Redox Modulation of Adjacent Thiols in VLA-4 by AS101 Converts Myeloid Leukemia Cells from a Drug-Resistant to Drug-Sensitive State

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Abstract

Interaction between the integrin VLA-4 on acute myelogenous leukemia (AML) cells with stromal fibronectin is a decisive factor in chemotherapeutic resistance. In this study, we provide a rationale for a drug repositioning strategy to blunt integrin activation in AML cells and restore their sensitivity to chemotherapy. Specifically, we demonstrate that the nontoxic tellurium compound AS101, currently being evaluated in clinical trials, can abrogate the acquired resistance of AML. Mechanistic investigations revealed that AS101 caused redox inactivation of adjacent thiols in the exofacial domain of VLA-4 after its ligation to stromal fibronectin. This effect triggered cytoskeletal conformational changes that decreased PI3K/Akt/Bcl2 signaling, an obligatory step in chemosensitization by AS101. In a mouse xenograft of AML derived from patient leukemic cells with high VLA-4 expression and activity, we demonstrated that AS101 abrogated drug resistance and prolonged survival in mice receiving chemotherapy. Decreased integrin activity was confirmed on AML cells in vivo. The chemosensitizing activity of AS101 persisted in hosts with defective adaptive and innate immunity, consistent with evidence that integrin deactivation was not mediated by heightening immune attack. Our findings provide a mechanistic rationale to reposition the experimental clinical agent, AS101, to degrade VLA-4–mediated chemoresistance and improve clinical responses in patients with AML. Cancer Res; 74(11); 1–12. ©2014 AACR.
Accumulated evidence suggests that much of the biologic activity of AS101 is directly related to its specific chemical interactions with cysteine thiol residues. The Te(VI)-thiol chemical bond may lead to conformational change or disulfide bond formation in a specific protein, possibly resulting in the loss of its biologic activity, if the thiol residue is essential for that function (19, 20). Indeed, we demonstrated that the specific redox-modulating activities of AS101 result in a variety of beneficial biologic effects: inhibition of interleukin (IL)-10 (20) resulting in tumor sensitization (18); neuroprotection in both Parkinson disease models (20); and ischemic stroke (21), all mediated by the Te(IV) redox chemistry of the compound. Likewise, the protective mechanism of AS101 against homocysteine toxicity was shown to be directly mediated by its chemical reactivity, whereby AS101 reacted with homocysteine to form homocystine, the less toxic disulfide form of homocysteine (22).

Vicinal thiols are closely spaced protein thiols in native proteins that undergo reversible conversions with disulfide bonds. Vicinal thiols do not necessarily have to be in close proximity to the primary amino acid sequence of a protein to be brought into close opposition by protein folding (23). Vicinal thiol-containing proteins regulate a variety of receptors and redox regulation proteins (24, 25). These thiols are also found on cell surfaces (26). Vicinal thiols in VLA-4 and, in particular on VLA on AML cells, have not been yet described.

On the basis of the thiol-Te interaction of AS101, the present study reveals a unique approach in regulating cellular VLA-4 activity, via redox modulation of vicinal thiols on VLA-4 by AS101, and explores its role in the conversion of resistant to chemotherapy-sensitive human myeloid leukemic cells both in vitro and in vivo.

Materials and Methods
Reagents, plasmid construction, and PCR analysis are described in Supplementary Materials.

Cells
The human AML cells (U937 and HL-60) and the mouse myelomonocytic leukemia cells (Wehi-3B) were obtained from the American Type Culture Collection. The DNA profiles characteristic of cells was authenticated by short tandem repeat analysis. Cells were cultured in RPMI-1640 containing 10% fetal calf serum at 37°C with 5% CO2 and 95% air. Patients’ leukemic cells were obtained from 14 newly diagnosed AML patients before chemotherapy, following approval by the Institutional Ethics Committee upon receipt of patients’ informed consent. Six-well plates (Costar) were coated with either 5 μg/mL fibronectin, 1 μg/mL rVCAM-1, or 2% bovine serum albumin (BSA).

