Phase I Dose-Escalation Study of VB-111, an Antiangiogenic Virotherapy, in Patients with Advanced Solid Tumors


Abstract

**Purpose:** VB-111 is an antiangiogenic agent consisting of a nonreplicating adenovirus vector (Ad-5) with a modified murine pre-proendothelin promoter leading to apoptosis of tumor vasculature by expressing a Fas-chimera transgene in angiogenic endothelial cells. In a phase I dose-escalation study, pharmacokinetics, pharmacodynamics, safety, and efficacy of a single dose of VB-111 in patients with advanced solid tumors were evaluated.

**Experimental Design:** VB-111 was administered as a single i.v. infusion at escalating doses from $1 \times 10^{10}$ (cohort 1) to $1 \times 10^{13}$ (cohort 7) viral particles (VP) in successive cohorts. Assessments included pharmacokinetic and pharmacodynamic profiles, tumor response, and overall survival.

**Results:** Thirty-three patients were enrolled. VB-111 was safe and well-tolerated; self-limited fever and chills were seen at doses above $3 \times 10^{11}$ VP. Transgene expression was not detected in blood but was detected in an aspirate from a subcutaneous metastasis after treatment. One patient with papillary thyroid carcinoma had a partial response.

**Conclusions:** VB-111 was safe and well tolerated in patients with advanced metastatic cancer at a single administration of up to $1 \times 10^{13}$ VP. Evidence of transgene expression in tumor tissue and tumor response was observed. Clin Cancer Res; 19(14): 3996–4007. ©2013 AACR.

Introduction

The development of adequate tumor vascularity is essential for tumor growth, invasion, and metastasis. Antiangiogenic agents, including VEGF and other tyrosine kinase receptor inhibitors, are now in common clinical use in the management of a variety of cancers, including colon, lung, kidney, thyroid, and glioblastoma (1). Several limitations have been recognized in the clinical application of these vascular-targeted agents. Only limited and transient antitumor activity has been shown, mostly as part of a chemotherapeutic regimen and not as a monotherapy. As current agents target factors that regulate a variety of key cellular processes, specificity to tumor vasculature is limited, resulting in various clinical toxicities (1,2).

VB-111, a novel antiangiogenic agent, targets the endothelial cells in the tumor vasculature, driving it to apoptosis. VB-111 comprises a nonreplicating adenovector (Ad-5, E1-deleted), which contains a proprietary modified murine pre-proendothelin promoter (PPE-1-3X) and a Fas-chimera transgene (Fas and human TNF receptor 1). This modified murine promoter is able to specifically target the expression of the Fas-chimera transgene to angiogenic blood vessels, leading to targeted apoptosis of these vessels (refs. 3, 4; Fig. 1). Via systemic i.v. administration, VB-111 can serve as a means to debulk tumor burden even in well-advanced rodent xenografts with extensive tumor burden (3).

The Fas pathway effectively mediates cell death in normal and in malignant cells. Its activation by Fas ligand, which is highly expressed in nontumoral normal tissues, is highly toxic. Hepatotoxicity is circumvented by the specificity of the PPE-1-3X promoter, which restricts activation of the Fas pathway to angiogenic endothelial cells. This specificity is further enhanced, as the Fas component of the chimeric transgene is activated via binding of the chimeric TNFR-1 receptor with TNF-α, which is less prevalent in nontumoral normal tissues (5). The transcriptional rate of endothelin-1 is also augmented in the presence of cytokines like TNF-α, which also contributes to tumor specificity (3,6).

Preclinical studies showed antitumor activity of VB-111 monotherapy in several models, including Lewis lung carcinoma in mice (3), mouse B16 melanoma model (3), and nude rat glioblastoma xenograft model (7). Synergistic effects were identified in combination with chemotherapeutic agents (8) and antiangiogenic agents (9).
We present here the results of a phase I, first-in-human, single-dose administration of a VB-111 in patients with advanced solid tumors. The primary objectives of the study were to evaluate the safety and find the maximal tolerated dose (MTD) or maximal feasible dose of VB-111. The secondary objectives were to assess the pharmacokinetic and pharmacodynamic profile of VB-111, to document any clinical responses, and to evaluate changes in angiogenesis biomarkers in response to VB-111 treatment.

