Immunology and Inflammation

Developing powerful and innovative new treatments

Advances in both basic and clinical immunology during the past few decades are now rapidly being translated into effective new treatments for a wide range of conditions. The immune response and inflammatory pathways play a key role in the pathogenesis of many diseases; thus, modifying the immune response can ameliorate — or in some cases, cure — these conditions. In some diseases (for example, cancer), the immune system can be turned into a powerful ally.

Because the immune system is highly complex, all new immune-based treatments must be evaluated thoroughly. Moreover, early clinical development of new immunologically active drugs requires both scientific and clinical expertise in order to ensure the subjects' safety and to maximise the resulting clinical and economic benefits.

CHDR is committed to overcoming the scientific and practical challenges associated with the early clinical development of these new treatments. Specifically, several new methods for evaluating immunologically active compounds have been developed and validated at CHDR. With access to *in vivo*, *in vitro*, and *ex vivo* systems, we can systematically evaluate the efficacy and safety of new test compounds in the earliest stages of development.

Practical answers to important research questions

Does our immunomodulatory compound reach its molecular target, and does it have its desired effect

Evaluating the pharmacological activity of a new immunomodulatory compound in early clinical testing is not always straightforward. For example, the specific physiological pathway that the compound is designed to target may not be sufficiently active in healthy volunteers. CHDR has the knowledge, resources, and facilities needed to induce and monitor the targeted pathway in *in vivo* and *ex vivo* systems. Using this approach, it is possible to quantify the drug's effects in the earliest clinical stages, showing whether an immunologically active compound reaches its designated target in healthy volunteers.

What is the optimal dose for testing our compound in patients?

At CHDR, we have developed — and we continue to develop — both *in vivo* and *ex vivo* challenge models that can be used to measure the putative effect of immunologically active compounds on various pathophysiological pathways. Using healthy volunteers, we can now use these robust models in the early phases of drug development to examine the correlation between drug concentration and the intended drug effect. The results of this early testing can then be used to better predict the ideal dosage for use in patients.

Does our biotherapeutic have any unintended side effects?

To investigate whether a new biotherapeutic compound has any unintended immunostimulatory properties (due either to impurities or its inherent mechanism of action), we have developed *ex vivo* incubation assays using human immune cells. Thanks to our extensive network, we have continuous access to fresh biological samples from both healthy volunteers and patients.

Immunology highlights

- At CHDR, we can study the effects of immunologically active compounds on several levels:
  - At the *in vitro* level, we use fresh human blood and/or tissue samples obtained from patients or healthy individuals;
  - At the *ex vivo* level, we use fresh blood and/or tissue samples obtained from subjects who have received the investigational compound, placebo, or an active control; and
  - At the *in vivo* level, we can measure the compound's effects in healthy volunteers and patients.

- On each level, we can study the compound's effects on basic physiology, or we can use a challenge model to induce an immune response and then measure the compound's effect on that response.

- In human subjects, in addition to performing routine laboratory tests (e.g. blood chemistry, haematology), we can also use advanced molecular techniques such as immunophenotyping, cell sorting, and *ex vivo* culture systems.

- Our robust *in vivo* challenge model can be either systemic or topical.

- At CHDR, we are actively involved in developing immunologically active drugs with a wide range of therapeutic applications, including haematology, dermatology, neurology, oncology, rheumatology, and pulmonology.

- In some cases, a new preparation may have unintended immunological effects. At CHDR, we have extensive experience analysing the immune response in order to determine the cause of these unintended effects.

Immunology and inflammation: a closer look

Bridging preclinical and clinical drug development

At CHDR, we are particularly interested in taking the step from preclinical research to clinical drug development. We carefully select, validate, and apply a wide range of biomarkers, challenge models, and clinical strategies to find answers to our sponsors' questions. A well-informed translational strategy also increases the safety of our subjects and the safety of participants in future clinical trials. Given the complexity of the immune response, our knowledge-intensive approach is particularly important in the field of immunology.

CHDR has its roots in academia, and our staff are closely involved in research. In addition, we have an extensive network of research groups and clinicians who share their expertise with us when needed. We also have direct access to several highly specialised laboratories, in addition to our own research facilities. This unique combination of resources and expertise allows us to offer each sponsor a tailor-made approach to translational drug development.

