Intravenous Safety and Pharmacokinetics of a Novel Dimerizer Drug, AP1903, in Healthy Volunteers

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AP1903 is a novel gene-targeted drug that is being developed for use in drug-regulated cell therapies. An intravenous, single-blind, placebo- and saline-controlled, ascending-dose study was performed to evaluate the safety, tolerability, and pharmacokinetics of AP1903. Twenty-eight normal healthy male volunteers were randomized into five dosage groups of AP1903 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg). Within each group, 4 volunteers received a single dose of AP1903, 1 volunteer received an equal volume of placebo, and 1 received an equal volume of normal saline. The only exception was in the 0.5 mg/kg group, in which 4 volunteers were dosed: 3 received AP1903 and 1 received normal saline. All dosages were administered as intravenous infusions over 2 hours. Clinical safety parameters were monitored, and serial blood and urine samples were collected for analysis of AP1903. No drug-related adverse events were observed at any of the dose levels with the possible exception of facial flushing in 1 volunteer at the 1.0 mg/kg dose level. AP1903 plasma levels were directly proportional to the administered dose, with mean C_{max} values ranging from approximately 10 to 1275 ng/mL over the 0.01 to 1.0 mg/kg dose range. Following the infusion period, blood concentrations revealed a rapid distribution phase, with plasma levels being reduced to approximately 18%, 7%, and 1% of the maximal concentration at 0.5, 2, and 10 hours postdose, respectively. AP1903 was shown to be safe and well tolerated at all dose levels and demonstrated a favorable pharmacokinetic profile at doses well above the anticipated therapeutic dose.

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High-dose radio-chemotherapy followed by allogeneic BMT is the recognized treatment of choice for many hematologic malignancies.[1-3] The therapeutic benefit of this procedure is based on two modalities. First, aggressive radio-chemotherapy directly destroys the abnormal malignant cells as well as normal cells in the bone marrow. Second, donor lymphocytes in the transferred bone marrow contribute a therapeutic graft-versus-tumor (GvT) immune response. However, in addition to this beneficial effect, donor lymphocytes are responsible for the major and potentially life-threatening complication of BMT, graft-versus-host disease (GvHD).[4-7] The incidence and severity of GvHD can be reduced by depleting T cells from the donor bone marrow prior to transplantation, but this also depletes the GvT response and can adversely affect engraftment of the bone marrow.[8] To recapture the beneficial effects of donor T cells, infusion of T cell–depleted BMT is generally supplemented with a delayed infusion of peripheral donor T cells. However, in patients receiving delayed lymphocyte infusions, GvHD remains a frequent and often lethal complication.[9-12] Innovative approaches that preserve the beneficial antitumor effects of donor lymphocytes while allowing control over GvHD would provide an important advance for the safe and effective use of allogeneic BMT in cancer patients.

A novel T cell suicide technology, the "AP1903/Fas" system, has recently been developed that addresses these concerns.[13-16] In this system, peripheral donor T cells are genetically engineered to express a condi-

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tional suicide gene that can be activated pharmacologically by administration of the drug AP1903 (Figure 1). Engineered T cells retain beneficial antitumor effects, but the ability to eliminate them with AP1903 provides a means to terminate GvHD should it occur. AP1903 is a member of a new class of compounds termed *dimerizer drugs* that act by inducing clustering of engineered proteins inside cells. AP1903-inducible cell death is achieved by expressing a chimeric protein comprising the intracellular portion of the human Fas receptor, which signals apoptotic cell death, fused to a drug-binding domain derived from human FK506-binding protein (FKBP). This chimeric protein is quiescent inside cells until administration of AP1903, which cross-links the FKBP domains, initiating Fas signaling and hence apoptosis.

In vitro studies have demonstrated that primary human T lymphocytes transduced with the AP1903/Fas suicide system (1) retain their immune potential, a relevant issue for the antitumor effect, and (2) can be eliminated by exposure to AP1903 with high efficiency, potency, and specificity.[16] Engineered human T lymphocytes were eliminated with approximately 70% efficiency following a single exposure to AP1903, increasing to approximately 90% following a second exposure. Maximal killing occurred at approximately 4.5 ng/mL AP1903, and the IC_{50} was about 0.3 ng/mL. This high potency is consistent with the in vitro affinity and selectivity of AP1903, which was engineered to bind tightly to the target FKBP fusion protein while binding minimally to the abundant natural FKBP.[14]

In the clinical setting, cancer patients will receive T cell–depleted BMT. Donor lymphocytes will be collected, transduced with the gene for the engineered Fas protein coupled to a cell surface marker, selected using antibody-coupled beads specific for the marker, expanded, and then administered to patients. Patients who develop GvHD will receive AP1903 intravenously to induce apoptosis of the causative T cells. The clinical rationale of this approach has been previously demonstrated using the HSV/tk suicide system: donor lymphocytes were transduced with the thymidine kinase gene (tk), derived from herpes simplex virus (HSV), and administered to leukemia patients in relapse after allogeneic BMT.[17] In those patients developing GvHD, administration of ganciclovir eliminated the engineered cells and ameliorated the complication. The Fas suicide system overcomes several limitations of the HSV/tk system. The HSV-derived tk gene has been shown to be immunogenic in patients,[18,19] whereas the Fas system is composed of human proteins and therefore less likely to elicit an immune response. In addition, the Fas system is compatible with the use of ganciclovir to treat the cytomegalovirus infections to which BMT patients are particularly prone.

