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Multiplexed Cytokine Comparison Across Two Species Dosed with the Same Drug

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Abstract

Preclinical studies provide a wealth of information for organizations looking to submit their Investigational New Drug for clinical trials. Questions often asked include which assay platform to use (Meso Scale Discovery (MSD) vs. Luminex xMAP®), and how relatable are the results between small animals (rats) and large animals (non-human primates, NHP). Here, we retrospectively examined two different drugs that were administered to rats and NHPs. Serum cytokine samples from Drug A (antibody-drug conjugate) were analyzed on the Luminex LX200 for both species, using a Millipore 23-plex assay for the NHP samples and a Millipore 27-plex assay for the rat samples. For Drug B (enzyme Fc fusion molecule), NHP serum cytokine samples were analyzed on the MSD SQ120 using a U-Plex 10-analyte assay, and the same Millipore 27-plex assay was used for the rat serum samples. Our analysis revealed commonalities and differences in cytokine responses across species, as discussed below.

The common analytes across all three assays were IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, MCP-1, and TNF-α. For Drug A, 336 NHP and 168 rat samples were analyzed. For Drug B, 312 NHP and 304 rat samples were analyzed. Drug A's time points in relation to dosing were similar for rats and NHPs.

For Drug A, most NHP samples were below the limit of quantitation (BLQ) for IFN-γ, IL-1β, IL-2, IL-4, IL-10, and TNF-α, whereas the rat samples showed elevated levels (2- to 29-fold increase) of cytokines over the course of dosing for most animals. Both species produced responses in all animals for MCP-1 over the course of dosing, with similar fold changes. Some animals of each species also produced IL-6, with NHP samples producing greater fold change in levels when compared to baseline.

For Drug B, the NHP samples were BLQ for IFN-γ, IL-1β, IL-2, IL-4, IL-10, and TNF-α, whereas the rat samples produced responses for these same analytes. For IL-6, both species had animals with elevated responses over the course of dosing, whereas all animals in both species showed elevated MCP-1 levels during dosing.

From our analysis, detectible levels of cytokines vary greatly between the species with certain cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-10, and TNF-α), regardless of the drug given. However, for IL-6 and MCP-1, both species had elevated levels for both Drugs A and B. By using two different species and multiplexed assays, this wealth of information provides a clearer picture of what could be expected as a drug moves forward into clinical trials.

Introduction

The decision to look at multiple cytokines and chemokines during preclinical testing can provide a wealth of information; however, the results from the testing can be misleading if not interpreted carefully. Sprague Dawley rats and Cynomolgus monkeys are two standard models used for preclinical testing, and which model is used depends on the goal of the drug being given. Here we examine two different drugs (Drugs A and B) that were given to rats and NHPs, with similar doses per kg body weight, and similar collection timepoints.

Materials, Methods, and Equipment

Drug A :
Drug A is an antibody-drug conjugate, given via IV. Whole blood was collected from both rats and NHPs and processed using serum-separating tubes. Thirty-five microliters of sample serum was used for analysis with the NHP and Millipore rat kits.

Table 1. Drug A Timepoint Collection Schedule

Drug A Collection Schedule, Timepoints						
NHP	Day -8	Day 1, 2 hr	Day 2, 24 hr	Day 15 Pre	Day 22, 2 hr	Day 23, 24 hr
Rat	Day -7	Day 1, 2 hr	Day 2, 24 hr	Day 15 2 hr	Day 22, 2 hr	Day 23, 24 hr

Drug B:
Drug B is an enzyme Fc fusion molecule. Whole blood was collected from both rats and NHPs and processed using Serum Separating Tubes. Ten microliters of sample serum was used for analysis with the MSD U-plex 10-plex Kits, and 35 microliters of sample serum was used for analysis with the Millipore rat kit.

