Direct targeting of $\alpha_v\beta_3$ integrin on tumor cells with a monoclonal antibody, Abegrin™

Kathy Mulgrew, Krista Kinneer, Xiao-Tao Yao, Beth K. Ward, Melissa M. Damschroder, Bill Walsh, Su-Yau Mao, Changshou Gao, Peter A. Kiener, Steve Coats, Michael S. Kinch, and David A. Tice

MedImmune, Inc., Gaithersburg, Maryland

Abstract
The humanized monoclonal antibody Abegrin™, currently in phase II trials for treatment of solid tumors, specifically recognizes the integrin $\alpha_v\beta_3$. Due to its high expression on mature osteoclasts, angiogenic endothelial cells, and tumor cells, integrin $\alpha_v\beta_3$ functions in several pathologic processes important to tumor growth and metastasis. Targeting of this integrin with Abegrin™ results in antitumor, antiangiogenic, and antosteolytic activities. Here, we exploit the species specificity of Abegrin™ to evaluate the effects of direct targeting of tumor cells (independent of targeting of endothelia or osteoclasts). Flow cytometry analysis of human tumor cell lines shows high levels of $\alpha_v\beta_3$ on many solid tumors, including cancers of the prostate, skin, ovary, kidney, lung, and breast. We also show that tumor growth of $\alpha_v\beta_3$-expressing tumor cells is inhibited by Abegrin™ in a dose-dependent manner. We present a novel finding that high-dose administration can actively impair the antitumor activity of Abegrin™. We also provide evidence that antibody-dependent cellular cytotoxicity contributes to in vitro and in vivo antitumor activity. Finally, we observed that peak biological activity of Abegrin™ arises at serum levels that are consistent with those achieved in clinical trials. These results support a concept that Abegrin™ can be used to achieve selective targeting of the many tumor cells that express $\alpha_v\beta_3$ integrin. In combination with the well-established concept that $\alpha_v\beta_3$ plays a key role in cancer-associated angiogenesis and osteolytic activities, this triad of activity could provide new opportunities for therapeutic targeting of cancer.

Introduction
Biological therapy is providing new weapons for oncologists that allow the selective targeting of cancer. In particular, monoclonal antibodies represent a successful and validated approach for targeting cells within the tumor environment (1). Examples include direct targeting of tumor cells (e.g., cetuximab) or components of the microenvironment (e.g., bevacizumab). Importantly, monoclonal antibodies have proven to be generally safe and effective.

In the search for potential targets for monoclonal antibodies, one could seek to identify either direct or indirect tumor targets. Examples of direct tumor targets include HER-2, epidermal growth factor receptor, CD20, and other molecules that are preferentially overexpressed in tumor cells. Alternatively, indirect tumor targets could include molecules within the tumor environment that nonetheless critically control tumor growth or survival, the most well-known being targets on angiogenic blood vessels.

Abegrin™ (etaracizumab; previously known as Vitaxin® or MEDI-522) is a monoclonal antibody that was chosen for its unique ability to selectively target multiple and different cell types, all of which are relevant to cancer pathophysiology. The target for Abegrin™ is $\alpha_v\beta_3$, an integrin that is overexpressed on tumor cells, angiogenic blood vessels, and osteoclasts. Antagonists of $\alpha_v\beta_3$ (including antibodies and small molecules) have been studied most extensively for their antiangiogenic properties (2–5). In addition, $\alpha_v\beta_3$ is expressed on tumor cells and osteoclasts and is believed to play an important role in bone metastasis and subsequent resorption (6). These findings suggest a potential role for $\alpha_v\beta_3$ in the pathology of osteolytic diseases, including breast cancer, prostate cancer, and multiple myeloma. Finally, $\alpha_v\beta_3$ is overexpressed on a variety of different tumor types. For example, a preponderance of data suggests that $\alpha_v\beta_3$ is found to be overexpressed in metastatic melanoma, glioma, multiple myeloma, ovarian, renal, and breast cancer (7–13).