Patients and Methods

Study design

This is a phase I, open-label, multisite, sequential dose-escalation "3 + 3" design study of a single dose of VB-111 in patients with advanced solid tumors. The study was conducted at Cleveland Clinic Foundation (CCF) in Ohio and at the Cancer Therapy and Research Center (CTRC) in San Antonio, TX, between November 2007 and December 2010 (clinicaltrials.gov identifier NCT00559117). All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice and was approved by the relevant Regulatory and Institutional Review Boards.

Patient selection

Patients eligible for this study were 18 years or older with a confirmed diagnosis of progressive and advanced solid tumors for which no effective treatment was available. Other key eligibility criteria included provision of written informed consent, a Karnofsky performance status of at least 70%, and an adequate hematologic profile and renal and hepatic function. Exclusion criteria included recently active cardiovascular disease, recent surgery, proliferative retinopathy, liver disease or central nervous system (CNS) metastasis, recent antiangiogenic therapy or immunosuppressive treatment, recent chemo/radiotherapy or use of an investigative agent (within 4 weeks), or an uncontrolled comorbidity.

Formulation, dosing, and concomitant medications

VB-111 was manufactured in a GMP facility. It was propagated in Per6 cells, purified, and formulated as a...
sterile vector solution. The vials were diluted with normal saline for infusion and administered as a single i.v. infusion. The starting dose, $1 \times 10^{10}$ viral particles (VP), represented a safety margin of 2,800-fold below the no-observed-adverse-effect-level in the mouse toxicology studies. Planned dose levels of $3 \times 10^{10}$, $1 \times 10^{11}$, $3 \times 10^{11}$, $1 \times 10^{12}$, $3 \times 10^{12}$, and $1 \times 10^{13}$ VPs [VP/plaque-forming units (PFU)] $\leq 30$ were examined in cohorts of 3 patients in the dose-escalation phase. If one patient experienced a dose-limiting toxicity (DLT; defined as any treatment-related, grade 3 toxicity), the cohort was to be expanded to 6 patients. If none of the remaining 3 patients in the expanded cohort experienced a DLT, escalation continued. The MTD was defined as the highest dose at which fewer than 33% patients experienced a DLT. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (Version 3). Dose-escalation continued until the MTD or the maximum-planned dose was reached. In the dose-expansion phase, 12 patients were enrolled at dose level 6 and 6 patients at dose level 7 ($3 \times 10^{13}$ VPs and $1 \times 10^{13}$ VPs) to obtain additional safety and efficacy data. To avoid fever following study drug administration, patients received 1 g of acetaminophen before dosing, then as needed for 72 hours after dosing.

**Pretreatment and follow-up visits: safety and tumor response**

The trial was initially designed as a 56-day follow-up after the single dose. It was later amended to include follow-up visits until disease progression, and, thereafter, bimonthly phone contacts for survival. Pretreatment evaluation included a complete history, physical examination, and routine laboratory studies including a complete blood count (CBC), chemistry [electrolytes, blood urea nitrogen, creatinine, uric acid, glucose, alkaline phosphatase, lactate dehydrogenase, alkaline aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, calcium, total protein, albumin, cholesterol, and triglycerides], urinalysis, electrocardiography (ECG), and tumor markers. Radiologic assessment was based on chest, abdomen, and pelvic computed tomographic (CT) or MRI scans. During the trial, radiologic studies for disease status were recorded at week 4 and 8 and every 8 weeks thereafter (cohorts 6–7 only, after protocol amendment) until disease progression. Tumor response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST 1.0) at these prespecified time points. Hematology (CBC, coagulation) and chemistry blood tests were collected at each study visit. Adverse events were recorded on an ongoing basis and up to 2 months following the administration of the test drug. Patients were observed for 6 hours after dosing. Upon disease progression, patients were followed every 2 months for survival.