Using cutting-edge immunological biomarkers to measure a drug's effects

At CHDR, we use *in vitro*, *ex vivo*, and *in vivo* approaches to study the effects of new test compounds. One powerful approach involves the use of biomarkers. We have an extensive library of biomarkers, which we are continuously expanding; do not hesitate to contact us if you have any specific questions or requests.
Our general approach is to build upon the groundwork laid by the preclinical scientists who originally developed a compound or treatment. Wherever possible, we attempt to include the tests and/or procedures that were used in preclinical testing. The primary goal in early clinical drug development is to demonstrate a pharmacological effect, ideally using highly sensitive biomarkers. However, because the setting in these early studies often differs somewhat from clinical practice, these biomarkers may not necessarily be the same biomarkers that are commonly used in a clinical setting. In this context, the primary goal when selecting a biomarker is to demonstrate that the drug reaches its intended target. Of course, if desired we can also include clinically relevant biomarkers.

In vitro measurements using human cells

Because CHDR has close ties with Leiden University Medical Center and other hospitals, it is relatively easy to obtain fresh human samples from specific patient populations and healthy volunteers. These biological materials can then be used in an *in vitro* setting to measure the pharmacological activity of a test compound. In some cases, the immunological effects of a compound on whole blood samples, isolated peripheral blood cells, or skin samples can be determined by measuring changes in the cells' physiology. Sometimes, however, this will not be sufficient to demonstrate that the compound has its intended effect. For example, a compound that was designed to modify the inflammatory response by acting on a specific pathway may not have any measurable effect on non-activated blood cells. In this case, we first need to activate the inflammatory response using the relevant pathway in our so-called 'challenge' model; the compound's effects on these activated cells can then be measured.

Showcase 1:

Neuroimmunology: the effects of choline on inflammation

One of the most striking examples of the direct link between the nervous system and the immune system is the cholinergic anti-inflammatory reflex. Stimulation of the vagus nerve causes a decrease in the release of pro-inflammatory cytokines in both laboratory animals and patients. The recent advent of compounds that act on the cholinergic system (for example, for treating some of the symptoms associated with Alzheimer's disease) has rekindled scientific interest in the cholinergic anti-inflammatory reflex.

To study this phenomenon — and the potential effects of new pharmacological compounds on the anti-inflammatory reflex — CHDR created and validated an innovative new *ex vivo* assay. In this assay, we culture whole blood drawn from healthy volunteers, including elderly subjects (65-80 years of age), who are likely to receive a cholinergic drug. We can then use these whole blood samples to measure the effects of choline on the response to a challenge with LPS and aluminium hydroxide ($Al(OH)_3$). In these experiments, choline caused a significant reduction in the release of both IL-1β, IL-6, and — to a lesser extent — TNFα. We also observed a circadian pattern in cytokine secretion: the inflammatory response was markedly lower in blood that was drawn in the afternoon compared to blood drawn in the morning. This robust model can now be used to examine putative modulators of cholinergic activity, and CHDR has developed an extensive set of *ex vivo* challenges, which we now use to measure the effect of compounds on a wide range of targets and pathways (see the table below). Moreover, we can develop new *ex vivo* challenges that are tailor-made to study the mechanism of action of a new test compound.

Table: Overview of stimuli for use in *ex vivo* challenge models, including the cellular target and pathway

Stimulus	Target	Pathway
endotoxin (LPS)	monocytes, lymphocytes, neutrophils	TLR4
endotoxin (LPS) plus ATP	monocytes, lymphocytes	TLR4, inflammasome
LPS plus Al(OH)3	monocytes, lymphocytes	TLR4, inflammasome
chemotactic peptide (fMLP)	basophils, neutrophils	broad host immune responses
coagulation	monocytes, neutrophils	IL-8 driven inflammation
superantigen (e.g. SEB)	T cells	T cell receptor-mediated responses
Pam3CSK4	monocytes	TLR1/2
double-stranded RNA (poly I:C)	dendritic cells, B cells	TLR3
flagellin	monocytes	TLR5
single-stranded RNA	dendritic cells, monocytes, B cells	TLR7, TLR8
CpG DNA	dendritic cells, B cells	TLR9

