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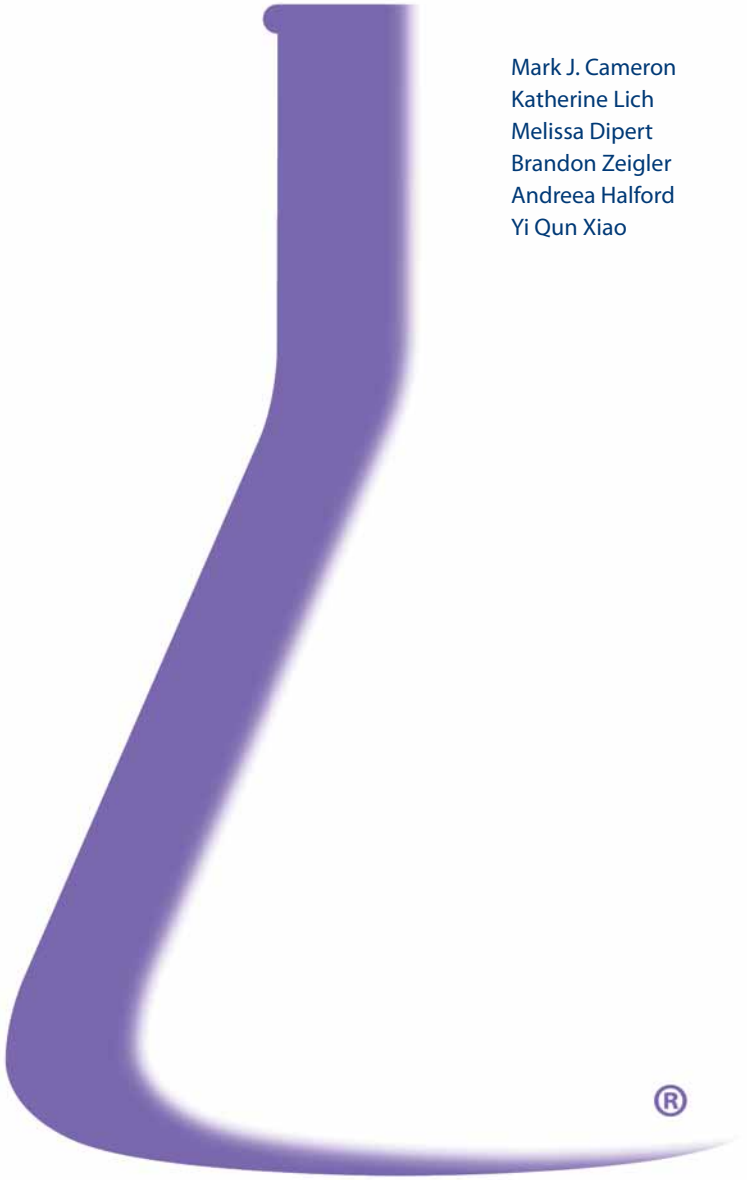

A Gyros Platform Pharmacokinetic Assay for Rituximab Using Microsampling with Simultaneous Analysis of a Pharmacodynamic Marker: An Animal-sparing Strategy

Mark J. Cameron, Katherine Lich, Melissa Dipert, Brandon Zeigler, Andreea Halford, and Yi Qun Xiao

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ABSTRACT

Pharmacokinetic studies in mice traditionally require one animal per time point. This strategy will result in euthanizing large numbers of mice placed on study. Dosing large numbers of animals may induce errors that may adversely impact the study. Animal-to-animal variation is inherent in this design. These studies are time-consuming, labor-intensive, and costly.

We describe a sampling strategy allowing for fewer animals placed on drug safety studies. This strategy allows both pharmacokinetic (PK) and pharmacodynamic (PD) sample analysis at each of 8 time points from serially drawn 25 uL blood samples from a single mouse. C-Reactive Protein (CRP) is a surrogate PD marker for this study and Rituximab is the test article for PK. The Gyros immunoassay platform was chosen for this evaluation as this technology features rapid assay development time, automation, assays with wide dynamic ranges, short assay run times, and minimal sample volume requirements

OBJECTIVES

1. To develop a micro sampling method that will allow multiple blood draws from a single mouse.
2. To develop a C-Reactive Protein (CRP) assay for mouse using commercially sourced critical raw materials on the Gyros in under 7 working days. CRP is a surrogate PD marker for this study.
3. To develop a "generic or universal" PK method for Rituximab using commercially sourced critical raw materials on the Gyros in under 7 working days.
4. To evaluate the Gyros platform for assay development time and assay performance in a demanding GLP environment of the preclinical CRO.

Figure 1. The Gyrolab workstation with Bioaffy CDs.

MATERIALS AND METHODS

Test Article
Pharmaceutical grade Rituximab was administered as a single intravenous dose of 5 or 20 mg/kg via the tail vein.

Blood Collection
Serial and non-terminal blood samples of approximately 25 uL per time point were collected from the submaxillary vein at protocol-specified time points using glass micro-hematocrit tube without anticoagulant.

Sample Processing and Storage
After collection, each tube was sealed at one end and the samples were allowed to clot at room temperature and then centrifuged to obtain serum. The samples were stored frozen at approximately -70°C until day of analysis.

CRP Assay
Antibodies and the recombinant mouse CRP protein were sourced commercially.

Generic or Universal Rituximab Assay
Antibodies were sourced commercially. Rituximab was used as the assay calibrator.

Antibody Labeling
The antibodies were labeled with biotin and Alexa Flour 647 prior to assay development. A biotinylation kit from Thermo Scientific (catalog number 21935) and an Alexa Fluor 647 labeling kit from Invitrogen (A21186) were used as directed by the manufacturer.

