Cytokines as Biomarkers of Immunotoxicity in Preclinical Safety Assessment: Navigating “Context of Use”

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EBF Autumn Focus Workshop, September 2020

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Agenda

1. Cytokines in Preclinical Development – Integration!
2. EBF 5 Pillars
3. The Challenges of Cytokines as Biomarkers
4. Analysis Platforms and Divergence
5. A Case Study: Establishing “Normal” Cytokine Ranges
6. Translating into Strategy

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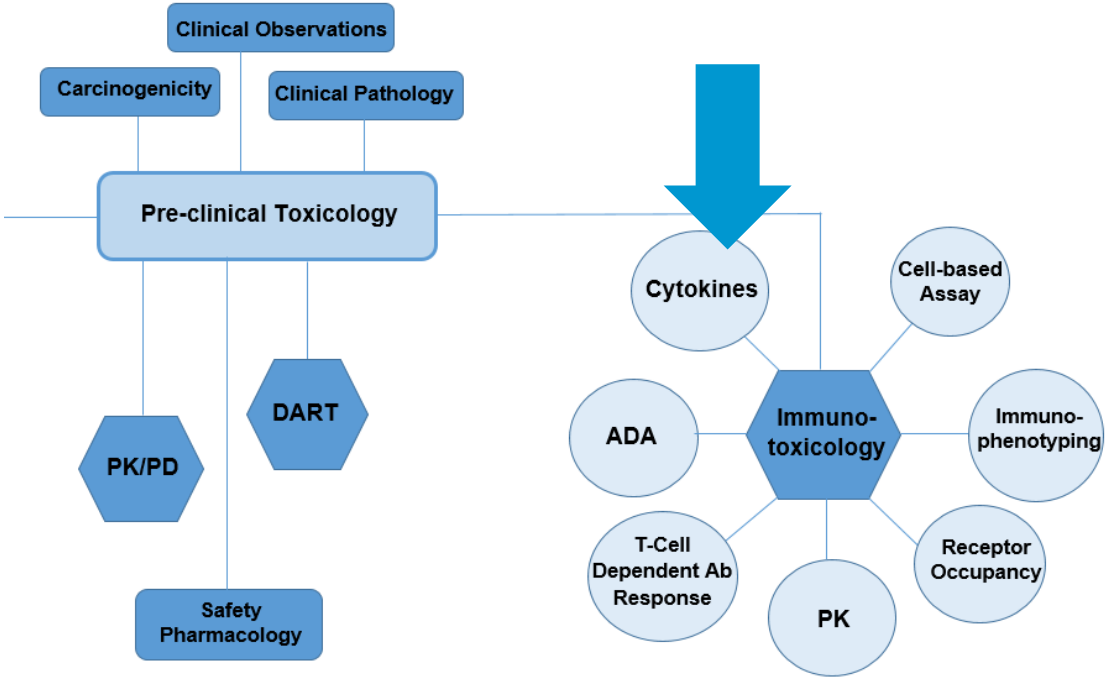
Cytokine Analysis in Preclinical Drug Development

- Analysis of systemic cytokine levels during the course of preclinical in-vivo toxicology studies
- Used as safety biomarkers to identify test article-related immunotoxicity



