Intracellular Activation of SGN-35, a Potent Anti-CD30 Antibody-Drug Conjugate

Nicole M. Okeley, Jamie B. Miyamoto, Xinqun Zhang, Russell J. Sanderson, Dennis R. Benjamin, Eric L. Sievers, Peter D. Senter, and Stephen C. Alley

Abstract

**Purpose:** SGN-35 is an antibody-drug conjugate (ADC) containing the potent antimitotic drug, monomethylauristatin E (MMAE), linked to the anti-CD30 monoclonal antibody, cAC10. As previously shown, SGN-35 treatment regresses and cures established Hodgkin lymphoma and anaplastic large cell lymphoma xenografts. Recently, the ADC has been shown to possess pronounced activity in clinical trials. Here, we investigate the molecular basis for the activities of SGN-35 by determining the extent of targeted intracellular drug release and retention, and bystander activities.

**Experimental Design:** SGN-35 was prepared with $^{14}$C-labeled MMAE. Intracellular ADC activation on CD30$^+$ and negative cell lines was determined using a combination of radiometric and liquid chromatography/mass spectrometry-based assays. The bystander activity of SGN-35 was determined using mixed tumor cell cultures consisting of CD30$^+$ and CD30$^-$ lines.

**Results:** SGN-35 treatment of CD30$^+$ cells leads to efficient intracellular release of chemically unmodified MMAE, with intracellular concentrations of MMAE in the range of 500 nmol/L. This was due to specific ADC binding, uptake, MMAE retention, and receptor recycling or resynthesis. MMAE accounts for the total detectable released drug from CD30$^+$ cells, and has a half-life of retention of 15 to 20 h. Cytotoxicity studies with mixtures of CD30$^+$ and CD30$^-$ cell lines indicated that diffusible released MMAE from CD30$^+$ cells was able to kill cocultivated CD30$^-$ cells.

**Conclusions:** MMAE is efficiently released from SGN-35 within CD30$^+$ cancer cells and, due to its membrane permeability, is able to exert cytotoxic activity on bystander cells. This provides mechanistic insight into the pronounced preclinical and clinical antitumor activities observed with SGN-35. Clin Cancer Res; 16(3); 888–97. ©2010 AACR.
of released drug are high over a prolonged time period, yet the amount of effluxed drug is sufficient to exert bystander activity on cocultured antigen-negative cells. These studies provide mechanistic insight into the activities and properties of SGN-35, and a basis for using this agent for the treatment of tumor cell populations that express the CD30 antigen in a heterogeneous manner.

**Materials and Methods**

Radiolabeled drugs and ADCs. 14C-labeled MMAE and vc-MMAE were prepared through custom synthesis by Perkin-Elmer (14C-MMAE and 14C-vc-MMAE). MMAE and vc-MMAE were labeled at the second valine (universally 14C-labeled, 270 mCi/mmol MMAE and 276 mCi/mmol vc-MMAE; Fig. 1). cAC10-14C-vc-MMAE was prepared with a method similar to that previously described using partial reduction by DTT (26). The remaining unreacted DTT was removed by PD10 size exclusion chromatography (GE Healthcare) and excess 14C-vc-MMAE was added to conjugate at 0°C. Any unreacted drug linker was quenched with cysteine and the conjugate was purified from unconjugated small molecule by PD10. The specific activity of the ADC was determined by UV/visible spectroscopy and liquid scintillation counting (LSC). The specific activity of the unconjugated drug linker was used to calculate ADC’s drug loading (7.9 μCi/mg, 4.4 drugs/antibody).

Cell culture. The CD30-positive cell line Karpas299 (anaplastic large cell lymphoma) and the CD30-negative line WSU-NHL (non-Hodgkin lymphoma) were obtained from the Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH. The CD30-negative line Ramos was obtained from American Type Culture Collection. The CD30-positive cell line L540cy, a derivative of the HL line L540 adapted to xenograft growth, was provided by Dr. Philip Thorpe (University of Texas Southwestern Medical Center, Dallas, TX). Cells were grown in suspension culture in RPMI 1640 supplemented with either 10% fetal bovine serum (FBS; Invitrogen, Carlsbad; Karpas299, WSU-NHL, and Ramos) or 20% FBS (L540cy) in a humidified environment of 5% CO2 at 37°C.