FACS analysis
VLA-4 or VLA-5 expression on AML cells was determined by fluorescence-activated cell sorting (FACS) after incubation with primary (mouse anti-human Cd49d and anti-cd49e) and secondary antibodies (fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G) and finally with phycoerythrin-conjugated CD45 antibodies. Blast cells were first identified by CD45/SSC gating in all cases of patients with AML as described by Lacombe and colleagues (27). Expression of active β1 on U937 leukemic cells was determined with anti-human HUTS-21 monoclonal antibody (mAb), which reacts with an activation-dependent epitope on the VLA-β1 chain. For cell-cycle distribution studies, cells were treated with RNase and stained with propidium iodide. DNA content was measured using a FACStar plus (Becton Dickinson) flow cytometer using Cell Quest software.

Cell surface labeling, immunoprecipitation, and Western blotting
Cells were pretreated with or without fibronectin, phenylarsine oxide (PAO), or AS101. After 60 minutes, cells were washed and incubated with 2,3-dimercapto-1-propanesulfonic acid (DMPS) or 2-mercaptoethanol for further 30 minutes. Washed cells were incubated with biotin-BMCC (1-biotinamido-4-[4′-(maleimidomethyl)cyclohexane-carboxamido]butane), the sulfhydryl group modification reagent, to tag unpaired cysteines, in serum-free RPMI-1640 medium at room temperature for 1 hour with rotation. After removal of unbound biotin-BMCC by centrifugation, cells were washed and lysed. Cell lysates were subjected to immunoprecipitation with anti-α4 or anti-β1 antibodies. For detection of biotin-BMCC, blots were incubated with horseradish peroxidase-conjugated monoclonal anti-biotin antibody. Immunoprecipitation and Western blot analysis was performed as described (20).

Attachment assay
Of note, 96-wells plates were coated with 80 μL of fibronectin or VCAM-1 or BSA. Cells with or without AS101 were incubated in the wells for one hour. Thereafter, cells were washed three times. The attached cells were tested by the XTT (2,3-bis[2-methoxy-4-nitro-S-sulfophenyl]H-tetrazolium-5-carboxanilide inner salt) assay at 450 nm.

Enrichment of human leukemic CD45+ cells
Human CD45+ cells were enriched from total spleen cells using the MACS magnetic cell sorter (Miltenyi Biotec) according to the manufacturer’s instructions, obtaining purity of about 95%. Purified cells were used freshly for attachment assays.

Actin shape index
A quantitative estimate of the actin shape changes was obtained as previously described (28). Briefly, actin shape index was calculated as P2/4πS, where P and S are the perimeter and the surface of the cell, respectively. These values were obtained by thresholding images of phalloidin staining to outline the polymerized actin. A perfectly circular shape of the polymerized actin yields a shape index of 1, and departure from a circle yields a shape index larger than 1. Actin reorganization was determined following 30 minutes of activation, which is the best time for observing this process in these cells.

Animals
Seven- to 8-week-old male SCID mice were obtained from Harlan Laboratories. NOD.CgPrkdc<scid> Il2rg<tm1Wjl>/SzJ (NSG) mice were purchased from The Jackson Laboratory. Animal experiments were performed in accordance with
approved Institutional protocols. Mice were irradiated at 2 Gy and were then administered patients’ leukemic cells ($1 \times 10^7$), intravenously (i.v.). SCID mice transplanted with patients’ leukemic cells were treated with AS101 (0.5 mkg/kg) or PBS intraperitoneally (i.p.) three times weekly as specified in figure legends. ARA-C was injected intraperitoneally at days 3 or 4 (40 mg).

**FRET analysis**

Fluorescence resonance energy transfer (FRET) was measured by the donor-sensitized acceptor fluorescence technique as previously described (29). Three sets of filters were used: one optimized for donor fluorescence (excitation, 468 nm; emission, 475–505 nm), a second for acceptor fluorescence (excitation, 514 nm; emission, 530 nm longpass; LP), and a third for FRET (excitation, 468 nm; emission, 530 nm LP). FRET was corrected, and the FRET efficiency was determined (Supplementary Methods).

**Statistical analysis**

Results are expressed as mean ± SE. Differences in cell viability between groups in response to chemotherapy were analyzed using two-way ANOVA. Differences in survival curves between groups were calculated by the Kaplan–Meier method. $P < 0.05$ was considered statistically significant.

**Results**

**AS101 sensitizes leukemic cells to chemotherapy-induced death via inhibition of VLA-4 activity**

In the present study, we show that AS101 increases the sensitivity of human AML cells to chemotherapy and prevents the minimal remaining of resistant cells in a xenograft mouse model. Furthermore, the mechanism of action of this phenomenon is defined.