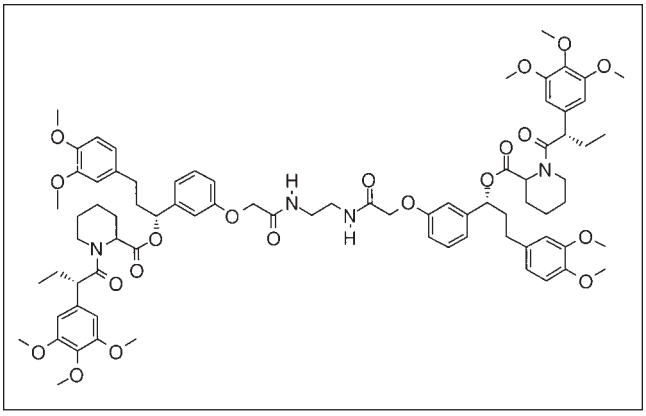

Figure 1. Chemical structure of AP1903.

Because AP1903 is a new chemical entity, its safety and pharmacokinetic profiles need to be evaluated alone in humans before moving forward with clinical studies on the AP1903/Fas suicide system. Preclinical intravenous toxicity studies in rodents and nonhuman primates (unpublished) established a favorable safety profile to support a phase 1 study of AP1903. This report describes the intravenous safety and pharmacokinetic profile of AP1903 when given to healthy volunteers.

METHODS

Study Population

Healthy male volunteers, between the ages of 19 and 45 years and within 15% of their ideal body weight range for height according to the Metropolitan Life Insurance Company, were enrolled in this investigation. The volunteers were nonsmokers or ex-smokers who had not smoked for at least the preceding 6 months. Prescription and over-the-counter medications were not permitted from 14 days prior to dosing until after the poststudy assessments had been performed. An exception was the use of acetaminophen during the study, if necessary. The study protocol and consent form were approved by the Covance Clinical Research Unit (Covance CRU) Independent Review Board. Written informed consent was obtained by all volunteers prior to their participation in the study.

Study Design and Procedures

This was a single-center phase 1, single-blind, randomized placebo- and saline-controlled, ascending single IV dose study. Five dose levels of AP1903 (0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg) were tested with each dose given as a continuous 2-hour intravenous infusion. This regime was chosen based on in vitro studies showing that a 2-hour exposure of engineered T cells to drug gives maximal killing.[16] The five treatment groups were studied in ascending order of dose with an evaluation of the safety, tolerability, and pharmacokinetic data preceding progression to the next higher dose level. The study was designed to include 30 volunteers randomly assigned to one of the five treatment groups (6 volunteers/group). Within each treatment group, the volunteers were further randomized such that 4 individuals received AP1903, 1 individual received placebo (the formulation vehicle), and 1 individual received normal saline for injection. However, because of recruitment issues, a total of 28 volunteers were studied; 6 volunteers per group were studied as planned with the exception of the 0.5 mg/kg group, which included only 4 volunteers. For this group, 3 volunteers received 0.5 mg/kg of AP1903, and 1 volunteer received normal saline. The volunteers arrived at the clinical investigational site on the day prior to dosing and remained at the site until at least 48 hours after treatment. The volunteers returned within 7 to 9 days after treatment for follow-up evaluation.

AP1903 was manufactured as a concentrated parenteral solution containing 5 mg drug/mL. Prior to administration at each dose level, the concentrated drug solution and the placebo solution were diluted 1:20 with normal saline for injection. Within each treatment group, equal volumes of drug solution, placebo, and normal saline were administered intravenously over the 2-hour period. Each IV infusion was given using an Imed Gemini PC-1 infusion pump, and the volunteers were in the supine position for 3 hours from the initiation of the infusion.

Volunteers were monitored for safety and tolerability, which included questioning for adverse events, clinical laboratory tests (hematology, serum biochemistry, and urinalysis), bleeding time, ADP- and collagen-induced platelet aggregation, blood pressure, pulse rate, body temperature, and 12-lead resting electrocardiograms (ECGs). Continuous cardiac monitoring via telemetry occurred from 1 hour predose to 3 hours postdose.

Blood samples (10 mL) for the determination of plasma and whole-blood levels of AP1903 were collected at predose (0 hour); 30 minutes; 1 hour; 1 hour, 30 minutes; 2 hours; 2 hours, 5 minutes; 2 hours, 15 minutes; 2 hours, 30 minutes; 2 hours, 45 minutes; and 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours after the start of the infusion. Urine samples were collected, and the volume was determined at –12 to 0 (predose), 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours after the start of infusion.

Analysis of Blood and Urine Samples

Ten mL blood samples were collected into 10 mL lithium heparin Vacutainer® tubes, gently inverted to mix, and then placed in a cool box containing a crushed ice/water mixture. Within 1 hour of collection, approximately 1 mL of whole blood was transferred to a separate 5 mL polypropylene tube and placed into a freezer set to maintain –60 to –80°C until time of analysis. The remaining portion of the sample was centrifuged at 1500 g for 10 minutes at 4°C within 1 hour of collection. Plasma was collected and stored at less than –60°C until analysis. Aliquots of each set of urine samples were stored at less than –60°C until analysis.

