Altasciences, 6605 Merrill Creek Pkwy, Everett, WA 98203, United States
Altasciences.com | contact@altasciences.com

Evaluating Cytokine Data in Nonhuman Primate Safety Assessment Studies: A Correlation to Toxicity Outcomes

Cassandra Johnson, Narine Lalayeva, Julie Forget
Altasciences, Seattle, WA, United States

Abstract

Cytokines are cell-signaling small proteins classified as either pro-inflammatory or anti-inflammatory. With the increasing number of biologics and immune-modulating drugs being developed, the evaluation of cytokine levels as potential biomarkers in safety assessment studies has become more common. However, cytokine evaluation can be challenging due to the short half-life and variable nature of the stimuli, thus establishing a clear correlation to toxicity responses between animals within the same dose group. The toxicity responses in clinical observations, body weights, clinical pathology, and anatomic pathology findings also often show high variability between animals within the same dose group. Previous studies were reviewed to determine whether animals with a larger magnitude of elevation and/or sustained elevation of inflammatory cytokines had worse prognoses when compared to the remaining animals within the same dose group. Below are examples from three studies, each evaluating a different compound. In one study, a single high-dose animal, with the highest circulating levels of IL-6 and MCP-1 (following Day 29 dose), was euthanized moribund on Day 31. In another study, the animal within a dose group with prolonged elevation of the inflammatory cytokines MCP-1, IL-6, and IFN-γ had altered clinical pathology parameters, decreased body weight, and/or abnormal clinical observations as compared to the other animals within the dose group. For example, a single animal within a dose group had a transient increase in BUN and creatinine three days after a persistent elevation of these inflammatory cytokines. In some studies, it can be difficult to correlate cytokine levels with other parameters when multiple clinical conditions are observed within the same dose group. For example, in a third study that was reviewed, clinical observations of excessive scratching, decreased activity, unresponsiveness, inappetence, and shivering were noted in the high dose group. Additionally, a single animal from this group had microscopic lesions in the kidney, lung, bone marrow, and spleen, and the highest circulating levels of the inflammatory cytokine IL-6, but not MCP-1. To conclude, the evaluation of cytokine data should be performed in the context of other findings within a study. The correlation of findings will likely depend on the pharmacological action of the test article, the cytokines being evaluated, and the timing of cytokine evaluation in relation to dosing.

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, likely because these molecules are produced transiently. 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 data for three toxicology studies were reviewed to determine whether animals with a larger magnitude of elevation and/or sustained elevation of inflammatory cytokines had worse prognoses when compared to the remaining animals within the same dose group.

Materials and Methods

Animals and Animal Care

Test System: *Macaca fascicularis*, male and female

Source: Cambodia

Approval for Research: All animal-related procedures were approved by the IACUC

Environmental conditions: Primary enclosure complied with the Animal Welfare Act and recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). Animals were housed in a temperature- and humidity-controlled environment with target ranges between 18 and 29 degrees Celsius, and 30 and 70%, respectively. A 12-hour light/dark cycle was set, and animals were kept in stainless steel metal cages.

Diet: PMI LabDiet® Fiber-Plus® Monkey Diet 5049 biscuits, and water was provided *ad libitum*. Treats were provided daily and included fresh produce, marshmallows, raisins, juice, etc.

Blood Collection

Blood was collected from awake, restrained animals into serum separator tubes (SST). Samples were processed to serum and analyzed.

Results

Study #1

Sex: Male
Origin: Cambodia
Age: 2.2 to 3.5 years
Body Weight: 2.0 to 2.6 kg

Compound Type: Fc fusion protein
Dose Route: IV infusion
Dose Frequency: Once weekly
Cytokine Assay: Meso scale diagnostic (MSD) U-PLEX

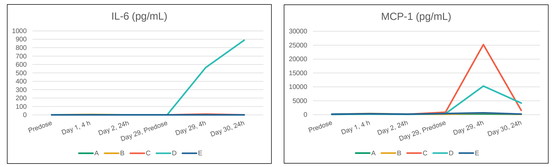

Figure 1. Circulating cytokine levels

Table 1. Summary of animal findings

Animal	Clinical Observations	Clinical Pathology	Summary of Microscopic Findings
A	-	-	Esophagus, kidney, liver, spleen
B	Swollen forelimb (Day 8)	-	spleen
C	Emesis (Day 15)	Decreased red cell mass – suggestive of iron deficiency, more pronounced decrease in albumin, more pronounced increase in cholesterol	Kidney, liver, spleen, thymus
D	Emesis (Days 22 and 29) Swollen forelimb (Day 29) Tremors (Day 30) Decreased activity (Day 30) Decreased body temperature (Day 30)	Kidney dysfunction, albumin and blood loss, inflammation, coagulopathy	Consistent with immune complex formation
E	Swollen forelimb (Day 29)	-	-