Figure 1 shows that AS101 significantly sensitizes the human AML leukemic cells U937 (Fig. 1A) and HL-60 (Fig. 1B) to

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**Figure 1.** Effect of fibronectin (FN) or VCAM-1 on chemosensitivity of AML cell lines. U937 (A) or HL-60 cells (B) were incubated with ARA-C on fibronectin (i), VCAM-1 (ii), or BSA (iii) in the presence of various concentrations of AS101, with or without ARA-C at $10^{-6}$, $10^{-5}$ mol/L, or DNR at 0.005 μg/mL. After 24 hours, the percentage of cell viability was assessed by the XTT assay. Data are shown as mean ± SE of three independent experiments.
chemotherapy-induced death in a dose-dependent manner only when cells are cultured on (i) fibronectin, (ii) but not on VCAM-1 (iii) or on BSA. The decrease in cell viability has apoptotic features (Supplementary Fig. S1) as reflected by increased annexin binding (Supplementary Fig. S1A) and increased caspase-3,7 activity (Supplementary Fig. S1B). U937 cells responded well to chemotherapy when cultured on BSA or VCAM-1 but were poorly responsive on fibronectin (Fig. 1 and Supplementary Fig. S1). Thus, AS101 disrupts the interaction between fibronectin and leukemic cells, resulting in enhanced sensitivity to drug-induced apoptosis.

Because fibronectin is a ligand for \( \alpha_\text{V}\beta_\text{4} \) (VLA-4), abundantly expressed on both leukemic cell lines examined, we studied the potential effect of AS101 on the activity of this integrin. For this purpose, we used the FRET technique to investigate the spatial proximity of \( \alpha_\text{v} \) and \( \beta_\text{4} \)-cytoplasmic domains in living cells in the presence or absence of fibronectin and AS101. Figure 2A shows that in the presence of fibronectin, U937 AML cell cultures exerted a significant decrease in FRET efficiency compared with its absence (21.8 ± 5.9 vs. 44.7 ± 3.9; \( P < 0.05 \)). Furthermore, treatment of AML cells with AS101 in the presence of fibronectin significantly increased FRET efficiency compared with fibronectin alone (43 ± 3.9 vs. 21.8 ± 5.9; \( P < 0.005 \); controls presented in Supplementary Fig. S1C). Moreover, treatment of AML cells with AS101 in the presence of fibronectin reduced the expression of the active \( \beta_\text{1} \) form in a dose-dependent manner (Fig. 2B). Collectively, these results suggest that in the presence of fibronectin, AS101 inhibits the activation of \( \alpha_\text{V}\beta_\text{4} \). Moreover, treatment of U937 cells, cultured on fibronectin-coated plates, with neutralizing anti-VLA-4 antibody, significantly enhanced leukemic cells sensitivity to chemotherapy-induced death (Fig. 2C), whereas addition of AS101 did not further enhance this sensitivity. Furthermore, neutralizing anti-VLA-5 antibodies did not affect cell sensitivity to chemotherapy (Fig. 2C). These results suggest that U937 cells acquire drug resistance via \( \alpha_\text{V}\beta_\text{3} \), but not \( \alpha_\text{V}\beta_\text{4} \), and VLA-4 is an important target for AS101’s sensitization of leukemic cells to chemotherapy-induced death. As further proof for integrin deactivation by AS101, Fig. 2D shows that AS101 prevents attachment of AML cells to both VCAM-1 (a VLA-4 ligand) and fibronectin (ligand to VLA-4 and VLA-5; Fig. 2D and E). With respect to cells cytoskeletal behavior in the presence of fibronectin, U937 cells exhibited normal actin rearrangement as shown in Fig. 2F. Cells were well spread and demonstrated radial morphology. However, cells preincubated with AS101 for 15 minutes displayed significantly aberrant spreading behavior (\( P < 0.0001 \); Fig. 2F), as indicated by the actin shape index and found to be similar to cells seeded over Poly-L-Lysine (PLL) only. These findings demonstrate that AS101 impairs normal cytoskeletal behavior. This is in line with the results shown in Fig. 2G showing decreased expression of pFAK in treated cells.