Cell volume determination. Cell volumes were determined by estimation of the average viable cell diameter using a Vi-Cell XR2.03 cell viability analyzer (Beckman Coulter) that provides the average cell size in microns. Volume calculation assumed a spherical cell with no correction made for nuclear volume.

ADC catabolism and drug distribution (constant exposure). Cells were seeded at 5 × 10^5 cells/mL. Cultures were examined in triplicate and typical culture volumes were 30 mL. On each day of the 72-h experiment, cell densities and viabilities were determined by trypan blue exclusion. Radioactive SGN-35 was added to each culture at a final concentration of ~200 ng/mL (6 nmol/L drug equivalent). Flasks were swirled to mix and aliquots were removed from each flask as a reference for the total amount of radioactivity added to the culture. The cultures were incubated at 37°C in a humidified 5% CO2 atmosphere. At the
indicated time points, cultures were mixed and 4 mL aliquots were removed and overlaid on FBS cushions (27) in 15-mL centrifuge tubes. The samples were centrifuged at 390 × g for 5 min at room temperature. From the separated sample, an aliquot of the upper medium phase was removed for further analysis (see below). The remaining supernatant was carefully removed. The pellet was resuspended in 4 mL of ice-cold PBS and 1 mL was removed for LSC quantitation of total cell-associated radioactivity. The remaining 3 mL of cells were pelleted and resuspended in 0.5 mL of proteinase K (5 μg/mL; Promega) in PBS to remove surface-bound ADC. After incubation at 37°C for 10 min, the enzyme was quenched by dilution with 1 mL of FBS-containing medium. The entire sample was overlaid on an FBS cushion (27) and centrifuged at 390 × g for 5 min. The pellet was resuspended in 100 μL of complete medium and was then treated with 900 μL of ice-cold methanol to precipitate protein and permeabilize the cells. An aliquot of this cell precipitation suspension (400 μL) was removed and examined by LSC to quantitate the total intracellular radioactivity. The remaining suspension was stored at −20°C for ≥30 min. The sample was then centrifuged at 16,000 × g for 5 min. The clarified supernatant (500 μL) was examined by LSC to quantitate the total intracellular radioactive small molecule.

A sample of the culture medium from each time point was diluted with nine volumes of ice-cold methanol. The resulting suspension was stored at −20°C for ≥30 min. The sample was then centrifuged at 16,000 × g for 5 min and the supernatant was counted by LSC for quantitation of the nonprotein-associated radioactivity present in the culture medium. All samples used for LSC were mixed with 4 mL of Ecoscint A scintillation fluid and were vortexed. L540c cells treated with chloroquine (Sigma) were preincubated with 100 μmol/L chloroquine at 37°C for 1 h followed by 5 h of incubation with radiolabeled SGN-35 and subsequent processing as described above.

**ADC catabolism and drug distribution (limited exposure).** To examine ADC catabolism and drug distribution in cells with limited ADC exposure, cultures were prepared as described above using ice-cold culture medium and were placed on ice before ADC addition. After incubation on ice for ~15 min, 14C-SGN-35 was added at ~200 ng/mL and the cultures were kept on ice for an additional 30 min. Cultures were then centrifuged at 390 × g for 5 min and were washed with ice-cold PBS followed by resuspension in fresh, warm culture medium. The cultures were placed at 37°C in a humidified 5% CO2 atmosphere and samples were removed at 24 h and processed as described in the constant exposure method.

