Sangivamycin-like Molecule 6 Exhibits Potent Anti-Multiple Myeloma Activity through Inhibition of Cyclin-Dependent Kinase-9

Nathan G. Dolloff1, Joshua E. Allen1,2, David T. Dicker1, Nicole Aqui3, Dan Vogl3, Jozef Malysz1, Giampaolo Talamo1, and Wafik S. El-Deiry1,2,4

Abstract
Despite significant treatment advances over the past decade, multiple myeloma (MM) remains largely incurable. In this study we found that MM cells were remarkably sensitive to the death-inducing effects of a new class of sangivamycin-like molecules (SLM). A panel of structurally related SLMs selectively induced apoptosis in MM cells but not other tumor or nonmalignant cell lines at submicromolar concentrations. SLM6 was the most active compound in vivo, where it was well tolerated and significantly inhibited growth and induced apoptosis of MM tumors. We determined that the anti-MM activity of SLM6 was mediated by direct inhibition of cyclin-dependent kinase 9 (CDK9), which resulted in transcriptional repression of oncogenes that are known to drive MM progression (MAF, CCND1, MYC, and others). Furthermore, SLM6 showed superior in vivo anti-MM activity more than the CDK inhibitor flavopiridol, which is currently in clinical trials for MM. These findings show that SLM6 is a novel CDK9 inhibitor with promising preclinical activity as an anti-MM agent.

Introduction
Multiple myeloma (MM) is the second most common hematologic cancer and accounts for approximately 10,000 deaths per year in the United States (1). The development of immunomodulatory drugs (thalidomide and lenalidomide) and the proteasome inhibitor bortezomib has revolutionized the clinical management of MM. Despite these advances, the disease remains largely incurable. It is therefore critical to develop new treatments, particularly ones with clinical activity in the refractory setting, to extend treatment options, prolong survival, and improve the quality of life for MM patients.

MM is characterized by the accumulation of plasma cells within the bone marrow and may spread to extramedullary sites in its late stages. Plasma cells are terminally differentiated B cells that are specialized for production and secretion of immunoglobulins (Ig). An early transcriptional event in the genesis of MM involves an erroneous class switch recombination involving the Ig heavy chain (IGH) gene (14q32). These illegitimate gene translocations juxtapose proto-oncogenes with potent enhancer sequences within the IGH locus leading to dysregulation of their expression (2). The most commonly dysregulated oncogenes in MM include FGFR3/MMSET, MAF, and CCND1 (3). Additional genetic events may be required to drive MM to a pathologic stage, as these translocations are also present in monoclonal gammopathy of undetermined significance, an asymptomatic precursor stage to MM (4). Whole genome sequencing analysis has revealed that MM is characterized by a diverse array of genetic abnormalities (5). Among these anomalies are somatic mutations in KRAS, NRAS, and TP53, as well as members of the NF-κB signaling pathway. In addition, rearrangement or translocation of the MYC gene is detected in 50% of MM patients and nearly all established MM cell lines (6).

The genetic heterogeneity of MM and the multitude of oncogenes and signaling pathways that drive MM development and progression pose a challenge to the development of molecular targeted therapies. Inhibitors of cyclin-dependent kinase-9 (CDK9) may simultaneously target multiple oncogenic pathways by disrupting gene transcription—a potentially advantageous therapeutic strategy in a heterogeneous disease such as MM. CDK9 is a subunit of the positive-transcription elongation factor b (P-TEFb) complex, which regulates mammalian gene transcription by phosphorylating the carboxy-terminus of RNA polymerase II at Ser2, a modification that initiates

Therapeutic Discovery

Mol Cancer Ther; 11(11); 2321–30. ©2012 AACR.
the elongation phase of transcription (7). Inhibitors of CDK9 and P-TEFb have shown preclinical activity in MM. For example, the bromodomain and extraterminal (BET) inhibitor JQ1 showed preclinical anti-MM activity through a mechanism that involves displacing BRD4 and blocking the recruitment of P-TEFb to c-Myc target genes (8). Also, broad-spectrum CDK inhibitors with activity against CDK9 have shown activity against MM and are currently in clinical development (9–11).

