Goal

Develop Methodology for Pharmacokinetic Screening of Monoclonal Antibodies (mAbs) in Mice and Rats using Small Volume Serial Sampling and Discovery Criteria.

Introduction

Monoclonal antibody therapy is an active research area for most pharmaceutical companies. mAb candidate selection includes pharmacokinetic screening as with small molecules, where the mAbs are dosed and blood samples are collected serially to determine the exposure and half-life.

This application note describes a generic discovery pharmacokinetic screening approach using mice or rats for mAbs screening. We use a small volume serial sampling scheme enabling determination of individual animal pharmacokinetics in mouse and rat, rather than using sparse sampling and composite data.

The advantages of this approach include a reduction in the numbers of animals used and a corresponding reduction in the amount of test article required, which is often important at the discovery stage. For example, serial sampling using mice (n = 3) requires approximately 100 µg per dose group vs. 1 mg per dose group with sparse sampling (n = 3 per time-point and 10 time-points). This 10X reduction in test article requirement can reduce the time necessary to generate sufficient test article if the mAb is of low yield.

Intravenous dosing via the dorsal metatarsal vein

Because blood samples are to be collected from the tail, we administer the IV dose at a different position. The rodent can be dosed *via* a jugular vein cannula. However, this means additional surgery is needed which increase the cost and the timeline from experimental planning to dosing. An alternative approach is to dose the rodent by injection into the dorsal metatarsal vein. In mice, the dorsal metatarsal vein is small (approximately 0.75 mm), so very small gauge needle, steady hands, and lots of practice are necessary to ensure reproducibility.

Sampling via tail snip

This is essentially a similar methodology being used in sample collection for dry blood spot analysis. The tails of the animals are warmed by using warm compresses to increase obtainable blood volume. The animals are put in a tubular restrainer, and the distal 1–2 mm of the tail then snipped, and "milked" to form a single drop of blood, which can be collected *via* capillary action into a small sampling capillary. With this method, the clot/scab can be gently pulled for repeated samples, or if necessary another 1–2 mm is snipped off.

Bioanalytical Method

In sandwich ELISA, Goat anti-Human IgG(Fc) is coated onto a microtiter plate, which is allowed to incubate overnight. The plate is then blocked for a period of time. Samples (calibration standards, QCs, and test samples) are added after dilution and incubated on the plate. Human IgG1 is detected by Goat anti-Human IgG(Fab)-Horseradish peroxidase.

Figure 1. *A Typical Calibration Curve of Human IgG1 in Rat Serum*

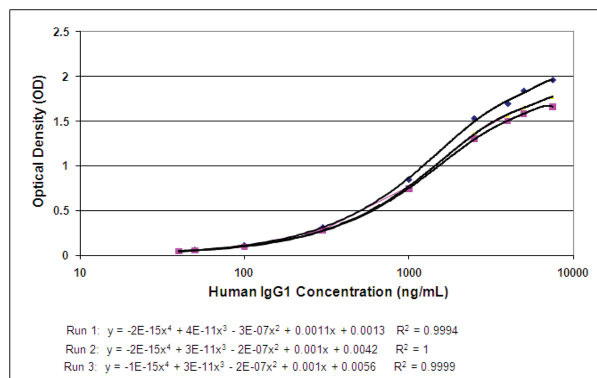
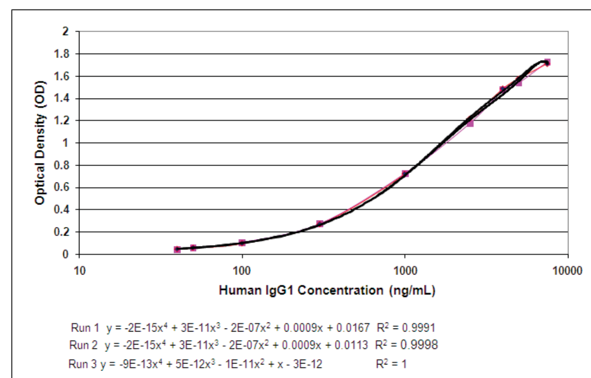


Figure 2. *A Typical Calibration Curve of Human IgG1 in Mouse Serum*



Methods

This study was conducted according to non-Good Laboratory Practice (non-GLP) criteria and employed good scientific pratices utilizing QPS standard operating procedures (SOPs), and a study protocol.