**Free drug retention.** For MMAE retention experiments, 25 × 10^6 cells were seeded at 5 × 10^5 cells/mL. Each cell type was treated in triplicate with a concentration of radioactive MMAE determined to provide a similar intracellular concentration for that cell type after 3 h of incubation at 37°C. The cells were washed twice into an equal volume of fresh medium. Each washed culture was split into three 15-mL centrifuge tubes. One-milliliter aliquots were removed immediately from each tube for LSC and a second 1-mL aliquot was layered over 2 mL of FBS in a 15-mL centrifuge tube, centrifuged at 390 × g for 5 min at room temperature. From the separated sample, 0.5 mL of the upper medium phase was removed for LSC. The remaining portion of each sample was frozen in a dry ice bath and the bottom of the tube containing the cell pellet was excised into a scintillation vial containing 0.5 mL of PBS. Samples were vortexed; Ecoscint A scintillation fluid (4 mL; National Diagnostics) was added followed by a
second vortex; and the samples were counted by LSC (Beckman LS6000IC, Beckman Coulter). Further aliquots (1 mL) were processed over FBS, as described, at the indicated time points. For the calculation of intracellular drug concentrations, cell densities were redetermined by trypan exclusion after washing into nondrug-containing medium and again after 24 h.

**Radioactivity calculations.** Calculations were made after subtracting the background from all the disintegration per second values. The triplicate background-corrected disintegration per second readings were averaged and the SD for those values was calculated using the STDEVPA function in Microsoft Excel. Average disintegration per second values were converted to μCi and subsequently to pmole of the drug using the specific activity of the radioactive drug or drug linker used in the experiment. For each mathematical manipulation, a propagation of error calculation was done using standard propagation of error formulas for propagation of SD values. The intracellular drug concentrations in pmole were calculated using the estimated volume of 1 × 10⁶ cells.

**Mass spectrometric drug quantitation.** Triplicate samples of cells were washed into fresh medium at ∼5 × 10⁵ cells/mL. ADC was added (200 ng/mL) and the cultures were incubated at 37°C in 5% CO₂. For intracellular drug quantitation, at 24 h, the cells were enumerated by trypan blue exclusion and a known volume of cells was harvested by centrifugation (360 × g rpm, 5 min, 4°C). The cells were washed with an equal volume of ice-cold PBS (360 × g, 5 min), repelleted, and resuspended in complete culture medium to provide final volumes of 150 μL. Two volumes of ice-cold methanol were added and the samples were stored at −20°C for ≥30 min. For released drug quantitation in the culture medium, cells were prepared in the same manner; however, aliquots were removed over 3 d. Culture medium was recovered by simple centrifugation of the samples and removal of the supernatant, taking care not to disturb the pellet. The culture medium samples (150 μL) were mixed with 150 μL of a 50-ng/mL internal standard in the same culture medium. These were precipitated with two volumes of ice-cold acetonitrile. Samples of medium and cell pellets not treated with ADC were used for standard curves and were prepared following the same precipitation procedures. Eight-point standard curves were made using varying amounts of MMAE plus constant internal standard in untreated medium samples. The resultant peak area ratios were plotted as a function of the standard concentrations and the data points were fitted to a curve using linear regression. The peak area ratios obtained for the released drug to internal standard in the experimental samples were converted to drug concentration using the derived equation.

**ADC-treated culture conditioned medium bioassay.** Samples of spent culture medium from SGN-35-treated cells were added to CD30-negative Ramos cell cultures in six different dilutions. In parallel, Ramos cells were incubated with eight MMAE concentrations as cytotoxicity standards. After a 96-h incubation at 37°C, the Ramos cultures were developed using resazurin (Sigma; relative fluorescence, excitation = 530-560 nmol/L, emission = 590 nmol/L). Using the average relative fluorescence unit (RFU) measurement for Ramos cells incubated with the MMAE standards, the percentage of viable cells relative to untreated Ramos cells (%) untreated) was plotted as a function of MMAE concentration (nmol/L). A four-parameter curve fit was used to generate an equation for the quantitation of released MMAE in the spent culture samples. Cell viability measurements of the spent culture dilutions were transformed into MMAE concentrations (assuming cytotoxicity is attributed solely to MMAE) using this equation. Only cell viability measurements falling between 15% and 85% of the untreated Ramos cells were used to calculate the concentration of MMAE.