Sangivamycin is a nucleoside analog that was isolated from Streptomyces rimosus (12), and subsequently found to possess potent antitumor and antiretroviral activity (13, 14). A phase I trial of sangivamycin in the 1960s showed the safety of this compound in humans, however, no follow-up clinical studies were conducted (15). The anticancer activity of sangivamycin has been attributed to pleiotropic effects including inhibition of protein kinase C (PKC; 16). We recently identified a class of small molecules with structural homology to sangivamycin [sangivamycin-like molecules (SLM)] in a high throughput cell-based drug screen for compounds that overcome hypoxia-induced resistance to apoptosis in preclinical models of colon cancer (17). The mechanistic effects of SLMs closely resembled those of dual GSK-3β/CDK1 inhibitors, although the precise molecular targets of SLMs have not been conclusively elucidated. Furthermore, the activity of SLMs in tumor types other than colorectal cancer has not been thoroughly examined. Here, we show a selective sensitivity of MM cells to SLMs, and in vivo screening of a panel of SLM-related structures identified SLM6 as an active and well tolerated lead compound for further development. A candidate approach led us to identify CDK9 as the critical molecular target responsible for mediating the potent anti-MM activity of SLM6. This work shows the mechanism, molecular target, and potential of SLM6 as a novel agent for the treatment of MM, a disease that recurs nearly 100% of the time and requires additional therapies to improve patient survival and therapeutic options.

Materials and Methods

Cell lines, reagents, and antibodies

Cancer cell lines were purchased from American Type Culture Collection and maintained in the growth media recommended by the supplier at 37°C and 5% CO2. Human fetal osteoblasts (hFOB) were kindly provided by Dr. Alessandro Fatatis (Drexel University College of Medicine, Philadelphia, PA) and were grown in DMEM/F12 media supplemented with 10% FBS and Geneticin (400 μg/mL). Cell lines were routinely verified using the CrystalGenetics Assay (Promega) as per the manufacturer’s instructions. Linear regression analysis was conducted to calculate inhibitory concentration 50 (IC50) values. For analysis of apoptosis, cells were collected, fixed, and stained with an active caspase-3-specific antibody (BD Pharmingen) or an antibody that specifically recognizes single stranded DNA fragments (Enzo Life Sciences) and analyzed by fluorescence-activated cell sorting (FACS) analysis.

In vivo studies

Hairless severe combined immunodeficient (SCID) mice were housed and maintained in accordance with the Institutional Animal Care and Use Committee and state and federal guidelines for the humane treatment and care of laboratory animals. NCI-H929 cells were injected subcutaneously into the rear flank of mice at a density of 5 × 10⁶ cells per injection in PBS/Matrigel (v:v) and 200 μL total volume. When tumors were between 300 and 400 mm³ in volume, tumor-bearing mice were randomized, and treatments were initiated. All drugs were delivered by intraperitoneal injection in PBS containing less than 0.1% dimethyl sulfoxide (DMSO). Tumor volumes were monitored over time by caliper measurements. For short-term in vivo experiments, animals were given 1 dose of SLM6, then tumors were harvested after 48 hours, fixed and embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) or immunostained with antibodies specific for cleaved caspase-3 and cleaved caspase-8 (both from Cell Signaling Technology). For RPMI-8226/NIH-3T3 tumors, 5 × 10⁶ RPMI-8226 cells stably expressing GFP were mixed with 1 × 10⁶ NIH-3T3 mouse fibroblasts in a PBS/Matrigel solution (v:v) and 200 μL total volume, and then injected subcutaneously. Treatments were initiated after 2 weeks when tumors were palpable and had grown to a volume of 100 to 200 mm³. For noninvasive fluorescence imaging studies, mice were anesthetized and imaged using the CRi Maestro system and related quantitative multispectral imaging software.

Reverse transcription PCR

RNA was isolated at the indicated time point using RNeasy Mini Kit (Qiagen) according to manufacturer’s
protocol. Reverse transcription (RT) was conducted with 1 μg of RNA using SuperScript II (Invitrogen) according to the manufacturer’s protocol. PCR was conducted using FastStart Taq DNA Polymerase (Roche) according to the manufacturer’s protocol. Primers were: cyclin D1 (sense, 5' - CCTGCTTTGCGGGCAAGAC-3'; antisense, 5'-ACGCCGTGGTGCACTGTAAG-3') and c-myc (sense, 5'-CGACCCGGACGACGAGACCT-3'; antisense, 5'-GTTCGGCCGTCCGTCTT-3'). PCR products were electrophoresed on a 2% agarose gel and visualized using ethidium bromide.

