



Rational testing of immune system biomarkers in drug development

FOCUS Immunology provides immunological consulting and laboratory services under GLP conditions for the development of novel medicines.

FOCUS Immunology offers distinct “testing packages”, which are purposefully set to address the major biomarkers of immune system function to characterize new drugs in development.

These proven testing packages can be applied as they are or they can provide a basis for the rational planning of a given project. Each testing protocol can be tailored to the specific needs of a given project.

For ease of use FOCUS Immunology has defined and grouped the following testing packages:

- 1. Specific immune system package**
- 2. Innate immune system package**
- 3. Immune activity screening package
for novel and established compounds**
- 4. Cytokine modulation package for immuno-modulators,
esp. immuno-suppressive compounds**
- 5. Immuno-stimulation package for immune-modulators,
vaccines and oncology drugs**
- 6. Biologicals testing package,
esp. for new biological entities (NBEs)**



1. Specific immune system package

The specific or adaptive immune system comprises T and B lymphocytes. FOCUS Immunology provides assays to analyze markers and activities of these cells.

Cytokine secretion or proliferation is assessed in bulk cultures, or at the single cell level by single cell analysis techniques, like ELISpot or intracellular Cytokine Staining (ICS) by flow cytometry. Proliferation at the level of individual T or B cells single is assessed by using the flow cytometry-based CFSE assay.

Multi-color flow cytometry allows exploring cell-type markers and activation markers in one assay on the single cell level. To this end, FOCUS Immunology operates a dual-laser FACScalibur from BD Biosciences. Up to four different biomarkers, be it intracellular markers or cell surface markers, can be analyzed in parallel.

This single cell approach provides refined information on the effects of test compounds on the functionality of immune cell subpopulations, since the activation status of defined immune cell subpopulations can be dissected and quantified in complex samples.

Prior to analysis the cells may be stimulated polyclonally by using phytohemagglutinin (PHA), phorbol myristate acetate (PMA) + Ionomycin, or stimulatory antibodies, like anti-CD3 plus anti-CD28.

Alternatively the cells may be stimulated in an antigen-specific manner by using CMV (Cytomegalovirus) antigens, Tetanus Toxoid or other antigens of choice.

For most of these assays it is possible to select certain cellular populations to start with. This ranges from whole blood, purified peripheral blood mononuclear cells (PBMC), or selected lymphoid subpopulations, such as B lymphocytes, T lymphocytes, CD4+ T lymphocytes, or regulatory T lymphocytes and so on.

2. Innate immune system package

The innate immune system provides immediate defense reactions against invading pathogens. Cells of the innate immune system, such as monocytes/macrophages, NK cells and others do not possess antigen-specific receptors but instead recognize conserved molecules shared by a various microorganisms.

These conserved molecules are called “pathogen-associated molecular patterns” (PAMP) and the corresponding receptors on cells of the innate immune system are called “pattern recognition receptors” (PRRs).

Toll-like receptors (TLRs) are a major group of pattern recognition receptors. TLRs recognize different types of molecules expressed by bacteria, viruses, fungi and

protozoa. For example TLR4 recognizes bacterial endotoxin (LPS), and TLR-9 recognizes bacterial DNA. Ligand binding to cellular TLRs leads to the activation of cellular defense reactions.

FOCUS Immunology has established a complete range of assays to analyse the triggering of TLRs and the subsequent activation of TLR-expressing cells.

Ligands for the 9 well characterized human TLRs (TLR1-TLR9) are used to stimulate peripheral blood mononuclear cells (PBMC) *in vitro* and analyze the secretion of the cytokine TNF-alpha. The assay allows analyzing the interference of any compound with TLR-mediated stimulation of cells of the innate immune system.

A comprehensive *FOCUS Expert Report* entitled "Biomarkers of Innate Immune System functions: Concepts and case studies with a special emphasis on their use in exploratory clinical studies" can be downloaded at www.focus-immunology.de.

3. Immune activity screening package for novel and established compounds

The aim of the immune screening testing package is to explore whether novel or known compounds affect basic immune functions. This may alert early on in development to potential side effects in clinical studies. On the other hand it may pave the way towards definition of new indications.

In the immune screening package FOCUS immunology provides a selection of assays which are purposefully set to address the major biomarkers of immune cell activation, like cell proliferation, cytokine release and expression of activation markers.

For cost efficiency we prefer to use bulk culture conditions the immune activity screening package. Under these conditions we can screen for cytokine secretion via ELISA in supernatants, activation marker expression by flow cytometry, apoptosis induction and proliferation.

4. Cytokine modulation package for immuno-modulators, esp. immuno-suppressive compounds

FOCUS Immunology has implemented *ex vivo* assays in order to detect compound effects on cytokine secretion. To this effect, whole blood cultures or purified blood cells are sub-optimally stimulated to activate both lymphoid and myeloid cells in the absence or presence of test compound. Suboptimal stimulation is used in order to test the compounds in the linear range of the dose-response curve of the stimulant which allows detection of compound-mediated effects.

At termination of the culture, the following model cytokines are determined from the supernatant by ELISA: IL-6 and TNF-alpha as prototype pro-inflammatory cytokines, IL-8 as mediator of chemotaxis, IL-2 as central regulator of immune cell functions, IFN-gamma for its involvement in monocyte/macrophage activation, and IL-10 as the major immune-suppressive cytokine.

The testing package was designed to answer many questions with a reasonable use of resources. It is of course possible to add or leave out any cytokine for a specific project.