**Pharmacokinetic and pharmacodynamic testing**

Testing was conducted under Good Laboratory Practice compliance at Southern Research Institute, Birmingham, AL. Blood and urine samples were collected before dosing, at the end of the infusion, at 3 hours and 6 hours, and on days 4, 7, 14, 28, and 56 for evaluation of viral DNA levels in blood and urine and the expression of the transgene (mRNA in blood). The presence of vector DNA was tested using a quantitative real-time PCR (qPCR) method. The presence of mRNA derived from transgene expression was determined using quantitative real-time reverse transcriptase PCR (qRT-PCR). Both assays were developed and validated at Southern Research Institute. Optionally, accessible tumor lesions were biopsied and analyzed for transgene expression before and after dosing.

**Immune response to VB-111**

Serum samples were diluted and analyzed for adenovirus-specific immunoglobulin G (IgG) for adenovirus 5 (Ad-5) by ELISA. For the ELISA, 96-well flat-bottomed, high-binding Immulon-IV plates were coated with 100 µL Ad-5 antigen (Penn Vector Core) at $5 \times 10^6$ particles/well in carbonate buffer (coating buffer, pH 9.3) overnight at 4°C, washed twice in PBS/0.05% Tween, blocked in PBS/1% horse serum albumin (HSA) for 1 hour at 24°C, and then washed twice in PBS/0.05% Tween. Appropriately diluted (3-fold) samples were added to antigen-coated plates and incubated for 2 hours on an orbital shaker at room temperature. Plates were washed three times with PBS/0.05% Tween and incubated with peroxidase-conjugated goat anti-human Ig (1:5,000 dilution; Jackson ImmunoResearch Laboratories, Inc.) for 1 hour at room temperature. Plates were washed three times as above, and 3,3′,5,5′-tetramethylbenzidine substrate (Sigma-Aldrich) was added at 100 µL/well for 30 minutes. Reaction was stopped when 100 µL 2 N H2SO4 was added, and optical densities were read at 450 nm on a VersaMAX tunable microplate reader (Molecular Devices). Tier was the dilution achieving 0.5 maximum optical density.

**Serum cytokines and receptors**

Serum levels of interleukin (IL)-6, IL-8, VEGF, TNF-α, soluble TNF receptor (sTNFR)-1, sTNFR-2, soluble intercellular adhesion molecule 1 (sCD54), soluble E-selectin (sCD106), and fibroblast growth factor (FGF) were measured by cytometric bead array (CBA) and measured in peripheral blood of patients at baseline and at 6 hours and 4, 7, 14, 28, and 56 days after dosing. Samples were diluted, and CBA Flex Sets (BD Biosciences Pharmingen) were used according to the manufacturer’s instructions. Flow cytometry was conducted using a BD LSR II System (BD Biosciences). Data were analyzed using the BD CBA software (FCAP Array Software, BD Biosciences). Standard curves for each cytokine/receptor were generated using the premixed lypophilized standards provided in the kits, and the concentrations in samples were determined from the appropriate standard curve.

**Statistical analysis**

The trial was planned to present a descriptive statistical data summary. Cohorts 6 and 7 were expanded as...
prespecified in the study protocol. From dose equivalence based on efficacy results in animal models, cohorts 1 to 5 were expected to be below efficacy levels, cohort 6 was expected to provide borderline efficacy, and cohort 7 was equivalent to efficacious doses in the animal model. As a post hoc exploratory analysis, we compared the time to progression of disease and overall survival, assessed with the use of Kaplan–Meier curves for cohorts 1 to 6 versus 7 and for cohorts 1 to 5 versus 7 and tested for significance by the log-rank test.

Results

Patient characteristics
A total of 33 patients [3 in each of cohorts 1–5 (total of 15 patients), 12 in cohort 6, and 6 in cohort 7] with progressive advanced and/or metastatic solid organ cancers were enrolled in the study. Cohorts 6 and 7 were extended to 12 and 6 patients, respectively, to enable detection of an efficacy signal. Patients’ baseline characteristics are detailed in Table 1. Most patients had received multiple lines of antitumor therapy, 60.6% had previous antiangiogenic therapy, 45.5% had previous radiation therapy, and 84.9% had previous cancer-related surgery.