**Coculture experiments.** Karpas299, L540cy, or Ramos cells in single culture were seeded at 2.5 × 10⁵ cells/mL in culture volumes of 1.5 mL, whereas cocultures of CD30-positive and CD30-negative pairs consisted of 1.25 × 10⁵ cells/mL of each cell type in 1.5 mL of culture medium (1:1 mixture of cells, RPMI 1640 + 10-15% FBS). The culture medium used in the coculture experiments adequately supports the growth of all three cell types. Cultures were treated with vehicle control, 1 μg/mL SGN-35, or IgG-vc-MMAE nonbinding control. After a 72-h incubation, cultures were fed with 60% medium containing the requisite treatment type. Cultures were examined for cell count and viability (Vi-Cell XR2.03 cell viability analyzer, Beckman Coulter) after 120 h and the surviving cells were stained with anti–CD30-Phycoerythrin (BD Biosciences) and anti–CD19-FITC (BD Biosciences) antibodies to determine the distribution of each cell type in the surviving cultures. Staining was accomplished by harvesting the cells by centrifugation at 1,200 rpm for 5 min, plating ∼5 × 10⁵ cells per well in a 96-well plate in 20 μL of fluorescence-activated cell sorting (FACS) buffer (PBS containing 2% FBS), and adding the labeled antibodies to the desired wells without dilution (5 μL/well). The plate was incubated on ice for 30 min before centrifugation at 1,500 rpm for 5 min and before the removal of the supernatant by tapping the plate. The cells were washed three times with PBS (200 μL) before resuspending in 250 μL FACS buffer and before storage at 4°C for subsequent analysis by flow cytometry on a BD FACScan instrument.

**Results**

**Drug release from SGN-35.** The kinetics of SGN-35 internalization, drug release, and the extent of intracellular, released drug retention were determined with a radiolabeled...
version of the ADC prepared using $^{14}$C-MMAE conjugated to cAC10 using the mc-valine-citrulline-PABC linker shown in Fig. 1. The resulting ADC contained an average of 4.4 MMAE molecules attached to interchain disulfides as previously described (18, 26). The fate of SGN-35 on cultured cells was examined by the incubation of cells with radiolabeled SGN-35 and the determination of the fraction of ADC in the medium versus associated with cells. ADC bound to the cell surface was removed by proteinase K to distinguish it from intracellular ADC. For the media and intracellular fractions, conjugated MMAE was distinguished from released MMAE by precipitation in organic solvent.

CD30-positive L540cy HL and Karpas299 anaplastic large cell lymphoma cells were treated with a constant exposure of $^{14}$C-labeled SGN-35 at ~200 times the IC$_{50}$ concentration. The total amount of cell-associated drug and intracellular drug are shown in Fig. 2A. Initially, most of the drug was associated with the membrane, but intracellular bound and released drug built up over the course of the assay. Beyond the earliest time point, the vast majority of intracellular drug was free, suggesting that upon internalization, the release of drug from SGN-35 is quite facile.

The concentration of intracellular and extracellular released drug in cell lines treated with constant SGN-35 exposure is shown in Fig. 2B. WSU-NHL cells are CD30 negative and did not release detectable levels of drug through the entire 3-day course of the assay. In contrast, both CD30-positive cell lines generated released drug, with high intracellular concentrations (>400 nmol/L) reached within 24 hours of treatment. This indicates not only that SGN-35 was processed in CD30-positive cells, but that the released drug accumulated and was retained within the cells at concentrations much higher than the initial treatment ADC concentration of 6 nmol/L. Appearance of free drug inside and outside the L540cy cells was greatly reduced at 4°C or when cells were treated with chloroquine before ADC exposure (Fig. 2C). Chloroquine raises lysosomal pH and reduces the activity of lysosomal proteases having optimal activities under acidic conditions (28). Taken together, these results are consistent with
intracellular drug generation by CD30-mediated internalization of SGN-35, and accumulation of extracellular drug in the culture medium through escape due to the inherent membrane permeability of MMAE (29, 30).

The combined intracellular and extracellular released drug over the 72-hour constant exposure incubation provides a basis for estimating the total number of SGN-35 molecules that were internalized by the cell and catabolized. This number can be compared with the number of CD30 receptors present in the cell culture at 72 hours and hence provide an estimate of the turnover of CD30 in each cell line (Table 1). The calculated number of ADCs internalized and catabolized is 2.5 and 3.4 ADCs per receptor for Karpas299 and L540cy cells, respectively, indicating that over the course of the 72-hour SGN-35 incubation, there was either recycling of CD30 to the cell surface or synthesis of new receptor. This is supported by the amount of MMAE (pmol/mL) observed in the CD30-positive cell culture in light of the fact that MMAE is observed to be accumulating in the culture medium (Fig. 2B).