Isolation of CD138-positive MM patient plasma cells

Aspirates from bone marrow needle biopsies were collected and deidentified for research use with approval from the University of Pennsylvania Institutional Review Board. White blood cells were isolated from whole bone marrow by Ficoll–Paque gradient separation. CD138-positive plasma cells were isolated using a magnetic bead-conjugated anti-CD138 antibody (Miltenyi Biotec) and MininMACS Separator (Miltenyi Biotec). The purity of CD138-positive populations was verified by FACS analysis. CD138-positive plasma cells were cultured in RPMI 1640 media supplemented with 10% FBS and gentamycin, and grown at 37°C under 5% CO2.

In vitro kinase assays

In vitro kinase assays with CDK1/cyclin B, CDK2/cyclin A, CDK4/cyclin D1, CDK7/cyclin H, CDK9/cyclin K, and CDK9/cyclin T1 recombinant protein complexes were conducted using the HotSpot assay platform as described previously (18).

Results

MM cell lines are most sensitive to the cytotoxic effects of SLMs

To test the activity of SLMs across multiple tumor types, we determined IC50 values for SLM3 in cell viability assays using human cell lines derived from multiple tumor types, including colon (HCT116, HCT116 p53−/−, HCT116 Bax−/−, SW620, HT29, Caco-2), esophageal (TE1, TE2, TE7, TE11, TE12), pancreatic (MiaPaca-2, Panc-1), lung (H460, H1299), breast (MDA-MB-231, MDA-MB-468, BT474, SKBR3), hepatocellular (SNU449, Hep3B, HepG2), glioma (A172, U87, T98G), and MM (RPMI-8226, NCI-H929, U266B1, MM.1S). We chose a 24-hour treatment time to focus the assay readout on the cytotoxic effects of SLM3 and minimize cytostatic effects. The potency of SLM3 varied considerably between tumor types of different origin (Fig. 1A; IC50 range: 0.2–>4 μmol/L). Breast, colon, lung, hepatocellular, glioma, and 1 of 2 pancreatic cancer cell lines were relatively resistant to SLM3, with
IC50's > 2 μmol/L (Fig. 1A and Supplementary Fig. S1). Esophageal cells were relatively more sensitive to SLM3 with IC50's of ≥1 μmol/L. Most notably, SLM3 exhibited striking cytotoxic effects in MM cells, where IC50's ranged from 200 to 400 nmol/L (Fig. 1A). We also confirmed this activity in primary CD138-positive plasma cells from MM patient bone marrow biopsies (Fig. 1B). SLM3 showed superior activity compared with other nucleoside analogs (5'-fluorouracil, gemcitabine, and cladribine; Fig. 1C), demonstrating a specific sensitivity of MM cells to SLM3 as opposed to a general sensitivity to cytotoxic agents of similar structure. We tested the anti-MM activity of other SLM molecules that were identified in the NCI DTP library. We found that SLM3 (NSC 188491), the HCl salt of SLM3 (NSC 742838, a.k.a. SMA-838), SLM5 (NSC 107512), SLM6 (NSC 107517), SLM7 (NSC 131663), and sangivamycin (NSC 65346) significantly reduced MM cell viability at concentrations less than 250 nmol/L (Fig. 1D).

**SLMs selectively induce apoptosis of MM cells but not other tumor or normal cell types**

The fact that SLMs rapidly and robustly reduced MM cell viability suggested they actively induced cell death, possibly by apoptosis. We tested this hypothesis by investigating the expression of apoptotic markers in response to SLM3 treatment. We found that SLM3 induced a dose-dependent increase in the apoptotic markers cleaved caspase-3 (Fig. 2A) and cleaved PARP (Fig. 2B). SLM3 activated caspase-8 and caspase-9 in a dose-dependent manner, further demonstrating the activation of apoptotic effectors (Fig. 2C). In comparison to MM cells, SLM3 induced negligible levels of apoptosis in cell lines derived from other tumor types (H460, A172, HT29, HepG2) and nontransformed cells [hFOB and normal human lung fibroblasts (MRC-5); Fig. 2D]. Furthermore, other SLMs and sangivamycin induced significant levels of MM cell apoptosis (60%–80%; Fig. 2E). Thus, SLMs as an entire class of small molecules with structural homology to sangivamycin have robust single agent anti-MM activity with the ability to selectively induce apoptosis in MM cells.

