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Translating Science to Medicine

The chemokine MCP-1/CCL2 is a key biomarker for respiratory diseases and lung tissue harm

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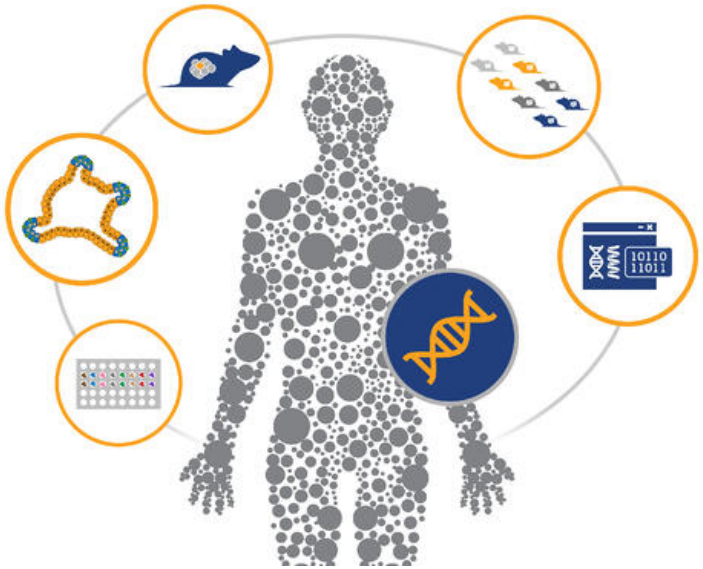
Biomarkers: Introduction

- What are Biomarkers?

A Biomarker is "*a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to an exposure or intervention*"

The FDA-NIH Biomarker Working Group



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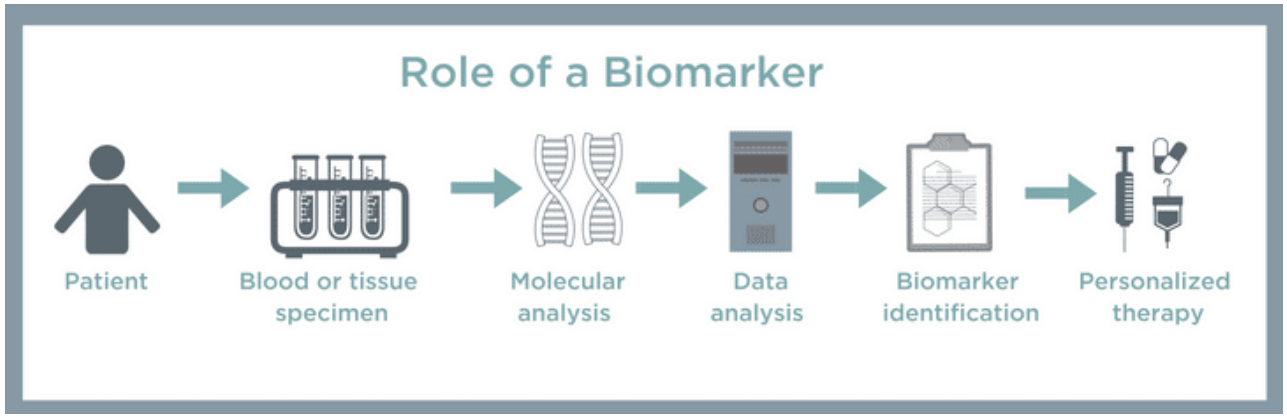
Biomarkers: Introduction

- Biomarkers can play a critical role in personalized medicine as an indicator of biological processes.



- Biomarkers become increasingly important therapeutic readouts as primary or secondary study end points.

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Key considerations for biomarker assays

- **Characterization of a Biomarker/Key Considerations are:**
  - Context of Use"
  - FFP - "Fit For Purpose"
- The **Context of Use (CoU)** for a Biomarker is defined by the FDA as "the circumstances under which the drug development tool is to be used in drug development and regulatory review"

The CoU is critically important to analysis and dictates the level of rigor required when developing and validating a method.