It is important to note, that FOCUS Immunology has experience in analyzing many more than the above listed cytokines and is also prepared to establish any biomarker assay for which reagents are available or if the need is dire enough to develop own assay reagents.

5. Immuno-stimulation package for immune-modulators, vaccines and oncology drugs

FOCUS Immunology has established in vivo as well as ex vivo assays to assess the immune-stimulatory capacity of compounds.

The ex vivo models allow to explore stimulatory activities at the level of proliferation, cytokine secretion and expression of activation markers in isolated lymphoid or myeloid cells. Defined cell populations may be purified from peripheral blood and used in the respective assay systems. The read-out systems may be at bulk culture level or single cell level.

FOCUS Immunology's in vivo model for immune-stimulation testing uses a suboptimal immunization schedule in mice. Animals are immunized with selected peptides in the presence or absence of the test-compound. At specified points in time the extent of the ensuing immune response is analyzed. This is done by measuring the frequency of responding antigen-specific cells upon in vitro re-stimulation with peptide in the EliSpot assay and by quantifying IFN-gamma from the serum of animals.

6. Biologicals testing package, esp. for higher risk new biological entities (NBEs)

The advent of biologicals has brought new challenges to drug development. Proteins with immune-modulatory activity such as monoclonal antibodies directed against major switches of the immune system or proteins such as receptor antagonists or agonists may have dramatic effects on immune cell function.

The recent past has shown the requirement for establishing *ex vivo* or *in vitro* models that properly reflect the *in vivo* situation.

The ensuing investigation of this incident resulted in committee recommendations among which the use of human *ex vivo* / *in vitro* cultures had a prominent place. This is based on the rationale that biologicals are very often species-specific not only with respect to ligand binding, but also with respect to downstream signal transduction. Thus traditional animal models are in all likelihood not adequate to investigate effects on the immune system.

FOCUS Immunology offers a range of assays to assess the effects of biologicals on different aspects of immune cell activity, which allows a comprehensive analysis of possible immune-modulatory activities.

Specifically, the following assays are provided by FOCUS Immunology : (i) cytokine release, (ii) cell proliferation, (iii) expression of activation markers, (iv) oxidative burst reaction, (v) chemotaxis, (vi) NK cell activity, (vii) cytotoxicity (CDC, ADCC), (viii) NO production, (ix) histamine release, (x) changes in the frequency of defined cell populations.

It may be advisable to test compounds not only on resting cells but also on cells after suboptimal stimulation. This reveals activation processes which may not suffice by themselves to activate resting cells but may strongly augment activation processes occurring independently (so-called “co-stimulatory activity”).

It also may be advisable to test different means of presenting the tested biological to the cells, from presentation in solution to different types of surface-bound presentations.

Our offer for you

In case you are developing a novel drug candidate or are planning to characterize your existing drug with respect to new characteristics, indications and potential uses FOCUS Immunology will be pleased to assist you in planning your best approach and problem solution.

Please categorize your molecule or question and select one of the above testing packages or get in touch with us regarding a tailor-made solution.

We would be happy to advise and cooperate with you.

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About FOCUS Immunology

FOCUS Immunology provides immunological consulting and laboratory services under GLP and non-GLP conditions for the development of novel medicines.

FOCUS Immunology' staff has extensive experience in cellular and molecular immunology. The head of the laboratory, Dr. Eddy Bruyns, has over 20 yrs experience in this area.

In its own > 170 sqm laboratories, FOCUS Immunology operates state-of-the-art equipment including a dual-laser flow cytometer (FACSCalibur), multi-platform reader (Fusion reader), real-time PCR (ABI 7900 HT), a fully equipped tissue culture lab as an equally well equipped lab for molecular biology and biochemistry work including the usual support facilities.

FOCUS Immunology is a wholly owned subsidiary of FOCUS Clinical Drug Development GmbH, a 120 employee full service CRO with headquarters in Neuss near Duesseldorf, Germany. At any time FOCUS Immunology can build on the broader drug development expertise of seasoned pharma experts at FOCUS CDD GmbH. Comprehensive information on FOCUS CDD GmbH can be found at the website www.focus-cdd.de.

The laboratories are located in the Heidelberg Biotechnology Park next to the Heidelberg University Medical Center, the University Campus, and renowned basic biomedical research institutions, like the German Cancer Research Center, the Max Planck Institut for Medical Research, The European Molecular Biology Laboratory and others. In this scientific cluster FOCUS Immunology can capitalize on outstanding immunology expertise.

If required for a given project, academic expertise can be easily contracted. We have successfully conducted several cooperative projects with Heidelberg research institutions and we have established contract templates in place that allow the timely project start and seamless project performance.

Internet links to helpful resources at the FOCUS Immunology homepage

FOCUS Immunology Homepage

www.focus-immunology.com

FOCUS Expert Report on “Biomarkers of Innate Immune System Function”

<http://focus-immunology-and-biomarker.com/expert-report-biomarkers-of-innate-immunity/>

FOCUS Expert Report on “Biomarkers in Exploratory Clinical Studies”

<http://focus-immunology-and-biomarker.com/free-expert-report-biomarkers-in-exploratory-clinical-studies/>

FOCUS Technical Brief on Innate Immunity Testing

<http://focus-immunology-and-biomarker.com/category/case-studies-and-technical-briefs/innate-immunity-testing/>

FOCUS White Paper on pre-analytics in immunological testing

<http://focus-immunology-and-biomarker.com/category/immunology-studies/pre-analytics/>

FOCUS Video slide presentation on immune monitoring in clinical trials

<http://focus-immunology-and-biomarker.com/video-presentation-on-immune-monitoring-in-clinical-trials/>