Al(OH)3, aluminium hydroxide; fMLP, N-formyl-methionyl-leucyl-phenylalanine; LPS, lipopolysaccharide; SEB, Staphylococcus enterotoxin B; TLR, Toll-like receptor

Showcase 2:

Testing for unintended immunostimulation

Drugs that are derived from biological sources (e.g. biologicals, biopharmaceuticals, and biosimilars) play an increasing role in the treatment of a growing number of diseases and conditions. However, because of their complex production process, the final product may contain small – yet biologically relevant – levels of contaminants. Moreover, some biologicals may form aggregates. These factors can have undesired effects in subjects, sometimes leading to the failure of a promising new product during clinical development. It is therefore important to be able to detect these unintended effects as early as possible and to determine the source of these effects.

CHDR has developed highly sensitive *in vitro* and *ex vivo* assays for detecting impurities in test compounds. A good example is a series of compounds based on a mutant recombinant form of apolipoprotein called Apolipoprotein A-1 Milano (ApoA-IM), which has been shown to reduce atherosclerotic plaques and provide a cardioprotective effect. The first attempt to develop an effective drug based on ApoA-IM was terminated due to a serious adverse reaction in a patient during a phase 3 trial. This severe reaction was likely caused by small amounts of residual proteins from the host cells that were not detected in the earlier phases of drug development.

A second drug based on ApoA-IM (MDCO-216) was developed, and CHDR investigated its immune effects using an *ex vivo* stimulation model. In this model, before a subject received MDCO-216, his/her blood was incubated with a relevant dose of the test compound. Using this model, CHDR showed that MDCO-216 did not significantly increase IL-6 levels or cause any other signs of an inflammatory response. When tested *in vivo*, MDCO-216 did not cause any signs of an inflammatory response. Thus, MDCO-216 is an excellent example of how CHDR can use an *ex vivo* assay to predict adverse immune responses in test subjects, including patients.

Ex vivo assays using cells obtained from study participants

In vitro research — in which a test compound is tested in human blood and/or tissue samples — is an important step in the development process, providing a unique opportunity to study the compound's effects in various cell types at physiological concentrations. During 'SAD' (single ascending dose) and 'MAD' (multiple ascending dose) studies, extremely valuable information can be obtained using blood samples.

In *ex vivo* studies, we can perform additional tests and challenges using the subjects' cells in the presence of the test compound, placebo, or active control. In many cases, practical and/or ethical issues prevent researchers from performing these tests *in vivo*. For example, triggering a massive inflammatory response in an *ex vivo* assay can be highly informative, whereas inducing the same response in a patient would be extremely dangerous. Because *ex vivo* studies provide information regarding the effects of the test compound under a wide range of conditions, they contribute to the safety of participants in future clinical trials that may include patients with a variety of clinical conditions.

Showcase 3:

Measuring bioequivalence in healthy subjects

Expiration of a patent is part of the normal 'life cycle' of all registered drugs, allowing companies to begin producing generic forms of the drug. In the case of biologicals (see Showcase 2), however, the company developing the generic biosimilar compound must demonstrate that the biosimilar has the same properties as the original compound. CHDR's array of biomarkers and bioassays can be used to test bioequivalence, through to providing the first tests in human subjects. In this respect, *ex vivo* assays can be extremely valuable, as they can be used to measure bioequivalence in blood cells obtained from healthy subjects.

For example, CHDR measured the pharmacokinetics (PK) and pharmacodynamics (PD) of ONS-3010, a biosimilar of the well-known TNFα blocker Humira®. In a randomised, double blind, single-centre study, our researchers found similar PK profiles between ONS-3010 and both the European and US versions of Humira®. Testing also showed that ONS-3010 has a similar safety profile as Humira®.

CHDR researchers also used the LPS-Al(OH)3 challenge model to compared the PD profiles of ONS-3010 and Humira® in an *ex vivo* assay, finding virtually no difference between the original compound and the biosimilar.

