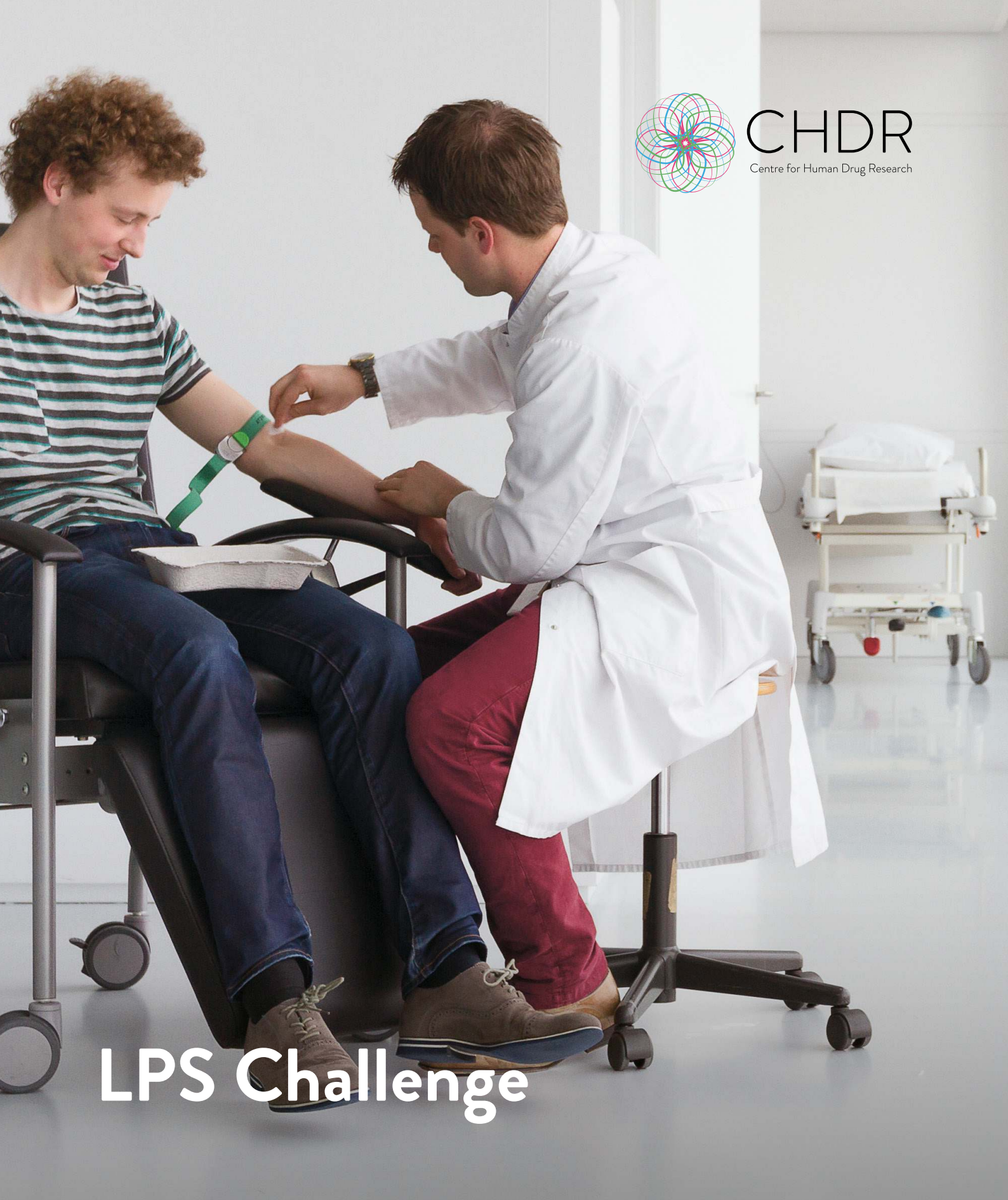

CHDR
Centre for Human Drug Research

LPS Challenge

Innovative tools for studying inflammation in healthy subjects

Inflammation is a primary pathogenic pathway in a wide range of diseases, including rheumatoid arthritis, Crohn's disease, cardiovascular disease, and neuropsychiatric disorders. To study the effect of compounds that target inflammatory pathways in the early phases of drug development, CHDR has developed a series of *in vivo*, *ex vivo*, and *in vitro* tools to induce inflammation in healthy subjects and cells. These tools – known as LPS (lipopolysaccharide) challenges – provide researchers with a robust and safe way to test anti-inflammatory compounds under controlled conditions, before moving on to testing in patients.

The LPS challenge at a glance

- Inflammatory cellular pathways downstream of TLR4 (Toll-like receptor 4) can be activated and measured *in vitro* using blood samples obtained from healthy volunteers.
- The *in vivo* LPS challenge is a safe method for obtaining valuable informative regarding the effects of anti-inflammatory compounds in healthy subjects.
- The *in vivo* LPS challenge can also be used to study specific aspects of the inflammatory response, including the vascular endothelium.
- CHDR has also developed *ex vivo* challenges and biomarkers for studying a variety of inflammatory pathways.

The *in vitro* LPS challenge uses whole blood obtained from a healthy subject. In the test tube, this blood is mixed with LPS, triggering an inflammatory response in the white blood cells. The test compound can be added to the blood sample either before or after the LPS challenge, and the effect on the LPS-induced inflammatory response is measured over time.