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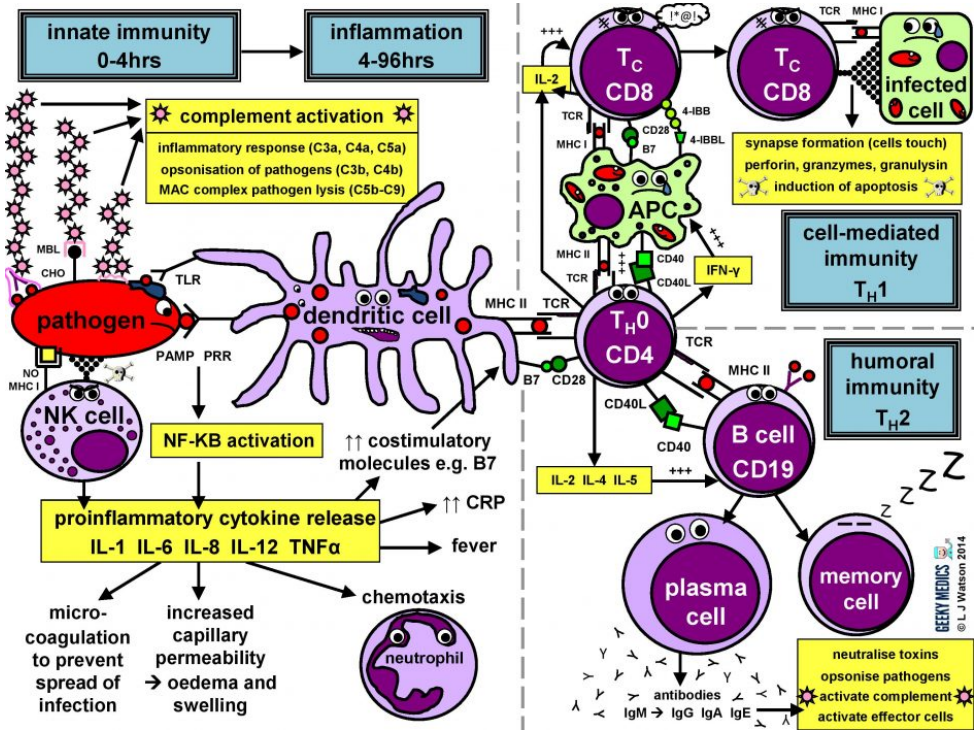
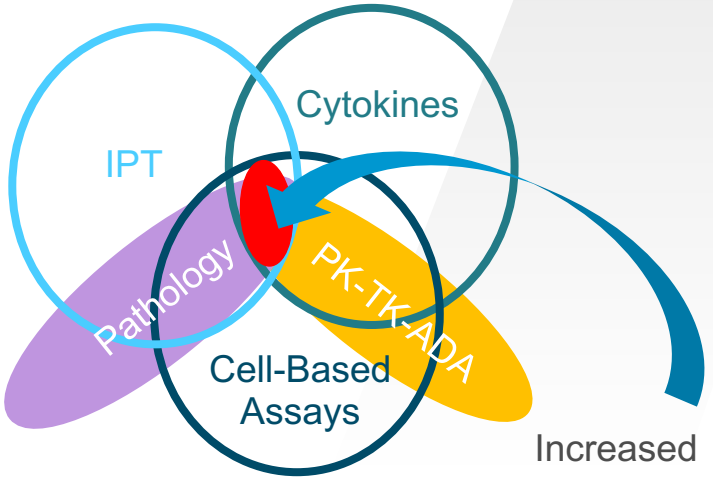
The Power of Integration…

