Measurement of decitabine levels using LC-MS/MS

The LC-MS/MS method has been previously described for determination of decitabine in human and rat plasma.[1] Modifications to published methods[1] for the measurements in baboons included use of a Finnigan TSQ Quantum EMR triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) instead of an API 300 triple quadrupole instrument, with a 4-fold improvement in sensitivity (LLOQ, 0.5 ng/mL). Validation of the assay validation was essentially as previously described.[1] The method was adapted and validated for quantitation of decitabine in baboon plasma in the presence or absence of THU. Briefly, a 20 µL aliquot of decitabine standards (5 to 10,000 ng/mL) in MeOH was mixed with 20 µL internal standard (5, 6-dihydro-5-azacytidine) stock solutions (1000 ng/mL) and 200 µL baboon plasma. Then, the above mixture was loaded onto an Oasis MCX cartridge (Waters Corporations, Milford, MA), which was preconditioned with 1.0 mL MeOH and 1.0 mL 0.1 N HCl. After sequential washings with 1.0 mL 0.1 N HCl, 1.0 mL 2% MeOH, 50% MeOH and 100 % MeOH, the samples were eluted with 500 µL 2% NH_4OH methanol solution. The fractions were collected and dried under a mild stream of nitrogen. The residues were reconstituted with 100 µL ice cold water, and 50 µL of the solution was injected for analysis. The temperature of the autosampler of the Shimadzu HPLC system (Shimadzu, Columbia, MD) was set at 4°C. Decitabine and the internal standard were separated on a Hypersil Aquasil C18 column (250 × 2.1 mm, 5µm; Thermo Hypersil-Keystone, Bellefonte, PA) coupled with a 2 µm Aquasil precolumn (Thermo Hypersil-Keytone). The mobile phase was 5% 10 mM ammonium formate aqueous solution in methanol. Isocratic elution was used with a run time of 15 minutes (solvent was diverted to the waste in the first two and the last minute). The mass spectrometer was operated in the positive ESI mode with a helium pressure of 27 psi, a typical electro-spray needle voltage of 4400 V and a heated capillary temperature of 325°C. The mass spectrometer was tuned to its optimal sensitivity by direct infusion of 10 µg/mL decitabine 50% methanol aqueous solution. All operations were controlled by Finnigan Xcalibur software on a Windows NT 4.0 system. Analysis was by multiple reaction monitoring, and the ion transition channels used for monitoring decitabine, THU and the internal standard were 229.00>113.13 (E=12%), 247.10>115.10(E=16%) and 247.10>115.10 (E=16%), respectively.