Just prior to analysis, plasma, whole blood, and urine samples were thawed and mixed, and a deuterated form of AP1903 was added as an internal standard. Samples (0.5 mL) were subjected to protein precipitation using a solution of $ZnSO_4$ (87 mM) in a mixture of methanol, acetonitrile, and water (50:30:20 v/v) (1 mL) followed by centrifugation. The supernatant was diluted with water (2 mL), then subjected to manual solid-phase extraction using Varian C18 extraction cartridges (200 mg resin/3 mL). The cartridges were washed with 50:50 v/v methanol water (1 mL) and hexane (1 mL), and then the extracts were eluted with acetonitrile (1 mL) and evaporated to dryness. The residues were redissolved in 50:50 v/v acetonitrile:0.1% formic acid, centrifuged, and analyzed using a validated liquid chromatography/tandem mass spectrometry method. Each extract (20 µl) was injected onto a Zorbax XDB-C8 column (5 cm × 2.1 mm, 45C), using an isocratic solvent system (70:30 acetonitrile:0.1% formic acid) flowing at 0.2 mL/min, with an analysis time of 2.75 minutes. The AP1903 and internal standard were detected using multiple-reaction monitoring in positive-ion mode on a PE Sciex AP365 mass spectrometer. The concentration range of the standard curves (lower and upper limits) for all three matrices (plasma, whole blood, and urine) was 0.5 and 50 ng/mL, respectively, using a sample volume of 0.5 mL. The precision of the assay (based on CV% data for quality control samples) ranged between 6.8% and 10.0% in plasma, 7.9% and 11.1% in whole blood, and 11.9% and 17.7% in urine.

Pharmacokinetic Analysis

Pharmacokinetic parameters for AP1903 were calculated using noncompartmental procedures by SAS version 6.12. The maximum plasma and whole-blood concentrations (C_{max}) and corresponding times (t_{max}) and the concentrations at the end of the infusion (C_{inf}) were obtained directly from the plasma and whole-blood concentration-time profiles. The area under the plasma and whole-blood concentration-time curve up to the last quantifiable plasma/whole-blood concentrations ($AUC_{(0\text{-}t_z)}$) were calculated using the linear trapezoidal rule from predose to 2 hours (end of infusion) and the log-linear trapezoidal rule from 2 hours to the last quantifiable plasma/whole-blood concentrations (C_z at time t_z). The area under the plasma/whole-blood concentration versus time curve from 0 hours to infinite time ($AUC_{(0\text{-}\infty)}$) was calculated as $AUC_{(0\text{-}\infty)} = AUC_{(0\text{-}t_z)} + C_z/\lambda_z$. The apparent plasma and whole-blood terminal elimination rate constants (λ_z) were calculated by log-linear regression analysis. The start of the terminal elimination phase for each volunteer was defined by visual inspection of the log-linear plasma/whole-blood concentration profiles. The terminal elimination half-life ($t_{1/2}$) was calculated from $t_{1/2} = \ln(2)/\lambda_z$. The intrinsic mean residence time (MRT_{int}) was calculated by $MRT = AUMC_{(0\text{-}\infty)}/AUC_{(0\text{-}\infty)} - IT/2$ (IT = infusion time, 2 hours). Area under the first moment curve to infinity ($AUMC_{(0\text{-}\infty)}$) was calculated by $AUMC_{(0\text{-}\infty)} = AUMC_{(0\text{-}t_z)} + t_z \bullet C_z/\lambda_z + C_z/\lambda_z^2$, and $AUMC_{(0\text{-}t_z)}$ was calculated by the linear trapezoidal rule. Total plasma and whole-blood clearance (CL) and volume of distribution during the terminal elimination phase (V_z) and volume of distribution at steady state (V_{ss}) were calculated from $CL = IV\ Dose/AUC_{(0\text{-}\infty)}$; $V_z = CL/\lambda_z$; $V_{ss} = MRT_{int} \times CL$.

Statistical Analysis

Descriptive statistics were used to analyze vital signs and ECG parameters. Changes from a baseline of > 30 mmHg for systolic blood pressure and > 20 mmHg for diastolic blood pressure were considered to be of potential clinical concern. Analysis of variance (ANOVA) was used to evaluate percent inhibition from baseline for platelet aggregation and the changes from baseline for bleeding time data at each postdose time point. The relationship between log-transformed C_{max}, C_{inf}, and AUC and log-transformed dose was examined using linear regression analysis and testing the slope for unity. All tests were two-sided. A p-value of < 0.05 was considered statistically significant.

RESULTS

Volunteers

Demographic characteristics for the 28 volunteers who participated in this investigation are summarized in Table I. All volunteers were males between 19 and 45 years of age. One volunteer was of mixed race, with all other volunteers being Caucasian. Body weights and heights ranged between 62.5 and 92.8 kg and 168 and 188 cm, respectively. The mean age, body weight, and height were similar for volunteers receiving saline, placebo, and each dose level of AP1903.

Safety and Tolerability Results

Adverse Events

No serious adverse events occurred during the study. The incidence of adverse events was very low following each treatment, with all adverse events being mild in severity. Only one adverse event was considered possibly related to AP1903. This was an episode of vasodilatation, described as "facial flushing" for 1 volunteer at the 1.0 mg/kg AP1903 level. This event occurred at 3 minutes after the start of infusion and resolved after 32 minutes' duration. All other adverse events reported during the study were considered by the investigator to be unrelated or to have improbable relationship to the study drug. These events included chest pain, flu syndrome, halitosis, headache, injection site pain, vasodilatation, increased cough, rhinitis, rash, gum hemorrhage, and ecchymosis.

