Pharmacodynamic Evaluation: Inflammation/Immunology

Jörg Schüttrumpf and Matthias Germer

Contents

Abstract

In any drug development including those for candidates in acute and chronic inflammation and any other immunological disbalance, distinct types of pharmacological studies are a regulatory requirement. Together clinical and nonclinical data are required to characterize a novel drug candidate adequately. These requirements were summarized, outlined, and exemplified in this chapter as follows:

- Primary pharmacology describes the mode of action of the drug candidate with respect to its therapeutic target.
- Secondary pharmacology: Studies on secondary pharmacology look deeper into the biochemical and physiological effects of a drug and the mode of action that is not directly related to the desired therapeutic target (general pharmacology studies).
- Safety pharmacology has a focus on the impact of a drug candidate in and above the therapeutic range on the function of organ systems.
- Immunological safety assessment: First-in-human studies.
- Immunotoxicology

As there is no international consensus on definitions of the terms, the attribution of either type of studies may sometimes be ambiguous. It is impossible to separate immunotoxicology studies required by regulatory agencies basically from the pharmacodynamics of a drug. An initial package of such pharmacological studies is a prerequisite for first-in-human studies for novel drugs.

J. Schüttrumpf (✉) · M. Germer
Corporate R&D, Biotest AG, Dreieich, Germany
e-mail: joerg.schuettrumpf@biotest.com; matthias.germer@biotest.com

F. J. Hock, M. R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_34-1

Primary Pharmacology

The intended mode of action of a drug candidate relevant for the intended immunomodulatory use is specific for each substance. Pharmacopoeias sometimes describe the relevant assays for common substances in some detail which are representative for the drug's mode of action and must therefore also be employed in the testing prior to batch release. For the majority of substances and in particular for any novel product, however no standardized and accepted tests are available. Rather the manufacturer starts to investigate the mode of action early in drug development and ideally develops specific functional assays. These should also be indicating stability and the starting point of the development of precise and robust assays for batch release. As every product is different in this respect, there is no general guidance on the required tests and data in preclinical and clinical research. Nevertheless it is important to understand the performance characteristics of any pharmacological test to allow for the interpretation of the data.

Predictive pharmacological in vitro or ex vivo models for disease play a limited role in immunology because this system critically depends on the interactions of a network of different soluble mediators and cell types and on the location of the cellular components in the organs. But also in vivo disease models often are of limited value because of substantial species-specific differences of the immune system in human and laboratory animals even including primates. Many disease models show some similarity to the human disease but differ substantially, e.g., in pathophysiology, disease progression, and therapeutic readout. Models for rheumatoid arthritis as an example for chronic inflammation and for tumors as an example for inadequate immune response are presented here.

Rheumatoid arthritis in humans is a chronic autoimmune disease typically progressing over many years before joints and cartilage become irreversibly damaged. In contrast the best studied animal model is arthritis in mice, rat, or even primates induced by the intradermal injection of collagen type II (Trentham et al. 1978). It has similarities in many clinical, histological, and immunological phenomena and was essential in the development on cytokine inhibitors, the current blockbusters in the arthritis therapy. Nevertheless the differences are also substantial: disease onset in this model is rapid (within weeks) and progression fulminant. The immune response in animal models is dominated from an innate immunity and by anti-collagen antibodies which are of little relevance in patients. In contrast to the response in patients, rodents benefit little from nonsteroidal anti-inflammatory drugs. Reversal of joint destruction is not observed. Alternative models have become available and can dissect various pathomechanisms in the disease initiation and progression. Also the brake of immunological tolerance is still poorly understood. HLA class II transgenic mice stains can differ in predisposition to develop rheumatoid arthritis. Mice with mutations in the T-cell receptor gene, ZAP70 (SKG mice), and with specific variants of the protein tyrosine phosphatase non-receptor type 22 need an additional environmental stimulus. Serum transfer models can be used to study the role of immune complexes in the inflammatory response. TNF-alpha transgenic mice develop chronic progressive polyarthritis spontaneously and very reliably within 6 weeks after birth. Their primary use is the investigation of the pathological production of TNF-alpha. No current model of arthritis can however fully represent the human disease. As all arthritis models are associated with pain and distress, the use of such models is debatable, and the implementation of the 3R principles is obligatory: replacement, reduction, and refinement. Despite of their limitations, the collagen-induced arthritis models have remained popular (Bessis et al. 2017; Benson et al. 2017).

