cooper et al., 1993). Whereas polyamine depletion leads to growth arrest, overexpression of these essential cations is cytotoxic (Pivin et al., 1993; Tobias and Kahana, 1995; Ray et al., 2001; Li et al., 2002). Therefore, tight regulation of intracellular polyamine levels is critical and is dependent on the proliferative state of the cell. Regulatory mechanisms include de novo synthesis, recycling via a back converting catalytic pathway and through transmembrane import and efflux (Gerner and Meyers, 2004; Casero and Marston, 2007). An overview of the polyamine pathway is shown in Figure 1.
FIGURE 1 | Regulation of the polyamines putrescine, spermidine and spermine by biosynthetic enzymes (shown in green) and catabolic enzymes (shown in red). Compounds and classes of compounds that target various aspects of polyamine regulation are shown in yellow: ODC1, ornithine decarboxylase; OAZ, antizyme; AZIN, antizyme inhibitor; SRM, spermidine synthase; SMS, spermine synthase; AMD1, adenosylmethionine decarboxylase; SAT1, spermine/spermidine N1-acetyltransferase; PAOX, polyamine oxidase; SMOX, spermine oxidase.

POLYAMINE BIOSYNTHESIS

The first rate-limiting enzyme in the polyamine pathway is ornithine decarboxylase (ODC1), which catalyzes the decarboxylation and conversion of ornithine, a product of the urea cycle, to the primary polyamine putrescine (Pegg, 2006). Putrescine is the precursor for spermidine and spermine synthesis, and is further processed into these more abundant polyamines by two aminopropyltransferases, spermidine synthase (SRM) and spermine synthase (SMS). The second rate-limiting enzyme, adenosylmethionine decarboxylase (AMD1), decarboxylates S-adenosylmethionine (SAM) to provide the aminopropyl donor for the conversions to spermidine and spermine. Both ODC1 and AMD1 are highly controlled at the transcriptional and post-transcriptional levels, and have among the shortest half-lives of any mammalian enzymes. In addition, ODC1 turnover is regulated by antizymes (OAZ1, OAZ2, and OAZ3) which in turn are controlled by antizyme inhibitors (AZIN1 and AZIN2). Antizymes initiate ODC1 degradation by binding the ODC monomer, inhibiting its activity and shunting ODC1 to the 26S proteasome for degradation (Li and Coffino, 1992; Murakami et al., 1992). Of the three antizymes, OAZ1 is the most effective at stimulating ODC1 degradation. Antizyme inhibitors antagonize the function of antizymes by mimicking ODC1 (Koguchi et al., 1997; Kanerva et al., 2008). They are highly homologous to ODC1, but lack enzymatic activity due to critical amino acid substitutions and bind antizymes with greater affinity than ODC1 (Albeck et al., 2008). Increased antizyme inhibitor activity therefore results in the release of ODC1 from the inactive ODC1-antizyme complex, which in turn increases the production of polyamines (Matsufuji et al., 1996; Mangold, 2006; Pegg, 2006). In addition, forced induction of AZIN1 in cell cultures has also been shown to increase polyamine uptake (Keren-Paz et al., 2006). Polyamine levels themselves act as down-regulators of both ODC1 and AMD1 and as up-regulators of antizymes by a feedback homeostasis mechanism.

POLYAMINE CATABOLISM

Polyamine catabolism allows for the re-utilization of polyamines as spermine is converted back to spermidine and spermidine back to putrescine. A number of key enzymes are involved in this process as shown in Figure 1. The degradation of polyamines depends on three enzymes: spermine/spermidine N1-acetyltransferase (SAT1), polyamine oxidase (PAOX), and spermine oxidase.
(SMOX). SAT1, a highly inducible cytosolic enzyme, acetylates spermine and spermidine (Casero and Pegg, 1993), which are then either exported from the cell, or oxidized by the peroxi-
somal enzyme PAOX, resulting in conversion to spermidine or
putrescine, H2O2 and 3-aminopropanol (Seiler, 1995). PAOX
preferentially catalyzes the oxidation of the N1-acetyl spermin/
sperridine produced by SAT1 activity, rather than spermine or
spermidine, whereas SMOX is a cytosolic enzyme which catalyzes
the oxidation of spermine directly to spermidine, without acetyl-
lation and produces H2O2 and 2-aminopropanol (Vujic et al.,
2002; Wang et al., 2003). Casero and Pegg, 2009, Pegg, 2009).
Mostly, PAOX is constitutively expressed and dependent on SAT1
as it is rate-limited by the availability of the acetylated spermi-
dine/spermidine (Casero and Pegg, 1993; Vujic et al., 2002). SAT1,
the rate limiting enzyme in polyamine catabolism, is therefore
extensively regulated at transcriptional and post-transcriptional
levels (Frogel-Petovic et al., 1993; Coleman et al., 1996), and
is a gatekeeper regulating flux through the polyamine pathway
(Kramer et al., 2008).