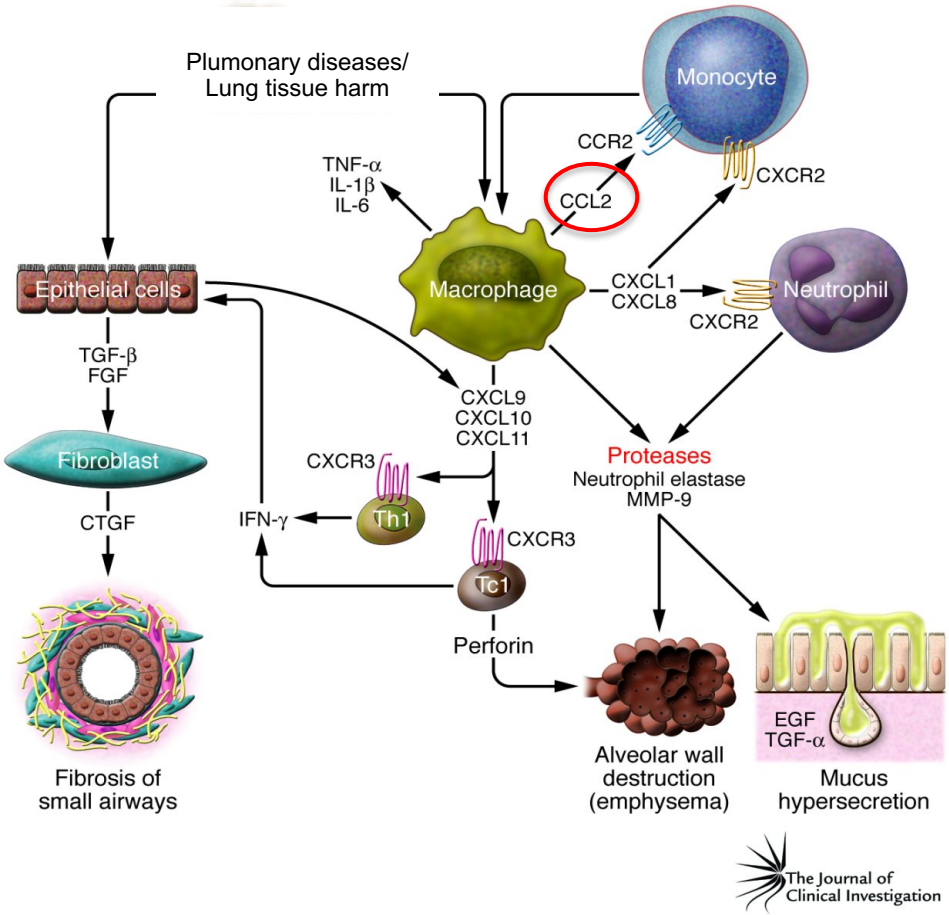
- The **Fit-For-Purpose (FFP)** concept states that the level of validation should be appropriate for the intended purpose of the study.

U.S. FDA, CDER, CVM, Bioanalytical Method Validation, Guidance for Industry, 2018.

Tiered approach to development

- Depending on the COU of the biomarker, sponsor requirements, and the availability of reference items and matrices, a tiered approach to assay development can be taken:
  - Tier 1 – Screening assay example
    - Qualitative method: Use of a non-certified reference item as well as surrogate matrices.
  - Tier 2 – Qualified assay example
    - Semi-quantitative method: Early screening and evaluation of the mechanism of action of a drug candidate or use of biomarker as a diagnostic tool to determine the disease state of an individual.
  - Tier 3 – Fully validated assay example
    - Quantitative method: Support claims for the safety/efficacy of dosing in a pivotal preclinical or clinical study, new drug application.

Respiratory Diseases and Lung Tissue Harm: Biomarkers



- Biomarkers for Respiratory Diseases often fall in the category of the Inflammatory Biomarkers:
- Inflammatory cytokines and chemokines:
  - IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-8,
  - EGF,
  - GM-CSF,
  - TNF-a,
  - ICAM-1,
  - MCP-1/CCL2
  - Endothelin,
  - Eotaxin,
  - IFN-γ

Used appropriately, biomarkers improve the assessment of respiratory tract infections and sepsis.