**SLM6 shows the most anti-MM efficacy in vivo**

To identify the SLM(s) with the most efficacy in vivo, we tested the antitumor activity of several SLM structures in an MM subcutaneous plasmacytoma model. For dose selection, we used a dose range based on available toxicity data provided by NCI DTP, and conducted pilot studies in a small cohort of mice to qualitatively assess toxicity. On the basis of body condition scoring, we observed no overt
toxicity after a single dose of 25 mg/kg SLM3 HCl, 5 mg/kg for SLM5 and SLM7, and 1 mg/kg for SLM6. These doses were then given by weekly intraperitoneal injections in tumor-bearing mice. SLM5 and SLM7 delayed tumor growth by 7 days relative to vehicle-treated controls, and although these responses reached statistical significance they were short lived (Fig. 3A). In comparison, SLM6 showed the most significant antitumor activity, which was sustained for 5 weeks after only a single dose on day 1. The HCl salt of SLM3 had no effect on the growth of MM tumors. We then tested for apoptotic markers in tumors from mice that received short-term SLM6 treatment (48 hours). We detected regions of intense cleaved caspase-3 and cleaved caspase-8 immu-

nostaining in tumors from SLM6-treated mice, whereas negligible staining was found in control tumors (Fig. 3B). Like SLM3, SLM6 showed selective killing and induction of apoptosis in MM cells in vitro compared with tumor cells from other tissues of origin (Fig. 3C and D) and CD34+ hematopoietic stem cells from normal human donors (Fig. 3E). SLM6 showed no signs of systemic toxicity, which we determined by serum biochemistry in immunocompetent mice (C57BL/6; Supplementary Fig. S2A). Furthermore, SLM6 showed no effects on normal hematopoiesis in vivo other than modest thrombocytopenia, which was determined by complete blood count with differential in C57BL/6 mice (Supplementary Fig. S2B).

Because of the superior in vivo profile of SLM6, we
chose to focus our subsequent mechanistic studies on this molecule.

**Inhibition of CDK9 is critical to the anti-MM activity of SLM6**

We next took a candidate approach to identify molecular targets of SLM6 that mediate its anti-MM activity. Previous work on the molecular effects of SLM3 and sangivamycin, by our group and others, suggested that the antitumor activity of these compounds may be mediated by inhibition of multiple molecular targets. Sangivamycin is known to inhibit PKC (16), and SLM3 (also known as 4-amino-6-hydrazinopyrazo[2,3-d]-pyrimidine-5-carboxamide (ARC; ref. 19) was shown to have modest activity against PKC and CDK1 (17). We first took a pharmacologic approach to test the role of GSK-3β and CDK1 in SLM6-mediated MM cytotoxicity. We measured the effects on MM cell viability of selective GSK-3β (lithium chloride, LiCl) and CDK1 (purvalanol A) inhibitors alone and in combination. In comparison to SLM6, neither LiCl nor purvalanol A reduced MM cell viability by themselves or in combination at concentrations that effectively inhibited their designated molecular targets (Supplementary Fig. S3). Similar results were obtained with GSK-3β inhibitor sc-24020 (data not shown). Because the combined inhibition of GSK-3β and CDK1 was not toxic to MM cells, we deduced that these molecular targets alone could not account for the anti-MM activity of SLM6.

To test the activity of SLM6 against PKC, we stimulated MM cells with phorbol-12-myristate-13-acetate (phorbol ester), an analog of diacylglycerol that directly binds to and activates PKC. We then measured the effects of SLM6 on PKC activity by analyzing the autophosphorylation of pan PKC isoforms (a site homologous to Ser660 on PKC βII) and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), which is activated downstream of PKC. SLM6 had no effect on phorbol ester-induced PKC or ERK phosphorylation, whereas sangivamycin significantly reduced phosphorylation of both kinases (Supplementary Fig. S4). Thus, SLM6 does not affect PKC signaling at concentrations that induce MM cell death, leading us to conclude that PKC was not a critical mediator of SLM6-induced MM cell death.