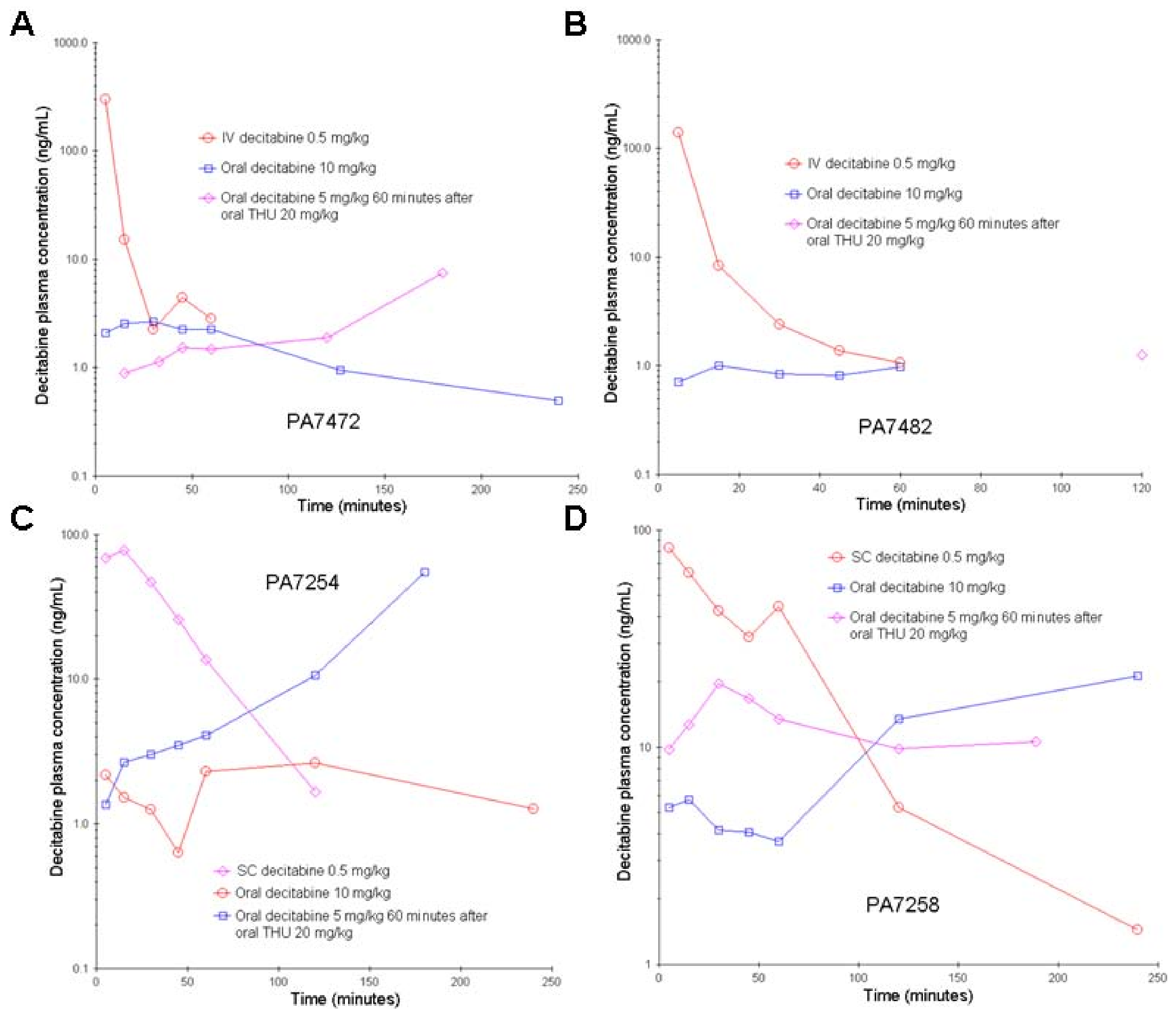

Figure S1. Plasma concentration-time curves following intravenous (IV) decitabine (DAC) 10 mg/m^2 (0.5 mg/kg), subcutaneous (SC) DAC 10 mg/m^2 (0.5 mg/kg) and oral gavage (Oral) DAC 200 mg/m^2 (10 mg/kg) administration to baboons

Some animals also received oral DAC 100 mg/m^2 (5 mg/kg) 60 minutes after THU 400 mg/m^2 (20 mg/kg). Blood was collected for up to 7 time-points after administration and plasma concentrations were determined using LC/MSMS. The data shows that administration by the oral route produces lower peak levels and a longer half-life than IV or SC administration. (A) IV and oral administration in PA7472. (B) IV and oral administration in PA7482. (C) SC and oral administration in PA7254. (D) SC and oral administration in PA7258.