The immune system uses a complex array of inter-linked protective mechanisms





Increased Confidence in Data Interpretation

Source: GeekyMedics.com

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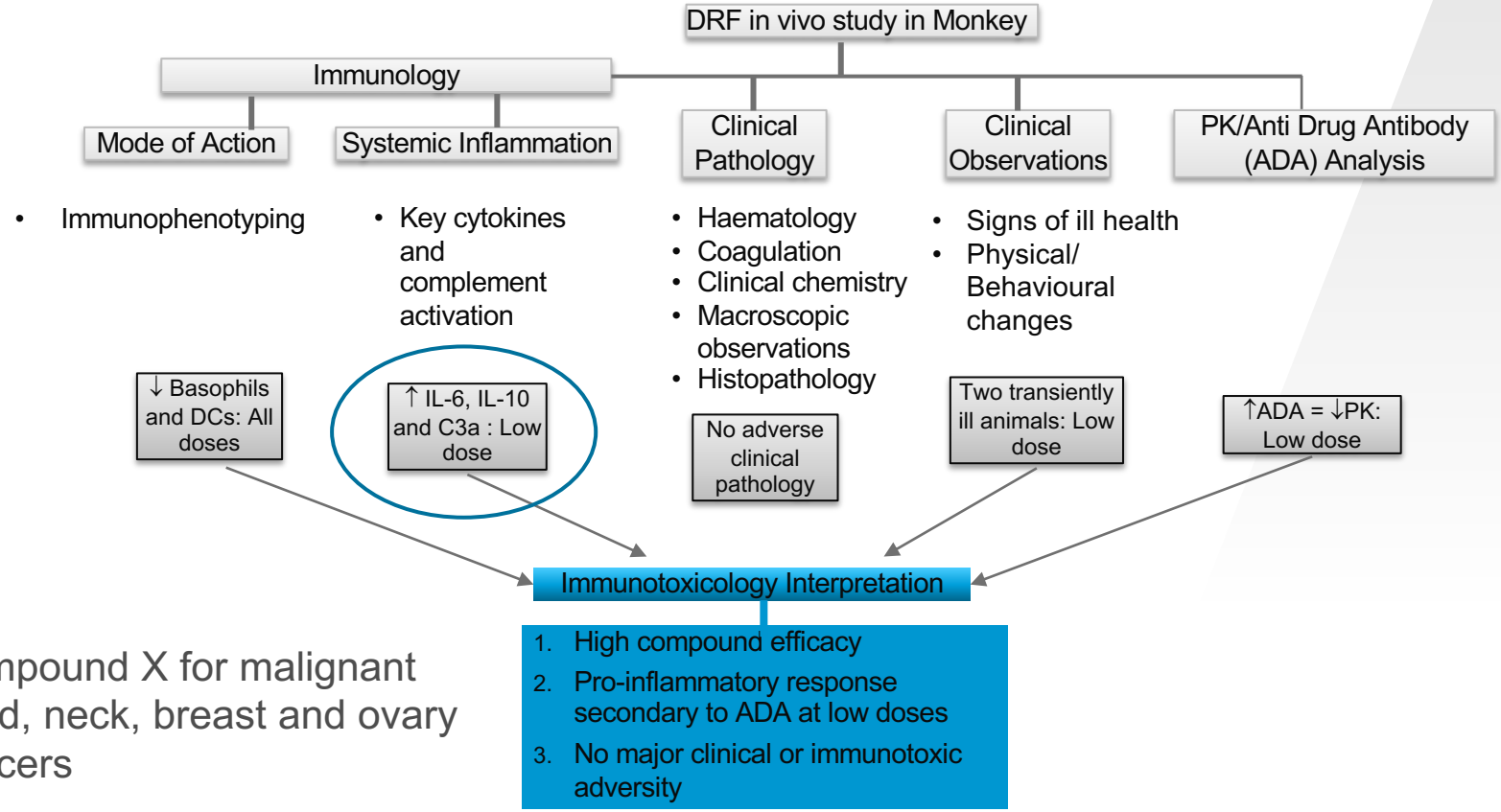
Into Practice…



- Compound X for malignant head, neck, breast and ovary cancers

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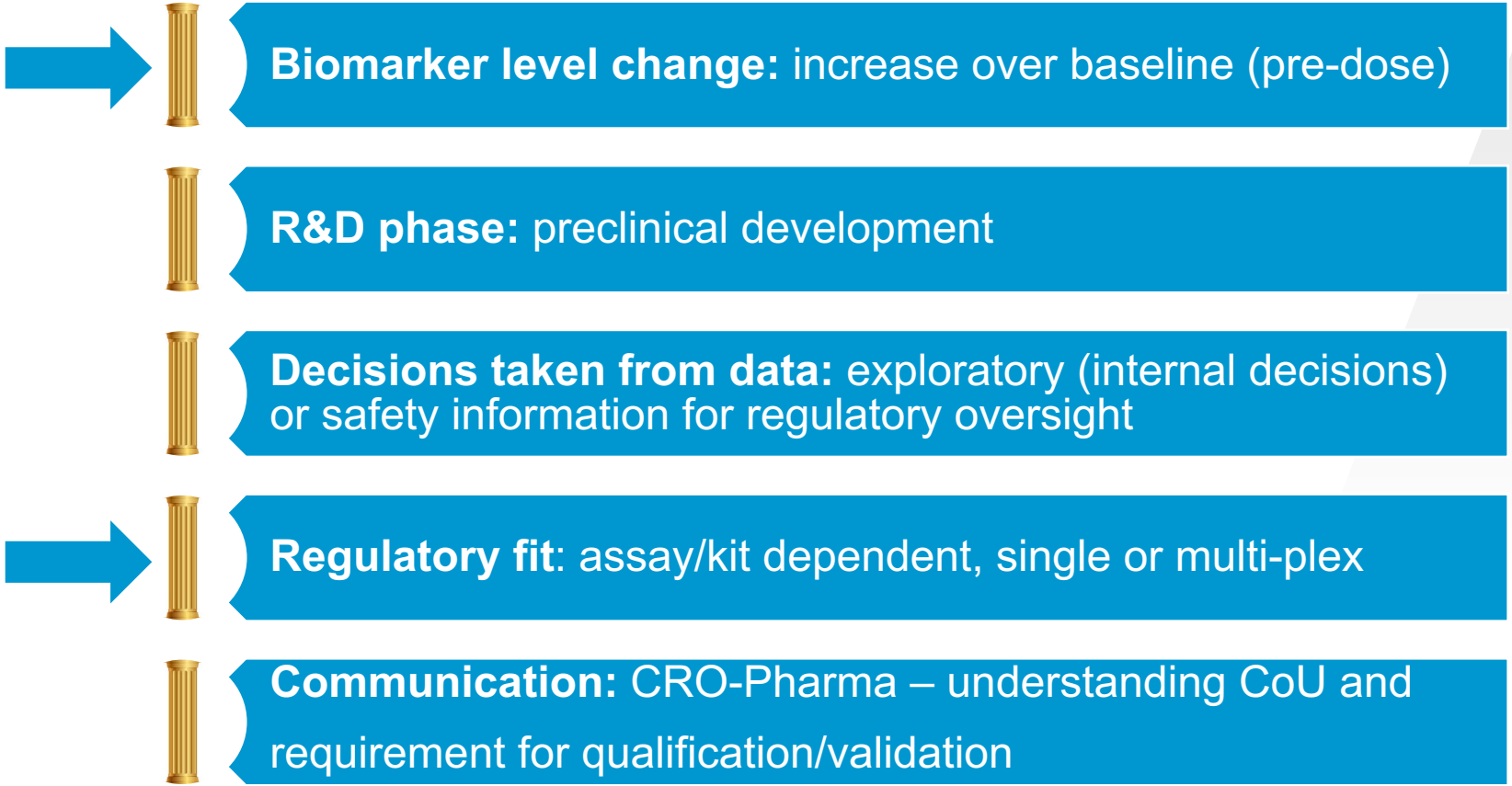
EBF Recommendation – 5 Pillars to Success