We next tested the activity of SLM6 against CDK9 signaling. We found that SLM6 inhibited phosphorylation of CDK9 at Thr186, an autophosphorylation site critical to the kinase activity of P-TEFb (21, 22). SLM6 also inhibited phosphorylation of RNA polymerase II at the CDK9-specific Ser2 site at concentrations as low as 250 nmol/L (Fig. 4A). Interestingly, SLM6 as well as flavopiridol, an inhibitor of CDK9 and other CDKs, more effectively blocked autophosphorylation of the 55-kDa isoform of CDK9 compared with the 44 kDa form. Unlike flavopiridol, SLM6 had no effect on the phosphorylation of RNA polymerase II at Ser5, a CDK7-specific site (Fig. 4A). In vitro kinase assays confirmed that SLM6 potently and directly inhibited of CDK9/cyclin K and CDK9/cyclin T1 kinase activity with IC50's of 280 nmol/L and 133 nmol/L, respectively (Fig. 4B and Supplementary Fig. S5). SLM6 also inhibited CDK1/cyclin B and CDK2/cyclin A with IC50's less than 300 nmol/L, but showed little activity toward CDK4/cyclin D1 and CDK7/cyclin H complexes (IC50 > 10 μmol/L; Fig. 4B and Supplementary Fig. S5). In comparison, flavopiridol inhibited all CDKs tested, with high potency against CDK1, CDK2, CDK4, and CDK9 (i.e., IC50 < 50 nmol/L) and less potency toward CDK7 (IC50 of approximately 4 μmol/L; Supplementary Fig. S5). We treated MM cells with various CDK inhibitors (i.e., alsterpaullone, roscovitine, purvalanol A, RO3306, flavopiridol, and SLM6) and found that only the inhibitors with activity toward CDK9 (i.e., SLM6, flavopiridol, and micromolar concentrations of alsterpaullone) were able to reduce the viability of MM cells (Fig. 4C and D). These findings show that inhibition of CDK9 is paramount to the selective anti-MM activity of SLM6.

**SLM6 represses oncogenic gene translation products**

Given the role of CDK9 in the regulation of transcription, we next investigated the impact of SLM6 treatment on the expression of oncogenes that are known to drive the progression of MM. With regard to the MM cell lines used in this study, U266B1 cells overexpress cyclin D1 due to an insertion of the IGH gene sequence upstream of the CCND1 transcriptional start site (23), and RPMI-8226 express high levels of c-MAF due to a gene rearrangement (24). In addition, c-MYC is highly expressed in NCI-H292 and RPMI-8226 cells, and L-MYC is highly expressed in U266B1 cells because of rearrangements of these respective genes (6). We found that treatment with SLM6 repressed the expression of cyclin D1 in U266B1 cells and c-Maf expression in RPMI-8226 cells in a time-dependent manner (Fig. 5A and B). SLM6 also repressed c-Myc protein levels in RPMI-8226 and NCI-H292 cells, and suppressed L-Myc expression in U266B1 cells (Fig. 5A and B, data not shown). RT-PCR analysis showed that SLM6 decreased c-Myc and CCND1 mRNA transcripts in RPMI-8226 and U266B1 cells, respectively (Fig. 5C), suggesting that SLM6 represses these oncogenes at the transcriptional level. Only CDK inhibitors with activity toward CDK9 were capable of downregulating c-Myc in RPMI-8226 cells (Fig. 5D), suggesting that CDK9 inhibition is critical to the repression of MM oncogenes by SLM6.