Furthermore, using the highly VLA-4–expressing mouse myelomonocytic leukemia cells, Wehi-3B, cocultured on syngeneic bone marrow stromal cells, we show that treatment with AS101 abrogates leukemic cells resistance to Ara-C (Supplementary Fig. S2A), suggesting a potential sensitizing effect of myeloid leukemic cells in vivo.

The VLA-4 integrin activity on AML cells can be directly affected by AS101 by redox rearrangements within vicinal thiols in the extracellular integrin domain, resulting in physiologic consequences

Many of the beneficial effects of AS101 were shown to be attributed to redox modulation by the compound (20–22). The following experiments show that the VLA-4 integrin activity on AML cells can be regulated by vicinal thiols redox rearrangements and that this mechanism of action exerted by AS101 has physiologic consequences both in vitro and in vivo. Supplementary Fig. S2B shows that treatment of U937 cells with AS101 or with the sulfhydryl blocker 5,50-dithiobis(2-nitrobenzoic acid) (DTNB), which is membrane impermeable, significantly increased leukemic cells sensitivity to chemotherapy-induced death. AS101 did not potentiate DTNB activity, suggesting that the membrane target of AS101 and DTNB responsible for increased drug sensitivity in the presence of the VLA-4 ligand may be similar. Importantly, when U937 cells were cultured in the presence of fibronectin and labeled with the sulfhydryl-binding chemical BMCC, the immunoprecipitated \( \alpha_\text{V}\beta_\text{4} \)-chain, but not the \( \beta_\text{1} \)-chain, contained exposed sulphydryl groups. These thiol groups were diminished following treatment with AS101. Furthermore, biotin-BMCC labeling was found on both \( \alpha_\text{V}\beta_\text{4} \) and \( \beta_\text{1} \)-peptides when the cells were pre-reduced with diithiothreitol (DTT). AS101 significantly lowered this thiol labeling on both chains (Supplementary Fig. S3A). Importantly, the thiol residues on the \( \alpha_\text{4} \)-chain were diminished in AML cells treated with the vicinal dithiol-binding agent, PAO either when thiols were labeled with the membrane impermeable sulphydryl-binding chemical 3-N-maleimidylpropionyl-biotin (MBP; Fig. 3A) or with BMCC (Supplementary Fig. S3B). The addition of the vicinal thiol containing reagent DMPS after incubation with PAO or with AS101 reversed the inhibition of both compounds labeling (Fig. 2F, lane 5 vs. lane 4 and lane 8 vs. lane 7). In contrast, the monothiol \( \beta_\text{1} \)-mercaptoethanol had minimal effect on reversing the inhibition of labeling. Overall, these data thus showed that ligand-binding conditions alter the sulphydryl group exposure on the \( \alpha_\text{V} \) but not the \( \beta_\text{1} \)-chain and that the exposure is different from nonspecific reduction by DTT. Furthermore, fibronectin binding results in exposure of vicinal thiols on the extracellular integrin regions of VLA-4 and these are diminished following treatment with AS101. Notably, the ability of AS101 to alter vicinal thiols on the VLA-4 chain had physiologic implications, because as shown in Fig. 3B, alteration of vicinal thiols, but not that of monothiols by AS101, resulted in the sensitization of AML cells to chemotherapy. Interestingly, no exposed sulphydryl groups were found on the immunoprecipitated \( \alpha_\text{V} \)-chain of U937 cells cultured in the presence of fibronectin (Fig. 3C).

The resistance of AML patients’ leukemic cells expressing high VLA-4 to chemotherapy may be converted by redox modulation of the VLA-4 integrin

We then asked whether the sensitizing effect of AS101 in the presence of fibronectin, as shown in two human AML cell lines, is also exerted in leukemic cells from untreated patients with AML. For this purpose, leukemic cells from 12 patients were analyzed for VLA-4 and VLA-5 expression. Although leukemic
cells from all patients expressed high levels of VLA-5 (≥80%), 8 patients exhibited high expression of VLA-4 (≥75% of cells), whereas four patients exhibited very low expression of VLA-4 (≤15% of cells; data not shown). Figure 3D–G shows that leukemic cells from patients with AML, that express high VLA-4, slightly respond to ARA-C (10⁻⁶ mol/L) in vitro when plated on fibronectin (D) as opposed to BSA (E). Treatment of AML cultures in the presence of fibronectin, with AS101 significantly increased their sensitivity to drug-induced apoptosis in a dose-dependent manner (A). On the other hand,
leukemic cells from patients with AML that express very low VLA-4 to chemotherapy, whereas AS101 did not further enhance this sensitivity (Fig. 4B). Moreover, overexpression of Akt in

