

Summer school 2007 – course abstracts

Theme 1: Drug discovery and drug targets: experimental approaches

1.1 Basic Medicinal Chemistry

Course leader(s): A. IJzerman (Leiden) M. Smit, I. de Esch (Amsterdam)
Duration: 1 day
Level: Basic

This course will provide a kaleidoscopic view on the multidisciplinary research domain of modern medicinal chemistry. G protein-coupled receptors will be the main theme during the day, as they are prototypic drug targets. During the course the participants will learn about these targets and their interaction with other proteins, and, equally important, how to intervene with receptor function through small molecules. Aspects of ligand design and pharmacological proof-of-principle will be main topics, leading to an appreciation of the early preclinical studies in both industry and academia.

1.2 Natural products as leads in drug research

Course leader(s): S. Christensen (Copenhagen) and R. Verpoorte (Leiden)
Duration: 2 days
Level: Basic

Progresses in the fields of synthetic organic chemistry and especially combinatorial organic chemistry have made most major medicinal companies neglect the potential of Nature as a source for new lead compounds for drug discovery. In spite of this development the number of newly registered drugs inspired from natural products or even being natural products does not decline.

The aims and objectives of the course are to provide an overview of drug development by exploration of biodiversity ("bioprospecting"). The lectures will cover bioactive natural products, basic aspects of chemotaxonomy, strategies for drug development from natural sources, biodiversity as source of leads for drug development, industrial use of natural products, and biodiversity treaties.

Everybody interested in the perspectives in using natural products in drug development is invited to participate.

1.3 Positron Emission Tomography (PET) in drug development

Course leader(s): A. Lammertsma and B. Windhorst (Amsterdam)
Duration: 2 days
Level: Basic

Molecular Imaging techniques are nowadays considered to be indispensable in academic clinical practice as well in clinical research. Moreover, in drug research these techniques provide the means for *in vivo* study of a large diversity of biological processes at a molecular level. Especially PET can provide the means to study biochemical processes *in vivo* in a non invasive manner. Using an appropriate radiolabelled ligand for a defined target, PET technology provides a selective method to quantitatively measure its distribution in time and place. PET is able to provide pharmacokinetic information with only a limited number of subjects, thus enhancing the pass through of clinical candidates in Phase I/II trials. Furthermore, working mechanism of

drugs or for instance the role of the studied biological target in the patho-physiology of a disease can be studied *in vivo* with the appropriate radiolabelled ligand. As a method, PET is not straightforward and easy to use, but once operational for the selected target it is a very valuable technique.

In this basic course feasibility, utility, application and prerequisites of PET are topics to be addressed via theoretical considerations and in depth case studies.

After following this course students should be aware of the possibilities and limitations of PET in general. In addition, they should be aware of the possibilities of radiopharmaceuticals development, prerequisites for clinical PET and the possibilities of PET in drug research. They will be able to judge if and how PET can be of added value for their research.

PET is typically operated in a multidisciplinary environment at the forefront of physics, medicine, pharmacology, biology and chemistry. Therefore, this course is aimed at (clinical) pharmacologists, medicinal chemists, drug delivery technologists and other pharmaceutical scientists.

Introduction to molecular imaging and application in drug research, the role of PET
Radiopharmaceutical chemistry / probe development
Pre-clinical evaluation, *in vivo* / *ex vivo* animal models and techniques, animal PET
Human PET studies and Pharmacokinetic PET data modeling
PET in oncology : monoclonal antibodies and response monitoring
PET in neurology : neuroreceptor studies

Theme 2: Drug discovery and targets: cheminformatics and in silico modeling

2.1 Pharmacogenomics – pitfalls and possibilities

Course leader(s):	J. Meerman (Leiden)
Duration:	1 day
Level:	Intermediate

Unravelling the 30,000 – 40,000 protein-coding genes in the human genome has been a scientific achievement comparable with landing on the moon or splitting the atom. Since that, in only a few years time, technology has advanced so fast that sequencing the whole genome of a human individual now takes only a few hours while the original effort took several years. But, as was the case with these former events, the possibilities of the future are just on the verge of unfolding; now they have to play out in real life. One of the most promising areas and maybe the first to play out in large scale is pharmacogenomics.

It is generally anticipated that pharmacogenomics will have an enormous impact on fundamental and applied research. For example, genome-wide screening for genes affected by disease may reveal novel molecular targets for innovative drug design, opening the way to tailored therapies targeting a range of diseases, such as diabetes and breast cancer. Identification of specific gene expression profiles in patients may also assist a more precise diagnosis leading to refined drug therapy in the near future. This has already proven to be very effective with several forms of cancer. Also, such research will have a tremendous impact on our understanding of the processes underlying disease and will have important spin-offs towards fundamental research. Thus, pharmacogenomics is seemingly an ideal cross point, linking fundamental and applied experimentation, cross-fertilising academic, industrial and medical research

This course will deal with the *theoretical aspects* and the *applications* of pharmacogenomics

The theoretical part of the course will deal with the different techniques that monitor the expression of a large number of genes i.e. DNA microarrays. Also new mechanisms for the control of gene expression (e.g. miRNAs) that have only very recently been discovered will be discussed. In addition, possible implications of the many single nucleotide polymorphisms (SNPs) for pharmaceutical experimentation will also be covered.

The application part of the course will focus on screening in the pharmaceutical industry and clinical trials using pharmacogenomic strategies.

2.2 Introduction to Bioinformatics

Course leader(s): M. Beukers (Leiden), F. Steen Jørgenson (Copenhagen),
A. Wilderspin, and C. James (London)

Duration: 1 day

Level: Basic

The vast amounts of data, that are generated with molecular biological techniques have provided a challenge for software development. Bioinformatics is a discipline that develops informatics tools to study these biological data. The majority of the resulting databases and tools can be found on the Internet.

The objectives of this course are:

- to give the participants an introduction to bioinformatics
- to illustrate the use of the methods by specific examples of pharmaceutical relevance
- to provide hands-on experience with Web-based tools

The morning lectures of this course will cover the following topics:

- Bioinformatics on the Internet
- Sequences and sequence function relationships
- Protein Structures and 3D databases
- Homology modelling
- Structure-based ligand design

During the afternoon practical exercises, the participants will work with specific problems related to protein sequence and structure using Web-based tools.