In the *ex vivo* challenge, the test compound is administered to the subject, after which blood is drawn and stimulated with LPS challenge as described above.

In the *in vivo* challenge, healthy volunteers receive a safe dose of LPS to induce a mild systemic inflammatory response that is monitored carefully. The effect of the test compound is then measured in the subjects.

Practical answers to important research questions

Does our test compound reduce the release of pro-inflammatory cytokines by immune cells?

Using a combined *in vitro/ex vivo* LPS challenge, CHDR can test whether a test compound affects the release of various cytokines by immune cells in the blood. The in vitro challenge can be used to optimise the dose of the compound, and the *ex vivo* challenge provides valuable information regarding the time course of the compound's effect.

How does our compound affect inflammatory responses involving endothelial cells and/or other vascular components?

By combining the *in vivo* LPS challenge with a set of biomarkers to measure endothelial integrity, we can induce and monitor a systemic inflammatory response in healthy subjects. We can then measure the pharmacological effect of the test compound on this inflammatory response.

The LPS challenge: a closer look

The CHDR approach

CHDR uses a combination of inducible challenges and biomarkers to study the pharmacological effects of test compounds as early as possible in clinical development. This approach provides a wealth of valuable information in the early phases of drug development and establishes a solid foundation for future studies in patients. Importantly, this approach can also reveal potential problems in the early stages of development.

The *in vitro* and *ex vivo* LPS challenges: cytokines

LPS binds to and activates the TLR4 receptor on the surface of specific immune cells, triggering downstream intracellular signalling pathways and leading to the production and release of cytokines, which can be measured easily in a blood sample. Measuring cytokines is therefore a simple, yet powerful way to measure the *in vitro* and *ex vivo* effects of a test compound. This approach can also be used to develop a dose-response curve, allowing researchers to optimise conditions before moving to the *in vivo* challenge.

The *in vivo* LPS challenge: measuring systemic effects

Both *in vitro* and *ex vivo* tests can provide a wealth of information, but how do they compare to the full-blown systemic response in the human body? To answer this question, CHDR developed the *in vivo* LPS challenge in healthy subjects. Because LPS is one of the key molecular players in sepsis, causing a severe systemic response in high doses, CHDR researchers developed a dosing regimen that triggers a relatively mild – yet reproducible and measurable – inflammatory response. In addition to providing important information regarding the effect of anti-inflammatory compounds at the systemic level, the *in vivo* LPS challenge also validated the *ex vivo* challenge, which provides a wealth of information without the need to administer LPS to subjects.

Using the *in vivo* LPS challenge, researchers can now study all aspects of systemic inflammation in healthy subjects under controlled conditions. We can monitor the immune response by measuring a comprehensive panel of cytokines and acute-phase proteins, and we can monitor the effects of the immune response in the vasculature by measuring biomarkers in the vascular epithelium, selectins, integrins, and thrombomodulin. The *in vivo* LPS challenge can therefore be used to answer specific questions regarding a test compound's effect on a wide range of systemic immune responses in healthy subjects before moving to patient studies.

'Using the in vivo LPS challenge, researchers can now study all aspects of systemic inflammation in healthy subjects under controlled conditions.'

Why choose CHDR?

The Centre for Human Drug Research specialises in early-phase clinical drug research. CHDR's overall mission is to improve the drug development process by collecting as much information as possible regarding the candidate drug in the early phases of development. This information helps sponsors make informed decisions regarding the course of clinical development for their product.

Why choose CHDR?

Research at CHDR covers a wide range of fields, including the central nervous system (CNS) and pain, the cardiovascular system, haemostasis, immunology, and dermatology. In addition, CHDR is at the forefront in developing novel biomarkers and methods for measuring drug-related effects in all of these research areas.

Pharmacology matters

Whether studying a new cognitive-enhancing drug, a next-generation painkiller, or a new monoclonal antibody designed to treat rheumatoid arthritis, the goal is to determine how the compound's effects correlate with both the dose and blood concentration at any given moment. In addition, understanding which biological systems are activated is an essential first step towards quantifying this relationship. At CHDR, our focus on pharmacology is reflected clearly in what we call question-based drug development.

Question-based drug development

CHDR actively uses question-based drug development - or QBD - as a more rational approach to drug development compared to conventional approaches. QBD can be best described as a series of questions that are addressed throughout the process. These questions often seem simple enough, but failing to answer even one question - or even addressing the questions in the wrong order - can have dire consequences. Thus, using this approach can potentially save companies millions of dollars by helping predict a catastrophic issue early in the development process, before the more expensive latter stages (for example, large-scale clinical trials or the marketing phase).

From a general perspective, the most important questions are:

1. Does the biologically active compound and/or active metabolite(s) reach the intended site of action?
2. Does the compound cause its intended pharmacological and/or functional effect(s)?
3. Does the compound cause any unintended pharmacological and/or functional effect(s)?
4. Does the compound have a beneficial effect on the disease and/or clinical pathophysiology?
5. What is the compound's therapeutic window?
6. How does any variability with respect to the drug response in the target population affect the product's development?

Contact

To learn about CHDR's full range of services, contact us today.

+31(0)71 524 64 00

info@chdr.nl

www.chdr.nl