Figure 3. Vicinal thiols on VLA-4, but not on VLA-5 are oxidized by AS101 and play an important role in AS101’s sensitization of leukemic cells to chemotherapy. A, U937 cells were cultured on fibronectin in the presence or absence of AS101 (1 μg/mL), PAO (5 μmol/L), DMPS (50 μmol/L), or jME (100 μmol/L) for 60 minutes. After washing, some cultures were supplemented with DMPS or jME for further 30 minutes and washed and labeled with biotin-MBP. The α4-chain was immunoprecipitated and blotted with either anti-α4 or anti-biotin. 1-control PBS; 2-control DMPS; 3-control jME. The results represent one of three experiments performed. B, U937 cells were cultured on fibronectin in the presence or absence of AS101 (1 μg/mL), PAO (5 μmol/L), DMPS (50 μmol/L), or jME (100 μmol/L) for 60 minutes. After washing, some cultures were supplemented with DMPS or jME for further 30 minutes and thereafter ARA-C (10⁻⁵ mol/L) or PBS. Cell viability was assessed by XTT. Data are shown as mean± SE of three independent experiments. *, P < 0.05; †, P < 0.001; ††, P < 0.0005. C, U937 cells were cultured on fibronectin or BSA in the presence or absence of AS101 (1 μg/mL) or DTT(10⁻⁵ mol/L) for 60 minutes and labeled with biotin-MBP. The α5-chain was immunoprecipitated and blotted with either anti-α5 or anti-biotin. The results represent one of three experiments performed. Leukemic cells from patients with AML with high VLA-4 are more resistant to chemotherapy and are sensitized by AS101. Patients with AML were monitored for VLA-expression by FACS. Cells from patients expressing high (n = 8) or low (n = 4) VLA-4 were cultured on fibronectin (D and F) or on BSA (E and G) with or without ARA-C (10⁻⁵ mol/L) and AS101. Cell viability was assessed by XTT. *, P < 0.001 versus the relevant doses of AS101 in the control group. FN, fibronectin.

Signaling alterations involved in enhanced drug sensitivity induced by AS101

Inhibition of VLA-4 activity by AS101 was associated with decreased PI3K/Akt/BCl-2 signaling and this effect was related to the enhanced drug sensitivity induced by AS101. Figure 4A shows that AS101 decreases the expression of pAkt in U937 cells plated on fibronectin in a dose-dependent manner. Pharmacologic inhibition of PI3K with LY294002 significantly increased drug sensitivity of leukemic cells when cells were plated on fibronectin, whereas AS101 did not further enhance this sensitivity (Fig. 4B). Moreover, overexpression of Akt in
leukemic cells (Fig. 4C) abolished the sensitizing effect of AS101 to drug-induced death when leukemic cells were plated on fibronectin as reflected by both cell viability (Fig. 4D) and sub-G1 accumulation assays (Supplementary Fig. S6). These data collectively suggest that inhibition of VLA-4 activity by AS101 decreases the expression of pAkt and this property, at least in part, mediates AS101’s sensitizing activity. Similarly, Bcl-2, a downstream substrate of Akt, was also shown to mediate AS101’s sensitizing activity. Overexpression of Bcl-2 abolished AS101’s sensitizing effect in the presence of fibronectin (Fig. 5A and B). Moreover, transient transfection of Bcl-2 and bronectin (Fig. 5C) partially but significantly restored leukemic cells’ sensitivity to chemotherapy, whereas addition of AS101 slightly but insignificantly further enhanced this sensitivity (Fig. 5D), suggesting Bcl-2 as a mediator of AS101’s activity.