Any progression of tumors can be seen as a failure of the immune system to eradicate aberrant cells. Unfortunately oncology is a field notorious for successful therapy of laboratory animal but failure to predict safety and efficacy in clinical trials (Mak et al. 2014). The process of human carcinogenesis, physiology, and progression is complex. Typical animal models in oncology include subcutaneous implantation of cultured human tumor cells (xenografts) into

immunocompromised mice. This procedure is easy and can be standardized. However the tumor environment is known to have a tremendous impact on treatment susceptibility and often is not the skin. Usually immunecompromised mice have to be employed to allow for adequate tumor cell survival and progression. Therefore, important interactions of the tumor and the host immune system will be missed in animal models. Also tumor cells are typically grown ex vivo before implantation and divide rapidly, whereas human tumor cells divide much slower. As a consequence the susceptibility to cytotoxic agents in the models can be overestimated. Tumor xenograft models remain valuable because of the established technology and the wide variety of tumor types that can be studied. But the ability of such models to mimic more than just single steps of the complex processes in patients and their predictive value are limited.

Individual limitations of the tumor models have been overcome in recent decades. When tumor grafts are directly explanted from patients, not only the malignant cells but also the correct anatomical structure can be transferred (e.g., stromal cells, bone chips). Variability of the response of a specific tumor entity can be assessed better. Because the immune system of mice and men are phenotypically and functionally too different, humanized tumor mouse models have been another major recent progress. By transplantation of CD34-positive human stem cells, a functional human immune system can be established in otherwise immunocompromised mice. When human tumor cells are transplanted in parallel or after establishment of the human immune system, the interaction of these systems can be taken into account. This has been particularly useful in the evaluation of immunomodulatory approaches (antibodies, tumor vaccines, cell therapy, cytokines). Genetically engineered mice may completely represent cancer development (initiation, progression, interaction with stroma and the immune system). Tumors from such animals can be transplanted in syngeneic animals and have been used to study chemotherapeutics and other small molecules and currently are probably the best available tool to study immunotherapeutics. But still species differences can be a major challenge. Future refined models might better address tumor variability, better control of genetic variability of the tumor and the host, clinically relevant endpoints, biomarkers, environmental factors, etc. (Day et al. 2015).

Other immunological indications have seen similar progress in pharmacological animal models. The pathomechanism, the initiation and progression of the disease in the animal model, and the patient should be understood and similar. For validation of an animal model, it can be helpful to demonstrate that a clinically effective intervention is effective in the model as well.

It is important to plan pharmacological animal studies with a clear rational and a well-characterized animal model. Also an adequate study design (e.g., group size) is required to verify or reject a hypothesis, although limited availability of novel drugs, chosen animal species, or ethical considerations make it often necessary to keep the number of animals low. Still the translation of preclinical pharmacological results to the clinical setting can be most reliable if common design flaws are minimized. In clinical practice therapy is started after the onset of symptoms and when diagnosis is clearly established. In animal models often a drug is given shortly after or even before the disease is initiated, and the therapeutic benefit is likely to be overestimated. Misleading results may also be obtained when the animal cohorts do not match the clinical situation in terms of age, gender, environmental factors, or concomitant diseases.

Today the use of irrelevant species in preclinical studies is often discouraged. Studies to proof the relevance of an animal model do not only include demonstration of reactivity with the relevant target structure. Also a good understanding of the target's primary structure, distribution and expression level, pharmacological effects, metabolism, and cross-reactivity with other structures is advisable.