2.3 Molecular modeling and quantitative SAR

Course leader(s): M. Mor and A. Lodola (Parma)

Duration: 2 days

Level: Basic

Computer-Aided Drug Design (CADD) had become a standard approach within medicinal chemistry projects aimed at drug discovery or lead optimization. While the availability of computational resources is growing quickly in both the industrial and academic environments, the tools employed are still based on some fundamental concepts, deriving from the pioneering quantitative structure-activity relationships (QSAR) or molecular modeling techniques.

In fact, the basics to understand and exploit many CADD tools reside in statistical analysis of multivariate data and in the complex equations of molecular models, for energy estimation or geometry optimization. Generally, after the application of CADD to their projects, medicinal chemists look for graphical outputs, to focus the attention on chemical interpretation. An in-depth consideration of numerical recipes underlying these outputs, however, helps to give a more realistic sight to CADD predictions or hypotheses.

This course will deal with the *theoretical aspects* and the *applications* of QSAR and molecular modeling. The first part will be focused on a critical presentation of the basics for the building of QSAR models and for multivariate regression analysis. In the second module, the theory of force fields and its application to drug discovery is discussed. Excel-based analysis of multiple regression models will be applied to typical QSAR data.

2.4 Computational drug discovery and design

Course leader(s): C. Oostenbrink and I. de Esch (Amsterdam)
Duration: 2 days
Level: Intermediate

In the post-genome era, an overwhelming amount of data describing the molecular characteristics of pharmaceutical targets is becoming available. For example, the structure of many proteins is being determined using crystallographic analysis and NMR techniques. Furthermore, high-throughput screening results in massive amounts of data that reveal the molecular properties of the ligands that are able to have interaction with the drug targets. In this course, several techniques that can help to translate this data into novel ligands will be discussed and demonstrated. The two-day course will be hands-on, making use of the modeling programme MOE.

At the end of this course you will be aware of the possibilities and pitfalls in computational drug design. Based on a specified target, you will be able to design a new ligand using the techniques that have been discussed and come up with a route leading to its synthesis.

Theme 3: Systems biology and bio-analytical chemistry

3.1 Modern concepts of bioanalytical chemistry

Course leader(s): Westerlund (Uppsala) H. Irth, and H. Lingeman (Amsterdam)
Duration: 2 days
Level: Basic to advanced

Sample preparation in many cases is the bottleneck in most chromatographic- / electrophoretic-based separations. In the present workshop a wide variety of methods and procedures enabling the (i) removal of interferences (low and high molecular weight, organic and inorganic), (ii) enrichment of the analytes, and (iii) phase-transfer of the analytes will be discussed. These approaches will be discussed in combination with the most frequently applied separation and detection techniques like liquid chromatography, capillary electrophoresis and mass spectrometry. The focus will be on advanced off-line, at-line, on-line and in-line methods to determine organic compounds in aqueous samples using examples from the pharmaceutical and biomedical field.

Topics that will be discussed are:

- (Bio-)analytical strategies (guidelines for choosing sample preparation techniques)
- Off-line sample preparation techniques in combination with CE, GC and LC (e.g., protein precipitation, liquid-liquid extraction, filtration)
- SPE: principles, method development, applications (showing numerous possibilities in combination with LC and CE)
- Membrane-based and 96-well plate-based SPE

Increasing the throughput for drug screening using membrane-based SPE techniques
The potential of special sorbent-based techniques (e.g., stir-bar sorptive extraction - SBSE, solid-phase micro extraction - SPME, matrix solid-phase dispersion - MSPD)
Dedicated semi-flexible automation using off-line and on-line techniques (e.g., SPE, dialysis, ultrafiltration)
Comprehensive two-dimensional liquid chromatography
Automation / integration of sample clean-up in bio-analysis using SPE and column switching
Instrumentation, operational procedures and practical guidelines for hyphenation of SPE, LC and UV / ECD / MS(MS)
General introduction to understanding, monitoring and elimination of ion suppression / matrix effects in bio-analytical LC-MS(MS)
MS adequate processing of biofluids by means of on-line SPE-(LC)
Properties and performance of tailor-made SPE packing materials (e.g., restricted access materials – RAM, molecularly-imprinted polymers – MIP, mix-mode sorbents)
Continuous-flow, on-line monitoring of biospecific interactions using ESI-MS
Applications (e.g., pharmacokinetics, therapeutic drug monitoring, biological monitoring, clinical-chemical analysis)
CE in all its variety for bioanalytical chemistry, including protein separations
Protein analysis in complex biological samples
Multidimensional SPE-(LC)-UV/MS(MS)

The primary goal of the workshop is to introduce a wide variety of analytical and sample preparation methods, to discuss their advantages and disadvantages and provide sufficient information how to develop new bioanalytical methods and procedures and to troubleshoot existing methods.

Participants should be scientists working in the field of pharmaceutical and bioanalytical analysis that are dealing with method development, and possess a basic level of knowledge of modern separation, detection and sample preparation methods.

3.2 Metabolomics

Course leader(s): T. Hankemeier (Leiden) and B. Griffiths (London)
Duration: 1 day
Level: Basic

The aim of the course workshop is to give insight into the concept of metabolomics, to discuss the analytical strategies and examples of applications to the biomedical field. Metabolomics involves the non-targeted analysis of all the metabolites at sub-cellular, cellular, tissue or body fluid level during changes of a biological system. In addition, metabolomics is an important part of the Systems Biology concept, i.e. the study of a biological system as an integrated system of genetic, protein, metabolite, cellular, and pathway events that are in flux and interdependent. The metabolome, the whole set of metabolites, is closest to the phenotype, and is therefore of increasing interest in medical research. Metabolites can be transported from certain cells and tissues to body fluids (blood, urine, saliva, tears etc), which gives opportunities for the discovery of various kind of biomarkers, e.g. prognostic markers, disease progression markers, safety markers, responder/non-responder markers, efficacy markers. In addition, system knowledge can be obtained and applied for target identification and prioritization and/or translation of the mechanism of action of a drug from animal to humans, and vice versa. For example, the onset of multi-factorial diseases can be the result of subtle changes in the communication and regulation network, in which metabolites play an important role.

In this course some state-of-the-art analytical techniques (GC-MS, LC-MS, CE-MS and NMR) will be discussed. A special focus will be given to lipids, also called lipidomics. Attention will be paid to data pre-processing, data analysis and knowledge extraction. Applications of metabolomics in various biological areas will be used to illustrate the possibilities and current challenges. Trends and future perspectives will be discussed.