Most adverse events resolved without treatment, but concomitant medication was administered to 2 volunteers during the course of the study. One volunteer receiving 0.1 mg/kg of AP1903 received acetaminophen (1 g) on three occasions and ibuprofen (400 mg) on one occasion, all for toothache, and phenoxymethylpenicillin (250 mg, four times daily) for treatment of a tooth abscess. One other volunteer receiving 0.1 mg/kg of AP1903 received acetaminophen (1 g) to treat cold symptoms.

Vital Signs and ECG

There were no clinically significant changes in the supine blood pressure, supine pulse rate, and body temperature during the study. In addition, there were no clinically significant changes in any of the 12-lead ECGs recorded during the study. There were no drug- or dose-related effects in PR interval, QRS duration, heart rate, or QTc interval. Continuous ECG monitoring

Table I Demographic Characteristics

	Treatment Group (mg/kg)						
	Saline	**Placebo**	**0.01**	**0.05**	**0.1**	**0.5**	**1.0**
Age (years)							
Mean (±*SD*)	27 (4.4)	32 (11.9)	32 (11.7)	27 (5.0)	26 (5.1)	25 (5.2)	36 (2.1)
Minimum/maximum	22/32	20/45	19/44	23/34	21/33	22/31	34/38
Weight (kg)							
Mean (±*SD*)	79.7 (3.84)	79.6 (9.69)	72.6 (7.90)	75.1 (5.83)	75.9 (9.34)	80.4 (7.17)	72.5 (10.45)
Minimum/maximum	76.3/86.2	71.8/92.8	64.8/82.7	70.5/83.5	67.0/88.3	75.3/88.6	62.5/85.3
Height (cm)							
Mean (±*SD*)	177 (2.3)	177 (4.5)	177 (2.9)	181 (5.3)	176 (7.0)	180 (0.6)	176 (3.1)
Minimum/maximum	174/179	172/183	174/181	176/188	168/184	179/180	172/179

from 1 hour predose to 3 hours postdose revealed no clinically significant arrhythmias.

Clinical Laboratory Evaluations, Bleeding Time, and Platelet Aggregation

There were no clinically significant changes in the individual serum biochemistry, hematology, and urinalysis parameters during the study. No drug- or dose-related changes were observed in bleeding time or platelet aggregation.

Pharmacokinetics

Plasma

Geometric mean plasma concentration versus time curves (linear and semi-logarithmic) are presented in Figures 2 and 3. During IV infusion of AP1903 at dose levels of 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg, plasma concentrations increased rapidly during the first 30 minutes of the infusion to geometric mean concentrations of 9, 39, 87, 473, and 1062 ng/mL, respectively. The plasma levels then increased more slowly during the infusion to reach geometric mean C_{max} concentrations of 11, 47, 107, 626, and 1273 ng/mL for the five dose levels, respectively. Maximal concentrations were attained at the end of the 2-hour infusion period for the 0.05 and 0.5 mg/kg dose levels and at the earlier time point of 1.5 hours for the 0.01, 0.1, and 1.0 mg/kg dose levels. It should be noted that the low dose level of 0.01 mg/kg provided a plasma concentration in the predicted therapeutic range based on in vitro killing data in engineered T cells.

Following the infusion period, plasma concentrations showed a rapid distribution phase, declining quickly in a consistent manner for all dose levels to reach values equivalent to approximately 18%, 7%, and 1% of the maximal concentration at 0.5, 2, and 10

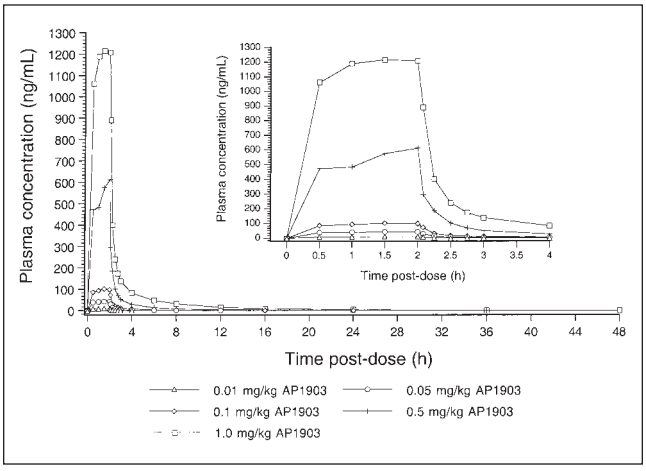

Figure 2. Geometric mean plasma concentrations versus time linear curves in healthy male volunteers receiving single 2-hour intravenous doses of AP1903 at 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg. Inset shows initial 4-hour profile expanded.

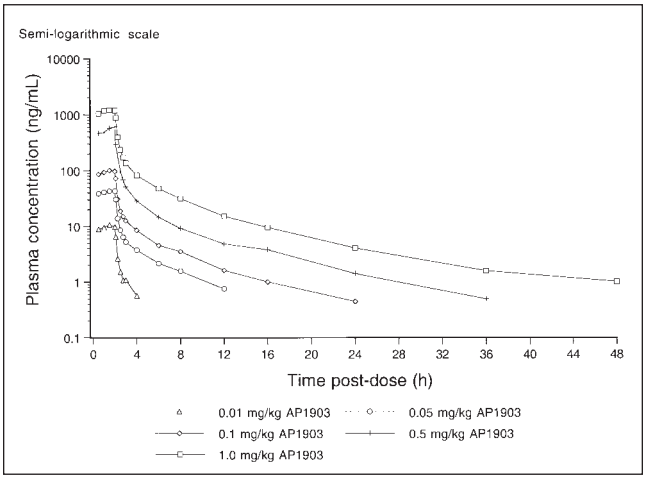

Figure 3. Geometric mean plasma concentrations versus time semi-logarithmic curves in healthy male volunteers receiving single 2-hour intravenous doses of AP1903 at 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg.