In clinical studies the primary pharmacological effect is often also investigated and correlation to clinical endpoints analyzed. The test systems are specific for each substance because of the unique mode of action of each drug candidate. Due to the

complex nature of the immunological, often cell-based, assays, it is not uncommon that only specialized laboratories can perform such assays reliably. In many cases robust results are only obtained when cellular assays are set up within a few hours after samples are taken. This can limit the use of central laboratory facilities in larger, multinational studies, and relevant data may only become available from a subset of patients/volunteers. Nevertheless they can be very helpful as the basis of pharmacometric models.

Secondary Pharmacology

During drug development secondary pharmaceutical effects are often not fully understood. Typically these studies are started early in development because they help to find an appropriate starting dose for first-in-men clinical trials. But the understanding of their biological relevance increases over time even after drug approval. Some of these effects can be consistently observed in experimental setups but remain inconspicuous in patients. In other cases they can contribute to the adverse event profile of a drug (Amantea and Bagetta 2016). They may also be crucial for upside indications or drug repurposing (for examples see Table 1).

Pharmacological effects are particularly relevant for patient safety in cases of unintended enhancement or suppression of immune responses. Investigations of these aspects are also often described as immunotoxicity studies. Novel chemical entities and biologicals somewhat differ in the complexity of the test strategy. The field has developed rapidly which is often not yet reflected in regulatory guidance documents. These investigations are driven by substance-specific characteristics which are different for every drug candidate. Current characterization of a drug candidate is very much science driven and rarely follow a standardized approach.

Immunological Safety Assessment: First-in-Human Studies

A sound understanding of the pharmacological effects is required in the development of any drug. In 2006 the particular importance of early availability of these data became apparent for novel drugs. A phase I clinical trial with the superagonistic CD28 antibody TGN1412 changed the development path not only for biologicals but also for novel chemical entities. Although a TGN1412 dose 500 times smaller than estimated safe from animal studies was infused in a first-in-men study, all healthy volunteers suffered from a life-threatening cytokine storm. Within an hour all participants were reported to suffer from severe fever, rash, rigors, etc. Within the first 4 h, they developed hypotension, tachycardia, and respiratory distress which rapidly developed into multi-organ failure. Due to massive swelling of the arms and legs, the study is often referred to the “Elephant Man” drug trial in the press. Pharmacology of TGN1412 had been investigated in vitro and in vivo. It induced proliferation of T cells without co-stimulation. Rodent CD28 was hardly bound, but nonhuman primates were reported to have sequence identity in the extracellular part and high binding affinity. Whereas rodents were regarded as irrelevant model, the pharmacology and toxicology of TGN1412 were investigated in rhesus and cynomolgus monkeys. No signs of toxicity and in particular neither immune dysregulation nor hypertension were observed. In addition, a rat CD28 antibody was investigated in relevant species, and the expected pharmacological effect was observed, i.e., an increase in T cells. Importantly, increases in cytokine levels were only moderate, and no signs of cytokine release syndrome, anaphylaxis, or autoimmunity were observed. At the time the first conclusion of the apparently unpredictable outcome of the clinical trial was that the pharmacological studies in animal models may show very different properties in humans.

After the incident extensive research has led to a number of hypotheses why the apparently adequate pharmacological data packages did not predict the activity in humans. Species differences in

Table 1 Examples of biologically relevant secondary pharmaceutical effects (Yan et al. 2016; Koo et al. 2010)

Contribution to adverse event profile		
Drug	Indication	Proposed mechanism
Kadcyla (anti-HER2/neu-DM1 antibody-drug conjugate)	Breast cancer	Thrombocytopenia due to cytotoxic effect on megakaryocytes; hepatotoxicity by uptake of the conjugate by hepatocytes
Orthoclone (CD3 antibody OKT3)	Transplant rejection	Cytokine release syndrome due to release by activated leukocytes potentially causing reversible renal function impairment and delayed graft function
TNF antagonists	Rheumatoid arthritis, psoriatic arthritis, Crohn's disease, ankylosing spondylitis	Opportunistic infections due to impaired macrophage activation and differentiation, neutropenia
Repurposing		
Drugs	Original use	New use
Methotrexate Cyclophosphamide	Solid tumors	SLE
Mycophenolic acid	Solid organ transplantation	ITP, pemphigus vulgaris, SLE
Alemtuzumab	Chronic lymphocytic leukemia	Multiple sclerosis