Safety and tolerability

VB-111 was found to be safe and well tolerated, except for transient febrile reaction after dosing in cohort levels 5 to 7 (dose levels $10^{12}$–$10^{13}$ VP$\cdot$s). One cohort 7 patient experienced a DLT of grade 3 pyrexia (temperature of 40.3°C) that was considered related to the study medication. It was not a serious adverse event and resolved with the use of acetaminophen with no sequelae. Three serious adverse events occurred; all were judged to be caused by progression of malignancy and unrelated to study medication. The most frequent adverse events were pyrexia, fatigue, and chills. Pyrexia and chills occurred mostly in the higher-dose groups and were usually considered related to study medication. Related adverse events are summarized in Table 2.

Several patients had declines in hemoglobin and hematocrit values after study treatment. Also, mild to moderately elevated activated partial thromboplastin time (aPTT) levels occurred (without clinical consequence) in some patients before day 28. No clinically significant posttreatment chemistry or urinalysis abnormalities occurred during the study with the exception of a patient with bile duct obstruction due to progression of cancer who developed markedly elevated alkaline phosphatase and elevations of ALT, AST, and total bilirubin. In cohort 7, transient mild elevations of ALT, AST, and alkaline phosphatase were observed after dosing in some patients, and 2 patients had transient thrombocytopenia; none of these events were considered clinically significant, and all were classified as Common Terminology Criteria for Adverse Events grade 1, except for one grade 2 ALT elevation (118 IU).

No clinically significant posttreatment changes in vital signs were observed, except for the occurrence of fever 6 hours after infusion in 83% (15 of 18) of the patients in cohort 6 to 7; these patients with fever also had increased heart rates, and some had transient blood pressure changes. All patients remained hemodynamically stable. No treatment-related changes were observed on physical examinations or ECGs.

Pharmacokinetic analysis

Mean levels of adenovirus vector DNA found in whole blood after dosing showed a dose-dependent effect (Fig. 2A). By day 56, levels of Ad-5 decreased by at least 2-log fold or were undetectable. No vector DNA was found in the predose samples from any patient. In most patients with detectable levels of Ad-5 in the urine, the presence was transient, with levels detectable only within the initial 24 hours after the i.v. infusion of VB-111. All of the blood samples were found to be negative for transgene expression. One patient (01-036 with esophageal cancer) underwent a fine-needle aspiration from a subcutaneous metastasis on three separate occasions: predose and days 4 and 28 after dosing. Although the predose sample was negative, detectable levels of transgene expression were found in the

### Table 1. Demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>59 (35–77)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (52%)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (48%)</td>
</tr>
<tr>
<td>Karnofsky, median (range)</td>
<td>90 (70–100)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
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<tr>
<td>Colorectal/adenocarcinoma</td>
<td>11</td>
</tr>
<tr>
<td>Carcinoid/neuroendocrine</td>
<td>4</td>
</tr>
<tr>
<td>NSCLC</td>
<td>3</td>
</tr>
<tr>
<td>RCC</td>
<td>3</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
</tr>
<tr>
<td>Merkel cell</td>
<td>1</td>
</tr>
<tr>
<td>Small cell lung</td>
<td>1</td>
</tr>
<tr>
<td>Bladder/transitional cell</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor</td>
<td>1</td>
</tr>
<tr>
<td>Testicular/sex cord</td>
<td>1</td>
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<tr>
<td>Esophageal adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Treatment history</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>24 (73%)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>15 (45%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>29 (88%)</td>
</tr>
<tr>
<td>1 prior regimen</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>2 prior regimens</td>
<td>9 (3%)</td>
</tr>
<tr>
<td>≥3 prior regimens</td>
<td>18 (55%)</td>
</tr>
</tbody>
</table>

Abbreviations: NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.
aspirate on days 4 (1.4 × 10⁵ copies/μg RNA) and 28 (3.9 × 10⁴ copies/μg RNA) after treatment, representing a 72% reduction in the transgene expression over the two time points.

