**Trial # XX**

**Note: Change sections in blue to reflect the current protocol then change font to black.**

**Title:**

Pharmacokinetics (PK) of nanoformulated prodrugs of cabotegravir (CAB) and rilpivirine (RPV) following a single intramuscular dose of each drug in rhesus macaques (

**Hypothesis:**

Nanoformulations of P407-stearoylated-CAB (NM2CAB) and P407-myristoylated-RPV (NMRPV14) at a dose of 45 mg/kg (native drug equivalents) for each formulation, given intramuscularly (IM), will yield sustained plasma levels above the PA-IC90 for 3 months or longer.

**Protocol:**

**Four** rhesus macaques (NHP) will be used for the study.

* Animals will be given NM2CAB and NMRPV14 on Day 0 by IM injection (volume: 0.5 ml/kg)
* Blood will be collected on Days 0, 1, 3, and 7.
* Whole blood, plasma, and PBMCs will be collected on Days 7, 14, 21, 28, 35, 42, 49 and every two weeks thereafter until no measurable drug is detected.
* Metabolic liver and renal panels and total blood counts (CBC) will be assessed on Days 0, 3, 7, 14, 21, 28, 42, 49, and every 4 weeks thereafter until the end of the study.

**Animal weights in kg (dd/mm/yyyy):**

* M1 (1841) \_**xxx**\_
* M2 (1793) \_**xxx**\_
* M3 (429) \_**xxx**\_
* M4 (801) \_**xxx**\_

**Food**

Rhesus macaques will be provided xxxxxxx

**Water**

Rhesus macaques will be provided xxxxxxx ad libitum

**Formulation preparations:**

The nanoformulations will be prepared by high pressure homogenization using aseptic technique to attenuate the potential for endotoxin contamination in the NNPP-GLP facility by Dr. Adam Szlachetka. This is now performed as per established GLP protocols developed in collaboration with Dr. Benson Edagwa and students James Hilaire, and Tanmay Kulkarni,. Samples will be taken at various stages of nanoformulation preparation and of the injection solution to assess physicochemical characteristics (particle size, polydispersity and zeta potential). Prodrug concentration of each nanoformulation will be determined by UPLC-TUV and UPLC-MS/MS (using analytical methods that meet FDA guidelines, developed by Dr. Nagsen Gautam in Dr. Yazen Alnouti’s lab). Endotoxin levels will be determined for the starting prodrug powders and the final nanoformulation suspensions. Formulation endotoxin concentrations of < 5 EU/kg will be considered acceptable for injection.

Formulations will be produced by high pressure homogenization using direct production. Dose volume will not exceed 0.5 ml/kg each site, which translates to 2.5 ml per injection site per 5 kg monkey.

* For NM2CAB and NMRPV14, to achieve a dose of 45 mg/kg (active drug equivalents), the formulation prodrug concentration will need to be 149.4 mg/ml. The total amount of prodrug given to each monkey would be 372.5 mg M2CAB or MRPV14 (74.5 mg/kg M1CAB or MRPV14).

Active drug doses for each prodrug formulation would be as follows:

**NM2CAB:** 74.7 mg M1CAB/kg = 45 mg CAB/kg (M1CAB MW: 671.83; CAB MW: 405.36)

**NMRPV14:** 74.7 mg MRPV14/kg = 45 mg RPV/kg (MRPV14 MW: 606.81; RPV MW: 366.42)

The monkeys will be weighed to prepare the appropriate dose. Animals will be anesthetized with ketamine, bled for study/CBC/met panel, and then injected with drug. The first set of nanoformulations will be given in the gluteus maximus on D0 (total dose volume will not exceed 0.5 ml/kg/per injection site). The dose nanosuspensions will be assessed for physicochemical characteristics and drug concentration before and after injections are completed. This will be completed in the NNPP-GLP lab and using UPLC-MS/MS for drug quantitation. For QCs of animal dosing solutions, collect two sets of three 10 µl aliquots into tubes containing 990 µl MS-grade methanol, one set just prior to dosing and the other at the end of animal dosing. Mix tubes well after addition of sample. Store at -80˚C for drug quantitation by UPLC-MS/MS.

Drug injections and sample collection will be conducted by Dr. Howard Fox’s lab in collaboration with Comparative Medicine veterinary staff.

**Sample collections:**

* Blood will be collected into K-EDTA coated tubes for CBC, metabolic panels and prodrug and parent drug quantitations.
* Follow the table below for dates for CBC and metabolic panel tubes (1 ml per/delivered to pathology and microbiology). An extra slide will be processed for CBC. (Contact information-hemotech 9-9113).
* Plasma will be separated by centrifugation (20’ RT; 2000RPM).
* Plasma will be stored at -80˚C.
* PBMCs will be collected, counted and pellets stored at -80˚C for prodrug and parent drug quantitations
* For parent drug and prodrug quantitations in whole blood, three 50 µl aliquots should be collected into tubes containing 1 ml MS-grade acetonitrile, mixed well, and stored at -80 ˚C for drug quantitation.
* Collected plasma will be analyzed for prodrug and parent drug concentrations in Dr. Gendelman’s lab (Dr. JoEllyn McMillan, Bhagya Laxmi Dyavar Shetty, Melinda Wojtkiewicz), PBMCs will be analyzed in Dr. Alnouti’s lab (Dr. Nagsen Gautam) for prodrug and parent drug concentrations.

**Projected start date:**

*Production:* June 25-29 for NM2CAB and NMRPV14

*Injection****:*** July 2 for NM2CAB and NMRPV14

**Projected Schedule:**

July 2 Day 0 Inject NM2CAB and NMRPV14 No blood collection

July 3 Day 1 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

July 5 Day 3 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

July 9 Day 7 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

July 16 Day 14 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

July 23 Day 21 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

July 30 Day 28 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Aug 6 Day 35 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Aug 13 Day 42 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Aug 20 Day 49 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Aug 27 Day 56 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 3 Day 63 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 10 Day 70 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 17 Day 77 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 24 Day 84 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 25 Day 85 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Sept 28 Day 88 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Oct 1 Day 91 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Oct 8 Day 98 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Oct 15 Day 105 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Oct 22 Day 112 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Oct 29 Day 119 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Nov 12 Day 133 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

Nov 27 Day 147 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)

**†Dec 11 Day 161 CBC, CMP and pharmacokinetics bleed (blood, plasma, PBMCs)**

\*Projected based upon previous PBMC quantitations

**†Will continue monthly until no detectable drug in blood/plasma**