The murine monoclonal antibody, LM609, binds human $\alpha_v\beta_3$ and was humanized and affinity matured based on its abilities to mediate antiangiogenic effects in preclinical models (14). The resulting antibody Abegrin™ has been tested in clinical trials of various solid tumors where it appears to be without significant toxicity. The trials also indicated that Abegrin™ may have effects on tumor perfusion and may exhibit clinical activity in renal cell cancer (15). Based on these findings, Abegrin™ is presently being investigated in clinical trials of androgen-independent prostate cancer and metastatic melanoma. In our present report, we seek to understand further mechanisms by which Abegrin™ functions in cancer. In particular, we have returned to preclinical investigation to address the question of whether Abegrin™ could provide additional...
opportunities to directly target cancer cells that over-express $\alpha_v\beta_3$. In addition, our studies reveal new opportunities for cancer cell targeting and identify novel mechanisms and dosing properties that could provide a wider understanding for targeting of cancer with monoclonal antibodies.

**Materials and Methods**

**Cell Lines**

All cell lines used in this study were obtained from the American Type Culture Collection (Manassas, VA) and cultured according to specifications.

**Radioligand Binding Assay**

For the concentration dependence of binding, $[^{125}I]$Abegrin$^\text{TM}$ was added at various concentrations to M21 cells at $10^5$ cells/mL and incubated with cells at 4°C for 30 min with shaking every 3 to 5 min. Duplicate 75 µL aliquots were layered over 200 µL phthalate oil mixture [Dibutylphthalate/bis(2-ethylhexyl)phthalate = 60:40; both from Sigma, St. Louis, MO] and immediately centrifuged for 1 min at 13,000 rpm in a microfuge. Two to 50 µL of the supernatant were removed and measured for $^{125}$I radioactivity to determine the concentration of free ligand. The tubes were then snap frozen in dry ice, and the tips were cut and counted for radioactivity. Duplicate samples were within 10% of the mean for the data reported. Nonspecific binding was measured in samples, in which $>$20-fold excess unlabeled Abegrin$^\text{TM}$ was added 20 min before adding $[^{125}I]$Abegrin$^\text{TM}$; it was typically $\leq$10% of the total bound. Average $K_D$ and binding sites per cell are calculated from nine independent experiments.

**Flow Cytometry**

Adherent cells were harvested using enzyme-free dissociation buffer (Invitrogen, Carlsbad, CA), washed once, and incubated with Abegrin$^\text{TM}$ (1 µg/10⁶ cells) at 4°C for 1 h in fluorescence-activated cell sorting buffer (PBS + 2% fetal bovine serum). Cells were then washed in fluorescence-activated cell sorting buffer and stained with a goat F(ab')₂ anti-human IgG R-phycocerythrin secondary antibody (used at 1:250 dilution; Southern Biotech, Birmingham, AL) at 4°C for 45 min. Subsequently, cells were washed twice and resuspended in 500 µL fluorescence-activated cell sorting buffer for analysis. Live whole cells were gated using propidium iodide (Roche, Indianapolis, IN) staining, and 10,000 events were acquired using a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using BD CellQuest software (BD Biosciences).

**Immunohistochemistry**

Placental tissue, a rich source of $\alpha_v\beta_3$, was collected either immediately after parturition or from freshly sacrificed, late-term pregnant animals. Tissue fragments, $\sim$1 cm³, were frozen in optimal cutting temperature, and thin sections were stained with Abegrin$^\text{TM}$ (10 µg/mL) at Pathology Associates, Inc. (Frederick, MD).

**Epitope Mapping**

Human, rat, mouse, and rabbit $\beta_3$ integrin sequences corresponding to residues 164 to 202 were aligned. Specific residues within human $\beta_3$ integrin were mutated to the corresponding residue from the rat sequence using QuikChange Site-Directed Mutagenesis kit from Stratagene (La Jolla, CA) as per the manufacturer's instructions. The resulting mutant human $\beta_3$ integrin-containing plasmids were transfected into HEK-293 cells, using the endogenous human $\alpha_v$ chain for dimerization of the integrin receptor. The mutant $\beta_3$ clones were analyzed for binding to Abegrin$^\text{TM}$ by flow cytometry as described above. A human $\beta_3$ integrin-specific antibody, clone PM6/13, from Chemicon (Temecula, CA) was used as a control to compare transient expression levels of the mutants.