**SLM6 has more potent anti-MM activity in vitro than flavopiridol and is highly active in combination with bortezomib**

The multi-CDK inhibitor flavopiridol potently induces apoptosis of MM cells in vitro and is being evaluated in human trials, although objective clinical responses have thus far been limited (25, 26). We next conducted in vitro studies comparing the anti-MM activity of SLM6 to...
flavopiridol. We developed a model whereby GFP-expressing RPMI-8226 MM cells were subcutaneously coinjected with NIH3T3 mouse fibroblasts. This coinjection strategy increased tumor take rate to more than 90% \((\text{n} = 100)\) compared with 0% for RPMI-8226 cells alone \((\text{n} = 20)\) or 40% for NCI-H929 cells alone \((\text{n} = 70)\). As GFP was stably expressed in RPMI-8226 cells, MM tumor burden could be visualized and distinguished from unlabeled fibroblasts using noninvasive fluorescence imaging techniques (Fig. 6A). Immunohistochemical analysis revealed that tumors were composed of \(>95\%\) CD138 \(^{+}\) MM plasma cells (Fig. 6A). We treated mice with weekly doses of SLM6 \((0.5 \text{ mg/kg})\), flavopiridol \((5.0 \text{ mg/kg})\), or vehicle \((\text{PBS})\) and measured tumor growth over time by caliper measurements and noninvasive fluorescence imaging. With results similar to what was observed in the NCI-H929 xenograft model, SLM6 significantly reduced the size of MM tumors relative to vehicle-treated controls, with anti-MM effects being evident within 7 days of the first treatment (Fig. 6B and C). Flavopiridol, in comparison, at a dose 10 times higher than SLM6 showed no anti-MM activity in this model. The repeated dosing of SLM6 had no effect on mouse body weight or body condition scoring (data not shown), suggesting that multiple doses of SLM6 at 0.5 mg/kg were efficacious as well as nontoxic to mice.

The proteasome inhibitor bortezomib is U.S. Food and Drug Administration-approved for frontline MM treatment. Despite high initial response rates, bortezomib eventually loses its efficacy. Therefore, we next determined if SLM6 could enhance the activity of bortezomib in MM cells. We treated NCI-H929 as well as CD138 \(^{+}\) MM patient bone marrow cells with increasing concentrations of SLM6 and bortezomib (Supplementary Fig. S6). MM cell lines and primary patient plasma cells were sensitive to both SLM6 and bortezomib as single agents, and the combined effects of the drugs was additive rather than synergistic. Nevertheless, the combination of SLM6 and bortezomib was highly toxic to MM cells, suggesting that this may be an effective therapeutic combination.

**Discussion**

The primary objective of this study was to continue the development of a new class of SLMs by testing their activity across tumor cell lines from different tissues of origin. Our cell screening approach revealed that MM cell
lines and primary patient plasma cells were remarkably more sensitive (3–10 times) to single-agent SLM3 than other tumor types, and we therefore focused our study on characterizing and investigating the molecular mechanism of activity of SLMs in MM. MM cells were relatively insensitive to other nucleoside analogs (5'-fluorouracil, gemcitabine, and cladribine) as well as drugs with specific molecular targets (LiCl, purvalanol A, RO3306, and roscovitine), demonstrating a unique sensitivity to SLMs rather than a general hypersensitivity to therapeutic agents. Our initial screening approach investigated the effects of SLM3, which was the focal molecule from our previous work (17). We expanded our drug candidates to include other structurally related SLMs and found that like SLM3, other SLMs effectively reduced viability and induced apoptosis of MM cells.

In vitro drug activity does not necessarily translate to in vivo efficacy. To prioritize individual SLM structures based on their drug-like properties, we tested each compound in an in vivo MM plasmacytoma model. SLM6 showed the most efficacy in vivo, producing robust and sustained antitumor responses with limited toxicity after a single dose of 1 mg/kg. Further in vivo testing revealed that repeated dosing of SLM6 at a dose of 0.5 mg/kg was also tolerable and active against MM tumors. Future studies should expand the dosing regimens of SLM6 to test more doses, schedules, and routes of delivery.

We took a candidate approach to determining the anti-MM mechanism of action of SLM6. We found that SLM6 potently and directly inhibited CDK9 and repressed the expression of oncogenes that are overexpressed in MM cell lines as a result of gene translocations involving the IGH gene locus (MAF, CCND1, and MYC). It is likely that the simultaneous downregulation of multiple genes contributes to the anti-MM activity of SLM6. However, given the known roles of c-Maf, cyclin D1, and c-Myc in the maintenance and progression of MM, their transcriptional repression likely plays a pivotal role in the anti-MM activity of SLM6. We found that SLM6 also inhibits CDK1 and CDK2, although pharmacologic studies using other CDK inhibitors revealed that only those with activity against CDK9 were capable of inducing MM cell death. This shows that the activity of SLM6 against CDK9 is critical to its apoptosis-inducing effects in MM cells.