Table 2. Drug B Timepoint Collection Schedule

Drug B Collection Schedule, Time Points			
NHP	Day 1, Pre	Day 1, 1 hr	Day 29, 8 hr
Rat	Day -6	Day 1, 4 hr	Day 29, 4 hr

NHP and Millipore Rat Kits (Cat. #PRCYMAG40PMX23BK and RECYMAG65PMX27BK):
The kit was removed from a refrigerator set to maintain 4°C and allowed to warm to room temperature for at least 30 minutes. Lyophilized serum matrix and lyophilized standards were rehydrated using deionized water. Antibody-coupled magnetic beads were sonicated, vortexed, and added to a white round bottom plate. Study samples were diluted using assay buffer. Calibration standards, QCs, and study samples were added to the appropriate wells. Study samples containing Sprague Dawley rat serum or NHP Serum had assay buffer added to the wells, whereas calibration standards and QCs without any Sprague Dawley rat serum or NHP serum had the kit's serum matrix added to the wells. The plate was sealed with an aluminum plate seal and stored overnight in a refrigerator set to maintain 4ºC, shaking on a plate shaker. The next morning, the plate was washed using 1x wash solution on a magnetic plate two times. The detection antibody solution was added to the wells, the plate sealed with an aluminum plate seal, and incubated for one hour with shaking. After an hour, the SAPE was added to each well, the plate was sealed with an aluminum plate seal, and incubated with shaking for 30 minutes. The plate was then washed two times using 1x wash solution. The magnetic beads were resuspended in sheath fluid and analyzed on the BioPlex 200 analyzer.

MSD U-plex 10-plex Kit for NHP (Cat. # K15068L-4):
Samples were thawed and diluted by a factor of 10, or as appropriate, in diluent 43. Standards, study samples, and quality controls (QCs) were added in duplicate to a microtiter plate containing pre-coated antibodies, then incubated for one hour while shaking. After a wash step, the SULFO TAG detection antibody solution was added to each well, and the plate was incubated for one hour while shaking. After a wash step, Read Buffer B was added to each well, and the plate was read in the MSD SQ 120 instrument.

Table 3. Analytical range for common analytes across the three kits

	MSD NHP		Millipore NHP		Millipore Rat	
Analyte	ULOQ, pg/mL	LLOQ, pg/mL	ULOQ, pg/mL	LLOQ, pg/mL	ULOQ, pg/mL	LLOQ, pg/mL
IFN-γ	248000	34.02	2500	12	15000	69
IL-1β	4260	5.84	2500	12	2500	12
IL-2	2050	2.81	2500	12	12500	58
IL-4	1900	2.61	5000	23	5000	23
IL-6	2040	2.80	2500	12	7500	347
IL-10	3990	5.47	12500	58	7500	35
MCP-1	5450	7.48	2500	5.8	30000	139
TNF-α	3680	5.05	2500	12	2500	5.8

MESO QuickPlex SQ 120:
The QuickPlex instrument offers affordable access to high-performance electrochemiluminescence immunoassays. This compact system has been engineered for reliability, ease of use, and low cost of ownership. The combination of rapid read times and the ability to perform multiple, simultaneous tests on a single sample increases productivity, saves sample, and delivers results quickly. The QuickPlex instrument has a wide menu of commercially available assay kits and a full line of components and reagents for developing your own assays.

Figure 1. MSD SQ 120

https://www.mesoscale.com/en/products_and_services/instrumentation/quickplex_sq_120

Bio-Plex Series 200 System:
The Bio-Plex 200 system is a suspension array system which offers protein and nucleic acid researchers a reliable multiplex assay solution that permits analysis of up to 100 biomolecules in a single sample.

Figure 2. Bio-Plex 200

https://www.bio-rad.com/en-us/product/bio-plex-200-systems?ID=715b85f1-6a4e-41b3-b5d9-80202d779e13

Results for Drug A

Here we compare the cytokine levels in NHPs (5mg/kg, highest dose) and rats (15 mg/kg, highest dose) dosed with Drug A, analyzed with Millipore NHP and rat kits, respectively.

For this drug, six of the eight common analytes (IFN-γ, IL-1β, IL-2, IL-4, IL-10, and TNF-α) were BLQ in most of the NHPs despite similar or lower sensitivity in the Mililipore NHP kits (Table 1), whereas rats had detectible levels of most cytokines, even in the lowest dosing group.

With regards to sensitivity, the Millipore NHP kit had a lower limit of quantitation of 12 pg/mL for MCP-1 when compared to the Millipore rat kit, which had a lower limit of quantitation of (139 pg/Ml, Table 1). For IL-6, the Millipore NHP kit had a lower limit of quantitation of 12 pg/mL compared to the Millipore rat kit, which had a lower limit of quantitation of 347 pg/mL (Table 1).