**Immunogenicity**

All patients had predose antibodies to Ad-5. Postdose total antibody titers to Ad-5 increased at least 5-fold; there was no correlation between a higher IgG predose titer and higher fold increase in level of IgG antibodies, and there was no apparent relationship of dose administered to posttreatment antibody response, as shown in Fig. 2B. Variability was observed in the level of neutralizing Ad-5 antibodies at baseline: 7 of 33 (21.2%) patients had highly elevated baseline levels (>titer of 620), 13 of 33 (39.4%) patients had moderately elevated levels (>15 and ≤620), and 13 of 33 (39.4%) patients had low levels (<15). No correlation was observed between neutralizing antibody levels at baseline and disease state following drug administration (data not shown). Peak anti-Ad-5 IgG titers were also not associated with preinfusion neutralization titer.

**Cytokine and angiogenic biomarker response**

Considerable variability in cytokine/cytokine receptor levels was observed in cytokine and angiogenic biomarker responses. An approximately 100-fold increase in IL-6 occurred in cohort 6 patients at 6 hours postdosing, correlating with the fever that occurred in cohort 6 patients at the same time. This elevation was transient, returning to normal levels by 24 hours. A 10-fold increase in VEGF was observed in cohort 6 patients at 6 hours postdosing, which was followed by a peak level on day 3.

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**Table 2. Summary of adverse events**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Cohorts 1–4 (n = 12)</th>
<th>Cohort 5 (n = 3)</th>
<th>Cohort 6 (n = 12)</th>
<th>Cohort 7 (n = 6)</th>
<th>Total (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse event grade</td>
<td>Grade 1–2</td>
<td>Grade 3</td>
<td>Grade 1–2</td>
<td>Grade 3</td>
<td>Grade 1–2</td>
</tr>
<tr>
<td>Nausea</td>
<td>0 0</td>
<td>0 0</td>
<td>2 0</td>
<td>0 0</td>
<td>4 0</td>
</tr>
<tr>
<td>Chills</td>
<td>0 0</td>
<td>1 0</td>
<td>6 0</td>
<td>2 0</td>
<td>9 0</td>
</tr>
<tr>
<td>Fever</td>
<td>1 0</td>
<td>2 0</td>
<td>9 0</td>
<td>5 1</td>
<td>18 1</td>
</tr>
<tr>
<td>aPTT prolonged</td>
<td>0 0</td>
<td>0 0</td>
<td>3 0</td>
<td>0 0</td>
<td>3 0</td>
</tr>
<tr>
<td>ALT increased</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>3 0</td>
<td>3 0</td>
</tr>
<tr>
<td>AST increased</td>
<td>0 0</td>
<td>0 0</td>
<td>1 0</td>
<td>3 0</td>
<td>4 0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>0 0</td>
<td>1 0</td>
<td>2 0</td>
<td>0 0</td>
<td>3 0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 0</td>
<td>3 0</td>
</tr>
<tr>
<td>Hyperhidrosis</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>3 0</td>
<td>3 0</td>
</tr>
</tbody>
</table>

**NOTE:** All events occurring in 3 or more patients and all grade 3–4 events.

Abbreviation: LFT, liver function test.
preinfusion levels by day 4. Cohort 6 results also showed transient but smaller increases in IL-8, sTNFR-1, and sTNFR-2 levels at the 6-hour postdosing time point ($P = 0.006$). There were small transient increases in sCD54 ($P = 0.03$) and TNFR-2 ($P = 0.002$) at several time points 28 days after dosing in cohorts 6 and 7. Other cytokines assayed did not show consistent or statistically significant changes from pre- to postinfusion at any of the time points assayed. Inadvertently, cytokine data were not available 6 hours after dosing for cohort 7 patients.