signal transduction might be responsible. Whereas in rat the mutation of PI3 kinase does not affect signaling of CD28 superagonists in humans, inhibition of this kinase blocked the production completely. Whereas the drug induced a rapid and marked calcium flux in humans, the calcium flux in cynomolgus was only weak. Some Siglec molecules which are receptors belonging to the immunoglobulin superfamily carry inhibition motifs (ITIMs). On human T cells, their expression is low, but in chimpanzees various members are expressed. Pharmacological in vitro models could be identified that may better mimic the situation in humans involving different cell densities, interaction with Fc receptors, and other cell types. Apparently, only rodents CD28 superagonists induced the expansion of regulatory T cells which can rapidly counteract an immune response.

Immediately after the TGN1412 incident, regulatory authorities (e.g., in Germany, France, the UK) gave recommendations on how to minimize the risk of phase I clinical trials with novel substances. Only 1 year after the incident, this was then covered by guidelines by the FDA (US Department of Health and Human Services, Food and Drug Administration 2005) and the EMA (currently updated, Committee for Medicinal Products for Human Use (CHMP) 2017) which are applicable not only to biologicals but also to new chemical entities. Before the TGN1412 incident, the starting dose was usually selected based on toxicological data. On the basis of a no-observed-adverse-effect level (NOAEL) of repeated-dose toxicology studies using the most sensitive species, the human equivalent dose and consequently the maximum recommended starting dose were calculated with the help of conservative conversion and safety factors (e.g., for species or route of application). Since the TGN1412 incident, pharmacological effects are also taken into consideration: the no-observed-effect level (NOEL), i.e., the highest dose tested in mice with no effects detected (e.g., on functional activity, morphology, life span) and the minimal anticipated biological effect level, i.e., the anticipated dose level leading to a minimal biological effect level (MABEL) in humans. For the determination of the MABEL, all relevant in vitro and in vivo pharmacological and if applicable pharmacokinetic data are considered, including, e.g., receptor occupancy, dose-response curves from in vitro and in vivo animal studies with relevant species, and also in vitro studies with human cells. In healthy volunteers the starting dose should be lower than the pharmacologically active dose (PAD). Also the importance of pharmacokinetic/pharmacodynamic models is

increasing (Yu et al. 2011; Diao and Meibohm 2015).

Whenever possible, alternatives to the use of live animals should be employed, e.g., in vitro studies using human-derived materials. When studies in animals are required, the relevance of the model should be demonstrated. Key parameters are target structure and expression and the interaction with the drug (affinities, receptor occupancy, and kinetics), pharmacodynamics, and pharmacokinetics including metabolism. Not only the molecular mechanism related to the intended therapeutic use should be understood, but also secondary pharmacodynamic characteristics are expected to be investigated. Typically, functional responses related to target binding are evaluated, e.g., cellular responses including their dose dependency and duration in relation to the turnover of the drug and its target. Pharmacometric models describing and predicting the relation between pharmacokinetics and pharmacodynamics have become an important routine tool for most stages of drug development.

Whereas the use of one relevant and one other species is acceptable for novel chemical entities, studies in irrelevant animal models should be avoided for biologicals. Instead studies in a single relevant species are often sufficient. In vitro human cellular test systems might be advantageous because of lack of species differences such as affinities for the target, expression patterns, signal transduction, regulation, and metabolism. Still pharmacological studies in animal models including disease models can provide additional understanding of the mode of action in vivo, on appropriate doses and dosing intervals, and safety.