3.3 Proteomics and peptidomics

Course leader(s): R. van der Heijden (Leiden), A. Wilderspin (London), and P. Andren (Uppsala)
Duration: 2 days
Level: Basic

Proteomics is the process of identification of all proteins expressed in a given cell or tissue. Sensitive and accurate measurement and identification of complex mixtures of proteins and peptides can be made using high-resolution protein separation techniques and identification by mass spectrometry. This allows an insight into the expression of proteins in complex biological systems under a variety of circumstances. For example, a normal cell can be compared with a diseased cell such as a tumor cell. The course will cover sample preparation and fractionation; theoretical and practical aspects of protein purification, crystallization, characterization and production; separation techniques such as two-dimensional gel electrophoresis and multidimensional liquid chromatography; mass spectrometry methods such as MALDI imaging, quantitative proteomics; bioinformatics; and different proteomics applications.

The aim with the course is to introduce the students into the field of proteomics and peptidomics, protein purification and characterization. The course is aimed at researchers with a background in biology and/or chemistry who wish to gain an insight into the most up-to-date methodologies used in proteomics.

Participants should have at least an intermediate level of biological and chemical knowledge.

Theme 4: Drug delivery systems

4.1 Protein pharmaceuticals: formulation and analysis

Course leader(s): W. Jiskoot, M. Sutter (Leiden), and M. van de Weert (Copenhagen)
Duration: 1 day
Level: Basic

With the advent of recombinant DNA and hybridoma technologies, the production of large amounts of highly pure recombinant human proteins has become possible. This has led to the introduction of a large number of therapeutic proteins for the treatment or prophylaxis of chronic and life-threatening diseases. The clinical importance of therapeutic proteins is still rapidly increasing.

Because of the structural complexity of proteins, pharmaceutical development requires approaches that differ in several aspects from the ways low-molecular-weight drugs are developed. Particular development challenges arise from the chemical and physical instability of proteins, their large molecular weight, and their often unfavorable pharmacokinetics. Moreover, practically all therapeutic proteins can cause immune reactions, which (apart from vaccines) is generally unwanted.

This course will give an introduction to pharmaceutical aspects of therapeutic proteins. Challenges in formulation, characterization, and delivery of this interesting class of drugs will be addressed in detail. Furthermore, strategies for avoiding immunogenicity will be presented.

4.2 Colloidal and cyclodextrin-based drug delivery system, basic concepts

Course leader(s): D. Duchene, D. Labarre, and C. Vauthier (Paris)
Duration: 1 day
Level: Intermediate

This course will introduce the colloidal and cyclodextrin-based drug delivery systems. After administration of a drug, its distribution depends upon its physicochemical characteristics. These result in distribution towards pharmacological target but also towards other tissues in which the presence of the drug can induce side effects. The aim of drug targeting is to increase distribution to specific tissues where it is needed. It can be obtained by using drug carriers systems. Thus distribution of the drug is rendered dependent on the characteristics of the carrier. Another role of drug carriers is to make possible distribution of "difficult" or fragile drugs.

Drug carriers can be classified into water-soluble drug conjugates and colloidal particulate systems, including liposomes, solid lipid nanoparticles, micelles, nanospheres, nanocapsules and polyplexes. Cyclodextrins constitute an interesting class of drug carriers, as inclusion complexes are easily obtained, and properties of their derivatives can make possible many possibilities.

The aim of the course is to present the different types of drug carriers, to show how to make and characterize them, to choose the best carrier adapted to a drug and to the route of administration, and finally to introduce the biological environment with which they interact and which determines their *in vivo* fate. Conventional applications of the different drug carrier systems in pharmacy and cosmetics will be presented.

4.3 Specific applications of colloidal and cyclodextrin-based drug delivery systems

Course leader(s): G. Barratt, A. Bochot and E. Fattal (Paris)
Duration: 1 day
Level: Advanced

The aim of the course is to illustrate the use of colloidal and cyclodextrin-based drug delivery systems by some recent developments.

Discussion will start with several applications in cancer treatment. Anticancer agents are one class of drugs for which drug delivery systems can offer a considerable advantage, since these molecules which interfere with cell division will also affect normal cells and produce side-effects. Delivery systems can give more specificity by re-routing the drug from sensitive sites and allowing better accumulation in the tumour. In particular, long-circulating carrier systems can accumulate in solid tumours by the "enhanced permeation and retention" effect. Further specificity can be achieved by targeting delivery systems to particular cell-surface ligands. Examples of targeting with antibodies and with small molecules will be given, as well as ones of physical targeting with magnetic particles.

The applications of colloidal carriers to the delivery of nucleic acids (antisense oligonucleotides and siRNA) will be discussed next. Vesicular systems (liposomes and nanocapsules) are able to protect these drugs against degradation and to enhance their intracellular penetration. Nucleic acids can also be delivered as complexes to cationic lipids and polymers as well. The efficiency of such systems will also be discussed.

New applications of cyclodextrins in dispersed systems will be discussed last: Emulsions, Beads, Microcapsules and Microspheres, Nanoparticles: Polymeric nanoparticles, Self-assembled polymeric nanoparticles, Amphiphilic cyclodextrin nanoparticles and Liposomes.

4.4 Oral, inhalation and transdermal drug delivery systems

Course leader(s): P. Colombo, P. Santi et al. (Parma)
Duration: 1 day
Level: Advanced

Drug delivery is considered an unavoidable element of every new medication. Today no new product comes out without its own delivery program. Oral delivery still remains the most frequent way of drug administration and drug delivery systems based on hydrophilic matrix technology are considered the most reliable.

The known biopharmaceutical constraints of orally administered drug products could be less stringent with other preparations such as inhalation, which however introduce other constraints linked to dose delivery and lung deposition. Inhalation dosage forms are unique since the combined formulation and delivery device constitutes the product since both affect the bioavailability of inhaled drug. For a limited number of substances the transdermal delivery is an option capable to prolong the control of drug level into body. For this reason transdermal delivery gained a large success in pharmacotherapy.

Here, the oral delivery through matrix system, the transdermal using new generation of patches and the pulmonary inhalation are illustrated, focusing on swelling for oral matrix, on permeation for transdermal and on aerodynamic behavior for the aerosols. Deep attention is given to metered dose inhalers (MDIs) and to dry powder inhalers (DPI). Nasal drug delivery is treated as well with respect to the variables affecting the efficiency of administration.