Showcase 4:

Combining *ex vivo* and *in vivo* assays during the development of a new TLR4 inhibitor

Ex vivo challenges such as the LPS challenge described above provide a safe way to study the pharmacological effects of compounds designed to modify the immune system. Sometimes, however, it may become necessary to validate the *ex vivo* response using an *in vivo* approach; this is particularly true when developing a new drug class. In other words, in addition to inducing an inflammatory response in isolated blood cells, it may also be necessary to induce a systemic inflammatory response in human subjects. This is the approach that CHDR researchers used to evaluate the first inhibitor of Toll-like receptor 4 (TLR4). TLR4 activates a variety of pathways that play a role in many acute and chronic inflammatory responses to tissue damage. Thus, an effective TLR4 inhibitor might be useful in treating rheumatoid arthritis and other autoimmune diseases.

Building on *ex vivo* data, we developed an LPS dosing regimen that triggered a relatively mild – yet reproducible and measurable – inflammatory response in our subjects, providing an *in vivo* setting in which we could examine the compound's anti-inflammatory properties. Based on these data, as well as PK/PD modelling and simulations, we were able to determine an effective dosing schedule for studying the compound's effects patients with acute or chronic inflammatory diseases.

Importantly, this *in vivo* study also helped us validate our *ex vivo* LPS challenge, showing that the *ex vivo* assay can provide a wealth of information without the need to administer LPS to subjects.

Using *in vivo* studies to quantify a drug's effects in human subjects

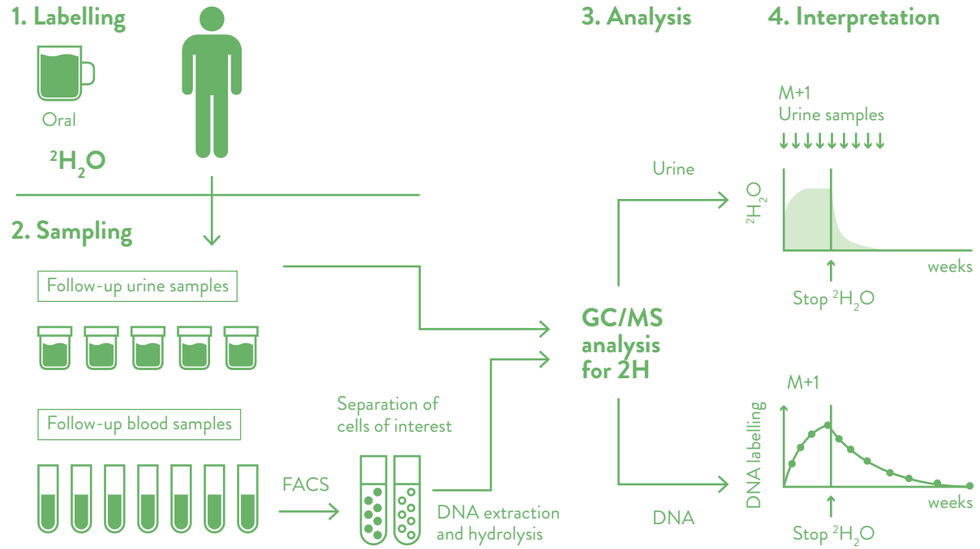
Early clinical drug development provides a unique opportunity to both intensively and extensively study the effects of a new test compound on human physiology and immunology. When we study a new compound for use in the field of immunology, we work closely with the sponsor, using the preclinical test results to develop a protocol that is tailor-made to match the characteristics of the test compound. In addition to the *in vitro* and *ex vivo* approaches discussed above, we can also measure a panel of relevant biomarkers in order to study the compound's pharmacological properties. These biomarkers can include routine blood chemistry and haematology, as well as more sophisticated molecular techniques for immunomonitoring and pharmacological challenges designed to mimic a specific immune reaction and measure the effects of the test compound. For example, to study the pharmacodynamics of a putative anti-inflammatory compound, we can induce an inflammatory response in subjects and examine how the test compound alters this response. In some cases, it may be necessary to induce a systemic response; however, in many cases, a local, confined response (for example, in a patch of skin) can be just as informative, with fewer risks and less discomfort for the subject. The choice of biomarkers and the approach used depend upon the specific questions that our sponsors want addressed, and — of course — the safety of our subjects always comes first.