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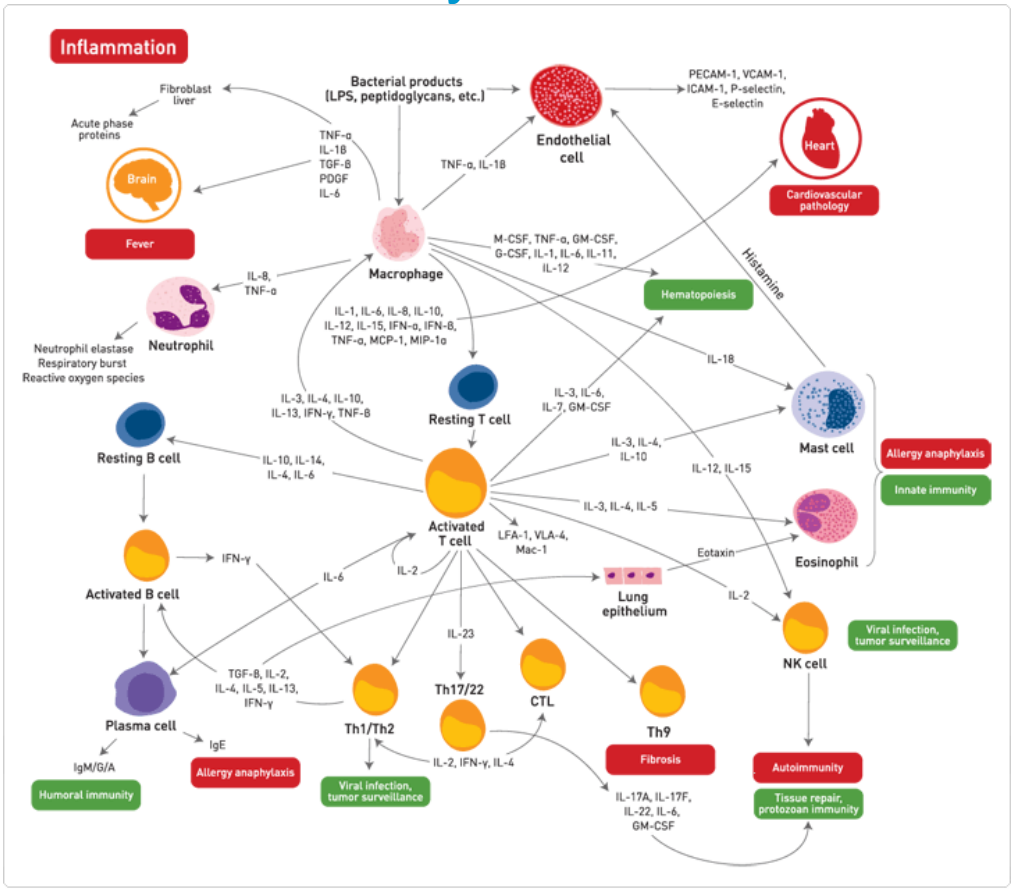
Cytokines as Inflammatory Mediators



Simplified representation of cytokine interactions during inflammation. BioProbes 67, June 2012.