**In vivo Tumor Xenograft Studies**

Athymic nu/nu (Harlan, Somerville, NJ), scid (Harlan), or scid/nod (Taconic, Germantown, MD) female mice 4 to 6 weeks of age were injected s.c. with $5 \times 10^6$ tumor cells. Treatments of PBS, IgG control, bevacizumab (McKesson Bioservices Corp., Rockville, MD), or Abegrin$^\text{TM}$ were injected two or three times weekly in the i.p. cavity as indicated in the figure legends and continued throughout the study. Only scid and scid/nod mice with $<100$ ng/mL of mouse IgG as assessed by ELISA (Pierce Biotechnology, Rockford, IL) were used for the study.

**Antibody-Dependent Cellular Cytotoxicity Assay**

Antibody-dependent cellular cytotoxicity (ADCC) was assayed in a 4-h nonradioactive lactate dehydrogenase release assay (Promega Corp., Madison, WI). Briefly, target cells were resuspended in RPMI 1640 containing 5% fetal bovine serum at 2 $\times$ 10⁵ per mL and distributed into 96-well U-bottomed plates (1 $\times$ 10⁴ per well). The target cells were preincubated with serial dilution of antibodies (50 µL/well) for 20 min on ice before the human effector cells (100 µL/well) were added. Human effector cells were peripheral blood mononuclear cells purified from healthy donors using lymphocyte separation medium (MP Biomedicals, Solon, OH). After a 4-h incubation at 37°C in 5% CO₂, plates were centrifuged. Fifty microliter supernatant was transferred to a 96-well flat-bottomed plate and incubated with 50 µL lactate dehydrogenase substrate for 30 min at room temperature. The reaction was stopped by adding a 50-µL stop solution. The samples were measured at 490 nm, and the percentage cytotoxicity was calculated according to the formula: % specific lysis = 100 $\times$ ($E_X - E_{\text{spon}} - T_{\text{spon}}$) / ($T_{\text{max}} - T_{\text{spon}}$), where $E_X$ represents the release from experimental wells, $E_{\text{spon}}$ is the spontaneous release of effector cells alone, $T_{\text{spon}}$ is spontaneous release of target cells alone, and $T_{\text{max}}$ is the maximum release from lysed target cells.

**Human IgG1 Quantitation ELISA**

Three mouse serum samples per treatment group were collected 4 days following the final treatment of the A498 tumor xenograft studies. They were diluted in PBS/T-BSA [1× PBS (pH 7.4) + 0.1% Tween 20 + 0.5% bovine serum albumin] and added in duplicate to wells of BD BioCoat anti-human IgG ELISA plates (BD Biosciences). Abegrin$^\text{TM}$ serially diluted in PBS/T-BSA served as the standard. Plates were incubated at room temperature for 60 min and then washed thrice with PBS/T [1× PBS (pH 7.4) + 0.1% Tween
3124 Targeting of $\alpha_v^3$ Integrin on Tumor Cells with Abegrin$^{TM}$

Goat anti-human IgG horseradish peroxidase conjugate (Pierce Biotechnology) diluted 1:60,000 in PBS/T-BSA was added to each well and incubated at room temperature for 60 min. Subsequently, plates were washed thrice with PBS/T and the binding of human IgG was detected using TMB substrate (BioFX Laboratories, Owing Mills, MD). Color development was analyzed by adding 0.18 mol/L (1%) H$_2$SO$_4$ to each well and reading the plate at 450 nm.