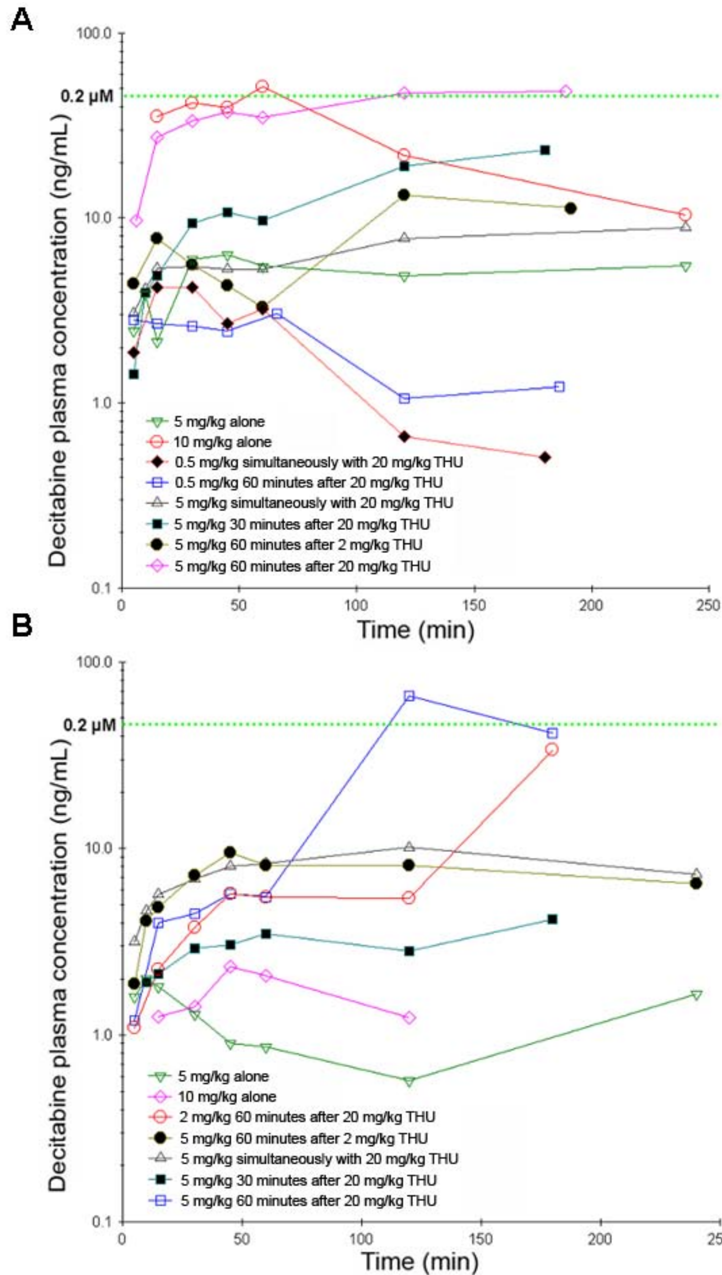

Figure S2. Identifying the optimal doses of THU and decitabine (DAC), and optimal timing between THU and DAC administration

(A) Baboon PA7470 treated with different doses of THU and DAC and different timing between the drugs. THU 400 mg/m^2 (20 mg/kg) produced higher DAC concentrations than THU 40 mg/m^2 (2 mg/kg). THU 400 mg/m^2 60 minutes before DAC produced higher DAC concentrations than simultaneous or 30 minute prior administration of THU. (B) Baboon PA7484 treated with different doses of THU and DAC and different timing between the drugs. THU 20 mg/kg produced higher DAC concentrations than THU 2 mg/kg. THU 20 mg/kg 60 minutes before DAC produced higher DAC concentrations than simultaneous or 30 minute prior administration of THU.