Study #2

Sex: Female
Origin: Chinese
Age: 2.7 to 3.6 years
Body weight: 2.4 to 2.9 kg

Compound type: Bispecific antibody
Dose route: IV bolus
Dose frequency: Once weekly (Days 1, 8, and 15)
Cytokine assay: Magnetic 29-plex panel for Luminex platform

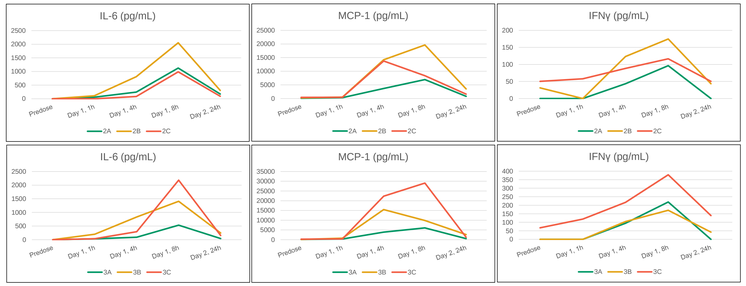

Figure 2. Circulating cytokine levels

Table 2. Summary of animal findings

Animal	Clinical Observations	Body Weight	Clinical Pathology
2A	Emesis (Day 2) Inappetence (Day 3)	-	-
2B	Emesis (Day 2) Inappetence (Days 3 and 15) Shaking (Days 4 and 5) Prostrate (Day 15) Decreased body temperature (Day 15)	Loss	Declining albumin Increased creatine kinase
2C	Emesis (Day 1 and 15) Decreased body temperature (Day 15)	-	-
3A	Emesis (Day 1) Decreased body temperature (Day 2)	-	-
3B	Emesis (Day 1) Hunched (Day 9)	-	-
3C	Emesis (Day 1) Decreased body temperature (Day 2)	Loss	Transient increase in BUN and creatinine on day 3

Study #3

Sex: Male
Origin: Cambodia
Age: 1.8 to 2.6 years
Body weight: 1.6 to 2.1 kg

Compound type: Enzyme Fc fusion protein
Dose route: IV Infusion
Dose frequency: Once weekly
Cytokine assay: MSD U-PLEX multiplex assay platform

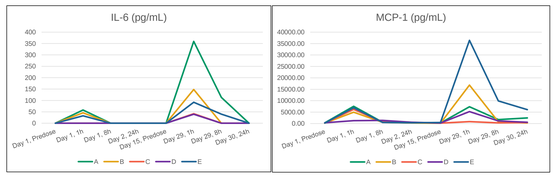

Figure 3. Circulating cytokine levels

Table 3. Summary of animal findings

Animal	Clinical Observations	Clinical Pathology	Tissues with Microscopic Findings
A	Decreased activity (Days 30 and 31)	Additional erythroid and leukocyte changes	Kidney, lung, bone marrow, eye, spleen
B	-	-	-
C	Decreased activity (Days 15 and 16) Cold to touch (Day 15)	-	-
D	Swollen forelimb (Day 15)	-	
E	Swollen forelimb (Day 2) Excessive scratching (Day 8)	-	-

Summary

Study #1: After the last dose administration, Animal D had the highest induction of circulating IL-6 levels and the second highest MCP-1 levels. This animal was euthanized moribund on Day 31 because of kidney dysfunction. Animal C had the highest circulating levels of MCP-1 and was the only animal with clinical pathology changes suggestive of iron deficiency.

Study #2: After dosing on Day 1, Animals 2B and 3C had the highest levels of IL-6, MCP-1 and IFNγ within their respective dose groups. Within each animals respective dose group, each animal was the only animal to lose weight through the duration of the study and have a worsened clinical prognosis.

Study #3: After the last dose administration, Animal A had the highest induction of circulating IL6 levels. Additionally, this animal had clinical pathology and microscopic findings that were not observed in the other animals of this dose group. Animal E had the highest levels of circulating MCP-1 levels, and excessive scratching was noted on Day 8.

Conclusions

The correlation of findings will likely depend on the pharmacological action of the test article, the cytokines being evaluated, and the timing of cytokine evaluation in relation to dosing.

Future Directions

Develop a scoring system for in-life findings to determine whether certain findings correlate with specific elevations in cytokine levels.