For both MCP-1 and IL-6 in NHPs, levels of both analytes peaked on Day 1, -8 hr, and trended downwards by Day 2, 24 hr. This peak was not observed in rats, as the collection was not performed on Day 1, 8 hr. Therefore, this dataset highlights the importance of timing when it comes to monitoring MCP-1 and IL-6 responses.

Furthermore, for both analytes in NHPs and rats, it was noted that control also had increases post dosing on Day 1, 2hr and Day 22, 2hr. This observation highlights the importance of including control animals in study design, and stresses the need for incorporating control animals when interpretating TA-related changes.

Table 4. Fold change of MCP-1 and IL-6 in NHPs and rats dosed with Drug A relative to baseline

Timepoints	Baseline	Day 1, 2 hr	Day 1, 8 hr	Day 2, 24 hr	Day 22, 2 hr	Day 23, 24 hr
NHP, MCP-1	1.00	1.90	4.24	1.85	2.32	1.99
Rat, MCP-1	1.00	1.17	-	2.00	5.42	6.14
NHP, IL-6	1.00	3.44	23.20	1.00	3.68	1.00
Rat, IL-6	1.00	1.28	-	1.89	1.56	0.89
NHP Control, MCP-1	1.00	1.71	2.58	1.72	1.63	1.55
Rat Control, MCP-1	1.00	1.22	-	1.05	1.11	1.03
NHP Control, IL-6	1.00	4.66	1.65	1.69	1.00	1.00
Rat Control, IL-6	1.00	3.96	-	2.92	5.41	4.30

Results for Drug B

For Drug B, most of the common analytes (IFN-γ, IL-1β, IL-2, IL-4, IL-10, and TNF-α) were BLQ for the NHPs (as seen with Drug A), whereas the rats had detectible levels of most cytokines, even in the lowest dosing group. For this drug, dosing Groups 2-4 for each species received the same amount of Drug B: Group 2 received 5 mg/kg; Group 3 received 30 mg/kg, and Group 4 received 100 mg/kg.
For MCP-1, both species had animals with elevated responses over the course of dosing, with the rats having on average nine times more MCP-1 during acclimation. As with Drug A, the concentration of MCP-1 in rats was higher, but the fold change across the species was greater in the NHPs than in the rats. For this analyte, the MSD U-plex 10-plex kit had a lower limit of quantitation of 7.48 pg/mL compared to the Millipore rat kit, which had a lower limit of quantitation of 139 pg/mL.

For IL-6, more variation across timepoints was seen in the rats compared to the NHPs, as seen with Drug A. However, greater fold changes were seen with the NHPs when samples had detectable concentrations compared to the rat samples during similar timepoints. MSD U-Plex 10-plex Kit had a lower limit of quantitation of 2.80 pg/mL for IL-6 compared to the Millipore rat kit, which had a lower limit of quantitation of 347 pg/mL.

Table 5. Fold change of MCP-1 and IL-6 in NHPs and rats dosed with Drug B relative to baseline

Fold Change Table, Animals dosed with 100 mg/kg of Drug B, mean of group			
Timepoints, NHP	Day 1, Pre	Day 1, 1 hr	Day 29, 8 hr
Timepoints, rats	Day -6	Day 1, 4 hr	Day 29, 4 hr
NHP, MCP-1	1.00	10.86	5.52
Rat, MCP-1	1.00	1.72	3.26
NHP, IL-6	1.00	8.92	1.00
Rat, IL-6	1.00	3.88	6.49
NHP Control, MCP-1	1.00	1.79	1.93
Rat Control, MCP-1	1.00	1.24	1.40
NHP Control, IL-6	1.00	7.00	1.00
Rat Control, IL-6	1.00	5.68	8.17

Conclusions

When comparing data across both species receiving the same drug, it appears that rats tend to produce higher levels of cytokines compared to NHPs (for these two drugs). However, the NHPs will have greater fold changes across timepoints overall when looking at immune response markers such as MCP-1 and IL-6. Researchers should not be alarmed at the levels of cytokines that the rats are producing, but should focus on the magnitude of the change, as that would indicate a potential cytokine storm.