Drug development from natural products: Exploiting synergistic effects

Gudrun Ulrich-Merzenich*a, D Panekb, H Zeitlerb, H Vetterc & H Wagnerc

*aMedical Policlinic of the Rheinische Friedrich-Wilhelms-University of Bonn, Wilhelmstr. 35-37, D-53111 Bonn, Germany
bInternal Medical Clinic I (CETA) of the Rheinische Friedrich-Wilhelms-University of Bonn, Sigmund-Freud-Str. 25, D-53227 Bonn, Germany
cDepartment of Pharmacy, Centre of Pharma Research, Ludwig-Maximilians-University, Butenandstr. 5-13, House B, D-81377 Munich, Germany

Drug development in phytomedicine has been focused in the past on the discovery and analysis of new structures from natural products. The search aimed at the determination of the single “active principle” in plants, based on the assumption that a plant has one or a few ingredients which determine its therapeutic effects. But traditional systems of medicines like Ayurveda, traditional Chinese medicine or the European phytotherapy generally assume that a synergy of all ingredients of the plants will bring about the maximum of therapeutic efficacy. This approach has for long been impossible to investigate since adequate methods to standardize complex plant mixtures as well as to rationalize complex mode of actions were lacking. The introduction of high throughput technologies provides the opportunity to determine profiles of plants and to systematically explore the mode of action of combinatory drug regimes. The present review highlights the concept of synergy and gives examples of synergistic effects of plant constituents. It elaborates on how the high throughput technologies can be used in drug development from natural products with the aim of creating evidence-based plant medications in prevention and treatment of different diseases in the form of new single treatments or new combinatory drug regimes while exploiting synergy-effects.

Keywords: High-throughput technologies, Phytomedicine, Synergy

Phytobotanical and ethnobotanical research have focused for decades mostly on the search for the single “active principle” in plants, based on the assumption that a plant has one or a few ingredients which determine its therapeutic effects. But traditional systems of medicines like Ayurveda, traditional Chinese Medicine (TCM) or the European phytotherapy generally assume that a synergy of all ingredients of the plants will bring about the maximum of therapeutic efficacy. This approach has for long been impossible to investigate since adequate methods to standardize complex plant mixtures (as drugs) as well as to rationalize the complex mode of actions were lacking. The recent development in synergy research and the omic-technologies have opened highly interesting perspectives for a new generation of phytopharmaceuticals. The present review is based on our recent articles1-3 updated with current data focusing on the concept of synergy, which has regained an increasing interest in drugs from natural resources for different reasons:

a) Large screening projects for the isolation and characterization of single bioactive compounds yielded only moderate results, e. g. The National Cancer Institute of the United States screened about 114,000 extracts from an estimated 35,000 plant samples against tumor systems4. Clinically significant cancer chemotherapeutic agents included Paclitaxel (taxol), topotecan and CPT-114. A provision for synergistic effects might elevate the yield.

b) The major burden to any health system is the treatment of multifactorial diseases with heterogenous disease courses (e.g. cardiovascular diseases, diabetes type 2). The efficacy of the monotarget therapies considering multitarget causes for these diseases is questioned.

c) Modern medical therapy presently uses more and more combination therapies in the treatment of several diseases like cancer, cardiovascular or rheumatic diseases often without observing the originally expected
cummulation of adverse events (ADS) of both single treatments. This has lead to an increasing interest in the synergy concept.

d) Ancient systems of medicine like the Ayurvedic and Chinese system provide an increasing amount of data demonstrating clinical efficacy in some of the known health challenges. The term “reverse pharmacology” has been coined by Patwardhan et al. and is proposed to be a new path for drug discovery.

e) The pre-clinical development of synthetic drugs has become extremely costly with costs calculated around $ 400 million.

f) The worldwide demand for phyto-pharmaceuticals grows steadily. In 2005 the herbal industry had a turnover of about US$ 62 billion. The world bank reports state that trade in medicinal plants, botanical drug products and raw materials is growing at an annual growth rate between 5-15 % (Ref. 7).

g) It is rather agreed upon that nature’s biodiversity has so far remained largely unexplored and thus still offers a tremendous potential.

h) The introduction of the “omic” – technologies as high-throughput methods opens the methodological possibility to investigate complex mixtures like whole plant extracts. They provide a new tool to investigate polyvalent pharmacological activity, synergy effects and thus multitarget treatments on a rational basis.

**Definition of synergy from a pharmacological perspective**

It is rather difficult to give an unequivocal universal definition for the term synergy effect. But the “isobole method” of Berenbaum seems to be one of the experimentally most convenient and also the most demonstrative method among all those so far proposed for the proof of synergy effects (Fig. 1); the x and y axes reflect the dose rates of the single individual components. Different dose combinations are investigated for the same effect. An isobole is understood to be a line or curve between points of the same effect.

- According to Berenbaum, the zero (0) – or additive interaction means that the effect of two substances a and b is a pure summation effect (equation 1).

- Correspondingly, the overall effect with antagonistic interaction is less than expected from the summation of the separate effects (equation 2). A convex curve will be obtained.

- With the existence of a real synergism with potentiated or over-additive effects, the overall effect of two drugs a and b that are applied together as a mixture must be larger than it would be expected by the summation of the separate effects. The result is then a concave curve (equation 3).

Equation 1: \( E (d_a, d_b) = E (d_a) + E (d_b) \)

Equation 2: \( E (d_a, d_b) < E (d_a) + E (d_b) \)

Equation 3: \( E (d_a, d_b) > E (d_a) + E (d_b) \)

E stands for observed effect; \( d_a \) and \( d_b \) are the doses of agents a and b.

