PNU-145156E, a Novel Angiogenesis Inhibitor, in Patients with Solid Tumors: A Phase I and Pharmacokinetic Study

Harry J. M. Groen, Elisabeth G. E. de Vries, Wim Wynendaele, Winette T. A. van der Graaf, Eelco Fokkema, Maria J. Lechuga, Italo Poggesi, Luc Y. Dirix, and Allan T. van Oosterom

INTRODUCTION

Our aim was to establish, in patients with solid tumors, the dose-limiting toxicity, maximum tolerated dose (MTD), and pharmacology of PNU-145156E, a new sulfonated distamycin A derivative that blocked circulating angiogenesis-promoting growth factors in animal studies and exhibited an antitumor effect in murine solid tumors. In a Phase I study, PNU-145156E was administered i.v. every 6 weeks. Included were patients with solid tumors; an Eastern Cooperative Oncology Group performance score ≤1; and normal bone marrow, renal, and liver functions and blood clotting tests. Excluded were patients with brain metastases or on steroid medication. Toxicity was scored with the National Cancer Institute Common Toxicity Criteria. Plasma and urine PNU-145156E were measured for pharmacokinetic analysis. The effect of PNU-145156E on serum basic fibroblast growth factor (bFGF) was measured by sandwich ELISA. Twenty-nine patients (median age, 54 years; range, 33–71 years; 19 males and 10 females; median performance score = 1) were treated at dose levels of 100–1050 mg/m². We observed, during 47 treatment cycles, erratic but short-lasting decreases of antithrombin III levels (>75%) at all dose levels. Other clotting tests remained normal except during thromboembolic events. Dose-limiting toxicity was thrombophlebitis, pulmonary embolism, and grade 3 dyspnea. PNU-145156E disappeared from the circulation, decreasing triexponentially with a long terminal half-life of 1 month. No significant change in bFGF and no objective tumor responses were observed. Disease stabilization was achieved in four patients. In conclusion, the MTD of PNU-145156E was 1050 mg/m². Serum bFGF level was not affected by PNU-145156E up to the MTD.

ABSTRACT

Our aim was to establish, in patients with solid tumors, the dose-limiting toxicity, maximum tolerated dose (MTD), and pharmacology of PNU-145156E, a new sulfonated distamycin A derivative that blocked circulating angiogenesis-promoting growth factors in animal studies and exhibited an antitumor effect in murine solid tumors. In a Phase I study, PNU-145156E was administered i.v. every 6 weeks. Included were patients with solid tumors; an Eastern Cooperative Oncology Group performance score ≤1; and normal bone marrow, renal, and liver functions and blood clotting tests. Excluded were patients with brain metastases or on steroid medication. Toxicity was scored with the National Cancer Institute Common Toxicity Criteria. Plasma and urine PNU-145156E were measured for pharmacokinetic analysis. The effect of PNU-145156E on serum basic fibroblast growth factor (bFGF) was measured by sandwich ELISA. Twenty-nine patients (median age, 54 years; range, 33–71 years; 19 males and 10 females; median performance score = 1) were treated at dose levels of 100–1050 mg/m². We observed, during 47 treatment cycles, erratic but short-lasting decreases of antithrombin III levels (>75%) at all dose levels. Other clotting tests remained normal except during thromboembolic events. Dose-limiting toxicity was thrombophlebitis, pulmonary embolism, and grade 3 dyspnea. PNU-145156E disappeared from the circulation, decreasing triexponentially with a long terminal half-life of 1 month. No significant change in bFGF and no objective tumor responses were observed. Disease stabilization was achieved in four patients. In conclusion, the MTD of PNU-145156E was 1050 mg/m². Serum bFGF level was not affected by PNU-145156E up to the MTD.