Theme 5: Drug transport and drug targeting

5.1 Drug transport to the brain

Course leader(s): A. de Boer (Leiden)
Duration: 1 day
Level: Basic

The brain is an intriguing organ that attains more and more interest in drug development due to the need for treatment of disorders related to the brain, like Alzheimer's disease, or for avoidance of side effects from the CNS. The blood-brain barrier (BBB) can be considered to be an organ of its own, regulating the flow of nutrients, waste products and drugs between the blood and the brain tissue. The properties of the BBB make drug development be difficult for targeting the brain tissue, in part due to the presence of active efflux transporters. There is also today a large interest in finding good, rapid methods to study brain drug delivery.

The aim of the course is to give knowledge on basic aspects of blood-brain barrier (BBB) function, transport of substances across the BBB, as well as on in vitro and in situ methods, as well as some information on in vivo methods to study this transport. The course contains lectures and training in the design of experiments on BBB transport. Participants are invited to bring ideas and examples to the course for discussion. After the course the participant will increase his/her ability to discuss physiological and methodological issues in BBB transport of drugs.

Participants should have basic knowledge on physiology and pharmacokinetics.

5.2 Methods in studies of drug delivery to the brain

Course leader(s): E. de Lange (Leiden) and M.Hammarlund-Udenaes (Uppsala)
Duration: 1 day
Level: Basic

The brain is an intriguing organ that attains more and more interest in drug development due to the need for treatment of disorders related to the brain, like Alzheimer's disease, or for avoidance of side effects from the CNS. The blood-brain barrier (BBB) can be considered to be an organ of its own, regulating the flow of nutrients, waste products and drugs between the blood and the brain tissue. The properties of the BBB make drug development be difficult for targeting the brain tissue, in part due to the presence of active efflux transporters. There is also today a large interest in finding good, rapid methods to study brain drug delivery.

This course can be taken as a continuation of Course 5:1 or on its own. The course will address more in vivo methods for studies of drug delivery to the brain. How can drug delivery to the brain be measured in vivo? Which techniques are suitable in early and later drug development? What are the advantages and disadvantages with different methods and what do they really measure? Which measurements should be made to answer questions? The main focus will be on in vivo methods like microdialysis and PET. Participants are invited to bring ideas and examples to the course for discussion.

After the course the participant will increase his/her ability to discuss physiological and methodological issues in BBB transport of drugs. They will become more aware of the effects of drug influx and efflux across the BBB on drug concentration-time profiles in the brain, and will have been trained to design an experiment to study BBB transport.

5.3 Drug delivery across the skin

Course leader(s): J. Bouwstra (Leiden) and P. Santi (Parma)
Duration: 1 day
Level: Intermediate

On first inspection, the skin does not seem to be the most favorite administration site for drugs, due to its strong barrier properties minimizing the exchanges with the external environment. However, if it is possible to overcome the skin barrier, transdermal administration has many important advantages. It is non-invasive, circumvents drug degradation and inactivation by the G.I. enzymes and constant plasma levels can be achieved over a long period of time.

It is well accepted that, without penetration enhancement, only low molecular weight and medium lipophilic molecules can cross the skin in therapeutically active amounts. For this reason several approaches have been developed successfully to increase the transport rate across the skin.

This course will deal with the structure of the skin, the mechanisms involved in skin penetration and the state of the art concerning enhancement procedures that can be employed to overcome the skin barrier properties. The enhancement methods that will be presented are the use of penetration enhancers, iontophoresis, carrier systems and microneedle arrays. Finally the future prospective of transdermal delivery will be discussed.

Theme 6: Preclinical drug development and safety evaluation

6.1 Drug Metabolism: interindividual variability and consequences for drug safety

Course leader(s): J. Commandeur (Amsterdam) and M. Lang (Uppsala)
Duration: 2 days
Level: Intermediate to advanced

This two-day course will consist of several supportive lectures which will provide participants with the basic knowledge necessary for the case-study. The latter will be carried during the rest of the time. The course will conclude with a plenary discussion of the findings of the case studies.

The role of drug metabolism in pharmacokinetics, drug failures due to pharmacokinetic defects, interindividual variability, bioactivation to pharmacologically active or toxic metabolites, general characteristics of drug metabolising enzymes, including: reactions, substrate selectivities, multiplicity, regulation (enzyme induction and tissue expression), genetic polymorphisms, computational and experimental approaches to study drug metabolism, tools to identify enzymes involved in drug metabolism, vitro-vivo extrapolations, high-throughput screening methodologies.

Groups of 3-4 participants will be provided with cases concerning drugs known to cause rare ('idiosyncratic') but fatal adverse drug reactions. Each case consists of a collection of experimental data which are illustrative for the various experimental approaches to study or to predict drug metabolism and bioactivation. From these data, the participants should try to elucidate which factors determine which patients will be susceptible to adverse drug reactions.

The aim and objectives of this course are to introduce the participants to: the roles (i.e. bioactivation/bioinactivation) drug metabolism plays in drug discovery and drug development, the recent advances in the knowledge of drug metabolizing enzymes, and various experimental approaches used drug metabolism studies.

Participants should have a basic knowledge of chemistry, biochemistry and drug metabolism. Basic knowledge of enzyme kinetics and some in vitro techniques is also advisable.

6.2 Cell signaling and drug safety I

Course leader(s):	B. van de Water and J. Meerman (Leiden)
Duration:	1 day
Level:	Intermediate

The balance between the pharmacological activity and the unwanted adverse drug reaction (ADRs) determines the safety margins of drugs. Generally the drug safety evaluation in pre-clinical models reflects the human situation. However, approximately 25% of all human adverse drug reactions (ADRs) are not predicted preclinically. This results not only in an economic loss but also impacts on social aspects including hospital admissions and patient discomfort. Therefore any future (bio)-pharmaceutical scientist should have a basic understanding of toxic stress response reactions, to allow for better and safer drug development. Many attrition-linked ADRs involve the low-level formation of reactive intermediates. Moreover, the immune system plays a central role in the exacerbation of such toxic responses. This is related to production of cytokines by damaged cells. Reactive metabolites may bind various macromolecules in cells, including lipids, proteins and DNA, which eventually may result in severe cell injury. Cells respond to this injury by activating a variety of signal transduction pathways that enable the recovery from the chemically inflicted cell damage or may ensure the onset of cell death. Over the last two decades much is known on the cellular signal transduction cascades that regulate cellular responses to injury/stress. This knowledge has a major impact on the field of toxicology and allows a better understanding of molecular mechanisms of cytotoxicity.

