Case Report

Clinical Binding Properties, Internalization Kinetics, and Clinicopathologic Activity of Brentuximab Vedotin: An Antibody-Drug Conjugate for CD30-Positive Lymphoid Neoplasms

Jonathan R. Fromm,[1] Julie A. McEarchern,[2] Dana Kennedy,[3] Anju Thomas,[1] Andrei R. Shustov,[4] Ajay K. Gopal[5]

Clinical Practice Points

- Brentuximab vedotin (b-vedotin), a CD30-directed antibody-drug conjugate, has shown activity in treating CD30-positive (CD30+) neoplasms (classical Hodgkin lymphoma [CHL] and systemic anaplastic large cell lymphoma [ALCL]); however, its in vivo saturation, internalization kinetics, and pathologic findings are unknown.
- We quantified antigen density on CD30+ cell lines cultured with b-vedotin and demonstrated the greatest expression of CD30 and bound b-vedotin at 24 to 48 hours, reaching a nadir by 120 hours. Similarly, serial tumor biopsies from a patient with relapsed ALCL revealed maximal CD30 expression (1.01x105 molecules/cell) at baseline. After treatment, CD30 (7.83x104 molecules/cell at 24 hours; 5.08x104 molecules/cell at 48 hours) and bound b-vedotin (2.26x103 molecules/cell at 24 hours and 1.40x103 molecules/cell at 48 hours) antigen density rapidly declined, consistent with internalization. Histologic evaluation showed a dense infiltrate of neoplastic CD30+ cells before treatment, apoptosis at 48 hours, and no neoplastic cells at day 21.
- These results demonstrate the potent and rapid antitumor properties and support the mechanism of action of b-vedotin. Additionally, as relatively few b-vedotin molecules are required for clinical efficacy, these results suggest that this drug should be evaluated in neoplasms with relatively low expression of CD30.

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Introduction

Brentuximab vedotin (b-vedotin), or SGN-35, is a CD30-directed antibody conjugated to the synthetic cytotoxic drug monomethyl auristatin E (MMAE) through a valine-citrulline dipeptide, designed to selectively target and kill CD30-expressing neoplasms.[1-4] Cell surface binding of b-vedotin to CD30 initiates internalization of the complex, which then travels to the lysosome, where MMAE is released by proteolytic cleavage. Binding of MMAE to

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[1]Department of Laboratory Medicine, University of Washington, Seattle, Washington
[2]Experimental Therapeutics, Seattle Genetics Inc, Bothell, Washington
[3]Clinical Affairs, Seattle Genetics Inc, Bothell, Washington
[4]Division of Hematology, Department of Medicine, University of Washington, Seattle, Washington
[5]Division of Medical Oncology, Department of Medicine, University of Washington; Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington

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Address for correspondence: Jonathan R. Fromm, MD, PhD, Box 357110, University of Washington Medical Center, Seattle, 1959 North East Pacific Street, Seattle, Washington 98195
E-mail contact: jfromm@u.washington.edu

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Table 1 Serial Quantification of CD30 Antigen and Bound b-Vedotin on Cell Lines and Tumor Samples from a b-Vedotin–Treated Patient With Systemic ALCL[a]

Time	Karpas 299		KM-H2		Patient		
	CD30	b-Vedotin	CD30	b-Vedotin	Serum b-Vedotin	CD30	b-Vedotin
0 h	9.30×10^5 (1.3×10^4)	–	1.08×10^5 (5.1×10^3)	–	0	1.01×10^5 (1.8×10^4)	–
24 h	3.16×10^5 (1.1×10^4)	1.62×10^5 (2.2×10^4)	2.64×10^5 (1.5×10^4)	6.07×10^4 (1.1×10^3)	10	7.83×10^4 (2.1×10^3)	2.26×10^3 (3.5×10^2)
48 h	1.60×10^5 (2.1×10^4)	2.97×10^4 (3.0×10^3)	1.51×10^5 (1.9×10^4)	9.83×10^4 (7.0×10^3)	7	5.08×10^4 (3.5×10^3)	1.40×10^3 (8.8×10^1)
120 h	9.49×10^4 (4.0×10^3)	1.21×10^4 (4.1×10^3)	9.45×10^4 (6.2×10^3)	5.50×10^4 (4.6×10^3)	1	–	–

[a] Values are given as ABC units/cell and are the average of 3 independent measurements, with standard deviation of the measurements in parentheses; serum concentrations of b-vedotin are given in micrograms/milliliter, and cell lines were incubated with b-vedotin at 15 μg/mL.