- **Test Article**
  - Fully human IgG1 isotype control (produced recombinantly in CHO cells under serum free conditions at 1 mg/mL in phosphate buffer solution) obtained from Eureka Therapeutics.
- **Test Species**
  - Six (6) male Sprague-Dawley rats (7 – 9 weeks, weighing approximately 250 – 300 g) obtained from Hilltop Research.
  - Six (6) male CD–1 mice (12 weeks, weighing approximately 30 g) obtained from Charles River Laboratories.
- **Group Designation**

Group Number	Species	Number/Sex	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Target Dose Conc. (mg/mL)	Blood Sampling Volume (µL)
1	rat	3 M	IV	1	1	1	50-70
2	rat	3 M	SC	1	1	1	50-70
3	mouse	3 M	IV	1	5	0.2	50-70
4	mouse	3 M	SC	1	5	0.2	50-70

- **In Life**
  - Animals were housed individually in standard plastic cages with bedding and had *ad libitum* access to food and water, and were not fasted prior to dosing.
  - During the study all animals were on a 12 hr light/dark cycle with a room temperature of 65 – 75°F, and relative humidity 20 – 70%.
  - Test article was administered either *via* single intravenous (IV) or subcutaneous (SC) dose.
  - Viability: Once daily observations for moribundity and mortality.
  - Cage side Observations: Prior to dosing and daily during treatment period.
- **Dosing Information**
  - For rats, IgG1 was dosed as supplied. For mice, a 5X dilution in PBS was used as the dosing formulation.
  - The IV doses were administered through a jugular vein cannula in rats or *via* injection into the dorsal metatarsal vein in mice.
  - The SC doses were administrated by injection in the mid-scapular area.

- **Sample Collection**
  - Mice Time-point: Pre-dose, 1, 4, 8 hours, and 1, 5, 8, 12, 19, 26, and 41 days.
  - Rats Time-point: Pre-dose, 1, 4, 8 hours, and 1, 6, 9, 13, 20, 27, and 42 days.
  - Whole blood was collected *via* tail snip using small volume sample collection capillaries, allowed to clot, and centrifuged in a hematocrit centrifuge at room temperature.
  - The resultant serum was stored at -70°C before bioanaylsis.

- **Bioanalysis**
  - The serum samples were analyzed using either a mouse or a rat sandwich ELISA assay developed by QPS.
  - 10 µL of either mouse or rat serum samples were diluted and analyzed in duplicate. The calibration curve included blank and standards ranging from 40 ng/mL (Lower Anchor) to 7500 ng/mL (Upper Anchor) and at least three levels of QC (150, 2000, and 3800 ng/mL). Calculations were done using "Logistic, Auto Estimate" regression in Watson™ LIMS (v. 6.4.0.04, Thermo Electron Corporation, USA).

- **Pharmacokinetics Calculation**
  - Non-compartmental pharmacokinetic analysis of the serum concentrations *vs.* time data was performed using WinNonlin™ (Pharsight Corporation).
  - IV: AUC_{last}, $t_{½}$, AUC_{inf}, CL, Vd_{ss}
  - SC: C_{max}, T_{max}, AUC_{last}, $t_{½}$, AUC_{inf}, F(%)

Results and Discussion

Both rat and mouse analytical methods were qualified using three (3) individual runs to establish precision and accuracy. Preliminary evaluation of specificity, dilution linearity, and room temperature stability indicated that these proprietary QPS human IgG methods can be validated as needed using the latest regulatory agency requirement.

Although the diameter of the metatarsal vein is small, dosing directly into the metatarsal vein in the mice is achievable with practice.

Individual animal serum IgG1 concentration *vs.* time profiles are shown in Figures 3 and 4. Pharmacokinetic parameters are reported in Tables 1 and 2. In both rats and mice, human IgG1 showed a biphasic serum concentration *vs.* time profile after IV injection. The mean terminal elimination half-life was 10.8 days in rats and 8.9 days in mice. Systemic clearance was low. In contrast after subcutaneous injection dosing, human IgG1 showed a slow absorption phase, with a T_{max} at 7 days post-dose in rats and at 1 day post-dose in mice. Subcutaneous bioavailability averaged 63% in rats and 121% in mice. The terminal elimination half-life after subcutaneous dosing averaged 2.8 days in rats and 9.0 days in mice.

In both rats and mice, some but not all animals in each group showed a rapid decline of serum IgG1 levels after approximately 8-12 days post-dose. This rapid elimination phase could be due to the formation of antihuman antibody in these animals and can be verified by measuring anti-drug antibodies (ADA). Alternatively a higher dose can be administered to determine the involvement of target mediated disposition. Because of the inter-animal differences in the terminal phase disposition of this human antibody, the characterization of individual pharmacokinetics is much more meaningful than composite pharmacokinetics.