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Exploring Cytokines as Biomarkers

Desirable Characteristics	Cytokines
Readily accessible in body fluids	Pursued because of specimen accessibility in blood
Sufficient in-vivo half-life and adequate in-vitro stability	Short serum half-life, rapid degradation in-vitro
Sensitive, dose-dependent change in response	Low to undetectable baseline levels (>pg/mL), not always dose-dependent
Low inter-animal (biological) variability	Inter-animal variability
Specificity to correlate biomarker level with toxicity	Lack of toxicity-specific expression, sensitive to study conditions
Reduction of biomarker to baseline levels following recovery period	Dependent on cytokine and cause of spike
Comparable biological response between nonclinical species and humans	Subjective translatability of preclinical cytokine data to clinical

Diversity in Context of Use

Biomarker Category	Context of Use	Suggested Validation Principle
Exploratory	General exploratory/research information used for information purposes only (internal decision making)	FFP, no min requirements
Monitoring	Indicate toxicity or assess safety; provide evidence of exposure	PM and/or BMV
Safety	Indicate the presence or extent of toxicity related to an intervention or exposure	PM and/or BMV
Susceptibility/ Risk	Indicate the potential for developing a disease or sensitivity to an exposure	PM and/or BMV

PM = Patient Management
BMV = Bioanalytical Method Validation

Diverse CoU = diverse validation strategy

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Analytical Aspects

- Standard single analyte ELISA or multiplex plate / bead-based
- “Off-the-shelf” assays are still research only
- Endogenous vs. recombinant
- LOD too high for the anticipated LLOQ
- Most **divergence** between assays is likely to occur for cytokines with **low to undetectable** concentrations in the blood of **healthy** animals

MSD® Sector S600

U-Plex

V-Plex

S-Plex

SpectraMax i3x

Multi-mode microplate reader

Bio-Plex® 200

xMAP technology

Ella™, ProteinSimple®
Microfluidic cartridge system

The right platform for the right analyte, CoU and sensitivity

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Case Study – Establishing “Normal” Cytokine Ranges

Objective: To examine the ranges and inter-subject variance of a panel of cytokines in healthy (control) Cynomolgus monkeys

Time period: 2014-2019

CoU: Various but mostly for safety information to support DRF and GLP toxicology

Assay: MILLIPLEX® MAP NHP Cytokine kit Luminex® xMAP® platform

Validation status: Fully validated

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Cytokine Control Data – Males

Cytokines Historical Control Data
Primate/Asian; Sex: Male; Supplier: Multiple; Time interval: 2014-2019

Parameter	N	Mean	Median	SD	Range	
					Lower Limit	Upper Limit
IFNγ	163	25.7	24.4	15.7	24.4	24.4
IL17a	92	2.4	2.4	0.0	2.4	2.4
IL1Bβ	163	2.4	2.4	0.1	2.4	2.4
IL4	121	19.6	19.5	0.8	19.5	19.5
IL-8	163	1817.0	1478.0	1172.5	405.0	4536.0
IL-10	163	58.4	48.8	86.0	48.8	48.8
IL-2	163	24.7	24.4	3.0	24.4	24.4
IL-6	163	14.1	2.4	108.7	2.4	23.6
MCP-1	121	479.0	384.0	301.5	181.0	1442.0
MIP-1α	121	9.8	9.8	0.0	9.8	9.8
MP1-β	163	27.1	24.4	34.7	24.4	24.4
TNFα	163	25.2	24.4	9.9	24.4	24.4

Cytokines Historical Control Data (Males): Ranges of TDAR-regulatory cytokine panel analysed using MILLIPLEX® MAP NHP Cytokine magnetic bead panel on the Luminex® xMAP® platform

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Cytokine Control Data – Females