Conversion of resistant to chemotherapy-sensitive human myeloid leukemic cells in vivo by AS101

We then asked whether the VLA-4 inactivation by AS101 translates into enhanced therapeutic effects in vivo. For this purpose, we inoculated leukemic cells from either U937 cell lines or from patients with AML intravenously to SCID mice, and monitored their organ distribution by PCR using the human α-satellite sequence. Eight days after U937 inoculation (Supplementary Fig. S7A) or 4 days after inoculation of leukemic cells from a patient with AML expressing high VLA-4 (Fig. 6A), leukemic cells resided only within the bone marrow. Although they persisted in the bone marrow of both AS101 or ARA-C–treated mice, they were totally eliminated from the bone marrow of mice subjected to combined treatment with AS101+ARA-C (Fig. 6A and Supplementary Fig. S7A). This therapeutic effect persisted at 60 days after U937 cell implantation (Supplementary Fig. S7A). At 14 days after implantation, leukemic cells from both origins were detected in all organs examined (Fig. 6B and Supplementary Fig. S7A). Nevertheless, no detectable human AML cells were found in organs of AS101+ARA-C–treated mice (Fig. 6B). Importantly, the improved sensitivity of leukemic cells to chemotherapy following treatment with AS101 was reflected by the increased survival of mice implanted with U937 cells (Supplementary Fig. S7B). Moreover, the combined treatment of mice inoculated with leukemic cells from a patient with AML expressing high VLA-4 significantly increased mice survival (Fig. 6C) as opposed to the relative insensitivity to ARA-C alone, whereas mice inoculated with leukemic cells from a patient with AML expressing low VLA-4, responded well to chemotherapy alone, yielding 80% survival (Fig. 6D). Cotreatment with AS101+ARA-C further insignificantly increased mice survival. To preclude the possibility that AS101 may potentially alter the innate immune response to the leukemia cells, which can be integrin α4β1 driven, we used the NSG (NOD SCID GAMMA) mice with profound immunologic multidysfunction in both adaptive and innate immunologic function. Figure 7A shows that at 14 days after implantation, leukemic cells were detected in all organs examined. Nevertheless, no detectable human AML cells were found in organs of AS101+ARA-C–treated mice (Fig. 7A). Furthermore, AS101 is shown to inhibit integrin activity in these mice in vivo. Figure 7B shows that treatment with AS101 inhibits attachment of leukemic cells, enriched from mice spleens, to both VCAM-1 and fibronectin. These data collectively suggest that high VLA-4 expression and activity in AML leukemic cells confer resistance to chemotherapy and VLA-4 is a target for AS101 in sensitization of AML cells in vivo.

Discussion

The novelty of this study resides within the concept that redox modulation of vicinal thiols on cellular VLA-4 on AML leukemic cells by agents such as AS101 alters VLA-activity, converting resistant to drug-sensitive cells, enabling the eradication of residual leukemic cells. In a xenograft model...
of AML, the combination of chemotherapy (cytarabine) with AS101 produced improved survival. This is the first time in which this mechanism of VLA-4 regulation is demonstrated to have physiologic meanings in AML both in vitro and in vivo. Furthermore, this is the first report in which AS101's activity is shown to be mediated by alteration of vicinal thiols.

Some of our data are consistent with the data previously described by Matsunaga and colleagues (3) showing that adhesion through the VLA-4 integrin engagement triggers chemoresistance of AML blasts to drug-induced apoptosis. The novelty of the present study resides within the mechanism of VLA inactivation. We show here that both established AML cell lines and leukemic cells obtained from patients with AML expressing high VLA-4, acquire resistance to chemotherapy-induced death as a result of the interaction of leukemic VLA-4, but not VLA-5, with fibronectin. This resistance is significantly reduced upon treatment with the tellurium small-molecule AS101 via redox modulation of vicinal thiols on VLA-4. Although a wealth of evidence shows that inside-out signaling via factors in the cytoplasm can control integrin activation state (30–32), an alternative concept now suggests that at least some integrin's activation could be controlled directly by a redox site in the extracellular domain, independent of factors in the cytoplasm (33–36). Thus, integrin disulfide exchange may be involved in aspects of integrin activation, altering...
integrin conformation, and increasing the ligand-binding affinity of the integrin (10, 37, 38). The two pathways to integrin activation, inside-out and redox modulation, may serve different purposes. The redox switch could regulate rapid and transient changes in activation state that require no "filtering" through the cytoplasm. Inside-out signaling may then play a larger role in persistent control of activation, a process that may benefit from filters provided by the interconnections among intracellular signaling paths.

Using a proteomic approach, Laragione and colleagues identified the α4-integrin as a molecular target susceptible to redox regulation (39). Furthermore, Liu and colleagues (40) found that ligand binding of VLA-4 induced exposure of sulfhydryl groups on the α4-peptide.