**TRANSMEMBRANE IMPORT AND EFFLUX**

Cellular polyamine levels are also regulated by transmembrane
transport where cells can take up polyamines from their sur-
roundings and also export them to the extracellular space, and this
can make a significant contribution to cellular polyamine levels.
Known polyamine transporters include SLCA2 (Uemura et al.,
2008) and SLCA2A16 (Aouida et al., 2010). SAT1 is co-localized
with the SLCA2 transporter and catalyzes the export of acetylated
polyamines via a polyamine/arginine exchange reaction, suggest-
ing a role for acetylation in polyamine efflux (Uemura et al.,
2008). SLCA2A16 has also been identified at a high affinity transporter
directing polyamine import in mammalian cells (Aouida et al.,
2010). Polyamine uptake by caveolar-dependent endocytosis has also
been identified (Roy et al., 2009). Polyamines are present in the
extracellular space from dietary intake, export from neigh-
boring cells and synthesis by intestinal bacteria. Such microen-
vironment polyamines provide a reservoir whereby polyamine
antagonized cancer cells can circumvent biosynthetic blockade
through augmented uptake.

**ABERRANT EXPRESSION WITHIN THE POLYAMINE PATHWAY IN NEUROBLASTOMA, AND THE ASSOCIATION WITH MYCN**

Polyamines are elevated in rapidly proliferating cells, including
cancer cells, and substantial evidence suggests cancer development
is associated with altered polyamine regulation. The biological
association between increased polyamines and tumor formation
is well established in numerous cancers including breast, prostate,
colon, skin carcinoma and neuroblastoma (Cipolla et al., 1993;
Leveque et al., 2000; Thomas and Thomas, 2003; Gerner and
Meyskens, 2004; Casero and Marton, 2007). There is also evidence
that increased polyamine biosynthesis is not just a consequence
of increased proliferation in these cells, but may be necessary
for the development of specific cancers (Gerner and Meyskens,
2004; Casero and Marton, 2007). The mechanism by which
MYCN amplification results in such a poor prognosis has yet
to be fully elucidated, and recent evidence suggests that its effect
on the polyamine pathway may play a critical role. A number of
polyamine genes have been shown to be c-MYC target genes
(ODC1, AMD1, and SMS) whereas others appear to be regu-
lated by MYC/MYCN (Bello-Fernandez et al., 1993; Fernandez
et al., 2003; Hogarty et al., 2008; Rounbehler et al., 2009; For-
shell et al., 2010). However, with the exception of ODC1 (Lutz
et al., 1996), the polyamine genes that are direct transcriptional
targets of MYCN remain to be established. It is highly likely
that polyamine synthesis may be specifically required to support
downstream MYCN-governed functions.

ODC1 is a well-established oncogene in its own right (Auvii-
nen et al., 1992), with high ODC1 activity associated with
tumor growth in several human cancers, including neuroblas-
toma (O’Brien et al., 1997; Janne et al., 1978; Scalabrin and
Ferlisi, 1981; Crozet et al., 1992; Mohan et al., 1999; Wallace
and Castale, 2001; Hogarty et al., 2008). The contribution of ODC1
activity to MYC-induced lymphomagenesis was examined in a
mouse model of B-cell lymphoma, the Eμ-Myc transgenic mouse.
In this model, ODC1 ablation inhibited lymphomagenesis, but
subsequent restoration of ODC1 activity promoted tumor onset
(Nilsson et al., 2005). In addition, enforced expression of ODC1
in the skin of transgenic mice led to increased tumor incidence
(O’Brien et al., 1997; Chen et al., 2000). In neuroblastoma there is
significant evidence that ODC1 is overexpressed in high risk dis-
ease. It is often co-amplified with MYCN or overexpressed, and
is associated with poor prognosis in both MYCN amplified
and non-MYCN amplified tumors (Hogarty et al., 2008; Rounbehler
et al., 2009; Geerts et al., 2010).