tubulin disrupts the microtubule network within the cell, induces cell cycle arrest, and results in apoptotic cell death.[2,5]

Two potential targets of this agent are classic Hodgkin lymphoma (CHL) and CD30-positive ($CD30^+$) T-cell non-Hodgkin lymphoma ($CD30^+$ T-NHL).[1,3] Approximately 30% to 40% of patients with advanced CHL will have relapse of disease.[6] $CD30^+$ T-NHL includes both primary cutaneous and systemic neoplasms. Although cutaneous $CD30^+$ T-NHL has a good prognosis,[7] systemic $CD30^+$ T-NHL often responds poorly to therapy.[8] Consequently, new therapeutic agents are needed for these neoplasms.

B-vedotin demonstrated antitumor activity in a phase 1 study of patients with relapsed or refractory Hodgkin lymphoma (HL) and systemic anaplastic large-cell lymphoma (ALCL; a $CD30^+$ T-NHL), with half of the patients responding at the 1.8 mg/kg dose.[3] To date, the binding properties, internalization kinetics, and clinicopathologic findings for b-vedotin have not been described in tumor specimens derived from treated patients. Therefore we investigated the activity of b-vedotin in a patient with cutaneous manifestations of systemic ALK-negative ALCL, and correlated these results with studies of the kinetics of b-vedotin on cultured $CD30^+$ cell lines.

Methods

Cell Culture

Karpas 299 and KM-H2 cell lines were cultured in RPMI 1640 (Gibco BRL, Life Technologies, Grand Island, NY)) supplemented with 10% fetal calf serum, 100 U/mL penicillin/streptomycin, and 2 mM L-glutamine, in 5% CO_2 at 37°C. b-Vedotin (Seattle Genetics, Bothell, WA) was added to the culture medium to a final concentration of 15 μg/mL at t = 0; aliquots were removed at t = 1, 2, and 5 days, and densities of CD30 and b-vedotin were measured in triplicate.

Processing of Skin Biopsy Specimens

Skin biopsy specimens (obtained with approval of the Fred Hutchinson Human Subjects Review Committee) were either fixed in formalin or disaggregated (as described previously[9]) for antigen density measurements. Immunohistochemical studies for CD30 were performed as described elsewhere.[10]