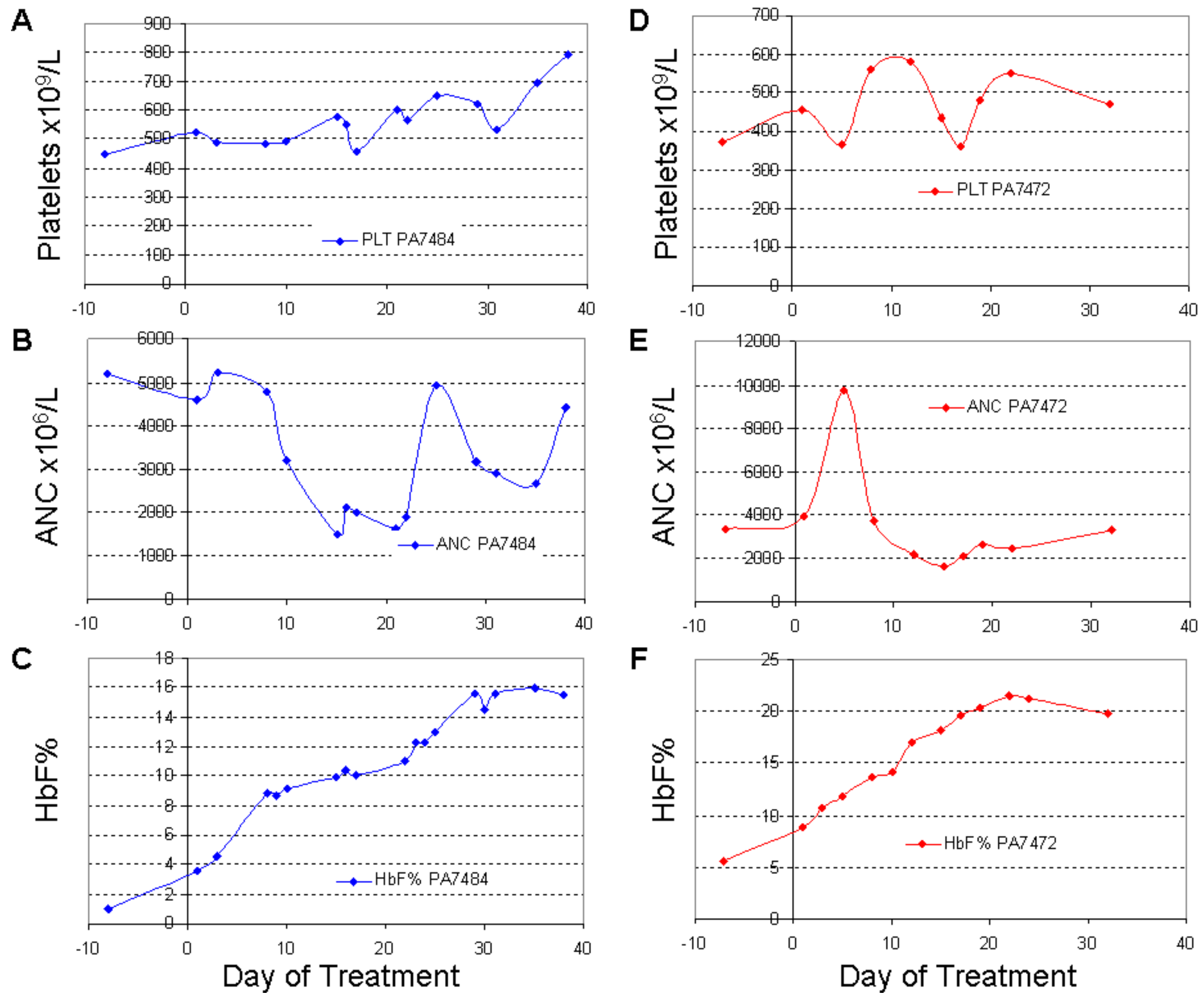

Figure S3. Pharmacodynamic effects of repeat dose oral THU-decitabine in non-human primates

Decitabine 5 mg/m^2 (PA7484) or 2.5 mg/m^2 (PA7472) 60 minutes after THU 400 mg/m^2 3×/week was administered for 5 weeks to PA7484 and for 3 weeks to PA7472. First dose day 1. Last day of treatment day 31 in PA7484, day 19 in PA7472. (A) Platelet counts in PA7484. (B) Absolute neutrophil counts (ANC) in PA7484. (C) HbF% in PA7484. (D) Platelet counts in PA7472. (E) ANC in PA7472. (F) HbF% in PA7472.

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Combination therapy with THU might offer other advantages, since treatment with cytidine analogues can select for cells that escape the effects of these drugs by upregulating CDA,[2–9] and cancer cells may find sanctuary from cytidine analogues in tissues expressing high levels of CDA.[10]

During normal adult erythropoiesis, the *HBG* promoter is transiently hypomethylated accompanied by transient HBG expression, indicating transcription factor binding at this locus.[11–13]

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