Table II Plasma Pharmacokinetic Parameters of AP1903

	Dose of AP1903				
Parameter	**0.01 mg/kg**	**0.05 mg/kg**	**0.1 mg/kg**	**0.5 mg/kg**	**1.0 mg/kg**
$AUC_{(0-t_z)}$ (ng•h/mL)[a]	20.8 (15.5)	104 (14.6)	247 (4.20)	1257 (6.55)	2954 (7.49)
$AUC_{(0-\infty)}$ (ng•h/mL)[a]	NC	109 (13.8)	253 (4.04)	1264 (6.49)	2972 (7.51)
C_{max} (ng/mL)[a]	11.2 (15.0)	46.8 (13.7)	107 (8.07)	626 (8.19)	1273 (9.23)
C_{inf} (ng/mL)[a]	9.90 (24.2)	43.5 (19.0)	98.3 (18.4)	612 (11.8)	1208 (12.7)
t_{max} (h)[b]	1.75 (1.00-2.00)	1.75 (1.00-2.00)	1.75 (1.50-2.00)	2.00 (1.50-2.00)	1.50 (1.00-2.00)
$t_{1/2}$ (h)[a]	NC	3.92 (21.4)	6.29 (33.9)	6.52 (8.03)	12.0 (12.6)
MRT_{int} (h)[a]	NC	1.84 (17.7)	2.35 (19.5)	1.74 (25.3)	2.41 (5.86)
CL (mL/min/kg)[a]	NC	7.64 (13.9)	6.60 (4.05)	6.60 (6.45)	5.61 (7.59)
V_z (L/kg)[a]	NC	2.59 (31.6)	3.60 (34.0)	3.73 (6.65)	5.82 (13.9)
V_{ss} (L/kg)[a]	NC	0.843 (27.1)	0.931 (19.5)	0.690 (24.9)	0.811 (10.8)

NC, not calculable.

a. Geometric mean (geometric CV%).

b. Median (minimum-maximum).

hours, respectively, after the end of the infusion. Plasma concentrations were quantifiable for longer periods of time with increasing dose, being quantified in all volunteers up to 1, 10, 14, 22, and 46 hours after the end of infusion for the 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg AP1903 dose levels, respectively. Overall, the semi-logarithmic plasma concentration versus time profiles revealed an apparent multiphasic decline following the end of the infusion, with more of the disposition profile becoming apparent with increasing dose level. Even at the highest dose level (1.0 mg/kg), a terminal monophasic decline in plasma concentrations was still not present.

The plasma pharmacokinetic parameters are presented in Table II. $AUC_{(0-t_z)}$, $AUC_{(0-\infty)}$, C_{max}, and C_{inf} increased in an approximate dose-proportional manner. Intervolunteer variability (assessed from the coefficient of variation [CV%]) was low, ranging from 4% to 16% for $AUC_{(0-t_z)}$, 4% to 14% for $AUC_{(0-\infty)}$, and 8% to 15% for C_{max}, being slightly greater for C_{inf} at 12% to 24%. Overall, the variability was lower at the three higher dose levels (0.1, 0.5, and 1.0 mg/kg).

The mean terminal half-life appeared to increase with dose level ranging from 3.92 hours at 0.05 mg/kg to 12.0 hours at 1.0 mg/kg. This was considered to be a consequence of more of the disposition profile being determined with increasing dose and the multiphasic disposition of AP1903 rather than a true dose-dependent change in the elimination kinetics of the drug.

The MRT_{int}, calculable for all but the lowest dose, was reasonably constant with dose. The total body clearance, again calculable at the four higher dose levels, showed a slight decrease with increasing dose, with the geometric mean ranging from 7.64 to 5.61 mL/min/kg, reflecting the slightly greater than dose-proportional increase in $AUC_{(0-\infty)}$ (CL = Dose/$AUC_{(0-\infty)}$).

The volume of distribution during the terminal phase (V_z) appeared to increase with dose; however, the data were influenced by the changing terminal rate constant value ($V_z = CL/\lambda_z$). The volume of distribution at steady state (V_{ss}) was more stable, reflecting the more constant MRT_{int} and CL ($V_{ss} = MRT_{int} \bullet CL$). The difference between the V_z and V_{ss} values (V_z being approximately three- to sevenfold higher than V_{ss}) probably reflects the marked multiphasic disposition kinetics of AP1903.