Results

Antibody Targeting of $\alpha_v^3$ on Tumor Cells

Based on evidence that $\alpha_v^3$ is overexpressed on many tumor types, we asked if $\alpha_v^3$ might provide a role in direct tumor targeting independent of its established role in angiogenesis. The successful accomplishment of these studies required further characterization of $\alpha_v^3$ binding sites per cell. Flow cytometric analyses revealed strong Abegrin$^{TM}$ immunoreactivity on multiple cell models that were representative of many different tumor types, including carcinomas of the breast, prostate, kidney, and skin (Fig. 1B). Melanoma models were emphasized based on evidence that has linked $\alpha_v^3$ overexpression in clinical specimens of metastatic melanoma. Flow cytometric analyses revealed that the estimated density of $\alpha_v^3$ receptors on melanoma cells ranged from 83,000 (SK-MEL-5) to >250,000 (A375) molecules per cell. The studies above confirmed that $\alpha_v^3$ is present on the surface of many tumor-derived cell lines. To focus on the role of $\alpha_v^3$ on tumor cells, it would be necessary to identify antibodies that would not interact with cells of the host microenvironment (e.g., endothelial cells). For this, the species specificity of Abegrin$^{TM}$ was determined via immunohistochemical staining of placental trophoblasts. This cell type proved to be particularly useful because $\alpha_v^3$ is ubiquitously expressed on mammalian placental trophoblasts. Immunohistochemical analyses revealed strong Abegrin$^{TM}$ immunoreactivity with human and moderate to weak binding to guinea pig, hamster, or rabbit $\alpha_v^3$ (Fig. 1C). In contrast, Abegrin$^{TM}$ was completely unable to react with either mouse or rat $\alpha_v^3$.

The species-dependent binding of Abegrin$^{TM}$ provided an opportunity to further characterize the epitope. Based on knowledge that binding to LM609 is imparted by residues 164 to 202 of the $\beta_3$ chain (16), the homologues of $\beta_3$ integrin were aligned from multiple species. Using the species specificity information, we were able to focus on several residues in the $\beta$ chain that distinguish human from mouse or rat $\alpha_v^3$. Each difference was introduced into human $\beta_3$ integrin by site-directed mutagenesis, and the resulting constructs were expressed in HEK-293 cells, which endogenously express the human $\alpha_v$. The human $\alpha_v^3$ integrin that contained a mutation in residue 171 of the $\beta_3$ chain was not recognized by Abegrin$^{TM}$, suggesting that this residue is critically important in the binding and species specificity of Abegrin$^{TM}$ (Fig. 1D). Equal cell surface expression of $\beta_3$ integrin was determined by staining transfected cells with a $\beta_3$ integrin-specific antibody (data not shown).

Abegrin$^{TM}$ Antitumor Activity and Mechanism of Action

Based on the studies above, we considered that human tumors implanted within a murine background could provide the opportunity to evaluate direct targeting of tumor cells by Abegrin$^{TM}$. The use of human tumor cells that expressed $\alpha_v^3$ in combination with the species specificity of Abegrin$^{TM}$, provided an opportunity to ask if Abegrin$^{TM}$ could confer antitumor effects independent of its role in angiogenesis. Biodistribution studies with labeled Abegrin$^{TM}$ showed specific targeting of the tumor type presented.

Figure 1. Binding characteristics of Abegrin$^{TM}$. A, the binding of Abegrin$^{TM}$ to $\alpha_v^3$ on human melanoma M21 cells was measured by radioligand binding using [125I]Abegrin$^{TM}$. Average $K_D$ and binding sites per cell are calculated from nine independent experiments. B, flow cytometry analysis of several cultured human tumor cell lines was performed with Abegrin$^{TM}$. Estimated number of $\alpha_v^3$ integrin receptors was determined by comparing the mean channel fluorescence of M21 cells with a calculated number of 223,000 receptors per cell with the mean channel fluorescence from an unknown cell line. C, immunohistochemical staining of placental trophoblasts with Abegrin$^{TM}$ scored on a 0 to 4 + scale. D, amino acids in human $\beta_3$ integrin were mutated to the corresponding rat residues and cloned into a mammalian expression vector. Transfection of human HEK-293 cells, which endogenously express $\alpha_v$, with the mutant $\beta_3$ cDNA resulted in surface expression of $\alpha_v^3$ that was detectable by flow cytometry with Abegrin$^{TM}$.
antibody to $\alpha_v\beta_3$-expressing human tumor cells in mouse xenografts (17). Analysis of xenografts revealed that Abegrin$^\text{TM}$ targeting of tumor cells is sufficient to decrease tumor growth in vivo. For example, treatment of mice with Abegrin$^\text{TM}$ prevented the subsequent growth of tumors by M21 melanoma cells (Fig. 2A). These effects were not unique to any particular model as Abegrin$^\text{TM}$ decreased the growth of A375 melanoma cells (Fig. 2B) as well as experimental models of renal and prostate cancer.