Evaluation of several polyamine genes included in the Neu-
roblastoma Prognosis Database (publically available at http://
home.cc.cancer.gov/oncology/oncogenomics/) revealed that
increased expression of biosynthetic SMS, AMD1, and AZIN,
and decreased expression of catabolic OAZ2 was associated with
decreased survival and poor prognosis as shown in Figure 2A.
The levels of SAT1 or SRF expression on the other hand, were
not prognostic of survival. However, all of these genes, including
SAT1 and SRF, were associated either positively or negatively with
MYCN amplification dependent on their biosynthetic or catabolic
role (Figure 2B). Since MYCN is upstream of the polyamine
biosynthesis pathway, this suggests a major role for MYCN in
regulating polyamine biosynthesis, and a mechanism by which
MYCN contributes to neuroblastoma development. Several stud-
ies support these findings. Geerts et al. (2010) found increased
ODC1 and reduced OAZ2 expression to be excellent predictors of
survival and poor prognosis in both MYCN amplified and non-
amplified neuroblastomas. OAZ1 and OAZ3 on the other hand
played no role in predicting survival. Transcriptome analysis of
101 primary neuroblastomas found several polyamine biosyn-
thetic genes, including ODC1, AMD1, SRF and SMS, to be
up-regulated in the MYCN amplified high risk cohort (and again
ODC1 expression was elevated in non-MYCN amplified high risk
group; Hogarty et al., 2008). OAZ2 was expressed at lower lev-
els in high risk MYCN amplified tumors but also significantly
reduced in non-MYCN amplified high risk tumors. In addition
the catabolic SMOX was decreased, while the level of SAT1 expres-
sion was not associated with any particular risk group (Hogarty
et al., 2008). These studies suggest a role for ODC1, and OAZ2,
FIGURE 2: Analysis of expression of the polyamine pathway regulators SMS, OAZ2, AMD1, AZIN1, SAT1, and SRM, and their association with neuroblastoma outcome. (A) Kaplan-Meier survival curves in the overall neuroblastoma cohort with dichotomization for high/low expression around the median. (B) Expression of polyamine pathway genes in the subsets of tumors with and without MYCN amplification. Data was obtained from the Neuroblastoma Prognosis Database (publicly available at http://home.ccr.cancer.gov/oncology/oncogenomics/).
independent of MYCN, in promoting an aggressive phenotype. Further evidence supporting this conclusion comes from the finding that ODC1 is not always co-amplified with MYCN in neuroblastomas, while copy number gain of ODC1 has been reported in half of high risk neuroblastomas without MYCN amplification, suggesting a mechanism by which the polyamine pathway is up-regulated in this subset (George et al., 1997; Mouss et al., 2007; Hogarty et al., 2008).

These data suggest that systemic alterations in polyamine metabolism correlate with MYCN amplification, but that polyamine enhancement in non-MYCN amplified tumors is also associated with high risk disease. Polyamine depletion strategies may be broadly effective against high risk tumors, rather than just MYCN amplified tumors.

**TARGETING POLYAMINE BIOSYNTHESIS AS A THERAPEUTIC APPROACH IN NEUROBLASTOMA**

Since elevated polyamines are sustained in rapidly proliferating cells and levels are increased in cancer tissues compared to surrounding tissues, suppression of polyamine biosynthesis provides an attractive therapeutic approach for many cancers. Inhibitors of the rate-limiting enzymes in polyamine biosynthesis, ODC1 and AMD1, have been developed and extensively tested in preclinical and clinical trials. a-difluoromethylornithine (DFMO) acts as a specific suicide inhibitor of ODC1 and is the most widely studied inhibitor of polyamine metabolism both as a chemotherapeutic and a chemopreventive agent (Meyskens and Gerner, 1999; Levin et al., 2000; Takahashi et al., 2000; Fabian et al., 2002; Levin et al., 2003). Exposure of a number of cancer cell lines, tumors and tissues to DFMO has shown a considerable decrease in intracellular putrescine concentrations, subsequent decreases in spermadine levels, and growth inhibition as a result of impaired synthesis of RNA, DNA, and proteins (Mamont et al., 1982; Sunkara and Rosenberger, 1987). Despite promising preclinical results, the anti-tumor activity of DFMO has to date failed to translate to the clinic. However, further investigations have shown additive and synergistic activities when used in combination therapies for the clinic. However, like DFMO, the novel second generation AMD1 inhibitor SAM486A treatment of p53 mutant neuroblastoma cells inhibited proliferation, and highly sensitive to SAM486A independent of their MYCN status (Koomoa et al., 2009). In these cells SAM486A functions by inducing p53, possibly through DNA damage induced by ATM, and by reducing Akt/PKB expression to induce apoptosis and inhibit cell proliferation (Koomoa et al., 2009). In addition, large increases in intracellular putrescine levels correlated with increased p53.