**Identification of released drug.** To determine if the identity of the released drug was exclusively MMAE, bioassay and mass spectrometry studies were undertaken on the soluble small molecules generated from CD30-positive cells treated with SGN-35. The data were compared with the total methanol-soluble radioactivity derived from the cells as shown in Fig. 3A and B. In the bioassay, antigen-negative Ramos cells were treated with the spent culture medium from SGN-35–treated antigen-negative cells. Using the cytotoxicity of Ramos cells to authentic MMAE standards, the effective MMAE concentrations in the spent culture samples were calculated. Over time, there was an increasing antigen-independent cytotoxin concentration that overlapped with the presumed MMAE concentration from LSC (Fig. 3A). Furthermore, quantitative mass spectrometric analysis of released drug in the cell culture supernatants, as well as inside cells (Fig. 3A and B), indicated that a species indistinguishable from MMAE by mass and retention time was present in concentrations that also overlapped with the presumed MMAE concentration from LSC.

**Cellular efflux of MMAE.** Because the escape of released drug from antigen-positive cells may affect therapeutic efficacy, we further established that this type of behavior can be observed with cells loaded with free MMAE by measuring

<table>
<thead>
<tr>
<th>Table 1. Catabolism of SGN-35 by CD30-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L540cy cells (HL)</strong></td>
</tr>
<tr>
<td>CD30*: 587,511/cell</td>
</tr>
<tr>
<td><strong>Limited,†</strong></td>
</tr>
<tr>
<td>1 × 10⁶ cells/mL</td>
</tr>
<tr>
<td>Intracellular released MMAE (pmol/mL)</td>
</tr>
<tr>
<td>Extracellular released MMAE (pmol/mL)</td>
</tr>
<tr>
<td>Total released MMAE (pmol/mL)</td>
</tr>
<tr>
<td>¹⁴C-SGN-35 catabolized per receptor‡</td>
</tr>
<tr>
<td><strong>1.2 ± 0.1</strong></td>
</tr>
</tbody>
</table>

Abbreviation: ALCL, anaplastic large cell lymphoma.

†Limited exposure refers to cells treated initially at 0°C with 200 ng/mL ¹⁴C-SGN-35 and subsequently washed to remove unbound ADC before incubation at 37°C. Continuous exposure refers to treatment of cells at 37°C with 200 ng/mL ¹⁴C-SGN-35.

‡Calculated using a drug loading of 4.4 drugs/mAb and the average number of cells remaining per milliliter at the time of sample collection.

www.aacrjournals.org
Clin Cancer Res; 16(3) February 1, 2010
893

Published OnlineFirst January 19, 2010; DOI: 10.1158/1078-0432.CCR-09-2069
the loss of intracellular drug over 24 hours, during which time the cells maintain their viability. To accomplish this, L540cy and Karpas299 cells were treated with sufficient \(^{14}\)C-MMAE to achieve intracellular concentrations of approximately 3 to 4 μmol/L and the rate of drug loss was measured, as shown in Fig. 3C. For both cell lines, 50% of the initial intracellular MMAE was retained for approximately 16 to 22 hours, and the remaining material was found in the culture supernatant. L540cy displays limited efflux of rhodamine dye whereas Karpas299 does not (Supplementary Fig. S1), indicating that the former but not the latter expresses an unidentified multidrug resistance protein. Despite this difference, the efflux of MMAE from the two cell lines is very similar (Fig. 3C), which suggests that MMAE can diffuse from viable cells regardless of whether a multidrug resistance protein is present. Because MMAE is only observed in the cell culture medium of SGN-35-treated CD30-positive cells and its appearance is inhibited by the inhibition of lysosomal proteases, the observation that MMAE can slowly diffuse out of cells bolsters the hypothesis that the extracellular-free drug found in SGN-35-treated culture medium originated from intracellular processing of the ADC, followed by subsequent passive or active drug efflux.