Cytokines Historical Control Data

Primate/Asian; Sex: Female; Supplier: Multiple; Time interval: 2014-2019

Parameter	N	Mean	Median	SD	Range	
					Lower Limit	Upper Limit
IFNγ	147	24.4	24.4	0.0	24.4	24.4
IL17a	92	2.4	2.4	0.0	2.4	2.4
IL1Bβ	142	2.5	2.4	0.2	2.4	2.4
IL4	121	19.6	19.5	0.4	19.5	19.5
IL-8	142	1625.0	1436.0	996.9	510.0	4307.0
IL-10	142	48.8	48.8	0.1	48.8	48.8
IL-2	142	24.4	24.4	0.0	24.4	24.4
IL-6	142	3.6	2.4	4.1	2.4	13.2
MCP-1	121	225.0	214.0	108.4	63.0	527.0
MIP-1α	121	9.8	9.8	0.0	9.8	9.8
MP1-β	142	24.4	24.4	0.0	24.4	24.4
TNFα	141	24.4	24.4	0.0	24.4	24.4

Cytokines Historical Control Data (Females): Ranges of TDAR-regulatory cytokine panel analysed using MILLIPLEX® MAP NHP Cytokine magnetic bead panel on the Luminex® xMAP® platform

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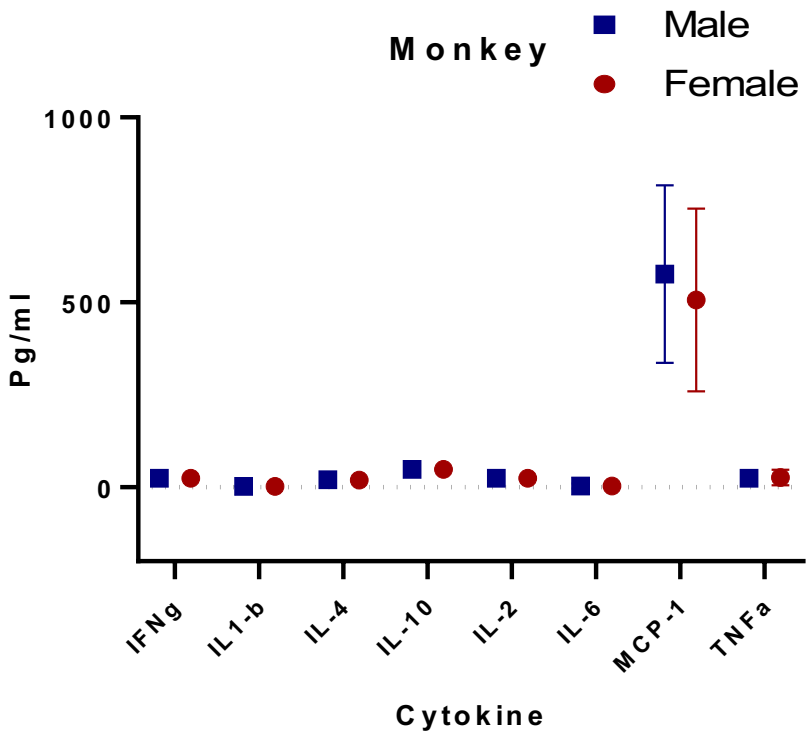
Establishing “Normal” Cytokine Ranges

Analyte	LLOQ (pg/mL)
IFNγ	24.42
IL-1β	2.44
IL-4	19.53
IL-10	48.83
IL-6	2.44
MCP-1	10.00
TNFα	24.42



Graphs are representative of means and SD. P values derived from unpaired, two-tailed t-test, **P=<0.001 and ***P<=0.0001.

Result: As expected, all serum cytokines analysed in control monkeys were generally lower than the LLOQ determined for each analyte or slightly above these thresholds, with the exception of MCP-1.

(IL-8 off the scale)

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Conclusions Drawn

- Industry-wide problem to establish baseline values for cytokines in monkeys
- LLOQ is often taken as baseline from which fold-changes are reported
- LLOQ acceptance criteria (± 30%) accuracy and precision (A&P)
- Is the current assay fit-for-purpose?
  - ✓ For safety context – to pick up spikes
  - ✗ Not for control group or as comparative
- Would we learn anything from detecting lower? – noise scenario

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Translating into Bioanalytical Strategy

Pre-determine assay performance requirements, platform technology & acceptance criteria

- CoU, cytokine biology, sensitivity, expected changes and biological variability

Are current multiplex assays appropriate?

- Sensitivity is a challenge
- Should we abandon assays that would otherwise be informative? – define utility & limits
- How is best to report the data?

Do we need alternative approaches?

- Reference range for baseline
- Titre-based approach (quasi-quantitative)
- Single-plex = more targeted

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