Showcase 5:

Monitoring the production rate and lifespan of immune cell subsets

Because some drugs can decrease the number and/or repertoire of lymphocytes in the blood, it is important to determine whether this effect is caused by a decrease in the production of these cells in the bone marrow, or by an increase in the loss of these cells from the circulation. Working with researchers at Utrecht University, CHDR developed a novel approach to study the production rate and half-life of lymphocytes and specific lymphocyte populations. During the course of several weeks, healthy volunteers drank 'heavy water' (D_2O, or ^2H_2O), a harmless form of water in which the hydrogen atoms are replace with deuterium atoms. Chemically, D_2O behaves exactly like H_2O; however, deuterium atoms can be measured using mass spectrometry. After drinking D_2O, the deuterium atoms are incorporated in newly synthesised molecules, including DNA in developing lymphocytes. Thus, lymphocytes that developed during a specific window in time can be tracked using mass spectrometry, and biometric modelling can be used to estimate the production rate and lifespan of these cells.

Figure: *In vivo* labelling of lymphocytes with D_2O (^2H_2O). Adapted from Westera et al. (2013) Methods Mol Biol. 979:107-31.



Showcase 6:

Monitoring the immunomodulatory effects of conventional therapies

Another elegant example is a study by CHDR researchers in the context of developing a therapeutic vaccine against cervical cancer. Cancer immunotherapy is nearly always combined with other treatment modalities such as surgery, radiotherapy, and/or chemotherapy. Given that both radiotherapy and chemotherapy have robust immunomodulatory effects, these treatments can potentially interfere with immunotherapy.

To measure the effect of radiotherapy on the immune system, we studied 30 patients with cervical cancer who received external beam radiation therapy (EBRT). Using serial blood sampling, we followed the effects of EBRT on lymphocyte and myeloid cell populations, as well as the expression of costimulatory molecules, T cell reactivity, and the function of antigen-presenting cells. We found that EBRT has specific effects on both CD4+ and CD8+ T cells, leading to the conclusion that conventional radiotherapy profoundly suppresses the immune system in cervical cancer patients and may reduce the effects of immunotherapy both during and for several weeks after radiotherapy.

Showcase 7:

Monitoring the immune system using RNA profiling in asthma patients

In asthma research, sputum induction is a relatively non-invasive procedure used to measure changes in the airways. In this procedure, the subject is instructed to inhale an aerosolised hypertonic salt solution, then expectorate sputum, which is processed and separated into a solid phase containing cells and a liquid phase that contains soluble biomarkers. At CHDR, we developed a method using the RNA profile measured in sputum to monitor the inflammatory response in the airways of patients with asthma in response to an allergen challenge. We also found that that corticosteroids induce a change in the inflammatory response that can be quantified my measuring the RNA profile. Importantly, the changes measured in the RNA profile were correlated with clinical data points measured in the same subjects. Thus, this new tool for measuring the immune response can help researcher study the pharmacodynamics of new immunomodulators for use in the field of pulmonology.

Showcase 8:

Using the imiquimod challenge to study the effects of TLR7 inhibitors

To evaluate new immunomodulatory therapies in healthy volunteers, we developed a model based on the pro-inflammatory effects of imiquimod on the skin. Imiquimod (Aldara®) is a topical treatment for genital warts, superficial basal cell carcinoma, and actinic keratosis. Imiquimod activates the body's innate immune system via Toll-like receptor 7 (TLR7), a signalling protein involved in recognising pathogens. CHDR researchers systematically examined the effects of applying increasing doses of imiquimod to the skin of healthy volunteers. Specifically, we studied the cytokine/chemokine cascades that were activated by imiquimod, as well as any resulting histological changes. Importantly, we also found that topical imiquimod does not reach clinically relevant systemic levels, even at the highest dose tested.

These results provided proof-of-concept that the imiquimod challenge model is safe, well tolerated, and fully reversible. Interestingly, the changes induced by imiquimod share several key biochemical characteristics with psoriasis, and CHDR is now using this model to investigate the effects of an immunomodulatory compound designed to treat inflammatory skin 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

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