**Overall survival, progression-free survival, and tumor response**

Assessment of RECIST responses at day 28 indicated that 17 of 32 (53%) patients in the entire study had stable disease at day 28. Of these, 5 of 14 (35.7%) cohort 1 to 5 patients had stable disease, compared with 12 of 18 (66.7%) patients in cohorts 6 to 7; whereas at day 56, 12 of 32 (37%) patients in the entire study had stable disease; of these, 3 of 14 (21.4%) patients in cohorts 1 to 5 and 9 of 18 (50%) patients in cohorts 6 to 7 (Table 3, Fig. 3A and B).
In a post hoc comparison between cohorts 1 to 5, 6, and 7, overall survival was greater in cohort 7: 187 days, 173 days, and not reached, in cohorts 1 to 5, 6, and 7, respectively ($P = 0.012$, Fig. 3C). Median time to progression was 31, 55, and 121 days in cohorts 1 to 5, 6, and 7, respectively ($P = 0.24$; Fig. 3D). In another post hoc exploratory analysis, overall survival was greater in cohort 7 ($1/3$ of $10^{13}$ VPs) than in cohorts 1 to 6: 173 days and not reached, in cohorts 1 to 6, and 7, respectively ($P = 0.0098$; Fig. 3E). Median follow-up in cohort 7 was 487 days (range, 122–599 days); 4 of 6 patients in cohort 7 remain alive to date. Median time to progression was 34.5 and 121 days in cohorts 1 to 6 and 7, respectively ($P = 0.10$; Fig. 3F).

One patient with metastatic papillary thyroid cancer who was resistant to radioiodine was dosed in cohort 6. Baseline CT scan showed a mass lesion in the neck with pressure on the airway. Day 28 and 56 scans showed stable disease, and follow-up scans 6 and 12 months after treatment showed greater than 30% reduction in the long diameter of the mass and no pressure on the airway; with
some central necrosis (Fig. 4). This patient received a second dose of VB-111 (3 × 10^{12} VPs) following disease progression after 18-month stability with VB-111; the patient’s tumor remained stable 8 months after the second dose. Tumor marker responses were observed in 2 patients: papillary thyroid cancer (thyroglobulin) and medullary thyroid cancer (calcitonin).

Discussion

We report results from a dose-escalation, first-in-human trial of VB-111, an antiangiogenic gene therapeutic for systemic administration, in 33 patients with refractory metastatic cancers. VB-111 is based on transcriptional targeting of tumor endothelial cells. By means of a proprietary PPE-1-3X promoter within a modified Ad-5 nonreplicating vector, a suicide Fas-chimera transgene is selectively expressed in tumor angiogenic endothelial cells, leading to apoptosis of angiogenic tumor vasculature.

Currently approved antiangiogenic agents are based on targeting mediators of angiogenesis involved in triggering signaling pathways that result in activation, proliferation, and migration of quiescent endothelial cells. The rationale for transcriptional targeting of tumor endothelium is based on advances in the understanding of the molecular biology of angiogenesis (10). Thus, recent exploration of gene expression patterns has shown that tumor-associated endothelial cells are molecularly distinct from physiologic endothelial cells. These differences are thought to reflect unique availability of specific promoters with specific and temporal combinations of transcription factors in the tumor angiogenic endothelial cells (11, 12). On the basis of such differences, it is conceivable to achieve specific and high transcription of therapeutic transgenes in the tumor endothelium.

Indeed, more than 10 different endothelial cell–specific promoters have been discovered and explored for transcriptional targeting of tumor angiogenesis in vitro and in vivo, with various degrees of success, including VEGF receptor 1 (VEGFR-1), VEGFR-2, von Willebrand factor, and others (13,14). One of these promoters is PPE-1-3X, a modified pro-endothelium promoter used in VB-111. The specificity of VB-111 toward the tumor microenvironment, as well as a robust antitumor effect, has been shown in several animal models (3,4,6,8). It is the first agent of transcriptional targeting of the tumor endothelium to be tested in a clinical trial.

In this phase I, single-dose clinical trial, VB-111 was found to be safe and well-tolerated up to the maximum administered single dose of 1 × 10^{13} VPs. MTD was not reached. There were no drug-related serious adverse events. A self-limited febrile reaction was frequent among patients receiving a dose of 1 × 10^{12} VPs and above, as has previously been described with adenovirus vectors (15). There was no evidence of significant hepatotoxicity, hypertension, bleeding or thrombosis. There were no infusion reactions, and all patients remained hemodynamically stable. A transient increase in IL-6 after infusion was observed; however, there was no suggestion of a cytokine storm. As expected, most patients had serologic evidence for prior exposure to adenovirus, and titers of anti-adenovirus antibodies increased after infusion, reaching a plateau at 14 to 28 days. In this trial, only predose samples were tested for neutralizing antibodies. Preliminary data from an ongoing phase II trial of VB-111 in patients with recurrent glioblastoma show a postdosing increase in Ad-5 neutralizing antibodies in all 5 patients with available data (increased range, 36- to 2,000-fold). An increase in PTT levels after dosing was seen in 12 of 33 patients, without any clinical sequelae. This finding is likely to be associated with a transient lupus anticoagulant (LAC), as was found subsequently in 4 patients enrolled in further VB-111 cancer trials. A similar phenomenon has previously been reported after adenovirus infections (16), as well as with other virotherapeutic agents based on Ad-5 vectors (17); for example, 6 of 11 patients developed transient prolongation of PTT and LAC positivity with another antitumor Ad-5–based agent in a prostate cancer trial. There too, no clinical adverse events of bleeding or thrombosis had occurred.