Whole Blood

Linear and semi-logarithmic geometric mean whole-blood concentration versus time curves are presented in Figures 4 and 5, respectively. Similar to the observations seen with the plasma samples, AP1903 whole-blood concentrations increased rapidly during the first 30 minutes of infusion with dose levels of 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg, resulting in geometric mean concentrations of 5, 26, 66, 360, and 710 ng/mL, respectively. The whole-blood levels then increased more slowly during the infusion to reach geometric mean C_{max} concentrations of 6, 33, 83, 432, and 1004 ng/mL for the five dose levels, respectively. Maximal concentrations were attained at the end of the 2-hour infusion for the 0.01, 0.5, and 1.0 mg/kg dose levels and at the earlier time point of 1.5 hours for the 0.05 and 0.1 mg/kg dose levels. On completion of the infusion period, whole-blood concentrations showed a rapid distribution phase, declining quickly to reach values

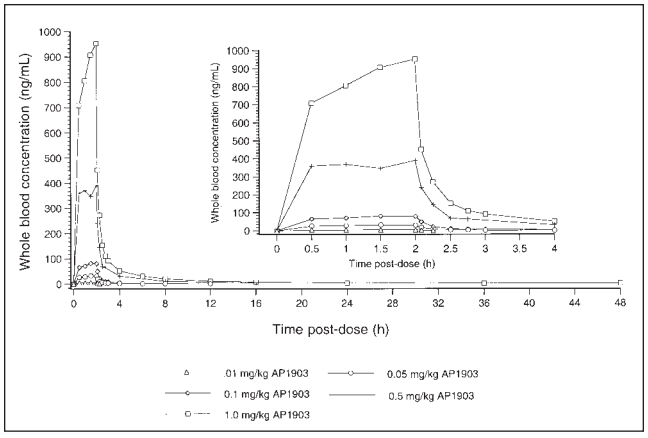

Figure 4. Geometric mean whole-blood concentrations versus time linear curves in healthy male volunteers receiving single 2-hour intravenous doses of AP1903 at 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg. Inset shows initial 4-hour profile expanded.

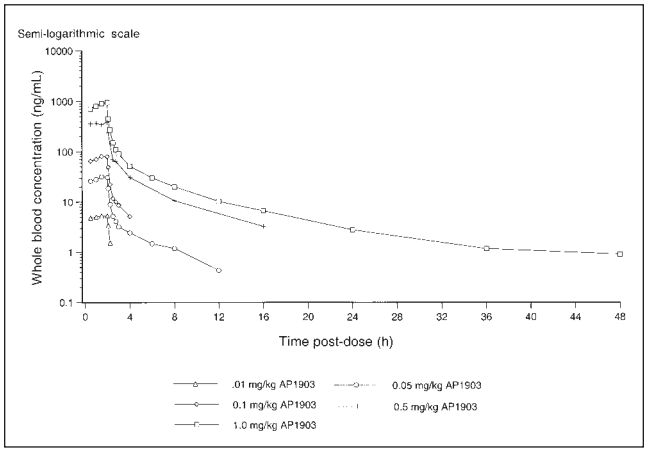

Figure 5. Geometric mean whole-blood concentrations versus time semi-logarithmic curves in healthy male volunteers receiving single 2-hour intravenous doses of AP1903 at 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg.

equivalent to approximately 16% and 7% of the maximal concentration at 0.5 and 2.0 hours, respectively, after the end of the infusion. As with plasma, whole-blood concentrations were generally quantifiable for longer periods of time with increasing dose; overall, the semi-logarithmic concentration versus time profiles revealed an apparent multiphasic decline following the end of the infusion. The lower limit of quantification for the assay, however, was variable and greatest (between 1 and 10 ng/mL) at the 0.1 and 0.5 mg/kg AP1903 dose levels, resulting in the terminal phase being undetermined. As a consequence, the associated pharmacokinetic parameters were not calculable for the 0.1 and 0.5 mg/kg AP1903 dose levels.

The whole-blood pharmacokinetic parameters are presented in Table III. Summary $AUC_{(0-\infty)}$ data were only calculable at the 0.05 and 1.0 mg/kg AP1903 dose levels. $AUC_{(0-t_z)}$, $AUC_{(0-\infty)}$, C_{max}, and C_{inf} increased in an approximate dose-proportional manner. Intervolunteer variability (CV%) was low, ranging from 5% to 19% for $AUC_{(0-t_z)}$, 10% to 13% for $AUC_{(0-\infty)}$, 6% to 15% for C_{max}, and 6% to 19% for C_{inf}. Variability was lower at the three higher dose levels. As seen in the plasma, the whole-blood mean terminal half-life (calculable only at the 0.05 and 1.0 mg/kg dose levels) appeared to increase with dose, that is, 3.63 and 13.9 hours at 0.05 and 1.0 mg/kg, respectively. Again, this is considered a consequence of more of the disposition profile being determined at the higher dose level and the multiphasic disposition of AP1903 rather than a true dose-dependent change in the elimination kinetics of the drug. Although only calculable for the 0.05 and 1.0 mg/kg dose levels, whole-blood observations comparable with those observed in plasma were noted for MRT_{int} and volume of distribution during the terminal phase and volume of distribution at steady state.

For each volunteer, the concentrations of AP1903 in whole blood were lower than those in plasma. The whole-blood/plasma ratios for C_{max} and $AUC_{(0-\infty)}$, where calculable, are shown in Table IV along with the ratio of the volumes of plasma to red blood cells. These data indicate that most of the AP1903 is present in the plasma, but there is evidence of some uptake into the red blood cells at dose levels of 0.05 mg/kg and higher. In addition, over the dose range of 0.05 to 1.0 mg/kg AP1903, the whole-blood/plasma ratios for C_{max} and $AUC_{(0-\infty)}$ were comparable, showing a similar extent of distribution of AP1903 into the red blood cell with increasing dose.

The fraction of administered dose excreted in urine as unchanged drug over the 48-hour period postdose was very low (geometric mean < 0.1%) following all dose levels of AP1903.