Once we had established a proof of principle that Abegrin$^\text{TM}$ can inhibit tumor growth, we then sought to identify mechanisms by which Abegrin$^\text{TM}$ acts on tumor cells. Not surprisingly, in vitro assays revealed that Abegrin$^\text{TM}$ treatment of tumor cells prevented them from interacting with underlying extracellular matrix proteins (e.g., fibrinogen and other RGD-bearing extracellular matrix proteins; data not shown) as has been shown with other $\alpha_v\beta_3$ antagonists (14, 18, 19). This finding is consistent with the well-established concept that $\alpha_v\beta_3$ integrin confers an important cell adhesion function.

We also considered that host effector mechanisms (e.g., ADCC and complement-dependent cytotoxicity) could contribute to the antitumor activity observed in xenograft models. To examine the relative importance of ADCC, xenograft analyses were done using scid/nod mice, which have a deficiency in monocytes and natural killer cells, thereby compromising their ADCC activity. Bevacizumab (Avastin) provided a control as it binds a soluble ligand and thus does not efficiently mediate ADCC. As expected, Abegrin$^\text{TM}$ treatment of scid mice decreased A375 melanoma tumor growth by at least 70% relative to isotype-treated controls ($P < 0.0001$; Fig. 3A). In contrast, tumor inhibition was marginal (<25%) when similar studies were conducted within a scid/nod background (Fig. 3B). As expected, the antitumor effects of bevacizumab were not altered in the nod background. Altogether, these results suggest that host effector mechanisms, and ADCC in particular, critically contribute to the antitumor activity of Abegrin$^\text{TM}$ in vivo.

**Dose-Limited Antitumor Activity**

In the course of conducting xenograft analyses with Abegrin$^\text{TM}$, we found that the tumor-inhibitory properties of Abegrin$^\text{TM}$ decreased when drug was given at relatively high doses (>20 mg/kg). We termed this behavior “dose-limited antitumor activity.” Whereas the antitumor activity of Abegrin$^\text{TM}$ steadily increased when animals were treated with 1 to 10 mg/kg, the biological activity of Abegrin$^\text{TM}$ decreased at doses >20 mg/kg (Fig. 4). This outcome was not limited to any particular cell line or tumor type as comparable findings were obtained using multiple and different models (melanoma, prostate, and renal; data not shown).

To begin addressing the mechanistic basis for “dose-limited activity,” we considered that ADCC critically contributes to Abegrin$^\text{TM}$ tumor inhibition. It was important to also determine whether “dose-limited activity” might simply represent an artifact of using a murine model system. To address both questions, ADCC assays were conducted ex vivo using human effector cells. The results of ADCC assays were consistent with the dose response from xenograft analyses. Specifically, Abegrin$^\text{TM}$ mediated ADCC of tumor cells increased with dose, with optimal killing observed at 1 $\mu$g/mL (Fig. 5A). With further increases in dose, however, ADCC activity decreased dramatically, with much less killing at 100 $\mu$g/mL. Comparable findings were observed at different ratios of effector to target cells (from 5:1 to 50:1; data not shown). The dose-limited activity was also observed regardless of whether peripheral blood lymphocytes or purified natural killer cells were used as effector cells or whether A498, A375, or M21 cells were used as target cells in the assay (data not shown). We then asked whether this...
“dose-limited activity” might be specific for Abegrin™ or could antibodies to other cell surface proteins elicit this same response. To test this, ADCC assays were done using 3F2, a human IgG1 antibody specific for human EphA2 (Fig. 5A). An identical pattern of dose-limited activity was observed, suggesting that this effect is not specific to Abegrin™ or \( \alpha v \beta 3 \) integrin.

We then asked whether inclusion of high-dose Abegrin™ would confer a “dominant-negative” phenotype to prevent other human IgG1 antibodies from mediating ADCC. This could arise if Abegrin™ saturated and inhibited effector cell function. To test this, ADCC assays were done using \( \alpha v \beta 3 \)-deficient A549 lung carcinoma cells. 3F2 was again used for ADCC assays in the presence or absence of various doses of Abegrin™. The inclusion of Abegrin™, even at concentrations as high as 100 \( \mu g/mL \), did not decrease 3F2-mediated ADCC (Fig. 5B). Controls confirmed that Abegrin™ itself also did not mediate ADCC, which is consistent with the absence of \( \alpha v \beta 3 \) on the target cells. These results indicate that high-dose Abegrin™ does not function as a dominant inhibitor of ADCC by acting on effector cells and that free unbound effector cells are in excess even at high antibody concentrations.