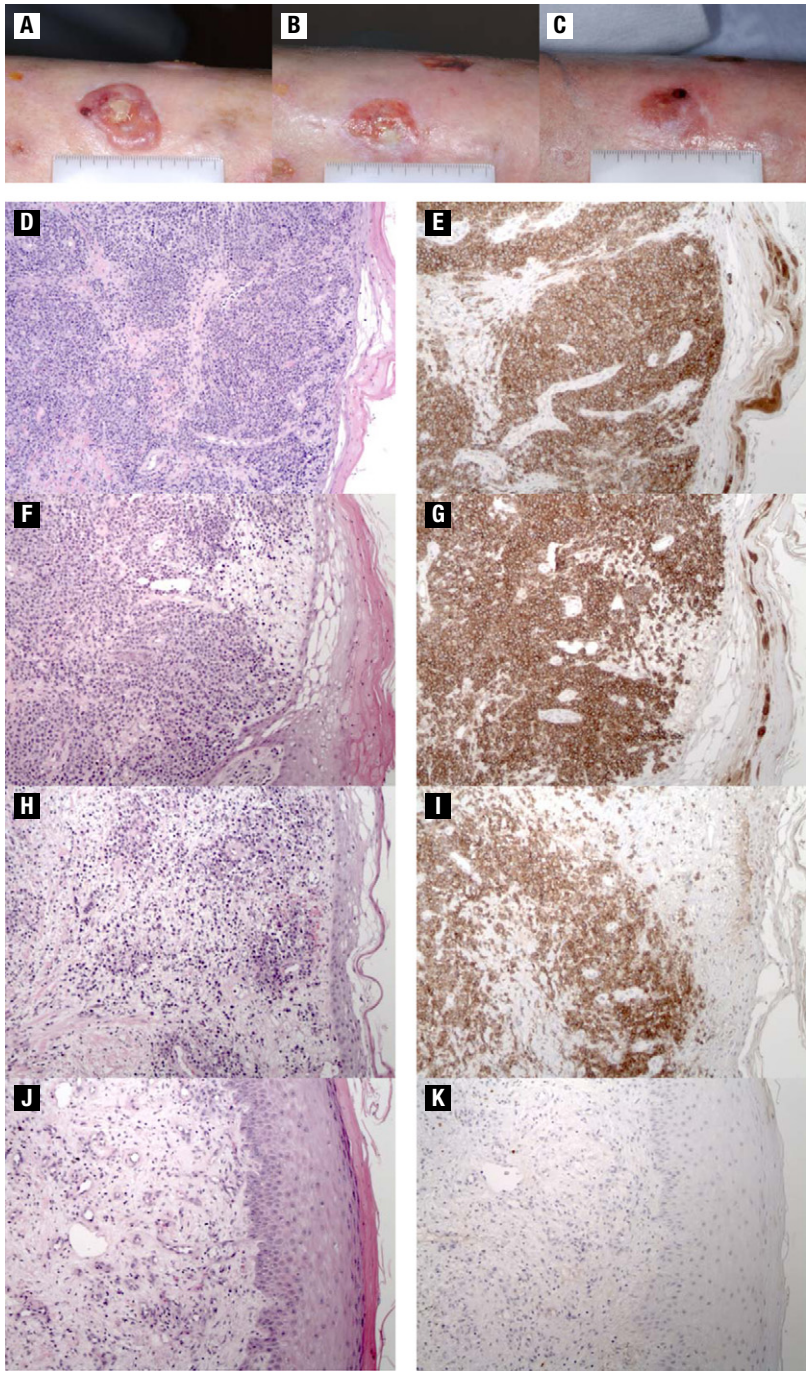
Antigen Binding Capacity Measurements

All samples were evaluated on a 10-color BD LSR II flow cytometer (Becton-Dickinson, Franklin Lakes, NJ). CD30 and b-vedotin antigen density (measured as antigen binding capacity [ABC] units/cell) on neoplastic cells from fresh skin biopsy specimens was measured according to the manufacturer's protocol. Briefly, the microbeads (Quantum Simply Cellular antimouse IgG, Bangs Laboratories Inc, Fishers, IN) used are coated with known quantities of goat antimouse IgG, which when mixed with saturating quantities of mouse anti-CD30-PE (Becton-Dickinson) or anti-b-vedotin-PE (Seattle Genetics), produced a standard curve to measure density of the respective antigen. Separately, mean fluorescence intensity from the skin biopsy specimen cell suspension or cell line suspension aliquot was measured for both CD30 and b-vedotin antigens using the anti-CD30-PE and anti-b-vedotin-PE, respectively, and the antigen densities were measured by comparison to the standard curve.

Results and Discussion

Saturation of CD30 binding sites by b-vedotin in cell lines was measured by using ABC beads and directly detecting the antigen on cells. We first confirmed that b-vedotin and the anti-CD30 antibody do not compete for binding to CD30 by increasing concentrations of b-vedotin (from 0 to 5 μg) added to Karpas 299 cells labeled with saturating amounts of anti-CD30-PE and quantifying the mean fluorescence intensity (data not shown). Next, using Karpas 299 (ALCL cell line) and KM-H2 (CHL cell line) cells incubated with 15 μg/mL b-vedotin, we quantitatively measured cell surface CD30 and bound cell surface b-vedotin over time (measured as ABC/cell), demonstrating maximum antigen expression at 24 to 48 hours, followed by a decrease at 120 hours, consistent with b-vedotin–induced internalization (Table 1). Similar studies were performed on sequential tumor biopsy specimens from a 68-year-old man with a 5-year history of ALK-negative ALCL relapsing after 4 previous systemic regimens and radiation, who now presented with multiple pink ulcerated tumorous lesions on his lower extremities (Figure 1A), as well as bone and nodal involvement. Hematoxylin and eosin (H&E) and anti-CD30 immunohistochemical stains of both before treatment (Figure 1D, 1E) and 24 hours after treatment (Figure 1F, 1G)