**Bystander effects.** Having shown that the released drug from SGN-35 is MMAE, that the drug is able to diffuse slowly out of the cell from which it was derived (Figs. 2B and 3C), and that SGN-35 is stable under these culture conditions with no generation of MMAE from antigen-negative cell lines (Fig. 2B), we explored whether the ADC elicits bystander activity in cocultures with CD30-positive and CD30-negative cell lines. We have previously shown (17) that Ramos Burkitt’s lymphoma cells are CD30 negative and are as sensitive to free MMAE (IC\(_{50}\), 0.04 nmol/L) as Karpas299 (IC\(_{50}\), 0.07 nmol/L) and L540cy (IC\(_{50}\), 0.21 nmol/L) cells, with IC\(_{50}\) values that are representative of the sensitivity of cancer cells to MMAE. Being CD30 negative, Ramos cells were relatively insensitive to SGN-35 (IC\(_{50}\), 3,300 ng/mL with eight drugs per mAb) compared with Karpas299 (IC\(_{50}\), 1.3 ng/mL with eight drugs per mAb) and L540cy (IC\(_{50}\), 9.9 ng/mL with eight drugs per mAb) cells (17). FACS analysis of cocultures of L540cy and Ramos cells showed that treatment with 1 μg/mL SGN-35 eliminated both populations of cells equally well, whereas a similarly treated mixed cell population with a nonbinding control ADC were unaffected (Fig. 4A and B). Similar treatment of the Karpas299/Ramos cell mixture with 1 μg/mL SGN-35 produced the same outcome (Fig. 4C and D). These results show an antigen-independent cytotoxic effect on the CD30-negative cells that is likely caused by the released MMAE from the cocultured CD30-positive cells. A 5-fold lower ADC dose was also examined under the same conditions and displayed a less potent reduction of the CD30-negative cells (data not shown), suggesting that the amount of small-molecule cytotoxic agent generated is dependent on the amount of SGN-35 added to the culture. Thus, SGN-35 has strong bystander activity on neighboring antigen-negative cells in culture.

**Discussion**

Recent advances in ADC research have led to the development of SGN-35, an agent that has shown considerable activity, both in preclinical models and in early clinical trials (7, 10, 13–16, 18). This molecule incorporates a highly potent payload, linker technology that is considerably more stable than earlier generation hydrazone and disulfide linkers (7, 8, 31, 32), and conjugation
methodology that provides high yields of well-defined ADCs with drugs attached at specific mAb sites that are distal to the variable region (18, 26). The results with SGN-35 have provided the basis for extending the findings to include such antigens as LeY (16), CD20 (33), CD22 (30), CD70 (19), CD79b (30), MUC16 (34), EphA2 (35), PSMA (36), and many others. In addition, in vivo model studies have shown that SGN-35 added to standard chemotherapeutic regimens leads to improved activity over either treatment group alone (19). Given the broad applicability of ADCs using this technology, it is of importance to have a molecular understanding of how this ADC functions at a molecular level, the subject of investigations reported here.

It has previously been shown that the nature of drug release from ADCs can have a profound influence on activity. For example, maytansinoid ADCs release lysine adducts if they are linked to mAb lysines through thioether linkers, but yield S-methylated derivatives if linked to lysines through disulfide linkers (37). These agents have distinct in vitro and in vivo activity profiles, and the charged lysine adduct was shown to be much less active than the thioether derivative as a free drug. It was therefore not surprising that the thioether adduct elicited a strong bystander effect, whereas the lysine adduct did not (38). We have also observed that the nature of the linker can influence the structure of the released drug, based on studies with thioether-linked auristatins that released cysteine adducts through mAb degradation (29).

