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Translatability of Non-Human Primate Cytokine Data to In-Life Parameters in Nonclinical Toxicology Studies

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Abstract

Cytokines are important immunoregulatory proteins that have gained focus in safety assessment. Interpreting cytokine data comes with challenges due to the variable nature of their stimuli and responses. Contributing factors to the variability in cytokine expression include species-specific reactions, individual variations, dose-response relationships, and unanticipated immunotoxicity. For these reasons, cytokine measurements should not be used as standalone biomarkers for immunotoxicity assessment. However, in conjunction with additional parameters such as clinical observations, body weight, and clinical pathology data, cytokine interpretation can be used to provide more definitive assessments in nonclinical safety studies. In several case studies, cytokines were evaluated for presence of a dose-response relationship. Multiplex platforms such as Luminex or MSD® were used in determining cytokine levels in non-human primates which included IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12/IL-23p40, MCP-1, IFN-γ, and TNF-α. In several instances, measurable levels of IL-6 or IL-12 correlated with clinical observations of bruising, injury, or abnormal feces which were not necessarily considered test article-related. Elevated TNF- α and IL-6 were detected in animals observed as dehydrated with elevated blood urea nitrogen (BUN), creatine, and decreased electrolytes. In cases of test article-related effects, animals becoming moribund also had elevated TNF-α and IL-6 levels. Therefore, when interpreting variations in cytokine values for assessment of potential toxicity, other measurements such as clinical observations, body weight, and clinical pathology parameters should be considered in addition to the test article-related effects.

Introduction

Cytokines are crucial orchestrators of the host immune response and have gained focus in safety assessment. Interpreting cytokine data comes with challenges due to the variable nature of their stimuli and responses. Therefore, evaluating cytokine measurements in conjunction with additional parameters such as clinical observations, and clinical pathology data, can be used to provide more definitive assessments in nonclinical safety studies. The purpose of this exploratory/pilot analysis was to demonstrate the value of assessing cytokines during drug development and to facilitate the interpretation of the data by examining other study parameters such as clinical observations, and clinical pathology measurements. The data for 13 animals were examined in this exploratory analysis. Individual animals were selected and represented in figures 1a-d.

Test Systems

- Cynomolgus monkeys - Chinese and Cambodian origin
- Naïve and non-naïve status
- 2-4 years old; 2-7 kg body weight
- Screening: Dental and physical examination including clinical pathology
- Treatments: Dewormed, vaccinated with MMR and Attenuvax
- Environmental Conditions: Group housed

Clinical Chemistry Parameter	Indication/Function
C-Reactive Protein (CRP)	Inflammation
Blood Urea Nitrogen (BUN)	Kidney
Alanine Aminotransferase (ALT)	Liver
Aspartate Aminotransferase (AST)	Liver
Neutrophil Count (PMN Count)	Immune cells
Creatinine (CRN)	Kidney

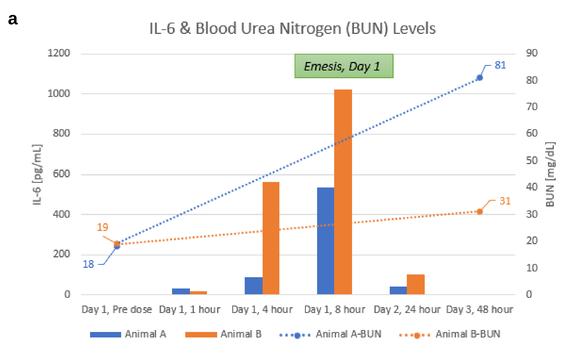
Table 1. Clinical pathology parameters and their indication and/or function. Commonly analyzed clinical chemistry parameters and their respective indications and/or functions.

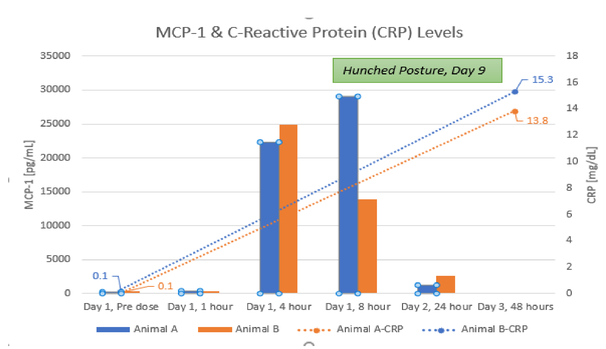
Cytokine/Chemokine	Function
IFN-γ	Pathogen Recognition/Anti-viral
IL-6	Pro-Inflammatory
TNF-α	Pro-Inflammatory
IL-2	Proliferation of T & B cells
IL-10	Anti-Inflammatory
IL-8	Attract Neutrophils
MCP-1	Attract Monocytes

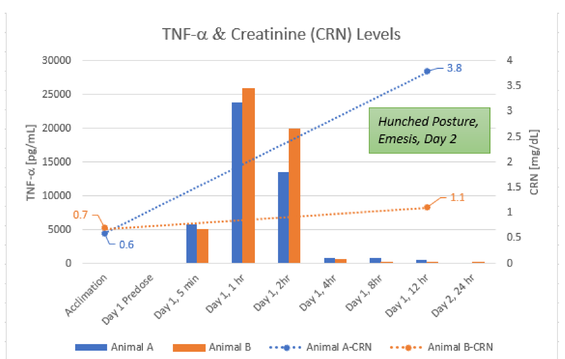
Table 2. Cytokine and chemokine function. Commonly analyzed cytokines and chemokines and their respective functions.