DISCUSSION

A phase 1, single-blind, placebo- and saline-controlled, ascending single intravenous dose study was performed in healthy male volunteers to assess the safety, tolerability, and pharmacokinetics of AP1903 at dose levels of 0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg. The 0.01 mg/kg dose led to C_{max} plasma levels (11 ng/mL) that are comparable with the drug concentration observed to cause maximal killing of engineered T cells in vitro,[16] implying that even this low dose may be therapeutic.

Table III Whole-Blood Pharmacokinetic Parameters of AP1903

Parameter	Dose of AP1903				
	0.01 mg/kg	0.05 mg/kg	0.1 mg/kg	0.5 mg/kg	1.0 mg/kg
$AUC_{(0-t_z)}$ (ng•h/mL)[a]	9.88 (18.5)	71.1 (15.2)	160 (5.23)	911 (13.4)	2051 (10.00)
$AUC_{(0-\infty)}$ (ng•h/mL)[a]	NC	74.6 (13.2)	NC	NC	2077 (10.3)
C_{max} (ng/mL)[a]	5.96 (15.1)	32.6 (11.9)	82.8 (7.76)	432 (5.78)	1004 (8.37)
C_{inf} (ng/mL)[a]	5.40 (18.9)	31.0 (13.8)	80.9 (5.91)	391 (10.4)	953 (10.4)
t_{max} (h)[b]	1.25 (1.00-2.00)	1.50 (1.50-2.00)	2.00 (1.50-2.00)	1.50 (0.500-2.00)	1.75 (1.00-2.00)
$t_{1/2}$ (h)[a]	NC	3.63 (5.41)	NC	NC	13.9 (40.0)
MRT_{int} (h)[a]	NC	1.74 (5.33)	NC	NC	2.76 (13.0)
CL (mL/min/kg)[a]	NC	11.1 (13.1)	NC	NC	8.03 (10.4)
V_z (L/kg)[a]	NC	3.50 (16.0)	NC	NC	9.67 (29.1)
V_{ss} (L/kg)[a]	NC	1.16 (17.1)	NC	NC	1.33 (4.08)

NC, not calculable.
a. Geometric mean (geometric CV%).
b. Median (minimum-maximum).

Table IV Summary of Whole-Blood/Plasma Concentrations of AP1903

Parameter	Dose of AP1903				
	0.01 mg/kg	0.05 mg/kg	0.1 mg/kg	0.5 mg/kg	1.0 mg/kg
Whole-blood/plasma ratio for C_{max}	0.53	0.70	0.77	0.69	0.79
Whole-blood/plasma ratio for $AUC_{(0-\infty)}$	NC	0.68	NC	NC	0.70
Plasma/whole-blood volume[a]	0.58	0.58	0.57	0.56	0.57

NC, not calculable.
a. Calculated as 1 minus the mean hematocrit value.

The highest dose tested (1.0 mg/kg) provided a C_{max} plasma concentration of 1273 ng/mL, which exceeds the predicted therapeutic dose by 2 logs. Although the effective concentration of AP1903 may be reduced in vivo by binding to plasma proteins, the in vitro studies reflect drug activity in the presence of 10% bovine serum.[16] Taken together, the in vitro data and the phase 1 clinical data therefore indicate that maximally effective AP1903 blood concentrations can be reached using low doses of the compound.

AP1903 was well tolerated by all volunteers at each dose level. Only 1 volunteer in the 1.0 mg/kg group experienced an adverse event (vasodilatation of short duration) that was considered possibly related to the drug. However, because the event only occurred during the first half-hour of the 2-hour infusion period and because, on questioning, the volunteer reported a similar response under another unrelated stressful condition, it is likely that the event was due to an emotional response of the experimental process rather than a drug effect.

AP1903 was shown to be safe at each dose level, with no clinically significant trends noted in any of the safety parameters, which included assessment of vital signs, hematology, serum biochemistry, urinalysis, ECG, and physical examination. AP1903 did not affect platelet function, with no clinically significant changes in bleeding time or ADP- and collagen-induced platelet aggregation.

Pharmacokinetic analysis was performed on whole-blood and plasma concentrations of AP1903. The concentration-time profiles of AP1903 in plasma and whole blood were very similar. During the intravenous infusion, plasma and whole-blood concentrations increased rapidly during the first 30 minutes, whereas for the remainder of the 2-hour infusion, any increase in the levels was relatively small. This indicated a rapid disposition phase of the drug from the plasma and whole blood, which was equally apparent at the end of the dosing period, where the plasma and whole-blood concentrations declined very rapidly at all dose levels. This was followed by a slower disposi-

tion phase, which was only evident at the higher dose levels, where the drug was quantifiable for a longer period of time. Based on a ^{14}C-labeled AP1903 study in rats using the clinical formulation of the drug, the rapid blood clearance is associated with a rapid distribution of the drug to the liver. In rats, 80% of the administrated radioactivity was found in the liver within 15 minutes of a bolus IV drug administration (Iuliucci JD, unpublished).

Assessment of dose proportionality for AUC, C_{max}, and C_{inf} in plasma and whole blood showed the parameters to be approximately dose proportional, although there was a trend for these parameters to increase in a slightly greater than dose-proportional manner over the dose range studied. Over the dose range 0.05 to 1.0 mg/kg, where the pharmacokinetics of AP1903 was well defined, a 20-fold increase in dose level resulted in a 27- to 28-fold increase in $AUC_{(0-\infty)}$, C_{max}, and C_{inf}. In whole blood, the corresponding increases were similar at 28- to 31-fold. These findings reflect a gradual decrease in plasma and whole-blood clearance with increasing dose. As the clearance of AP1903 appears to occur by nonrenal mechanisms, the trends seen in clearance may be due to saturation of hepatic biotransformation. The similar trend in the plasma and whole-blood pharmacokinetics of AP1903 suggests that there are no marked dose-dependent changes in the distribution of AP1903 between plasma and the blood cells. This is supported by the similar whole-blood/plasma ratios (0.7 to 0.8) for $AUC_{(0-\infty)}$ and C_{max} over the dose range 0.05 to 1.0 mg/kg. The data suggest only a limited but consistent uptake of AP1903 into the blood cells.