For our final set of studies, we considered that high-dose antibody administration might alter the serum levels of Abegrin™. For example, bolus administration might cause...
antibody aggregation in vivo, thereby accelerating its clearance and limiting its availability. We determined that the concentrated Abegrin™ stocks used for these experiments were free of aggregates (data not shown) but could not exclude the possibility that such an event might occur in vivo. A greater understanding of the serum levels was also important as Abegrin™ is currently being investigated in clinical trials of melanoma and prostate cancer. To ask how “dose-limited activity” in xenograft models (at 30 mg/kg, given twice weekly) relates to the situation encountered in the clinic (at 8 mg/kg given weekly), the serum level of Abegrin™ in mice was compared with the known pharmacokinetic profile in humans. The trough serum concentration of human IgG (Abegrin™) is defined by the serum concentration just before the administration of the next dose and was measured by ELISA in mice during week 8 of a xenograft study using A498 tumor cells (Fig. 6). The circulating levels of Abegrin™ were proportional to the dose administered, with mean trough concentrations of 11 and 192 μg/mL in animals that had been treated with 1 or 10 mg/kg. Trough levels of 574 μg/mL were attained in animals treated with 30 mg/kg Abegrin™, indicating that lack of antitumor activity at this dose was not due to increased clearance of the antibody. Tumor homogenates also showed a comparable increase in IgG levels with increasing dose, suggesting that diffusion into the tumor was not altered at the high dose (data not shown). Notably, the serum levels of Abegrin™ from human trials at a dose of 8 mg/kg (peak and trough of approximately 300 and 100 μg/mL, respectively) coincided with doses associated with optimal antitumor activity in our in vivo tumor studies (15).

Discussion

The major finding of our present study is that antibody targeting of αvβ3 integrin on tumor cells can provide usefulness for targeting cancer independent of, and in addition to, antibody targeting of αvβ3 on angiogenic blood vessels. We have also increased our understanding of the distribution of αvβ3 receptors and the mechanisms of action for Abegrin™. In particular, we provide evidence that Abegrin™ invokes host defense mechanisms and that ADCC critically contributes to Abegrin™ antitumor activity. Finally, we present a novel finding that high-dose administration can actively impair the antitumor activity of Abegrin™. This new information has important implications not only for the future development of αvβ3 integrin inhibitors but also for understanding the mechanism of action for monoclonal antibody-based therapies.

Our present findings are novel, in part, because they shed new light about potential therapeutic opportunities for antibody targeting of αvβ3 in cancer. Previously, much of the interest surrounding this integrin centered on the overexpression and functional relevance of αvβ3 on tumor-associated endothelial cells. This information triggered considerable interest in αvβ3 as a target for antiangiogenic cancer therapy and, indeed, this was the original rationale supporting Abegrin™ as a cancer therapeutic. αvβ3 is also known to be overexpressed on...
osteoclasts and functionally relevant to bone resorption. This has stimulated considerable interest in the application of antagonists of αvβ3, including Abegrin™, to osteolytic tumor types, such as melanoma, multiple myeloma, and breast cancer. Our present study widens this knowledge by presenting evidence for Abegrin™ activity against tumor cells themselves. In some ways, the “triad” of Abegrin™ activity may be likened to combination targeted therapy through simultaneous blocking of tumor growth and survival, angiogenesis, and bone metastasis. All of these strategies can be achieved with a single agent that shows minimal toxicity. Consequently, future studies will be focused on the potential for combining Abegrin™ with known cytotoxic agents to evaluate potential opportunities for synergistic therapies and to ask if Abegrin™ might have applications for drug sensitization in treatment-refractory indications.