INTRODUCTION

PNU-145156E (FCE 26644) is a polyanionic, polysulfonated derivative of distamycin A consisting in a skeleton of 4-methyl pyrone rings ending in two naphthalene rings with four sulfonic groups in the 1,3 positions (Fig. 1). PNU-145156E shows structural similarities to suramin. This compound is able to form complexes with different growth factors involved in the angiogenic process, in particular bFGF. Through this mechanism PNU-145156E inhibits the binding of bFGF to its receptor. bFGF is involved in angiogenesis and neovascularization, and some growth factors even seem an essential survival factor for tumor endothelial cells. PNU-145156E has also anti-insulin-like growth factor-1 activity in NSCLC cell lines (3). This factor elevates urokinase-type plasminogen activator-1 in vitro, suggesting facilitation of angiogenesis (4). Another feature of PNU-145156E is the inhibition of proliferation and motility of endothelial cells induced by bFGF.

No cytotoxic effect was observed in bovine aortic and capillary endothelial cells, whereas in animal models, antitumor activity has been observed in M5076 reticulosarcoma, in bFGF-producing sarcoma 180 sarcoma, and in MXT fibrosarcoma (5). Preclinical studies with single and 5-day administration schedules showed a consistent toxicity pattern of vacuolization of macrophages and hepatocytes, lymphoid depletion, and thrombosis in coronary and lung vessels (6). Renal changes in the proximal part of the convoluted tubules and hepatic changes, including thrombotic events, were the main toxicities at high dose levels. In animal studies, drug distribution from the systemic circulation into the tissues was limited just after administration, but increased at later times, and plasma clearance was low, leading to a long half-life. Elimination of the drug was primarily via feces, with approximately one-third eliminated via urine.

The present study describes a Phase I and pharmacokinetic study with PNU-145156E in which we estimate the maximum tolerated dose and conducted pharmacodynamic studies with serum bFGF and blood clotting factors.

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The abbreviations used are: bFGF, basic fibroblast growth factor; NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PT, prothrombin time; PTT, partial thromboplastin time; DLT, dose-limiting toxicity; CTC, Common Toxicity Criteria; AT III, antithrombin III; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor.
PATIENTS AND METHODS

Patients. Included in the study were patients with advanced and refractory solid tumors for whom no standard treatment was available. The patients were 18–75 years of age and had an ECOG performance score \( \leq 1 \), a life expectancy of at least 3 months, normal blood cell counts, normal renal and hepatic function tests, serum albumin \( \geq 30 \) g/L, normal PT, normal PTT, and normal thrombin time. Excluded were patients with hemorrhagic diathesis, coagulation disorder, peripheral neuropathy, and brain or adrenal metastasis and patients who received steroids or who required therapeutic doses of anticoagulants.

All patients gave written informed consent, and the study was approved by the medical ethics committees of the three university hospitals.

Drug Administration. A starting dose of 100 mg/m\(^2\) PNU-145156E, which corresponded to \( \frac{1}{10} \) of the dose that produced no mortality and acceptable toxicity in animal studies, was chosen. Considering the prolonged half-life of PNU-145156E, the compound was given every 6 weeks. We therefore decided to administer 50% of the first cycle dose of PNU-145156E in the second and subsequent cycles. PNU-145156E (supplied by Pharmacia & Upjohn, Milan, Italy) was formulated in vials containing 100 mg of active drug with mannitol as excipient. Vial contents were dissolved in 2 ml of sterile water to obtain a concentration of 50 mg/ml and were administered as an i.v. infusion over 1 h. In the absence of toxicity, subsequent dose levels were increased by 100% unless pharmacokinetic data or toxicity indicated a slower increase in dose. DLT was defined as National Cancer Institute CTC grade 4 granulocytopenia or anemia, thrombocytopenia higher than CTC grade 3, abnormal coagulation tests (\( \geq 75\% \) of control), neurological toxicity of CTC grade 1 or higher, or other nonhematological toxicities higher than CTC grade 2. If one of three patients had at least one DLT, then an additional three patients were studied before dose escalation. The maximum number of cycles per patient was not fixed and depended on the clinical benefit for the patient. At each dose level, the first patient was observed for 4 weeks or until recovery from toxicity before two new patients were entered. Those two patients at each dose level were followed for at least 6 weeks or until recovery from acute toxicities before proceeding to the next dose level. Maximum tolerated dose was defined as the dose level in which two of three or three of six patients experienced DLT.