This favorable safety and tolerability profile is attributable to the selective expression of the chimeric TNFR/Fas transgene in the tumor environment, coupled with the safety and tolerability of the Ad-5 vector. The adenoviral vector is stable in vivo and is an efficient gene delivery vehicle that rarely causes any significant disease by itself (14, 18); it has been widely used without safety concern, including large clinical trials of Ad-5–based HIV vaccines (19). One of the limitations of gene therapy using the adenoviral vector is the nonspecific expression of therapeutic genes in normal cells causing toxicity to nontumorous tissues. Targeted expression of therapeutic genes is essential to prevent this toxicity. The proprietary PPE-1-3X promoter restricts the expression of the Fas-chimeric transgene to the angiogenic vasculature in the tumor environment, providing a safeguard against unwanted expression in normal cells and tissues and allowing for systemic administration. TNF-α, which activates Fas-c in minimal quantities, is abundant in the tumor microenvironment and is sufficient to activate the gene. Findings reported in this phase I trial confirmed the selective expression of the chimeric transgene, as was previously shown in animal models (3,6). Thus, transgene was found to be expressed in a subcutaneous metastasis following dosing and was detectable by RT-PCR up to 28 days after therapy, whereas all whole blood samples were negative for transgene expression for this index case as well as all other treated patients.

Although efficacy was not a primary endpoint of this trial, we found evidence of antitumor activity after a single-dose administration as well as evidence of a dose response. One patient (with papillary thyroid carcinoma) developed a partial response at 6 months, which persisted for 18 months after dosing. In an exploratory post hoc analysis, a statistically significant increase was seen in overall survival in cohort 7 (1 × 10^{13} VPs) compared with lower doses (cohorts 1–6; \( P = 0.0098 \)), as well as a trend toward improved progression-free survival in this cohort (\( P = 0.10 \)). Four of 6 patients in cohort 7 remain alive,
with a median follow-up of 487 days. This dose response is consistent with the results seen in the Lewis lung carcinoma model, in which robust reduction in tumor burden was reached at a dose of $10^9$ to $10^{11}$ VPs per mouse (25 g), a dose/kg equivalent of $2.8 \times 10^{12}$ to $2.8 \times 10^{14}$ VPs for an average 70-kg patient. Accordingly, we hypothesized that more potent antitumor activity would occur in patients in cohort 7 ($n = 6, 1 \times 10^{13}$ VPs), and we therefore compared the outcomes of this cohort to those of cohorts 1 to 6 ($n = 27$, doses of $10^{11}$ to $3 \times 10^{12}$ VPs), who received subtherapeutic or borderline therapeutic drug levels and served as an internal control. These findings should be interpreted with caution considering that this is a single-arm trial in which the comparisons of time-to-event endpoints in different non-prespecified endpoints is exploratory and the outcome could be dependent on differences in patient and tumor characteristics. It should be noted that all patients had advanced cancer with evidence of recent progression. Also, all patients in cohort 7 had tumor types that are known to be responsive to antiangiogenics [2 cases of renal cell carcinoma (RCC), 2 neuroendocrine tumors (NET), 1 medullary thyroid, and 1 NSCLC]; however, 2 of them enrolled after failure of prior antiangiogenic therapy.