Figure 2. An example of micro hematocrit tubes used for the collection of small amounts of blood from mice.

RESULTS

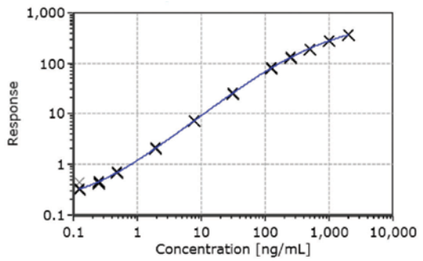

Figure 3. Typical standard curve for mouse CRP assay.

Nominal Conc ng/mL	2000	1000	500	250	125	31.3	7.8	1.95	0.49	0.24	0.12
Mean	1980	1050	472	260	125	31.1	7.8	1.97	0.49	0.024	0.12
%CV	0.7	1.4	2.7	2.7	2.6	2.1	0.6	0.9	1.5	6.1	3.3
Ave. Bias	-0.9	4.5	-5.5	4	0.1	-0.4	0.1	0.2	0.1	0.1	0.1

Table 1. Typical data for mouse CRP standard curve.

RESULTS (CONTINUED)

Dilution	Calculated Concentration	Percent of Previous Dilution
1:10	10,700	--
1:40	9,630	90
1:160	9,730	101
1:320	9,630	98
1:640	9,220	96
1:1280	8,890	96
1:2560	8,880	100
1:5120	8,810	99

Table 2. Dilution assessment: A mouse serum sample containing a detectable concentration of CRP was diluted serially from 1:10 to 1:5120 for analysis. The concentration was interpolated from the standard curve and multiplied by the dilution factor.

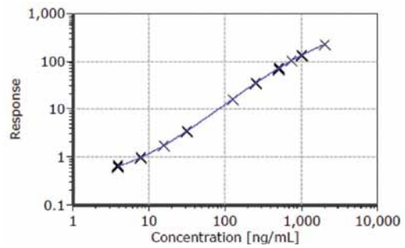

Figure 4. Typical standard curve for mouse PK assay for Rituximab.

Nominal	2000	1000	750	500	250	125	31.3	15.6	7.8	3.9
Mean	1999	998	756	503	251	125	31	17	8.0	86
% CV	0.7	1.2	1.2	0.5	1.3	1.6	2.7	16.5	4.3	213
% Recovery	99.9	99.8	101	101	100	100	99	110	103	2202

Table 3. Typical data for mouse PK assay.

Dilution	300	1000	5000
Mean Recovery ng/mL	75,311	70,215	73,374
% CV	2.5	1.2	5.8
% Recovery	116	108	113

Table 4. Dilutional linearity of a 65,000 pg/mL Rituximab spike into mouse matrix.

Nominal Concentration ng/mL	% CV Rituximab	% CV CRP
1	5.3	8.3
5	--	4.7
10	--	6.1
12	8.5	--
50	7.4	--
125	2.1	--
600	3.8	--

Table 5. Inter-assay variability of the Rituximab and CRP quality control samples.

RESULTS (CONTINUED)

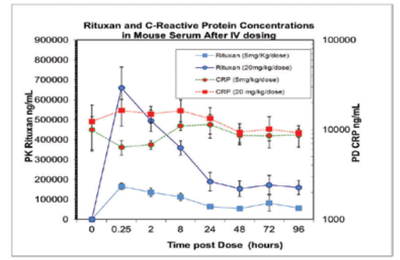

Figure 5. PK and PD profile for Rituximab and CRP from serially drawn 25 uL samples.

Function	CRP	PK	Disks Used CRP	PK
Sourcing of Reagents	7 Days	7 Days		
Labeling of Antibodies	1 Day	1 Day		
Best Pair Determination	1 Day	1 Day	2	3
Titer of Detection Antibody, Dilution Series, Typical Standard Curve	2 Days	2 Days	3	3
Minimum Required Dilution, Spike, and Recovery	1 Day	1 Day	2	2
Intra- and Inter-Assay Precision and Accuracy	3 Days	3 Days	3	3
Total Bioaffy CDs Used in Development (minus stability)			10	11
Total Number of Days for Assay Development (minus stability and antibody sourcing)	7 Days	7 Days		

Table 6. Milestones of timely immunoassay development mouse CRP and Rituximab on the Gyros platform.

CONCLUSIONS

The micro sampling strategy used for individual mice with Gyros platform sample analysis resulted in sufficient sample volume to permit quantitation of at least two analytes, allowing determination of pharmacokinetics and pharmacodynamics of a biologic drug.

The Gyros platform facilitated rapid assay development for both PK and PD markers. The Gyros platform is well suited for the demanding GLP environment supporting studies with challenging timelines and limited sample volumes.

REFERENCES

Jonsson, O, Rodrigo PV, Nilsson, LB, Norsten-Höög,C, Brogren J, Eriksson, M, Königsson K, and Samuelsson, A. Capillary microsampling of 25 µL blood for the determination of toxicokinetic parameters in regulatory studies on animals. *Bioanalysis:* March 2012, Vol. 4, No. 6, Pages 661-674.

Hoff, J. Methods of Blood Collection in the Mouse. Lab Animal 2000; 29(10):47-53.

Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brien PJ, Sailstad J, Khan M, Ray C, Wagner JA. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res.* 2006 Feb;23(2):312-28.

Kelley M., DeSilva B. Key elements of bioanalytical method validation for macromolecules. *AAPS J.* 2007 May 18;9(2):E156-63.

Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R. Quantitative bioanalytical methods validation and implementation: Best practices for chromatographic and ligand binding assays. *Pharm Res.* 2007 Oct;24(10):1962-73.

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