Renal clearance was very low (< 0.1%) over the 48-hour period postdose for all dose levels of AP1903. Although fecal specimens were not collected in this investigation, it is expected that the major portion of the administered drug is excreted in the feces. This is based on further observations in the previously cited ^{14}C-labeled AP1903 study in rats. Approximately 1% of labeled drug was excreted in the urine of rats, while 90% and 96% of the administered drug were recovered in the feces at 24 and 48 hours, respectively.

CONCLUSIONS

AP1903 was safe and well tolerated when administered to healthy male volunteers at dose levels up to 1 mg/kg over a 2-hour infusion period. The 1 mg/kg dose provided plasma concentrations of AP1903 > 250 times higher than the drug concentrations shown to be effective in inducing apoptosis in Fas-engineered human T lymphocytes. These observations support further investigations on AP1903 in conjunction with the AP1903/Fas suicide system in patients with hematologic and solid malignancies.

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REFERENCES

1. Devergie A, Apperley JF, Labopin M, Madrigel A, Jacobsen N, Carreras E, et al: European results of matched unrelated donor bone marrow transplantation for chronic myeloid leukemia; impact of HLA class II matching. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transpl* 1997;20:11-19.

2. Klingemann HG, Phillips GL: Immunotherapy after bone marrow transplantation. *Bone Marrow Transpl* 1991;8:73-81.

3. Thomas ED, Clift RA, Fefer A, Appelbaum FR, Beatty P, Bensinger WI, et al: Marrow transplantation for the treatment of chronic myelogenous leukemia. *Ann Intern Med* 1986;104:155-163.

4. Apperley JF, Mauro FR, Goldman JM, Gregory W, Arthur CK, Hows J, et al: Bone marrow transplantation for chronic myeloid leukemia in first chronic phase: importance of a graft-versus-leukemia effect. *Br J Haematol* 1988;69:239-245.

5. Apperley JF, Jones L, Hale G, Waldmann H, Hows J, Rombos Y, et al: Bone marrow transplantation for patients with chronic myeloid leukemia: T-cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukemic relapse. *Bone Marrow Transpl* 1986;1:53-66.

6. Korngold R, Leighton C, Manser T: Graft-versus-myeloid leukemia response following syngeneic and allogeneic bone marrow transplantation. *Transplantation* 1994;58:278-287.

7. Ferrara JLM, Deeg HJ: Graft-versus host disease. *N Engl J Med* 1991;324:667-674.

8. Mitsuyasu R, Champlin RE, Gale RP, Ho WG, Lanarsky C, Winston D, et al: Treatment of bone marrow with monoclonal anti-T-cell antibody and complement for the prevention of graft-versus-host disease: a prospective, randomized, double-blind study. *Ann Intern Med* 1986;105:20-26.

9. Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G, et al: Donor leukocytes transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990;76:2462-2465.

10. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, et al: Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. *Blood* 1995;86:1261-1268.

11. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al: Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 1995;86:2041-2050.

12. Drobyski WR, Keever CA, Roth MS, Koethe S, Hanson G, McFadden P, et al: Salvage immunotherapy using donor leukocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose. *Blood* 1993;82:2310-2318.

13. Spencer DM, Belshaw PJ, Chen L, Ho SN, Randazzo F, Crabtree GR, Schreiber SL: Functional analysis of Fas signaling in vivo using synthetic inducers of dimerization. *Curr Biol* 1996;6:839-947.

14. Clackson T, Yang W, Rozamus LW, Hatada M, Amara JF, Rollins CT, et al: Redesigning an FKBP-log and interface to generate chemical dimerizers with novel specificity. *Proc Natl Acad Sci U S A* 1998;95:10437-10442.

15. Amara JF, Courage NL, Gilman M: Cell surface tagging and a suicide mechanism in a single chimeric human protein. *Hum Gene Ther* 1999;10:2651-2655.

16. Thomis DC, Marktel S, Magnani Z, Traversari C, Gilman M, Bordignon C, Clackson T: A humanized Fas receptor-based suicide switch expressed in human T lymphocytes for the induction of controlled graft-versus-leukemia. *Blood* 2001;97:1249-1257.

17. Bonini C, Ferrari G, Verseletti S, Servida P, Zappone E, Ruggieri L, et al: HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997;276:1719-1724.

18. Verzeletti S, Bonini C, Marktel S, Nobili N, Ciceri F, Traversari C, Bordignon C: Herpes simplex virus thymidine kinase gene transfer for controlled graft-versus-host disease and graft-versus leukemia: clinical follow-up and improved new vectors. *Hum Gene Ther* 1998;9:2243-2251.

19. Ciceri F, Bonini C, Marktel S, Zappone E, Servida P, Rossini S, et al: Long term follow-up in 30 patients receiving HSV-tk transduced donor lymphocytes after allo-BMT [abstract 2963]. *Blood* 1999;94:668a.