Pretreatment Evaluation and Follow-Up. Pretreatment evaluation was performed within 7 days prior to the start of treatment. A complete history and physical examination, including ECOG performance status, weight, complete blood cell count with WBC differential count, serum bilirubin, alanine aminotransferase, aspartate aminotransferase, creatine kinase, creatinine, urea, glucose, electrolytes (including magnesium, calcium, and phosphorus), total protein, albumin, amylase, lipase, and a lipid profile. Plasma cortisol and renin activity, HIV1, HIV2, hepatitis B surface antigen, and hepatitis B core antigen were measured. Creatinine clearance was calculated from a 24-h urine collection. PT, PTT, thrombin time, and AT III were measured with standard methods. A resting 12-lead electrocardiogram was obtained for all patients. Tumor evaluation using WHO criteria was performed by chest X-ray or any other appropriate imaging test.

During treatment, patients were evaluated weekly for toxicity according to CTC with physical examinations and hematology, blood chemistry, and coagulation factors. Creatinine clearance, plasma cortisol, and renin activity were measured every 3 weeks during the first 6 weeks. At the end of each cycle, all baseline evaluations, including tumor evaluations, were repeated, except for viral antigen or antibody tests.

Angiogenic Growth Factor. Blood samples were collected at 0, 1, 24, 48, 96, 168, 336, and 672 h after PNU-145156E infusion, and samples were immediately stored at \(-20^\circ\)C until assayed. Serum bFGF was measured in duplicate during the first cycle by Quantikine human bFGF ELISA (R&D Systems Inc., Minneapolis, MN). Values were compared with normal controls.
PNU-145156E Pharmacokinetics. Blood samples were collected from an indwelling venous catheter placed in the arm contralateral to the drug infusion and drawn in heparinized polypropylene tubes just before infusion, at 20 and 60 min during infusion, and after each treatment administration at 10, 20, and 30 min and 1, 2, 4, 8, 24, 48, 96, 168, 336, and 672 h; for patients who did not receive a second cycle, blood was also collected at 1344 and 2688 h. The tubes were immediately centrifuged (10 min at 1200 g) and stored at −20°C. Urine samples were collected only after the first administration of PNU-145156E at the following time intervals: 0–8, 8–24, 48–72, and 72–96 h.

PNU-145156E levels in plasma and urine were measured by reversed-phase ion-pair high-performance liquid chromatography with isotropic elution after ion-pairing extraction from plasma. PNU-145156E and the internal standard, bromphenol blue, were extracted from plasma samples with methylene chloride after deproteinization with acetonitrile and addition of the ion-pairing compound, tetrabutylammonium hydroxide. Quantification was performed with UV detection at 323 nm (7). The lower limit of quantitation was 0.3 μg/ml.

Pharmacokinetic Analysis. Pharmacokinetic analysis of PNU-145156E was performed using a noncompartmental model. The area under the curve was calculated using the linear trapezoidal rule up to the last quantifiable concentration and extrapolated to infinite time (AUC0–inf). The half-life of the terminal decay phase, t1/2,γ, was determined by linear regression analysis of the natural-log concentration versus time curve, where t1/2,γ = ln(2)/slope of the regression line. Plasma clearance (CL) was calculated as dose/AUC0–inf. The volumes of distribution of the terminal phase (Vd) and at steady state (Vss) were calculated as CL × t1/2,γ/ln(2) and CL × AUMC/AUC0–inf, respectively, where AUMC is the area under the first moment curve [calculated with the same methods as for AUC on (c × t) versus time plots]. Pharmacokinetic parameters were summarized with descriptive statistics.

Statistics. Toxicity data were described in frequencies per dose level. Pearson’s correlation was used to evaluate the relationship between PNU-145156E and AT III levels. Changes in serum bFGF and blood clotting factors were estimated with ANOVA.

RESULTS

Patients. From October 1995 until September 1998, a total of 29 patients were enrolled. Patients characteristics are given in Table 1. Three patients were treated at 100 mg/m2, four at 200 mg/m2, three at 400 mg/m2, seven at 600 mg/m2, three at 675 mg/m2, six at 840 mg/m2, and three at 1050 mg/m2. We decreased the PNU-145156E dose increments after 600 mg/m2 because of a thrombotic event at that level. At the 675 mg/m2 level, we did not have any thrombotic event and proceeded with a 25% increase in dose thereafter. All patients received at least one cycle. Overall, 47 cycles were administered, and 12 patients received >1 cycle.