Another novel aspect of our present study is the demonstration that ADCC is a major mechanism that contributes to the activity of Abegrin™ directed against the tumor cells. Before this report, αvβ3 was generally understood to play an important role in cell adhesion and signaling. Indeed, αvβ3 has been shown to critically control many different aspects of malignant character, including cell migration, invasion, and survival (e.g., via anoikis) and antagonists of αvβ3 (both antibodies, such as Abegrin™ and small molecules) are presently being investigated in clinical trials (5). Although our present studies do not preclude that these mechanisms contribute to antitumor activity, they do identify an important role for ADCC for targeting αvβ3. This information is consistent with findings with other antibodies (rituximab and trastuzumab) and also has potential importance for identifying improved means of αvβ3 targeting (20). Specifically, small-molecule antagonists of αvβ3 function are incapable of mediating ADCC and this may limit their therapeutic potential.

Perhaps, the most intriguing finding of our present report is the demonstration of “dose-limited” antitumor activity. The decreased activity of Abegrin™ at the highest dose levels has important implications for our understanding of basic mechanistic activities of monoclonal antibodies and conveys potentially important information that is highly relevant to clinical applications. Importantly, this dose-limited activity was not unique to Abegrin™ or αvβ3 as we have observed comparable effects with other antibodies and with different antigens. The dose-limited activity described herein is reminiscent of the prozone-like effect observed in passive immunization approaches against human pathogens (21). Several studies have shown that sera or purified IgG from patients or infected animals elicited a prozone-like response in ADCC (22–25), complement-dependent cytotoxicity (26), and neutralization (27) assays in vitro. Furthermore, prozone-like effects have been observed in vivo following infection with various pathogens, such as Streptococcus pneumoniae and Cryptococcus neoformans (28, 29). Much less is known about how this phenomenon affects passive immunization approaches in other disease areas, such as oncology.

Regardless of why dose-limited activity occurs, the outcome could have particular importance for designing and applying antibody-based therapeutics. Historically, biologics generally have relatively few side effects and many clinical trials fail to reach a maximum tolerated dose. Consequently, the dose selected for further clinical application may be defined by other considerations (cost and availability). If the findings herein have a more general application to other antibodies, then important changes will be necessary to identify optimal doses, particularly with biological agents that function via ADCC. Within this context, we have conducted extensive pharmacokinetic analyses with Abegrin™ and are fortunate in that the clinical doses presently under investigation in clinical trials seem to fall within the optimal zone of preclinical activity. Nonetheless, our present findings indicate a need to determine and apply similar criteria to other biologics-based therapeutic agents.

Acknowledgments

We thank the animal barrier facility staff at MedImmune, Inc. (Gaithersburg, MD) for their careful attention and assistance in animal handling and the clinical department for their helpful discussions and review of the manuscript.

References

1. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005;23:1147–57.
2. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin αvβ3 for angiogenesis. Science 1994;264:569–71.
3. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994;79:1157–64.
4. Brooks PC, Stromblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. Antitumor activity of vitaxin, an antibody to αvβ3 integrin, is associated with suppression of angiogenesis. J Clin Invest 1995;96:1815–22.
5. Kumar CC. Integrin αvβ3 as a therapeutic target for blocking tumor-induced angiogenesis. Curr Drug Targets 2003:4:123–31.
6. Teti A, Migliaccio S, Baron R. The role of the αvβ3 integrin in the development of osteolytic bone metastases: a pharmacological target for alternative therapy? Curr Tissue Pathol 2002;71:293–9.
7. Natali PG, Hamby CV, Felding-Habermann B, et al. Clinical significance of αvβ3 integrin and intercellular adhesion molecule-1 expression in cutaneous malignant melanoma lesions. Cancer Res 1997;57:1554–60.
8. Van Belle PA, Elens S, Satyamoorthy K, et al. Progression-related expression of α3 integrin in melanomas and nevi. Hum Pathol 1999;30:562–7.
9. Gingras MC, Roussel E, Bruner JM, Branch CD, Moser RP. Comparison of cell adhesion molecule expression between glioblastoma multiforme and autologous normal brain tissue. J Neuroimmunol 1995;7:143–53.
10. Ria R, Vicca A, Ribatti D, Di Raimondo F, Merchionne F, Dammacco F. αvβ3(3) integrin engagement enhances cell invasiveness in human multiple myeloma. Haematologica 2002;87:836–45.
11. Carreiras F, Denoux Y, Staedel C, Lehmann M, Sichel F, Gauduchon P. Expression and localization of αv integrins and their ligand vitronectin in normal ovarian epithelium and in ovarian carcinoma. Gynecol Oncol 1996;62:260–7.
12. Wechsel HW, Petri E, Feil G, Nelde HJ, Bichler KH, Loeser W. Renal cell carcinoma: immunohistological investigation of expression of the integrin αvβ3. Anticancer Res 1999;19:1529–32.
13. Lipins H, Flath A, Kitazawa S. Integrin αvβ3 expression by bone-resident cancer metastases. Diagn Mol Pathol 1998;5:127–35.
14. Wu H, Beuerlein G, Nie Y, et al. Stepwise in vitro affinity maturation of Vitaxin, an αvβ3-specific humanized mAb. Proc Natl Acad Sci U S A 1998;95:8037–42.
15. McNeel DG, Eickhoff J, Lee FT, et al. Phase I trial of a monoclonal antibody specific for \( \alpha_\text{v}\beta_3 \) integrin (MEDI-522) in patients with advanced malignancies, including an assessment of effect on tumor perfusion. Clin Cancer Res 2005;11:7851–60.