Immunotoxicology

When pharmacological mechanisms of a drug are supposed to provoke dysfunction of the immune system, often the term immunotoxicity is used. The immunotoxic effect refers to any unintended modulation of the immune response and is not limited to exaggerated pharmacological effects. These effects also include, e.g., cytotoxic effects on immune cells and interactions with surface structures of immune cells or on corresponding, interacting tissues. This immunotoxic effect may either manifest as an insufficient or excessive immune response (immunosuppression or, alternatively, allergy, autoimmunity, chronic inflammation). The extent, type, and timing of such studies depend on the specific, functional properties of the drug but also on the characteristics of the patient population (e.g., immunocompromised patients). They are typically required before large patient populations are exposed. Due to the nature of the drugs, the requirements for small molecule and biologicals somewhat differ (see guidelines ICH S8 and ICH S6 (R1), respectively). For biologicals immunogenicity is a concern that may be of major clinical relevance. As human proteins are immunogenic in laboratory animals, relevant immunogenicity data are typically obtained in clinical development.

Discussion

The pharmacodynamic evaluation is essential for the selection of appropriate doses and designs in clinical trials for indications of inflammation and immunological disorders. Understanding of the pharmacodynamic properties is relevant to develop effective and safe drugs. Still the translation of preclinical results to the clinical situation must be made with caution. Testing of molecular pathways, cellular components, cells, or even tissues is only moderately predictive. The complexity of the human organism cannot be reduced to an array of individual in vitro assays. Also in vivo studies in animal models are limited in their predictive value. Especially in the field of immunology, numerous species differences can lead to misinterpretation of the data. Disease models often differ substantially both in the pathomechanism and the progression of the disease from their human counterparts. For biologicals species specificity of the drug is a further major challenge. Last but not least, patients are diverse. Their genetic heterogenicity can have an impact on drug metabolism and transport, disease

characteristics, and many other parameters. Furthermore, patients change over their life, e.g., with age or with their diseases. Nevertheless, preclinical pharmacological evaluation is still a scientific valuable basis to design good clinical development strategies to minimize patient safety risks, to optimize efficacy aiming to maximize the benefit for the patient, and to best show the full potential of a new drug.

References and Further Reading

Amantea D, Bagetta G (2016) Drug repurposing for immune modulation in acute ischemic stroke. Curr Opin Pharmacol 26:124–130

Benson RA, McInnes IB, Garside P, Brewer JM (2017) Model answers: rational application of murine models in arthritis research. Eur J Immunol. https://doi.org/10.1002/eji.201746938

Bessis N, Decker P, Assier E, Semerano L, Boissier MC (2017) Arthritis models: usefulness and interpretation. Semin Immunopathol 39(4):469–486

Committee for Medicinal Products for Human Use (CHMP) (2017) Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. EMEA/CHMP/SWP/28367/07 Rev. 1

Day CP, Merlino G, Van Dyke T (2015) Preclinical mouse cancer models: a maze of opportunities and challenges. Cell 163(1):39–53

Diao L, Meibohm B (2015) Tools for predicting the PK/PD of therapeutic proteins. Expert Opin Drug Metab Toxicol 11(7):1115–1125

ICH S6(R1) (1997) Preclinical safety evaluation of biotechnology-derived pharmaceuticals. CPMP/ICH/302/95

ICH S8 (2005) Immunotoxicity studies for human pharmaceuticals EMEA/CHMP/167235/2004-ICH

Koo S, Marty FM, Baden LR (2010) Infectious complications associated with immunomodulating biologic agents. Infect Dis Clin N Am 24(2):285–306

Mak IWY, Evaniew N, Ghert M (2014) Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 6(2):114–118

Trentham DE, Townes AS, Kang AH, David JR (1978) Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats. J Clin Invest 61 (1):89–96

U.S. Department of Health and Human Services, Food and Drug Administration (2005) Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers

Yan H, Endo Y, Shen Y, Rotstein D, Dokmanovic M, Mohan N, Mukhopadhyay P, Gao B, Pacher P, Wu WJ (2016) Ado-trastuzumab emtansine targets hepatocytes via human epidermal growth factor receptor 2 to induce hepatotoxicity. Mol Cancer Ther 15(3):480–490

Yu J, Karcher H, Feire AL, Lowe PJ (2011) From target selection to the minimum acceptable biological effect level for human study: use of mechanism-based PK/PD modeling to design safe and efficacious biologics. AAPS J 13(2):169–178