Clinical Toxicities. Hematological toxicity was very mild. Grade 1 and 2 hemoglobin toxicity was observed in four and two patients, respectively. No significant abnormalities were observed for leukocytes, neutrophils, or platelets.

The main nonhematological toxicities were thrombotic events. Pulmonary thrombosis was confirmed with perfusion studies with 99mTc-labeled human albumin and with xenon ventilation studies. It occurred in one patient with NSCLC during the second cycle (50% dose) treated at a dose level of 600 mg/m2 (AT III = 61%) and in one patient with adenocarcinoma of unknown origin during the first cycle treated at a dose level of 1050 mg/m2. The first patient also had a venous thrombosis in his right leg, and the second patient had a concomitant thromboembolitis in both legs. The two patients recovered. We decided after the first thrombotic event at the dose level of 600 mg/m2 that patients should receive anticoagulant therapy when AT III was <75%. One other patient had a severe decrease in AT III level to 17% at a dose level of 840 mg/m2 during the first cycle.

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>29</th>
</tr>
</thead>
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<tr>
<td>M/F</td>
<td>10/19</td>
</tr>
<tr>
<td>Median (range) age (yrs)</td>
<td>54 (33–71)</td>
</tr>
<tr>
<td>Median (range) ECOG performance score</td>
<td>1 (0–1)</td>
</tr>
<tr>
<td>Number of patients per tumor type</td>
<td></td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>3</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>13</td>
</tr>
<tr>
<td>Head and neck carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma of unknown origin</td>
<td>4</td>
</tr>
</tbody>
</table>

Two patients developed grade 3 dyspnea at different dose levels. One patient with advanced NSCLC and a history of pulmonary emphysema had dyspnea at a dose level of 675 mg/m2. Another patient with NSCLC had increasing severity of left leg paresis attributable to perineal nerve dysfunction after the first PNU-145156E cycle with 840 mg/m2. Grade 1 paresis occurred in 11 patients. Transient cold and pale fingers attributable to peripheral vasoconstriction (grade 1) occurred in four patients at different dose levels. Grade 1 headache and dyspnea were occasionally reported.

Seven patients showed an increase up to grade 1 for alanine aminotransferase, and three patients had a transient elevation of aspartate aminotransferase. Serum bilirubin increased significantly during the first week, reaching the upper limit of normal, but decreased to baseline levels within 1 week. Lipid profiles, plasma cortisol, and renin activity did not change. A slight increase (grade 1) in serum creatinine was observed in five patients. Three patients receiving 200, 675, or 840 mg/m2 PNU-145156E had a significant short-lasting increase in serum creatine phosphokinase during the first cycle. No episodes of infection or local toxicity or hemolysis were observed. DLTs were pulmonary embolism and grade 3 dyspnea occurring at the 1050 mg/m2 dose level in two of three patients.

No deaths were attributable to PNU-145156E during the study. Two early deaths occurred at the 840 mg/m2 dose level (one patient had an AT III level of 39%), and one early death occurred at the 1050 mg/m2 dose level after the second cycle, all as a result of tumor progression.
Coagulation Studies. A prominent feature during the study was the erratic AT III levels. The time from PNU-145156E administration to AT III nadir was quite variable for the 20 patients in whom the nadir was observed. The nadir occurred in the 1st week of treatment in four patients, in the 2nd week in two, in the 3rd week in six, in the 4th week in three, in the 5th week in two, in the 6th week in one, and in the 7th week in two patients. The median time to AT III nadir was 3 weeks, and levels became normal within 1 week in all patients. The mean AT III nadirs at different PNU-145156E dose levels are given in Table 2. AT III levels decreased to <75% in 15 of 29 patients during the first cycle and in 3 of 12 patients during the second cycle. Minimal and average AT III levels were not related to PNU-145156E dose levels, c max , or AUC values. Other clotting tests (PTT and PT) remained normal in all patients.