In the work described here, we show that CD30-positive cell lines process SGN-35 by releasing MMAE in a chemically unmodified form. The results are consistent with the proteolytic cleavage at the citrulline-PABC amide bond, which leads to the release of MMAE after spontaneous fragmentation of the PABC spacer (Fig. 1). This is supported by inhibition studies with chloroquine (Fig. 2C), a lysosomotropic agent that reduces lysosomal protease activity through pH modulation. Upon release, the MMAE diffuses through cell membranes and accumulates in culture media, albeit at concentrations that were ∼250 times lower than that found inside the cells, presumably due to dilution into the relatively larger volume of medium (Fig. 2B). Despite the great differential, extracellular drug was able to kill CD30-negative cells cocultured with CD30-positive cells. Thus, SGN-35 has the potential to act on cells within a heterogeneous tumor cell population that do not bind sufficiently high amounts of ADC for effective direct cytotoxic activity. In general, this is of importance because mAbs have been shown to distribute within tumors in an uneven manner and many tumors are heterogeneous with respect to antigen expression (22, 39, 40). In targeting HL with antibodies directed against CD30 in particular, the ability to eradicate antigen-negative cells within the tumor mass may be useful because only a small percentage of the cells are CD30 positive and thought to be a part of the clonal malignancy (25). Because this bystander cytotoxic effect would be localized to the tumor

---

**Fig. 4.** Bystander activities of MMAE containing ADCs. Coculture experiments included L540cy and Karpas299 (CD30+, CD19−) and Ramos (CD30−, CD19+) cells. A, cell count of L540cy/Ramos cocultures treated with 1 μg/mL SGN-35 and a corresponding nonbinding control ADC (IgG-vc-MMAE). B, L540cy/Ramos cocultures were stained with anti–CD30-PE and anti–CD19-FITC following treatment, allowing the determination of the number of surviving CD30-positive and CD30-negative cells. C, cell count of Karpas299/Ramos cocultures treated with 1 μg/mL SGN-35 and a corresponding nonbinding control ADC (IgG-vc-MMAE). D, Karpas299/Ramos cocultures were stained with anti–CD30-PE and anti–CD19-FITC following treatment, allowing the determination of the number of surviving CD30-positive and CD30-negative cells.
microenvironment, with cells near the CD30-positive cells exposed to a concentration gradient of MMAE that decreases with increasing distance; it is likely to minimize toxic systemic exposure of diffusible tumor-released MMAE. In summary, targeted in vitro delivery of MMAE to CD30-expressing cells with SGN-35 leads to high and sustained intracellular MMAE levels, and successfully ablates both CD30-expressing malignant cells and neighboring malignant cells that do not express the target antigen. This may be of significance in treating tumors that are heterogeneous with respect to both antigen presentation and ADC distribution (9, 41). The results reported here provide insight into the pronounced activities associated with this promising ADC.

References

29. Doronina SO, Mendelsohn BA, Bovee TD, et al. Enhanced activity of monomethylauristatin F through monoclonal antibody delivery:

Disclosure of Potential Conflicts of Interest

All authors are employed by and have ownership interest in Seattle Genetics.

Acknowledgments

We thank Charles G. Cerverny, Damon L. Meyer, and Vajira Nanayakkara for the assistance with several of the experiments described in this article. 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 8/3/09; revised 10/9/09; accepted 12/1/09; published OnlineFirst 1/19/10.


Correction: Intracellular Activation of SGN-35, a Potent Anti-CD30 Antibody-Drug Conjugate

In this article (Clin Cancer Res 2010;16:888–97), which was published in the February 1, 2010, issue of Clinical Cancer Research (1), there was an error in the labeling of the legend in the right panel of Fig. 2B. The legend labels for L540cy and Karpas299 in the extracellular MMAE concentration graph were inadvertently switched.

Reference


Published OnlineFirst August 2, 2011. ©2011 American Association for Cancer Research. doi: 10.1158/1078-0432.CCR-11-1753
Clinical Cancer Research

Intracellular Activation of SGN-35, a Potent Anti-CD30 Antibody-Drug Conjugate

Nicole M. Okeley, Jamie B. Miyamoto, Xinquin Zhang, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-2069

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2010/01/19/1078-0432.CCR-09-2069.DC1

Cited articles
This article cites 38 articles, 19 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/3/888.full#ref-list-1

Citing articles
This article has been cited by 48 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/16/3/888.full#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, use this link
http://clincancerres.aacrjournals.org/content/16/3/888.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.