In this course concepts of cellular stress responses induced by pharmacological agents in the context of adverse drug reactions are introduced. This includes an overview of the different types of cytotoxicity, the methods to identify cytotoxicities, the signal transduction routes involved in the onset of adverse drug reactions, and the role of such signal transduction pathways in either the onset of toxicity or recovery from the initial insult, including the regulation of the immune system. **This course is essential before entering course 6.3.** The course will be in the format of lectures that are given in the morning. In the afternoon, there will be case-studies of how to apply state-of-the-art molecular and cellular biological approaches to unravel mechanisms of cytotoxicity. A round-table discussion will finalize the day.

6.3 Cell signaling and drug safety II

Course leader(s): J. Meerman and B. van de Water (Leiden)
Duration: 1 day
Level: Advanced

The last two decades of biological research have witnessed dramatic progress toward understanding the molecular basis of life. Technological advancements have resulted in various (gen)-omics techniques and high throughput methods that have enabled molecular biological databases to grow at rates never seen before. This also has had a large impact in Toxicology. Much more has become known about intracellular signaling pathways that are activated or shut-down during toxic insult. This data is currently exploited on a large scale for making new (automated) classifications for toxic compounds based e.g. on gene expression profiles rather than on organ specificity, LD₅₀, or chemical structural alerts. There is also a promise towards detecting cellular toxicity profiles that are universal and valid beyond the cell type. Already it has resulted in a better understanding of the relationship between exposures to toxins and human disease susceptibility and the identification of useful biomarkers of disease and exposure to toxic substances. Another focus is on detecting gene polymorphisms characteristic of an increased susceptibility to the pathology of environmental health diseases and adverse drug effects. As a future perspective, compounds may be selected with a gene expression profile that is opposite to that of a toxic compound, which may possibly be used as antagonists of the toxin.

Course 6.2 is a requirement for course 6.3 and provides an introduction on topics such as cellular stress responses and signaling pathways activated by toxicity. Course 6.3 is an advanced course and will introduce DNA microarrays and (phospho-)proteomic technologies as methods to understand mechanisms of toxicity as well as to provide predictive tools for adverse drug reactions. Moreover, bio-informatics tools for data mining and establishing correlations between databases with information on gene expression profiles, chemical compounds and diseases will be discussed. This will be in the format of lectures that are given in the morning. In the afternoon, there will be case-studies of applications and progress in toxicology of large-scale data production and mining in cell signaling, that will be discussed in a round-table session at the end of the day.

Theme 7: Clinical drug development and PK-PD modeling

7.1 Introduction in clinical drug development

Course leader(s): Z. Diamant and A. Cohen (Leiden)
Duration: 1 day
Level: Intermediate

Clinical drug development involves several phases of *ex vivo* and *in vivo* intervention trials each with a specific study protocol. In addition, each development phase (I-IV) has specific requirements, in terms of (the right) subject population in combination with local expertise, employment of standardized procedures according to good clinical practice (GCP), and other onsite skills such as data management and analysis. Another mandatory aspect is a supportive infrastructure, requiring an active Committee of Medical Ethics (CME), a scientific board, a dedicated pharmacy, and other specified research facilities or labs. Beyond these more or less local requirements, national (or in multi-center trials: international) legislation is a major determinant in the process of clinical drug development.

In this course we aim to discuss several characteristics and procedures of clinical drug development, including the set up of a study protocol, timelines, logistics, sponsoring, including the ethical and legal aspects. The practical part will consist of composing an informed consent, based on a study synopsis.

7.2 Introduction to pharmacokinetics

Course leader(s): M. Danhof and E. de Lange (Leiden)
Duration: 1 day
Level: Basic

This course will deal with the basic concepts of pharmacokinetics and its application in drug discovery, drug development and clinical practice. Following a general introduction we will focus on the pharmacokinetics following intravascular administration. Here the concepts of the primary pharmacokinetic parameters volume of distribution and clearance will be introduced followed by a discussion of elimination rate constant and elimination half life. Next the course will focus on pharmacokinetics after extra vascular administration focusing on the concepts of bioavailability and bio-equivalence. This will then be followed by a discussion of the pharmacokinetics of intravenous infusion and repeated administration in relation to the pharmacokinetic optimization of dosing regimens. The course will be concluded with a discussion of inter-individual variability in pharmacokinetics.

7.3 Introduction to mechanism-based PK-PD modeling

Course leader(s): M. Danhof and O. Della Pasqua (Leiden)
Duration: 1 day
Level: Intermediate

In recent years important progress has been made in the field of pharmacokinetic-pharmacodynamic (PK-PD) modeling. Specifically, mechanism-based PK-PD models have been developed with much improved properties for extrapolation and prediction. Meanwhile mechanism-based PK-PD modeling concepts are increasingly applied in drug discovery and development.

Mechanism-based PK-PD models differ from traditional descriptive models in that they contain specific expressions to characterize processes on the causal path between drug administration and effect. This includes expressions for the pharmacokinetics, the target site distribution, the target binding and activation, the transduction and homeostatic feedback mechanisms. Finally also the effects on disease processes and disease progression are described.

This course will deal with the *theoretical aspects* and the *applications* of mechanism-based PK-PD modeling. The applications include the pre-clinical to clinical extrapolation of PK-PD, the application of PK-PD in early clinical development and in the design and evaluation of phase-3 clinical trials.

7.4 Determinants of drug response and variability in early clinical development

Course leader(s): O. Della Pasqua (Leiden)
Duration: 1 day
Level: Intermediate

The objective of this one-day workshop is to provide an overview of how PKPD modeling can be used to characterize sources of variability in clinical drug development and support decision-making on study design and dose selection.

The course deals with:

- Issues in Drug Development Strategy
- Determinants of Drug Response
- Drug-receptor interaction & *In vivo* signal transduction
- Dose versus concentration Delivery Rate as determinant of drug response
- Linking pharmacokinetics, pharmacodynamics and disease
- Intrinsic & Extrinsic Determinants of Variability in Drug Response
- Time-dependent processes
- Disease Progression
- Compliance and Drug-drug interaction

At the end of this training course, participants will understand the determinants of drug response, and how the interaction between pharmacokinetics, pharmacodynamics and delivery affects therapeutic response. They will be able to identify and understand determinants of variability, and will know now the principles of (population) pharmacokinetic-pharmacodynamic modeling.