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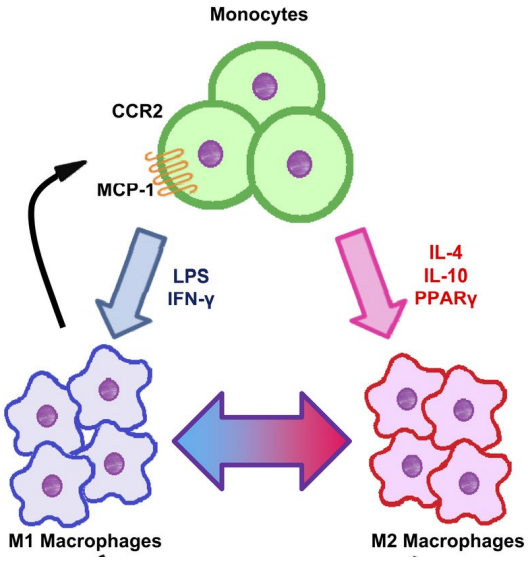
Biomarkers for Respiratory Diseases and Lung Tissue Harm : MCP-1/CCL2

- **Monocyte chemoattractant protein-1 (MCP-1/CCL2)** is one of the key chemokines regulating infiltration of monocytes/macrophages in lung tissue thereby progressing inflammation.

- As a consequence a CCL2 key factor of lung diseases and lung tissue harm for chronic diseases like COPD but also after exposure to cigarette smoke.

- Close monitoring of CCL2 in patients suffering from lung disease or damage is key for adequate treatment. To this end a robust biomarker assay for measuring CCL2 is mandatory.



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Development of a bioanalytical assay to assess the key respiratory biomarker/ biomarker of lung tissue harm MCP-1

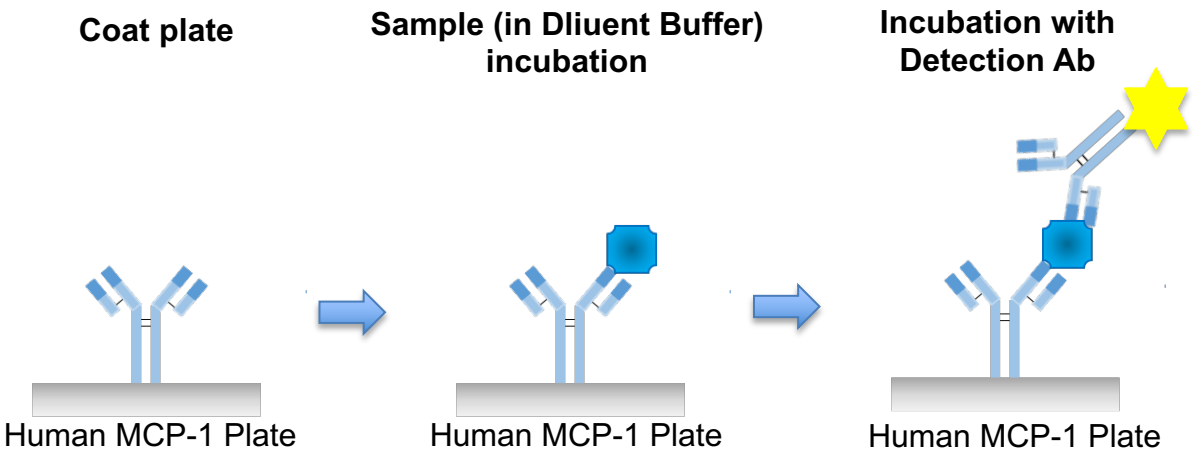
Qualification/Validation parameters

1. Calibration curve
2. Precision & Accuracy (P&A)
3. Selectivity
4. Parallelism
5. Stability

Analyte	MCP-1
Endogenous	Yes
Species	Human
Matrix	Serum
Assay Format	MSD
Minimum Required Dilution	4
Quantitation range	40 – 1500 pg/mL

Assay principle: determination of total MCP-1 in human serum using MSD technology