After the first thrombotic event at the 600 mg/m²2 level, we decided to better characterize the effect of PNU-145156E on blood coagulation. Additional blood coagulation tests were performed weekly in seven patients at the highest PNU-145156E dose levels. However, no changes were observed in the levels of protein C activity and antigen, protein S antigen, and tissue plasminogen (Table 3) in the first 6 weeks after infusion of PNU-145156E, except in the patient with pulmonary thrombosis at the 1050 mg/m²2 level, where clotting levels temporarily decreased. In addition, tPA and plasminogen activator inhibitor measured in four patients were unchanged. Median pretreatment fibrinogen levels (range) were elevated, 6.6 (4.3–9.1) g/L (normal values, 2.0–4.0 g/L), and remained unchanged during the treatment period in all seven patients.

Angiogenic Growth Factor. Mean (SD) pretreatment serum bFGF (n = 19) was elevated compared with normal, to 14.3 (13.1) pg/ml. Slight decreases in bFGF were observed in 13 of 19 patients at 1 h after PNU-145156E infusion and in only 7 of 19 patients after 96 h. Higher PNU-145156E dose levels did not prolong decreases in bFGF levels. The level of bFGF after treatment was not related to dose level.

Pharmacokinetics. Mean plasma PNU-145156E at different dose levels after the first 60-min infusion are shown in Table 4. After the end of the infusion, drug levels were still increasing, with the maximum concentration, ~40% higher than c end infusion , occurring ~20 min after the end of infusion. PNU-145156E disappeared from the systemic circulation, decreasing triexponentially with a terminal half-life of ~750 h (31 days; Fig. 2). The AUC of the terminal phase accounted for 85% of the total AUC 0–inf . The interindividual variability, presented as coefficient of variation for dose-normalized indicators of systemic exposure, was 20% (n = 19). The systemic exposure, as described by c max and AUC 0–inf increased in direct proportion with the dose. In addition, the data obtained in a number of subjects during the second cycle of treatment indicated that PNU-145156E has time-independent pharmacokinetics. Plasma PNU-145156E was still measurable in all patients after 4 weeks (>10 μg/ml). The clearance of the drug was much lower than the hepatic blood flow, indicating a slow extraction from the plasma compartment. Cumulative amounts of PNU-145156E excreted unchanged in urine accounted for ~2% of the dose in the first 4 days after administration. At higher doses, daily urinary excretion of PNU-145156E accounted for ~0.3% of the dose on day 8 and 0.1% on day 29.

Antitumor Activity. No objective tumor responses were observed. In two patients with previously untreated NSCLC (840 mg/m²2 dose level), disease stabilization lasted 4 and 7 months, and in one patient with previously untreated mesothelioma and one patient with renal cell carcinoma previously treated with immunotherapy, stable disease lasted for 6 months (previous time to progression was 4 months).

DISCUSSION

PNU-145156E was developed for clinical studies because it inhibited angiogenesis as measured with different blood vessel-sprouting assays (endothelial cell assay, sponge assay, chorioallantoic membrane assay, and mouse embryo growth assay) and because animal studies showed antitumor activity. New blood vessel growth is required for solid tumors to expand beyond a volume of ~2 mm³. In smaller tumors, proliferation and apoptosis seem to be in balance (8). Immunohistochemical studies of tumor sections from margins of growing tumors show a preponderance of blood vessels irrespective of tumor type. Angiogenesis factors released from tumor cells migrate to nearby blood vessel endothelial cells and activate these cells to undergo morphological changes. There is strong evidence that in angiogenesis in solid tumors, VEGF and bFGF are signaling through the endothelial restricted receptors Flk-1 and Tie-1, respectively (9). VEGF is mitogenic for endothelial cells in vitro. In patients with solid tumors, VEGF has shown a positive correlation with stage of disease (10, 11), formation of metastases (12, 13), and progression of disease (12). Unfortunately, serum is not the ideal matrix for measuring VEGF because the aggregation of platelets results in a release of VEGF. Platelet-poor plasma would be preferable (14). Nevertheless, all of these arguments do suggest that reducing circulating angiogenic growth factors could inhibit tumor growth. We assumed that both VEGF and bFGF could act as surrogate markers for efficacy, but unfortunately, PNU-145156E up to a dose of 1050 mg/m²2 did not reduce serum levels of bFGF.