Participants are required to have a basic knowledge of pharmacokinetics, pharmacology and physiology.

Theme 8: Pharmaceutics and pharmaceutical technology

8.1 The Role of Thermal Methods in the Design and Development of Medicines

Course leader(s):	Simon Gaisford (London)
Duration:	1 day
Level:	Intermediate

Modern medicines contain numerous components (an ibuprofen tablet, for instance, contains around 17 individual ingredients) and each plays a significant role in the performance of the product. It is thus not easy to ensure the stability of such a formulation, because it must be ensured that each component is stable on its own and also in combination. These processes can be classified as chemical changes.

Furthermore, the physical form of a material is often critical to the performance of the final product. An example would be ensuring the correct polymorph (crystal form) of a drug is manufactured. Increasingly, the role of amorphous materials in pharmaceuticals is being understood (and exploited). Again, controlling the physical form of a material through manufacture, quantifying its presence and demonstrating its stability is central to getting a license to produce a new medicine. These processes are classified as physical changes.

Few analytical tools are as adept at studying chemical and physical change as thermal methods (specifically calorimetric methods, such as differential scanning calorimetry and isothermal calorimetry); because of this, thermal methods are widely used at all stages of the development and manufacture of a medicine. This course will look at a variety of these roles, using real case examples to highlight applications and underpinning all discussion with the theory of instrument design and operation. Although the subject matter is focused primarily on design of medicines, the principles learnt will apply to any type of sample.

8.2 Process analytical technology

Course leader(s):	J. Rantanen (Copenhagen) and G. Frenning (Uppsala)
Duration:	1 day
Level:	Intermediate

Getting relevant information from multi-component systems, such as pharmaceutical unit operations, is not a straightforward task. Consider a typical solid dosage form with numerous sequential processing steps. There are many possible pitfalls during processing that may critically affect the final product performance. Focusing analysis on the end product will not enable the early detection of problems or further, the complex relations between them. Process analytical technology (PAT) is a system for developing and implementing new efficient tools for use during pharmaceutical development, manufacturing, and quality assurance while maintaining or improving the current level of product quality assurance.

This course will introduce recent developments in utilization of PAT in manufacture of solid dosage forms. Students will also work in the groups and summarize the PAT elements of selected publications.

Those attending are expected to prepare themselves by reading the following three documents: [1](#), [2](#), and [3](#)

Theme 9: Novel concepts of drug treatment – CNS disorders

9.1 Translational research in neuropsychopharmacology

Course leader(s):	M. Oitzl (Leiden) and F. Nyberg (Uppsala)
Duration:	1 day
Level:	Basic

Part 1: The stress system and glucocorticoids: from emotion and cognition in animals to humans.

Stress represents a crucial health and welfare problem for Western societies. Its economic burden already induces costs as high as 3-4 % of the European gross national products. In addition, there are unfavorable stress effects on social behavior, mood, learning and memory, which are difficult to quantify with economic means. During the last years our knowledge of stress mechanisms has grown substantially, but much regarding the psychoneuroendocrinology of stress remains to be elucidated.

Cognition, emotion and other behaviors are targets of glucocorticoids and other stress hormones. Research in animals allows to dissect cause from consequence and detect underlying molecular mechanisms. Animals provide the possibility to (i) define the stress event, (ii) study the emotional and cognitive consequences in time-lines; e.g., early life events, (iii) examine concomitant molecular changes in the brain and (iv) detect targets for therapy. What are the contributions of genetic and environmental factors determining resilience or vulnerability to stress? Which therapeutic (pharmacological) tools do we have to counteract or prevent stress?

This course deals with the translation of animal models to human conditions – and *vice versa*. Following aspects will be highlighted and covered in interaction with the students: stress and (i) multiple memory systems; (ii) genetic markers of aggression; (iii) polymorphism of the stress-receptors.

Part 2: Mechanisms involved in drug addiction - The brain reward system: Animal experiments translated into human/treatment of addictive diseases

The past years have seen an increase in abuse of addicted drugs among adolescence. Also, a number of new drugs have been introduced on the illegal market. Many of these drugs are taken in combination with other addictive drugs and this has led to severe problems in the society. An important target for addictive drugs is the brain reward system and studies have revealed that one drug may sensitize the reward system for other drugs of abuse.

This part of the course is directed to the description of the function of the mesocorticolimbic reward system, dealing with molecular mechanisms essential for its function. It also relates the importance of stress and cognition to the development of drug addiction. The impact of abuse of anabolic androgenic steroids as a gateway to addiction of alcohol and narcotics will also be highlighted, as well as adverse effects observed while combining androgenic steroids with central stimulants. Data obtained from studies carried out in experimental animal will be translated to human and new strategies for the treatment of addicted diseases will also be described.

9.2 Silent pharmacogenomics: Gene silencing using siRNA

Course leader(s):	E. Vreugdenhil, C. Fitzsimons and R. De Kloet (Leiden)	Duration:	1 day
Level:	Intermediate		

Years 2005 and 2006 have been difficult for pharmaceutical industry. Notably, some of the few achievements accomplished have been in the field of RNA interference. The use of small-interfering (si)RNAs is a very promising strategy to circumvent serious drawbacks of current methods of gene silencing. siRNAs are capable of down-regulating gene expression in a highly selective fashion without affecting other closely related genes. Beyond its use as target-validation tool, a great lot of attentions is currently been put on the development of siRNA into a completely new class of drugs. Though its *in vivo* application is still in its infancy, it is generally anticipated that **(I)** the specificity of siRNA-based drugs will superior to that of conventional drugs, **(II)** its potency will be higher than for example antisense DNAs, **(III)** its window of applicability will be much broader than that of conventional drugs. However, appropriate delivery has remained the biggest issue for its therapeutic application. siRNA molecules pass poorly lipid-rich cell membranes and transfection agents, optimized to deliver siRNA molecules to cultured cells, are toxic.