V-PLEX human MCP-1 kit



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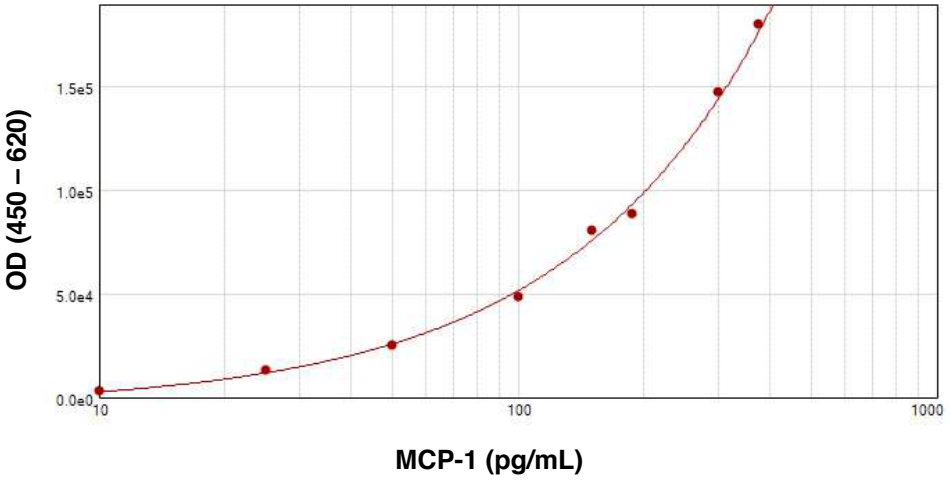
1: Calibration curve



Regression model	4PL
Weighting factor	1/y2
Unit	pg/mL

- The quantitation range of the assay is 40 – 1500 pg/mL .
- Precision and accuracy has been confirmed at Quality Control levels.

2: P&A

	QC LLOQ	QC Low	QC Med	QC High	QC ULOQ
Nominal [pg/ml]	**58.8**	**139.3**	**186.3**	**919.9**	**1500**
Mean Observed Conc. [pg/ml]	58.8	139.3	186.3	919.9	1412.9
Nominal [%]	100.0	100.0	100.0	100.0	94.2
Number of Runs	3	3	3	3	3
Number of replicates	6	6	6	6	6
Between Run Precision (%CV)	**5.2**	**4.8**	**4.0**	**2.2**	**2.5**
Within Run Precision (%CV)	**1.7**	**2.0**	**1.8**	**2.1**	**2.5**
Total Variation (%CV)	**5.5**	**5.2**	**4.4**	**3.0**	**3.5**

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3: Selectivity

	Blank (pg/ml)	Low Spike [MCP-1] (pg/ml)	Nom %	High Spike [MCP-1] (pg/ml)	Nom %
IND. 1	156	294	96.1	425	93.2
IND. 2	176	308	94.7	453	95.3
IND. 3	182	315	94.9	455	94.3
IND. 4	188	312	92.2	429	87.8
IND. 5	160	302	97.5	431	93.8
IND. 6	175	309	95.3	445	93.7
IND. 7	179	309	94.0	436	91.0
IND. 8	187	319	94.8	465	95.5
IND. 9	159	294	95.2	410	89.4
IND. 10	153	288	94.8	411	90.7
Pool	138	267	92.9	390	89.2
mean	171		95.0		92.5
SD	13.2		1.4		2.6
CV [%]	7.7		1.4		2.8
n	10	10	10	10	10

Low spike: Individuals spiked with 150 pg/mL,

High spike: Individuals spiked with 300 pg/mL

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Parallelism and Stability

- Parallelism – the ability to dilute a sample without introducing bias
- Sample Stability – specifically the stability of the endogenous compound.

4. Parallelism

	IND. 1	IND.2	IND.3	IND.4
MCP-1 (pg/mL)	152	163	171	194

	% Recovery			
	IND. 1	**IND.2**	**IND.3**	**IND.4**
DF2	111	104	110	112
DF4	BLOQ	100	94.7	108
DF8	BLOQ	BLOQ	BLOQ	BLOQ

Parallelism was evaluated by diluting four individual samples with buffer. Four dilutions were prepared. Concentrations within the analytical range were considered. They are presented here are % of recovery considering the first concentration in range as 100%.

5. Stability

	LLOQ 58.8	QC High 920
BenchTop, undiluted 3 hours	3/3 acceptable	3/3 acceptable
Freeze / Thaw 3 cycles	3/3 acceptable	3/3 acceptable
Freeze / Thaw 6 cycles		3/3 acceptable

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Conclusions

- The here presented data, summarizes key development steps in order to establish a robust biomarker assay for the assessment of CCL2 from patients suffering from different lung diseases and other sources of lung tissue harm.

- This essay allows for appropriate patient monitoring and taking key treatment decisions.

Questions

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