16. Takagi J, Kamata T, Meredith J, Puzon-McLaughlin W, Takada Y. Changing ligand specificities of \( \alpha_\text{v}\beta_1 \) and \( \alpha_\text{v}\beta_3 \) integrins by swapping a short diverse sequence of the \( \beta \) subunit. J Biol Chem 1997;272:19794–800.

17. Cai W, Wu Y, Chen K, Cao Q, Tice DA, Chen X. In vitro and in vivo characterization of \( ^{64}\text{Cu} \)-labeled Abegrin\textsuperscript{TM}, a humanized monoclonal antibody against integrin \( \alpha_\text{v}\beta_3 \). Cancer Res 2006;66:9673–81.

18. Cheresh DA. Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc Natl Acad Sci U S A 1987;84:6471–5.

19. Felding-Habermann B, Ruggeri ZM, Cheresh DA. Distinct biological consequences of integrin \( \alpha_\text{v}\beta_3 \)-mediated melanoma cell adhesion to fibrinogen and its plasmic fragments. J Biol Chem 1992;267:5070–7.

20. Iannello A, Ahmad A. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. Cancer Metastasis Rev 2005;24:487–99.

21. Taborda CP, Rivera J, Zaragoza O, Casadevall A. More is not necessarily better: prozone-like effects in passive immunization with IgG. J Immunol 2003;170:3621–30.

22. Gomez-Roman VR, Patterson LJ, Venzon D, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 2005;174:2185–9.

23. Parker SJ, Sadlon TA, Gordon DL. Enhancement of NK cell-mediated antibody-dependent lysis of recombinant gp120-coated CD4 cells by complement. J Infect Dis 1995;171:186–9.

24. Sinclair AL, Habeshaw JA, Muir L, et al. Antibody-dependent cell-mediated cytotoxicity: comparison between HTLV-I and HIV-1 assays. AIDS 1988;2:465–72.

25. Holmes MJ, Callow KA, Childs RA, Tyrrell DA. Antibody dependent cellular cytotoxicity against coronavirus 229E-infected cells. Br J Exp Pathol 1986;67:581–6.

26. Ball ED, Kadushin JM, Schacter B, Fanger MW. Studies on the ability of monoclonal antibodies to selectively mediate complement-dependent cytotoxicity of human myelogenous leukemia blast cells. J Immunol 1982;128:1476–81.

27. Asano Y, Albrecht P, Stagno S, Takahashi M. Potentiation of neutralization of Varicella-Zoster virus to antibody to immunoglobulin. J Infect Dis 1982;146:524–9.

28. Ramisse F, Binder P, Szatanik M, Alonso JM. Passive and active immunotherapy for experimental pneumococcal pneumonia by polyvalent human immunoglobulin or F(ab')_{2} fragments administered intranasally. J Infect Dis 1996;173:1123–8.

29. Taborda CP, Casadevall A. Immunoglobulin M efficacy against Cryptococcus neoformans: mechanism, dose dependence, and prozone-like effects in passive protection experiments. J Immunol 2001;166:2100–7.