SAM486A is a derivative of the first generation AMD1 inhibitor mitoguazone (MG1661), and exerts potent and specific inhibition of ODC1 (Regenass et al., 1992, 1994). Its efficacy has been assessed in a number of cancer cells and animal systems, and has been tested in phase I and II clinical trials in adult cancers. However, like DFMO, when used as a single agent, results have been disappointing. In neuroblastoma, in vitro studies found p53 wild-type cells to be highly sensitive to SAM486A independent of their MYCN status (Koomoa et al., 2009). In these cells SAM486A functions by inducing p53, possibly through DNA damage induced by ATM, and by reducing Akt/PKB expression to induce apoptosis and inhibit cell proliferation (Koomoa et al., 2009). In addition, large increases in intracellular putrescine levels correlated with increased p53.

SAM486A treatment of p53 mutant neuroblastoma cells inhibited polyamine-dependent cell growth and caused a G2 arrest, which was further enhanced upon combination with DFMO. Neither compound, either alone or in combination, induced apoptosis (Walllick et al., 2005). Following removal of these inhibitors in the p53 mutant cells, the proliferative capacity of the cells was slow and only partially restored, but this was shown to be largely due to DFMO and not SAM486A. DFMO has been shown to induce cell cycle arrest in a p53 mutant neuroblastoma cell line via induction of two contradictory pathways: cell survival via PI3K/PKB signaling, and cell cycle arrest through p27kip1 phosphorylation (Walllick et al., 2005; Koomoa et al., 2008).

The disappointing clinical trials with either DFMO or SAM486A as single agents are likely due to activation of compensatory mechanisms following DFMO or SAM486A exposure. This allows intracellular polyamine levels to be maintained in a
cell upon loss of a single biosynthetic enzyme activity. Polyamines may be imported from extracellular pools, and compensatory induction of other biosynthetic enzymes or reduced polyamine catalysis may be involved.

AMDI has been shown to be up-regulated following ODC1 inhibition (Wallick et al., 2005) and it has been reported that combined DFM0 and SAM486A therapy is synergistic in neuroblastoma (Evangelou and Hogarty, 2009). This indicates that attacking the polyamine synthesis pathway with multiple compounds may be a more effective approach, particularly if the two rate-limiting enzymes are simultaneously inhibited. DFM0 and SAM486A are of particular interest because clinical trials in other cancer types have shown both inhibitors to be well tolerated, even at high doses, with only the occasional occurrence of reversible ototoxicity (DFMO only), nausea and mild neutropenia. In addition, DFM0 is already FDA approved as it is used in the treatment of African trypanosomiasis (Van Nieuwenhove et al., 1985; Spoerdena and Schechter, 1999).

As well as being tested as a chemotherapeutic agent, DFM0 has demonstrated promising results in human trials as a chemopreventive agent. This polyamine inhibitor has been shown to suppress skin carcinogenesis in patients with moderate to severe actinic keratoses (Alberts et al., 2000), and also slowed prostate cancer growth in men with a family history of prostate cancer (Simonsson et al., 2008). In addition, DFM0 in combination with sulindac, a SAT1 inducing COX2 inhibitor, resulted in a remarkable decrease in colon adenomas in patients with previous disease (Meyslens et al., 2008). There were no significant toxicities in any of these studies. Whilst chemopreventive approaches are not currently practical in neuroblastoma, the use of polyamine antagonists could prove useful in managing minimal residual disease (ABD) post-autologous stem cell transplantation in order to reduce the risk of relapse.

Another compound that targets polyamine biosynthesis is the SRM inhibitor, trans-4-methylcyclohexamide (4MCHA). This inhibitor has been tested in a B-cell lymphoma mouse model where 4MCHA had chemopreventive effects in vivo, but was not effective against established lymphomas (Shirahata et al., 1993; Forshell et al., 2010). SRM is a MYC target and interestingly, it was found to be more potently induced by MYC than ODC1 suggesting it may be important in MYC-induced oncogenesis.