The Te(IV)-thiol chemical bond formed between AS101 and the α4-chain leads to conformational change in the VLA-4 integrin as seen in this study. Our study suggests that the inactivation of VLA-4 is due to binding of AS101 to the thiol groups of vicinal cysteines on the α4-chain. At least some tellurium derivatives are known to interact with specific vicinal thiols within certain proteins (41). We thus assume that these dithiols reversibly interconvert to a disulfide bond under physiologic conditions, providing sites where changes in the redox environment can regulate protein function.

We cannot exclude the possibility that AS101 also modifies sulfhydryls on other membrane cell proteins (including other integrins) that could be involved in chemoresistance. Nevertheless, the consistency between the results obtained with α4β3 expressed on cell surface leukemic cultures and on patients’ leukemic cells, with regulation of chemosensitivity, and the lack of free thiols on the exofolial region of α5 strongly suggests a mechanistic link between the decrease in free cysteine residues in α4β3 and the decrease in chemotherapy-induced cell resistance following treatment with AS101. Yet, a differential reactivity of various cysteines toward AS101 may exist, possibly re...
treated with the compound for several years. This suggests that the compound is probably relatively specific.

VLA-4 has emerged as a promising therapeutic target in AML. Still, its role as a clinical marker to define remission induction, disease recurrence, or prognosis remains controversial. Initially, anti-VLA-4 antibodies were shown to chemosensitize human AML cells and to eradicate minimal residual disease (MRD) in experimental mice when combined with chemotherapy, implicating VLA-4 in acquired chemotherapy resistance and MRD (3). These findings suggested that high VLA-4 expression might reduce chemosensitivity, resulting in poor remission induction, MRD, disease recurrence, and short survival (3). Another study supported this prediction. Tavernier-Tardy and colleagues (43) showed that high VLA-4 expression was associated with shorter survival of patients with AML. Furthermore, although non-significant ($P = 0.058$), there was a trend for lower VLA-4 expression in samples from patients in remission as compared with refractory patients. Moreover, they found a high significant correlation between the expression of CXCR4 and VLA-4. Overexpression of CXCR4 on AML cells has been previously described to predict adverse overall survival (44–46). Our results are in line with those of Matsunaga and colleagues. Nevertheless, a recent study (47) failed to confirm the prognostic role of VLA in AML as suggested by Matsunaga and colleagues, and reported that functional VLA-4, but not merely VLA-4 expression, is associated with longer overall survival of adult AML patients. This apparently controversy may be hypothetically explained by the fact that high VLA-4 expression and function may merely be a surrogate for certain favorable factors that affect prognosis, and they do not contradict the principle that inhibition of VLA-4 activity on AML leukemic cells is advantageous with respect to increased chemosensitivity and improved outcome.

The ability of AS101 to sensitize leukemic cells from patients expressing high VLA-4 to chemotherapy-induced death was associated with the inhibition of focal adhesion kinase (FAK) phosphorylation at Tyr-397, a residue that is critical for its function. FAK is a downstream signal that follows $\alpha_2\beta_1$-integrin engagement. Recently, Recher and colleagues (48) have shown that FAK is frequently expressed and activated in AML cells, and that FAK expression correlates with enhanced migratory properties, drug resistance, high leukocytosis, and reduced survival.

Besides our prototype tellurium compound AS101, the investigation of therapeutic activities of other tellurium(IV) compounds is scarce in the literature, although tellurium is the fourth most abundant trace element in the human body. Our integrated results show that eradication of residual leukemic cells in a xenograft model of AML can be achieved by a unique alternative approach to existing strategies of controlling the VLA-4 integrin activation using AS101, currently being tested in clinical trials. In light of these results, clinical studies involving patients with AML refractory to chemotherapy treated with AS101 and chemotherapy have been approved and will be soon initiated. Such treatment might be particularly beneficial for patients with high functional VLA-4 expression.

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

Authors’ Contributions

Conception and design: A. Berrebi, D.L. Longo, Y. Kalechman, B. Sredni
Development of methodology: E. Noy, M. Albeck
 Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Berrebi, E. Noy, M. Albeck
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Writing, review, and/or revision of the manuscript: D.L. Longo, Y. Kalechman, B. Sredni

Study supervision: B. Sredni
Other (Designed and performed the in vitro and in vivo experiments): A. Layani-Bazar
Other (Design and performance of the experiments that were concerned with modulation of vicinal thiols): A. Silberman

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