Figure 1 Effects of b-Vedotin on Cutaneous Tumors in a Patient With Systemic Anaplastic Large-Cell Lymphoma (ALCL). (A-C) Appearance of Skin Lesions From the Patient's Leg Before Treatment (A), and After the First (B) and Second Doses (C) of B-Vedotin (doses given every 21 days). The Raised Lesions Have Flattened and Ulcerated With Therapy. (D-K) Histologic Sections of Sequential Skin Biopsy Specimens From Leg Lesions Biopsied Before Treatment and at 24 Hours, 48 Hours, and 21 Days After Treatment With B-Vedotin. Sections are Stained With H&E *(left)* or Anti-CD30 Antibody *(right):* (D) H&E Before Treatment, (E) CD30 Immunohistochemical (IHC) Testing Before Treatment, (F) H&E 24 Hours After Treatment, (G) CD30 IHC Testing 24 Hours After Treatment, (H) H&E 48 Hours After Treatment, (I) CD30 IHC Testing 48 Hours After Treatment, (J) H&E 21 Days After Treatment, (K) CD30 IHC Testing 21 Days After Treatment. Decreased Numbers of Neoplastic Cells and Single-Cell Apoptosis Begins at 24 Hours and Peaks at 48 Hours. No Neoplastic Cells are Apparent on H&E or CD30 Immunostaining at 21 Days



after the first dose of b-vedotin (1.8 mg/kg; http://ClinicalTrials.gov identifier NCT01026415), skin punch biopsies showed a dense dermal infiltrate of large $CD30^+$ neoplastic cells. Biopsy at 48 hours after treatment demonstrated a decreased density of cells and apoptosis (Figure 1H, 1I).

Antigen density experiments on the patient biopsy specimens and clinical findings correlated with these morphologic results. CD30 antigen density was highest on the patient's cells from the pretreatment biopsy specimen (1.01×10^5 ABC units/cell) and then decreased after 24 hours (7.83×10^4 ABC units/cell) and 48 hours (5.08×10^4 ABC units/cell). Bound b-vedotin was greatest at 24 hours (2.26×10^3 ABC units/cell) and decreased at 48 hours (1.40×10^3 ABC units/cell) (Table 1). The corresponding measured blood concentrations of b-vedotin at 24 hours, 48 hours, and 21 days were 10, 7, and 1 μg/mL, respectively. No $CD30^+$ cells were present in the biopsy specimen from day 21 (Figure 1J, 1K), precluding evaluation of CD30 antigen density and bound b-vedotin. Clinically, the size and prominence of the skin lesions were reduced at day 21 after treatment and biopsy (Figure 1B) and at this time showed no morphologic or immunohistochemical evidence of the neoplastic population (pathologic complete remission). After the second dose of b-vedotin (Figure 1C), the patient achieved an 81% reduction in all radiographically measurable lesions and achieved a radiographic and cutaneous complete remission after the fifth infusion of this agent.

These data suggest in both cell lines and patient-derived tissues that the internalization kinetics of b-vedotin are rapid, with resultant reduction in CD30 expression within the first 48 hours and concurrent apoptosis induction within the targeted cells. Although the measured b-vedotin occupancy of CD30 binding sites in a cutaneous lesion (~3%) is lower than that in cell lines (13%-65%), these data highlight the potent antitumor activity of this agent. (The b-vedotin occupancy of CD30 binding sites in nodal disease is currently unknown.) The results imply that even with subsaturating occupancy of CD30, hundreds or thousands of molecules of b-vedotin (each with approximately 4 molecules of MMAE) are likely to be internalized by, and potentially kill, each targeted cell and are sufficient to yield pathologic remissions and clinical activity.

Few studies have examined the density of therapeutic monoclonal antibodies in tumors. Epenetos and coworkers examined the localization of radiolabeled therapeutic antibodies (AUA1 and HMFG2) in solid tumors, demonstrating 0.17 and 0.2 times as much drug in the tumor as in the blood for AUA1 and HMFG2, respectively, at 1 day after drug injection.[11] Measurements of the density of the $CD30^+$ cells at the center of the patient's cutaneous lesion suggest a concentration of b-vedotin of 0.44 μg/g tissue in the tissue or 0.044 times as much drug in cutaneous lesions as in the blood at 1 day, comparable to the aforementioned antibodies, suggesting similar initial binding kinetics. Furthermore, as b-vedotin density decreased from day 1 to 2 after therapy, peak b-vedotin cell surface density may occur at less than 24 hours and may be greater than observed.

In conclusion, this is the first study to measure binding properties, internalization kinetics, and clinical and pathologic activity of b-vedotin. Cell kill was rapid and durable, highlighting the antitumor potential of this antibody-drug conjugate.

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JRF and AKG designed the experiments, ARS evaluated the patient clinically, AT performed the experiments, all authors analyzed the data, JRF and AKG wrote the manuscript, and all authors edited the manuscript for scientific content.

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Disclosure

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