In this course the various concepts and aspects of the use of siRNA molecules in pharmaceutical research will be discussed. Different aspects and concepts regarding RNA interference and its applications will be highlighted in the form of seminars in the morning. At the end of the morning, different questions/tasks will be formulated by the teachers. Participants will be divided in groups of four and each group will address a specific question/task. Based on literature handouts, each group of participants will prepare a 15 minute oral presentation in which a particular topic/question will be discussed.

The course aims to give insight in follow-up strategies of pharmacogenomic experimentation, into the possibilities of silencing gene expression, to understand the action of siRNA application, and to discuss the possibilities of using siRNA as a drug.

9.3 Central pain mechanisms

Course leader(s):	F. Nyberg (Uppsala)
Duration:	1 day
Level:	Basic

During the past decade research directed to endogenous mechanisms for pain perception and modulation has seen a significant progress. Clarification of mechanism underlying the action of opiates and other analgesic compounds has contributed new knowledge essential for the development of rationales for the treatment of various pain disorders.

This course is intended to deal with neuronal mechanisms of pain sensation and analgesic drugs. It includes description of basics with regard to nociceptive afferent neurons and mechanisms modulating the nociceptive pathway. Furthermore, it addresses the gate control theory, descending inhibitory control and highlights chemical mediators in nociceptive pathways. A particular focus is directed to neurotransmitters and neuromodulators in pain processing pathways (e.g. tachykinins and opioid peptides).

A certain part of this course will be directed to pain diseases and pain managements. Pharmacology of opioid analgesics and also to genetic factors underlying response to opioid and vulnerability to pain disorders will be highlighted. In addition to attending lectures the students are supposed to work with particular tasks related to treatment of various pain disorders.

Theme 10: Novel concepts of drug treatment – Cardiovascular disorders

10.1 Modulation of lipoprotein metabolism for cardiovascular disease prevention

Course leader(s): T. van Berkel (Leiden) and F. Bernini (Parma)
Duration: 1 day
Level: Intermediate

The incidence of atherosclerotic related cardiovascular diseases (CVD) in the industrialized countries represents a medical and social problem of wide relevance. Since altered lipoprotein plasma levels represent a common risk factor for atherogenesis, target for CVD prevention is to correct conditions of dyslipidemia.

Beyond the classical treatment with HMGCoA reductase inhibitors, total and LDL-cholesterol reduction can be achieved by the block of the intestinal transporter NPC1L1 or ACAT enzyme thus reducing cholesterol absorption. Moreover the raise of HDL has emerged as a novel, promising approach able to stimulate the antiatherogenic processes named reverse cholesterol transport (RCT). Whereas fibrates and nicotinic acid have been used for years, new classes of compounds affecting different steps of RCT are currently under investigation. Among them, LXR activators, CETP inhibitors, inhibitors of lipases.

Aim of the course is to describe the role of genes/proteins in lipid metabolism and RCT and the effects of their pharmacological modulation on cellular cholesterol homeostasis and atherosclerosis development. This course will provide knowledge on both basic science and clinical applications for the prevention of cardiovascular disease through the modulation of lipid and lipoprotein metabolism.

Note: This course can not be taken without taking course **10.2:** Identification of novel therapeutic targets for atherosclerosis.

10.2 Identification of novel therapeutic targets for atherosclerosis

Course leader(s): T. van Berkel (Leiden) and F. Bernini (Parma)
Duration: 1 day
Level: Intermediate

Atherosclerosis is the major process underlying cardiovascular disorders, and accounts for the majority of mortality and morbidity in the western world.

This course will give more insights into the mechanism and ontogenesis of vessel narrowing (atherosclerosis). Special emphasis will be put on the contribution of inflammatory processes and lipid fluxes in the development of atherosclerotic lesions. Also the clinical end-points of atherosclerosis will be discussed. This course will provide knowledge on both basic science and clinical applications for the prevention of cardiovascular disease through the modulation of lipid and lipoprotein metabolism. Therapeutic targets will be defined and future approaches will be discussed.

The Program includes lectures on atherosclerosis in general, on the role of macrophages, on Inflammation in atherosclerosis, on plaque stability and infarction on HDL and reverse cholesterol transport, and on modulation of lipoprotein metabolism and atherogenesis.

Note: Course 10.1 is a requirement for taking course 10.2

Theme 11: Novel concepts of drug treatment – Cancer

11.1 Novel classes of drugs for anti-cancer chemotherapy

Course leader(s): E. Danen (Leiden)
Duration: 1 day
Level: Intermediate

Cancer is a major world-wide health problem. Cancer development and progression involves the accumulation of genetic lesions ultimately leading to uncontrolled growth, angiogenesis, tissue invasion, and metastasis. Through surgery, radiotherapy, and chemotherapy several types of cancer can be cured to date. However, there are still many examples of cancers that escape current therapeutic strategies. Therefore, big investments are being made in finding novel drug targets and developing novel anti-cancer drugs.

In the first part of this course scientists and clinicians working at the forefront of clinical cancer research will discuss major novel classes of anti-cancer drugs that are tested in pre-clinical models or have already entered clinical trials. These will include multiple tyrosine kinase inhibitors, Farnesyl-Transferase inhibitors, Statins, and anti-angiogenic drugs. Some of these therapeutic strategies are highly promising whereas others have proven disappointing or problematic in patients. The possibilities to improve their effectiveness will be discussed.

In the second part of the course we will move to basic tumor cell biology and explain how the above-mentioned strategies target overlapping signal transduction cascades. We will then discuss how advances in basic cancer research can lead to the discovery of novel drug targets. Here, we will also enter the laboratory and see examples of innovative *in vitro* and *in vivo* pre-clinical cancer models, including various advanced imaging systems.

11.2 Apoptosis in drug discovery and safety

Course leader(s): F. Nagelkerke and B. van de Water (Leiden)
Duration: 1 day
Level: Intermediate

This course aims to instruct on:

- how molecular mechanisms of cell death work in relation to cytotoxicity
- the complexity of regulatory networks in cell biology (apoptosis as an example)
- how defects in these networks may result in disease
- how basic science leads to the identification of potential therapeutic targets
- how to define molecular targets for therapeutic intervention of apoptosis
- various strategies to target the apoptotic proteins
- how to apply obtained knowledge in paper evaluation and discussion

The goal of the afternoon projects during the course is to use the information on molecular mechanisms of apoptosis obtained in the morning lectures, to define and discuss possibilities to utilize proteins that are part of the apoptotic machinery or (in)directly regulate the onset of apoptosis, as drug targets to modulate apoptosis *in vivo*.