Figure 3. *Serum IgG1 Concentration vs Time Profiles in Individual Rats*

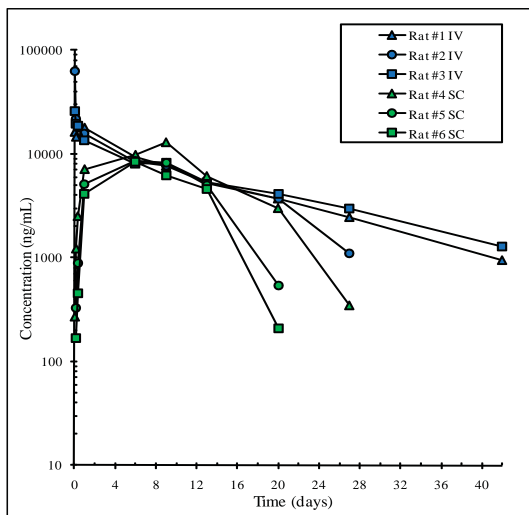


Figure 4. *Serum IgG1 Concentration vs Time Profiles in Individual Mice*

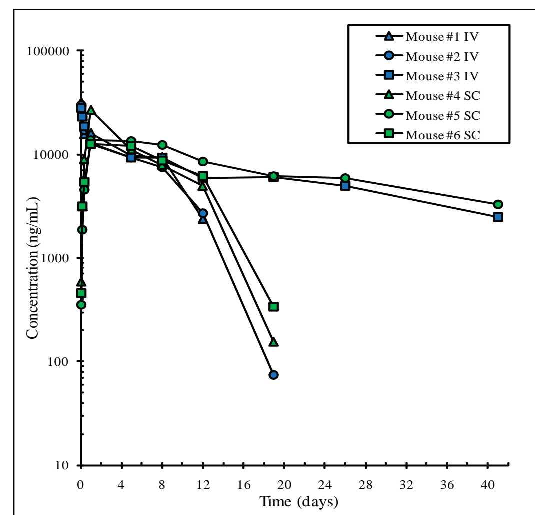


Table 1. *Human IgG1 Pharmacokinetics in Rats after a 1 mg/kg Dose*

IV	Rat #1	Rat #2	Rat #3
AUC_{last}(d*ng/mL)	216078	180859	213338
AUC_{inf}(d*ng/mL)	231872	191501	239165
t½ (d)	11.6	6.8	13.9
CL (mL/h/kg)	0.18	0.22	0.17
Vd_{ss} (mL/kg)	63	51	76

SC	Rat #4	Rat #5	Rat #6
C_{max} (ng/mL)	12970	8581	8449
T_{max} (d)	9	6	6
AUC_{last}(d*ng/mL)	162278	107044	93334
AUC_{inf}(d*ng/mL)	163982	109147	93975
t½ (d)	3.4	2.7	2.1
F (%)	75	50	43

Table 2. *Human IgG1 Pharmacokinetics in Mice after a 1 mg/kg Dose*

IV	Mouse #1	Mouse #2	Mouse #3
AUC_{last}(d*ng/mL)	122254	118430	256464
AUC_{inf}(d*ng/mL)	133547	118605	334600
t½ (d)	3.3	1.6	21.7
CL (mL/h/kg)	0.31	0.35	0.12
Vd_{ss} (mL/kg)	40	42	85

SC	Mouse #4	Mouse #5	Mouse #6
C_{max} (ng/mL)	26833	13629	12489
T_{max} (d)	1	1	1
AUC_{last}(d*ng/mL)	161416	304115	139922
AUC_{inf}(d*ng/mL)	161922	409489	141245
t½ (d)	2.2	22.2	2.7
F (%)	78	209	68

Conclusions

The data contained within this application note clearly demonstrated that IV dosing in the mouse *via* metatarsal vein is a *via*ble alternative to IV dose *via* the tail vein or cannula. Using tail snip blood collections, it is feasible to serially sample individual mice at minimally 7 time–points (5 time–points within the first 24–hour in this study), which will provide superior PK data compared to composite PK using multiple animals. Without serial sampling, inter-animal differences in the elimination profiles cannot be examined. This advantage of serial sampling is in addition to the advantage of minimizing the amount of test article required.

In addition, QPS proprietary human IgG methods in mice and rat are qualified to use for chimeric or humanized mAbs screening. Both methods can be validated under the latest regulatory agency criteria.

Coupling IV dosing in mice *via* the metatarsal vein and tail snip sampling, with a sensitive and selective ELISA method, can result in pharmacokinetic screening for monoclonal antibody drug candidates being as routine as PK screening of small molecule drug candidates.