The findings from this trial suggest a durable effect of VB-111 after a single administration. Pharmacokinetic findings show that presence of the Ad-5 in whole blood declines gradually after drug administration, reaching at least 2-log decline 2 months after dosing. The durability of this effect may be explained by VB-111 transfection and activation patterns. After the drug is infused, VB-111 transfects many tissues, including quiescent endothelial

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**Figure 3.** Tumor response, overall survival, and progression-free survival. A, cohorts 1–5 tumor response at day 56. B, cohorts 6 and 7 tumor response at day 56. C, overall survival: 187 versus 173 days versus not reached in cohort 1–5 versus 6 versus 7, respectively ($P = 0.012$). (Continued on the following page).
Figure 3. (Continued) D, time to progressive disease: 31 versus 55 versus 121 days in cohort 1–5 versus 6 versus 7, respectively ($P = 0.24$). E, overall survival in cohort 1–6 versus 7: 173 days versus not reached ($P = 0.0098$). F, time to progressive disease in cohort 1–6 versus 7: 34.5 vs. 121 days ($P = 0.10$).
In the current clinical trial, no relationship was seen even in the presence of neutralizing antibodies (22). The efficacy signal seen in this trial suggests primarily a disease stabilization effect of VB-111, rather than robust tumor regression. Similar effects have been seen with other antiangiogenic agents such as bevacizumab and tyrosine kinase inhibitor (TKI) drugs (1,2). Moreover, in some indications (e.g., colorectal cancer, lung cancer, and others), antiangiogenics were found to be efficacious only when combined with cytotoxic agents. Currently available antiangiogenic agents have several limitations due to primary refractoriness or development of resistance through a variety of mechanisms and compromised tolerability and safety, which are probably related to their effect on a variety of targets associated with normal physiologic processes unrelated to angiogenesis (1,20). The transcriptional targeting of VB-111 limits effects to proliferating endothelial cells, enhancing the selectivity to the tumor microenvironment. By directing gene expression to angiogenic endothelial cells, this tissue-specific gene therapeutic may be less toxic to normal tissues than TKIs and anti-VEGF antibodies. Targeting of host cells rather than tumor cells provides an additional measure against the development of resistance. The host endothelial cells have inherent genetic stability, with a central role in the endothelin pathway.

A potential limitation of Ad-5 vector is the development of neutralizing antibodies (21). In the presence of such antibodies, Ad-5 is cleared primarily by hepatic sequestration (21). This may compromise efficacy among patients with prior exposure to adenovirus or upon repeat administrations. Nevertheless, the target of VB-111 is the endothelial lining of the blood vessels, which is the first cellular layer this agent will encounter after systemic i.v. administration. It is plausible that a first-pass effect with higher local concentration of transduction at the endothelium may suffice for transfection of endothelial cells, even in the presence of neutralizing antibodies (22). In the current clinical trial, no relationship was seen between prior levels of neutralizing antibodies and anti-tumor effects.

To conclude, we report results of a first-in-human clinical trial of VB-111, a novel viral therapy agent for systemic administration, which targets tumor vasculature. Although several antitumor gene therapeutics have been explored, to the best of our knowledge, this is the first clinical trial of a gene therapeutic agent targeting tumor vasculature (13,18). This trial confirms preclinical findings in animal models and suggests targeted expression of the Fas-chimera transgene selectively in tumor vasculature. The drug showed a favorable safety and tolerability profile. Tumor activity was shown. In a post hoc exploratory analysis, a dose response was seen, with greater overall survival at the highest dose level cohort.

Now that safety has been established, VB-111 is being evaluated to confirm its activity in further clinical trials, with repeat dose administration in several indications, including glioblastoma multiforme, differentiated thyroid cancer, NETs, and RCC.

Disclosure of Potential Conflict of Interest
A. Brenner has a commercial research grant from and is a consultant/advisory board member of Vascular Biogenics. Y.C. Cohen, E. Breitbart, L. Bangio, and D. Harats are employed by and have ownership interest in VBL Therapeutics. P. Triozzi has a commercial research grant from and is a consultant/advisory board member of VBL Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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Figure 4. Partial response (RECIST criteria) in a patient with metastatic papillary thyroid cancer, radioiodine resistant. At 6 months, there is a 30% reduction in the lesion diameter, central necrosis, and no pressure on trachea.
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