As has been discussed in the morning session there are several important groups of proteins that can be used as drug targets to modulate apoptosis in disease. We will discuss caspases, Bcl-2 family members, death receptors, and p53.

Each group will receive a set of papers that is focused around one of the above proteins. After briefly evaluating the documentation the group should discuss the findings. This discussion could include mechanism of action, upstream and downstream in the apoptosis cascade, targets and target sites, disease application, pro/cons (long-term/short-term therapy) and dosage forms, etc. Each group should prepare a brief presentation (15 min) of their findings. These presentations will be followed by a general discussion at the end.

Theme 12: Medicines in children and the elderly

12.1 Development of medicines for use in children

Course leader(s):	C. Knibbe (Leiden), C. Tuleu (London), and J. van den Anker (Washington)
Duration:	1 day
Level:	Basic

In order to define effective and safe dosing regimens for children of different ages, detailed information is needed on the pharmacokinetics (the drug-concentration versus time profile) and pharmacodynamics (the drug-concentration versus effect relationship). Both the pharmacokinetics (PK) and pharmacodynamics (PD) may differ between children and adults and between children of different age groups and between children in a different state of health. Differences in PK may be caused by differences in absorption, distribution, metabolism (as a result of differences in genetic background and maturation of drug metabolizing enzymes) or excretion. Differences in PD can be the result of differences in receptor expression and target tissue sensitivity at different ages and/or in different diseases.

The design of clinical trials in children should therefore consider variability in both PK and PD simultaneously. Properly designed studies in children, however, are difficult to perform. Specific problems are the availability of limited patient numbers and restrictions with regard to the volume and the frequency of blood sampling. The application of the so called 'population' approach opens new avenues for drug research, especially for children. Population pharmacokinetic and pharmacodynamic modeling involves the application of Nonlinear Mixed Effects Modeling to data from a group of patients, to obtain detailed information on the intra- and inter-individual variability in PK and PD of a drug.

This course will deal with all these aspects of designing and conducting pediatric clinical pharmacology trials including pediatric formulation aspects and also will focus on the new EU regulation on pediatric drug research.

Theme 13: Scientific communication

13.1 Scientific writing, a framework for writing a scientific paper

Course leader(s): R. Watt (London) and S. Ghouti (Paris)
Duration: 1 day
Level: Intermediate

The aim is to give an understanding of the requirements to have a scientific paper or abstract published in the scientific press and to give practice in developing the writing skills to meet these requirements.

The course consists of interactive lectures, exercises in writing and group discussion sessions to critique published abstracts. The topics include: practical exercises in writing abstracts, writing an introduction, describing methodology, presenting data in tables and figures, writing a summary of a participant's project and getting a list of references in the required style. There are group discussions on what makes a good (and bad) paper or abstract.

It is preferable, but not essential, that participants have written a conference abstract before attending the course. Participants should bring with them an example of a well written an informative paper in their field and be able to give reasons for its selection.

13.2 Presentation skills

Course leader(s): Ian Bates (London)
Duration: 1 day
Level: Basic

The course will provide an opportunity to improve and develop skills in data interpretation and presentation. The seminar will address and engage the abilities of the individual and provide practice in scientific presentation skills, for both small and large audiences.

Topics to be covered:

- a) Evaluation of technical language and material.
- b) Distinction between comment, opinion and fact
- c) The oral format for presenting data.

The course will offer a series of opportunities to discover and discuss. Your colleagues on the course will have differing views about writing and data presentation; to share these within the seminar group will be a valuable exercise.

There will be guidance provided on presentation skills and media selection, but remember that these are skills, and can only be understood and improved by guided practice. We will examine ways to improve data illustration and simplify the presentation of research results. Later sessions will focus on oral presentation skills, with group work directed towards presenting material.

The day is designed for the participants to develop their presentation skills. You may already have well developed skills - this is a good thing and you can share your insights with others in addition to having the opportunity to concentrate on more subtle aspects. Or you may feel you do not have sufficient skills in this area - in which case you will have the opportunity to develop some insight into this area of communication in a non-threatening environment.

13.3 Teaching and learning skills

Course leader(s): Ian Bates (London)
Duration: 1 day
Level: Basic

The aim of the seminar is to provide an opportunity to develop skills and understanding of curriculum design and development, learning methods and effective teaching.

Objectives of the Course:

- to enable a participant to describe the principal features of effective learning designs.
- to enable participants to discuss the successful determinants of a teacher, tutor or mentor.
- to enable participants to relate learning objectives with learning methods and learning assessment methods.
- to facilitate participants to exchange experience which can be utilized by others.

Topics to be covered:

a) Curriculum design and development.

The design of any course (from 3 hours to 3 years) is dependent on the successful design of the "curriculum" for the course, which paradoxically tends to be overlooked or even omitted in many courses! This session will examine curriculum design from a theoretical and pragmatic viewpoint, drawing on the experiences of the tutors.

b) Learning objectives and learning methods.

Discussing methods to construct effective learning objectives, and examine the relationship with learning objectives and effective teaching methods and assessment protocols.

c) Effective teaching

The learner, the teacher, teaching processes, and outcomes.

d) Problem based learning methods.

An examination of the nature of problem based learning and application to vocational courses.

Theme 14: Health policy

14.1 European patenting and intellectual property rights (IPR)

Course leader(s):	M. Johansson (Uppsala) and AM Lademann (Denmark)
Duration:	1 day
Level:	Basic

Traditionally, patenting within the pharmaceutical field is of utmost importance in order to ensure a return of investment of the research and development costs invested by the medicinal industry. Therefore, the course will deal with the importance of identifying patentable developments in the daily research and development work, and it will deal with patenting as a strategic tool at management level. This course will provide the participants with an overview of the basic principles for patenting especially within the pharmaceutical field.

The course will also provide the participants with an overview of important legal aspects related to IPR such as laboratory notebooks, data protection, extension of patent term, experimental use, etc.

At the end of the course the participants will have a general knowledge of:

- The basic principles for patenting and claims drafting
- How to read and use the patent literature in research and development

The importance of avoiding infringing others' patent rights

The importance of ensuring (at a very early stage in the research and development work) the freedom to enter the market without infringing others' patent rights

The importance of an interaction between the different departments in a medicinal company with respect to the overall patent policy of the company