OTHER MECHANISMS OF POLYAMINE DEPLETION

POLYAMINE ANALOGS AND INDUCTION OF POLYAMINE CATABOLISM

Polyamine analogs have a multistep role in depleting polyamine pools. They function by mimicking natural polyamines and subsequently lowering intracellular polyamine levels by feedback inhibition. The result is down-regulation of synthetic enzymes such as ODC1 and AMD1, and also induction of catabolic enzymes such as SAT1 and SMOX. Elevated levels of SAT1 increases export of acetyl-polyamines due to co-localization with a polyamine transporter (such as SLC3A2), and induction of SMOX results in polyamine salvaging activity (Pegg, 1988; Seiler et al., 1996), another mechanism of inhibiting this pathway includes antagonizing polyamine uptake. A number of compounds are under preclinical development, including t-hyline spermine (MQT-1426), N1-spermyl-L-hyamine (OR1202), and a spermine analog di-Lys(c4,acyl)-Spm (AMXT1301; Weeks et al., 2008;
intracellular polyamine levels (Xie et al., 2011b). A putrescine conjugate with anthracycles, Ant 4, was shown to induce cytotoxicity and subsequent apoptosis in a promyelocytic leukemia cell line (Palmer et al., 2009). Putrescine uptake was significantly reduced, demonstrating that this conjugate could successfully compete with its native polyamine for uptake. The spermine-podophyllotoxin conjugate F14512 has shown exceptional cytotoxicity in vitro, as well as inhibiting breast carcinoma in a xenograft model (Barret et al., 2008). Whilst preclinical data using this class of compound look promising, to date no clinical trials have taken place. It is attractive to speculate that combining a polyamine-chemotherapy conjugate with other polyamine depleting agents will facilitate the uptake of these conjugates and provide a more active targeted approach in reducing polyamines.

CONCLUSION
Many compounds targeting the polyamine pathway have been developed or are under development. However, those that have made it to clinical trials have produced limited effects, most likely as a result of compensatory mechanisms that allow a cell to circumvent polyamine depletion. Polyamine depletion compounds have been well tolerated clinically, and in combination with chemotherapeutic agents may have significant clinical potential in improving the outcome of patients with aggressive neuroblastoma. Furthermore, these compounds are likely to be effective in both MYCN amplified and non-MYCN amplified patients since polyamine deregulation has been observed in both tumor groups. A phase I clinical trial, coordinated by the New Approaches to Neuroblastoma Therapy (NANT) consortium, for the treatment of refractory neuroblastoma using high dose DFMO and celecoxib in combination with standard chemotherapy (cyclophosphamide and topotecan) is in development, and results from this study will be invaluable in determining the potential use of polyamine depletion for the treatment of neuroblastoma.

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cating that D-Lys(C16acyl)-Spm in combination with DFMO is successful in reducing intracellular polyamines (Burns et al., 2009). Available evidence indicates that high levels of expres-
sion of SLC22A16, a polyamine transporter, are prognostic of poor outcome in MYCN non-amplified metastatic neuroblastoma (http://pob.abcc.ncifcrf.gov/cgi-bin/K). Targeting this polyamine transporter may be required to effectively reduce intracellular polyamine levels.

The use of NSAIAs such as celecoxib and sulindac, has also been investigated, which function by influencing polyamine acetylation and export through up-regulation of SAT1 (Babbar et al., 2003). Celecoxib combined with anticancer agents induces synergistic and anti-proliferative effects (Shirode and Sylvester, 2010), exerting their chemopreemptive action by affecting SAT1. DFMO in combination with NSAIAs has been shown to suppress colorec-
tal carcinogenesis in murine models and in phase II clinical trials (Fischer et al., 2003; Gerner et al., 2007).

POLYAMINE-CHEMOTHERAPY CONJUGATES
Polyamines conjugated to cytotoxic drugs, such as naphthylim-
ides, anthracene, or anthraquinone, can be transported into cancer cells via the polyamine transporter system, and have been shown to exert potent anti-tumor effects (Tian et al., 2009; Xie et al., 2011a). Since the polyamine transporter is up-regulated in many tumor cells, these compounds may provide a targeted therapy, inhibiting cell proliferation through simultaneously delivering a cytotoxic drug, and also depleting intracellular polyamine content. A number of preclinical studies have produced promising results in a variety of cancers, although no studies have been carried out in neuroblastoma. In colorectal cancer cell lines, a naphthylmide-polyamine conjugate (NPC-16) in combination with DFMO produced enhanced apoptosis as a result of elevated SAT1 activity, and decreased NPC-16 uptake due to up-regulated SAT1 activity, and decreased
Gamble et al. Targeting polyamine metabolism in neuroblastoma

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