Measuring response rates with imaging techniques may not be the best way to evaluate angiogenic drugs because tumor control, e.g., stabilization of disease, is a more realistic goal of this new approach. Therefore, time to progression seems a more reasonable parameter to assess these drugs.

After observing pulmonary embolisms, thrombophlebitis events, and erratic AT III deficiencies, we concentrated on the unique toxicity profile of PNU-145156E. An increased inci-

Table 2

<table>
<thead>
<tr>
<th>Dose level (mg/m²2)</th>
<th>No. of patients</th>
<th>AT III nadir (%)</th>
</tr>
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<tr>
<td>100</td>
<td>3</td>
<td>77</td>
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<tr>
<td>200</td>
<td>4</td>
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<td>400</td>
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<td>600</td>
<td>7</td>
<td>65</td>
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<tr>
<td>675</td>
<td>3</td>
<td>76</td>
</tr>
<tr>
<td>840</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>1050</td>
<td>3</td>
<td>69</td>
</tr>
</tbody>
</table>

Mean Range

Angiogenic Growth Factor. Mean (SD) pretreatment serum bFGF (n = 19) was elevated compared with normal, to 14.3 (13.1) pg/ml. Slight decreases in bFGF were observed in 13 of 19 patients at 1 h after PNU-145156E infusion and in only 7 of 19 patients after 96 h. Higher PNU-145156E dose levels did not prolong decreases in bFGF levels. The level of bFGF after treatment was not related to dose level.

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The evidence of coronary artery thrombosis has been observed in animal studies of PNU-145156E. Interaction with blood clotting enzymes is a possible feature of PNU-145156E, although we did not find any correlation between AT III levels and PNU-145156E plasma levels.

In vitro, single-chain tPA, cathepsin B, and elastase were inhibited by PNU-145156E in doses up to 78 \( \mu \)g/mL, whereas double-chain urokinase-type plasminogen activator and tPA were not. Fragments of plasminogen enzyme were shown to be polypeptides with antiangiogenic properties.

In the present study, we did not observe any changes in plasminogen, tPA, and plasminogen activator levels during treatment and no consistent changes in AT III levels during different cycles. Down-regulation of coagulation proteins, such as AT III, protein C, plasminogen, and fibrinogen, has also been observed in patients with acute lymphoblastic leukemia treated with L-asparaginase during chemotherapy, which explains the thromboembolic complications in 1.1–14.3% (15, 16). The explanation involved inhibition of protein synthesis in the liver, with subsequent decreases in multiple plasma proteins, especially AT III. However, when incubated with L-asparaginase, blood samples from healthy male donors also showed significant decreases in fibrinogen, plasma AT III, protein C, and plasminogen compared with controls (17). In contrast, increases in plasma von Willebrand factor antigen and plasma thromboglobulin were observed, which suggested that L-asparaginase may also directly attack proteins of the coagulation system. Treatment with L-asparaginase also reduced the levels of plasma D-dimer and thrombin-antithrombin complex.

### Table 3
Additional blood coagulation tests measured weekly during 6 weeks after the first administration of PNU-145156E in seven patients with solid tumors

<table>
<thead>
<tr>
<th>Coagulation test</th>
<th>Normal values</th>
<th>Baseline Median</th>
<th>Range</th>
<th>Coagulant nadir Median</th>
<th>Range</th>
<th>Nadir reached (days)</th>
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<tr>
<td>PTT (s)</td>
<td>24–38</td>
<td>30.6</td>
<td>26.2–36.2</td>
<td>29.8</td>
<td>24.4–35.4</td>
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<tr>
<td>PT (s)</td>
<td>11–16</td>
<td>13.3</td>
<td>11.7–16.6</td>
<td>13.2</td>
<td>11.9–15.4</td>
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<tr>
<td>AT III (%)</td>
<td>70–130</td>
<td>93</td>
<td>72–128</td>
<td>81</td>
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<tr>
<td>Protein C activity (%)</td>
<td>70–130</td>
<td>93</td>
<td>34–149</td>
<td>90</td>
<td>67–100</td>
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<td>Protein C antigen (%)</td>
<td>75–161</td>
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<td>68–150</td>
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<td>62–147</td>
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<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Thrombin time (s)</td>
<td>13.0–19</td>
<td>15.6</td>
<td>14.0–22.0</td>
<td>15.1</td>
<td>14.0–17.8</td>
<td>14</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>70–130</td>
<td>129</td>
<td>117–136</td>
<td>76</td>
<td>76–76</td>
<td>14</td>
</tr>
</tbody>
</table>

\(^a\) nAPC-SR, normalized activated protein C-standard ratio; NA, not available.