Analyte	Avg Difference from Predose [pg/mL]	Clinical Pathology	Avg Difference from Predose	Clinical Observation	n =
IL-6*	1374.13	CRP	33.1 [mg/dL]	Emesis, Hunched Posture	3
MCP-1*	14818.30	BUN	23 [mg/dL]	-	
IFN-γ	233.4	PMN Count	5.29 [10^3/mL]	-	
IL-6	809.945	ALT	126.5 (Units/L]	Emesis, Inapetent	2
MCP-1	16059.01				
IFN-γ	166.3				
IL-6	6340	AST	660 [Units/L]	Petechial Bruising, Abnormal Color on Chest	6
MCP-1	32272	ALT	236 [Units/L]		
		PMN Count	5.91 [10^3/mL]		
TNF-α**	24845	CRN	3.2 [mg/dL]	Hunched Posture, Moribund	2
IL-6**	36580.5	BUN	169 [mg/dL]		

Table 3. Cytokine and chemokine changes and the associated clinical chemistry changes relative to pre dose levels, as well as the clinical observations for the 13 animals.*Represented in figure 1a, and 1b respectively.**Represented in figure 1c, and 1d, respectively.







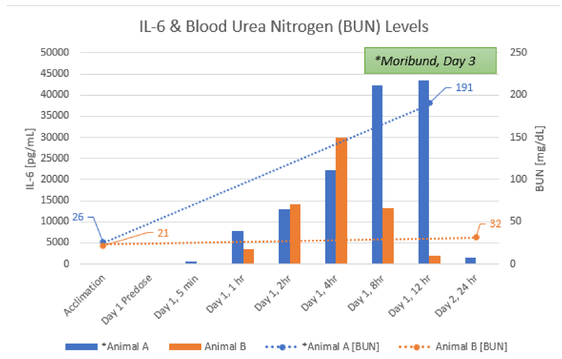


Figure 1a-d. Cytokine and chemokine changes and the associated clinical chemistry changes, as well as the in-life observations. Graphs show changes in measured analytes over different time points for the selected animals. Bars represent cytokine and chemokine elevations which are transient and return to predose levels after day 2. Dotted lines represent clinical chemistry parameters measured over time (two data points). In-life observations for the animals are described within the graph in a green textbox.

Serum Cytokine and Blood Chemistry Analytical Method

Serum Collection: Whole blood was collected in serum separator tubes and then divided into aliquots for analysis.

Luminex Method: Cynomolgus serum cytokines and chemokines were analyzed using the Invitrogen Cytokine Magnetic 29-Plex Panel Kit (Product Number: LPC0005M). Data was acquired using the BioRad BioPlex 200 Instrument and BioPlex Manager Software.

MSD Method: Cynomolgus serum cytokines and chemokines were analyzed using an electrochemiluminescence (ECL) assay by Meso Scale Discovery (MSD). Data was acquired using the MSD Sector™ Imager and WATSON LIMS™ for processing.

Blood Chemistry: Blood was collected for serum chemistry analysis using an AU680 chemistry analyzer.

Results

Data for 13 animals were analyzed and represented in Table 3. Individual animals were selected and illustrated in figures 1a-d, to show changes in the measured analytes over time. Cytokine and chemokine increases started one hour after dosing and peaked at eight hours. The increases were transient and returned to predose levels within 48 hours after dosing. Dosed animals showed increases in IL-6 and MCP-1 and corresponded with blood chemistry parameters: C-Reactive Protein (CRP), and BUN (Blood Urea Nitrogen) levels (Figure 1a, b). Moreover, clinical observations such as emesis and hunched posture were observed in the same animals on Day 1. In a second set of animals (Figure 1c, d), a more robust increase in TNF-α and IL-6 was observed. The levels peaked at one hour and eight hours after dosing, respectively. These cytokine increases corresponded with increases in BUN and CRN. Animals displayed hunched posture and emesis on Day 2, one animal was euthanized due to moribundity on Day 3.

Conclusion

The aim of this exploratory analysis was to facilitate the interpretation of cytokine data by examining other study parameters such as clinical pathology data and clinical observations. In general, increased cytokines were associated with changes in ALT, AST, BUN, CRN, CRP, and neutrophil counts for the thirteen animals examined. It is important to note that this was not an exhaustive analysis, but a starting point to aid in the interpretation of safety assessment data in nonclinical drug development.

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