A biologic explanation for the high sensitivity of MM cells to SLM6 may be related to the instability of the oncoproteins that are overexpressed in MM. We found the protein half-lives of c-Maf, cyclin D1, c-Myc, to range between 15 minutes and 1 hour (Supplementary Fig. S7), whereas the half-lives of nononcogenic housekeeping proteins, such as glycogen synthase, the nuclear GTPase Ran, ribosomal S6 protein (S6), and the retinoblastoma tumor suppressor gene (Rb), all exceeded 24 hours.
This suggests that MM-associated oncoproteins are in a constant state of turnover and require perpetual replenishment by de novo synthesis, and therefore their expression and activity may be altered more rapidly and significantly by an agent that disrupts transcription. We propose a model for the anti-MM activity of SLM6 (Fig. 6D), where unstable oncoproteins are expressed at high rates due to recombination errors involving the IGH gene. These oncogenes induce transformation and drive cell proliferation while preventing the differentiation and apoptosis of MM plasma cells. Inhibition of CDK9 function by SLM6 interrupts gene transcription, quickly leading to degradation of MM oncoproteins because of their instability and short protein half-lives. As MM cells are highly dependent on these oncogenic signals, their loss of expression results in rapid apoptosis.

Our study focused on a new class of experimental small molecules, SLMs, and the observation that these compounds were highly cytotoxic to MM cells at low nanomolar concentrations. We determined that SLM6 was the most promising molecule of this class given its favorable activity in vivo. Our mechanistic studies found CDK9 was the critical molecular target of SLM6 that was responsible for mediating its anti-MM activity. In support of the role for targeting CDK9 in the treatment of MM, other CDK9 inhibitors have shown similar activity against MM cells. These compounds include flavopiridol, SNS-032, AT7519, and the BET bromodomain inhibitor JQ1, which seem to indirectly inhibit CDK9 by disrupting the recruitment of
the P-TEFb to c-Myc target genes (8–11). We show that through CDK9 inhibition, SLM6 represses multiple oncogenic gene translocation products in MM cells, a favorable therapeutic strategy in a disease that is driven by a diverse array of genetic abnormalities involving numerous oncogenes. In conclusion, SLM6 is a novel CDK9 inhibitor and a promising agent for the treatment of MM that warrants further development—efforts that are currently underway by our group.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: N.G. Dolloff, D. Vogl
Development of methodology: N.G. Dolloff
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.G. Dolloff, J.E. Allen, D.T. Dicker, W.S. El-Deiry
Writing, review, and/or revision of the manuscript: N.G. Dolloff, J.E. Allen, D. Vogl, J. Malyasz, G. Talamo

References


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N.G. Dolloff, D.T. Dicker, N. Aqui, G. Talamo
Study supervision: N.G. Dolloff

Interpreting immunohistochemical verification of myeloma cells: J. Malyasz

Acknowledgments

The authors thank the Penn State Hershey College of Medicine Microscopy and Histology Core Facility for technical assistance with our histo logic analyses.

Grant Support

The work was funded in part by support from NIH grants CA141395, CA123528, CA105008, and CA123273, as well as from Penn State Hershey Cancer Institute laboratory start-up funds to W.S. El-Deiry.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 6, 2012; revised July 18, 2012; accepted August 20, 2012; published OnlineFirst September 10, 2012.
Molecular Cancer Therapeutics

Sangivamycin-like Molecule 6 Exhibits Potent Anti-Multiple Myeloma Activity through Inhibition of Cyclin-Dependent Kinase-9

Nathan G. Dolloff, Joshua E. Allen, David T. Dicker, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-12-0578

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2012/09/10/1535-7163.MCT-12-0578.DC1

Cited articles
This article cites 26 articles, 11 of which you can access for free at:
http://mct.aacrjournals.org/content/11/11/2321.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/11/11/2321.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.