### Table 4
Pharmacokinetic parameters [mean (SD)] in patients with advanced solid tumors following i.v. administration of PNU-145156E at different dose levels

<table>
<thead>
<tr>
<th>Dose(^b) (mg/m(^2))</th>
<th>AUC(_{0-\infty}) (mg*h/ml)</th>
<th>(c_{\text{max}}) ((\mu)g/ml)</th>
<th>CL (ml/h/m)</th>
<th>(t_{1/2,\gamma}) (h)</th>
<th>(V_e) (liters/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>7.29 (2.10)</td>
<td>52.0 (24.0)</td>
<td>13.4 (3.3)</td>
<td>664 (2)</td>
<td>13.4 (3.8)</td>
</tr>
<tr>
<td>200</td>
<td>17.6 (5.3)</td>
<td>99.5 (23.9)</td>
<td>11.4 (3.8)</td>
<td>730 (207)</td>
<td>12.5 (7.3)</td>
</tr>
<tr>
<td>400</td>
<td>28.5 (5.2)</td>
<td>236 (4)</td>
<td>13.3 (2.6)</td>
<td>NA(^c)</td>
<td>13.4 (1.2)</td>
</tr>
<tr>
<td>600</td>
<td>51.4 (5.1)</td>
<td>329 (97)</td>
<td>10.9 (1.1)</td>
<td>765 (112)</td>
<td>12.1 (2.7)</td>
</tr>
<tr>
<td>675</td>
<td>67.8 (3.8)</td>
<td>371 (30)</td>
<td>9.2 (0.5)</td>
<td>NA</td>
<td>10.0 (0.5)</td>
</tr>
<tr>
<td>840</td>
<td>74.4 (10.6)</td>
<td>512 (173)</td>
<td>10.7 (1.6)</td>
<td>564 (191)</td>
<td>10.7 (2.7)</td>
</tr>
<tr>
<td>1050</td>
<td>145.0 (95)</td>
<td>629 (263)</td>
<td>8.5 (5.6)</td>
<td>NA</td>
<td>9.2 (6.0)</td>
</tr>
</tbody>
</table>

\(^a\) Doses expressed as tetrasodium salt; concentrations as the free acid.

\(^b\) The number of patients per dose level is at least three.

\(^c\) NA, not available.
which are considered early markers of a hypercoagulability state (18). PNU-145156E and suramin, a structurally related drug, did not show such interactions with the blood clotting system.

In this study, only mild thrombocytosis and elevated fibrinogen levels were indicative of the hypercoagulable state. Although low-grade coagulopathy in cancer is well recognized and many other factors, such as tissue factor, cancer procoagulant, and cytokine release of interleukin-1β and tumor necrosis factor α, which in turn regulate procoagulants (19), may explain coagulation events, none of the studied factors explained the thrombotic events. The apparent propensity for patients with NSCLC to develop thromboses or embolisms and dyspnea during PNU-145156E therapy is in some ways reminiscent of other angiogenesis inhibitors. Whether these agents act through tumor necrosis remains speculation. Future studies with angiogenesis inhibitors should monitor the blood clotting system carefully to predict thrombotic events in time. The recommended dose of PNU-145156E for future studies is 860 mg/m² i.v. once every 6 weeks with a 50% dose reduction in subsequent cycles. However, the unique toxicity profile and its inability to change its surrogate end points must be taken into account when further studies are planned.

In conclusion, PNU-145156E shows dose-dependent pharmacokinetics with a long half-life. The promising angiogenic properties of PNU-145156E evaluated with serum bFGF as the surrogate end point are not achievable with i.v. administration in patients with solid tumors.

REFERENCES


PNU-145156E, a Novel Angiogenesis Inhibitor, in Patients with Solid Tumors: A Phase I and Pharmacokinetic Study


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