In case of a synergism, lower amounts of agents a and b (doses=d) are necessary to achieve the effect. The achieved synergy effect can amount to doubling or even greater multiplication of the expected effect. Connected with the option of a dose reduction, it could be expected that at correctly chosen combination of a natural product (a) with a strongly effective synthetic product (b), the potential of side effects of agent (b) can be reduced simultaneously.

Mechanisms of synergy effects based on classical pharmacological, molecular biological and clinical work can be divided into at least following four mechanisms:

a) synergistic multitarget effects: natural products affect not only one target, but several targets and can cooperate in an agonistic and synergistic way;
b) pharmacokinetic and physiochemical effects: natural products improve solubility and/or the resorption rate and thereby the bioavailability;

c) interference with the resistance mechanisms of bacteria; natural product antagonize the resistance to antibiotics; and

d) elimination and neutralization effects; natural products by itself or after treatment (e.g. heating) eliminate or neutralize within a drug preparation or in combination with synthetic drugs adverse events, so that altogether efficacy improves.

Examples for each mechanism are given in Table 1. A list of herbal drugs with evidences for synergistic effects or polyvalent activities has been given earlier.

**Synergism in the context of the multitargeting approach**

Investigations of synergism as described by Beerenbaum (E(a,b)>Ea+Eb) have so far examined the combinatorial effects on one target at a single time point in one experimental setup. But the phenomenon of synergism of drug action in a human body or any living organism is likely to be a dynamic process and a multitarget phenomenon. Imming et al. already described that it ultimately would be desirable to move away from a static to a dynamic target definition of drug action.

The developing “omic”-technologies provide us now with the possibility to detect the interaction of a drug with several targets and have indeed already demonstrated multitarget effects. The term “omic”-technologies relates to high-throughput technologies. They are capable to determine a multitude of molecules on the gene (genomics, transcriptomics) or the protein level (proteomics, metabolomics) at the same time. They include different platforms of gene and protein microarrays. The commonly used gene microarray platforms over the past 10 years like Affimetryx, Agilent or PIQOR™ allow to study the gene expression levels of the complete genome. The ability of these arrays to simultaneously interrogate thousands of transcripts has led to important advances in a widerange of biological problems including pharmacogenomic responses. Nevertheless, these techniques have certain limitiations (e.g. background levels of hybridisation). Thus, sequencing based approaches measuring gene expression have evolved. Ultra-high-throughput sequencing is emerging as an attractive alternative to microarrays for genotyping, analysis of methylation patterns, the identification of transcription factors, but also as platform to study mRNA-expression levels. First studies comparing the sequencing data from an Illumina sequencing platform with data obtained from a “conventional” Affimetryx microarray revealed comparable results. But the ultra-high throughput sequencing allowed for additional analysis like a detection of low-expressed genes, alternative splice variants, and novel transcripts. It may be predicted from this that the sequencing approach will be over time widely adopted for measuring mRNA expression levels.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Example</th>
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<tr>
<td>Multitarget effects</td>
<td>Cannabis</td>
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<td></td>
<td>Antispastic effects of Tetrahydrocannabinol + Cannabis extract (Cannabidiol increases the transport of anandamide through the membrane)</td>
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<td></td>
<td>Hypericum perforatum</td>
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<td>Hypericum, Hyperforin, Hypericin, Amentoflavon target different receptors in the pre- and postsynaptic neuron</td>
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<td>Pharmacokinetic effects</td>
<td>Atropa belladonna</td>
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<td>Resorption of 1-Hyoscyamin is increased in the presence of flavonol-triglycosides</td>
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<td></td>
<td>Ammi visnaga</td>
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<td>Khellin as part of the extract is better absorbed than Khellin alone.</td>
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<tr>
<td></td>
<td>Hypericum perforatum</td>
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<td>Hypericum combined with Polyphenols (Epicatechin, Procyanidin, Hyperosid, Rutin) enhances bioavailability of Hypericum</td>
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<tr>
<td>Elimination of resistance mechanisms</td>
<td>Penicillin + clavulicin acid: antagonization of penicillinase</td>
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<tr>
<td>of bacteria</td>
<td>β-lactam antibiotics +Epigallocatechin gallate (EGCg) combined attack on the peptidoglycan part of the bacterial wall</td>
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<tr>
<td></td>
<td>Inhibition of lactam- or ester-cleaving enzymes by EGCg to maintain penicillin activity</td>
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<tr>
<td></td>
<td>Norfloxacin, Amphotericin + Origanum vulgare, Pelargonium graveolens or Melaleuca alternifolia</td>
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<td>⇒ reduction of the antibiotics through synergy</td>
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<td>Elimination, neutralisation of</td>
<td>Radix Aconiti</td>
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<tr>
<td>toxically acting constituents</td>
<td>Four methods to reduce toxic Aconitin to ~0.2%</td>
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Nevertheless the “conventional” gene microarrays provided already new evidences in the context of synergy research and multitargeting\(^7\). E.g. the gene-expression profile of \(\alpha\)-tocopherol showed the targeting of genes related to the immune system, as well as the activation of genes related to the lipid metabolism and to inflammation interestingly with no significant change in the expression of classical antioxidant genes\(^{15}\). Similarly methotrexate (MTX) or mercaptopurin targeted a multitude of genes involved in apoptosis, mismatch repair, cell cycle control and the stress response\(^{16}\). This demonstrates convincingly that “single synthetic drugs or natural single component/substances” have a multitude of targets on the genetic level (Table 2). Simultaneous proteomic analysis demonstrates that at least part of the genomic regulation is translated into proteins (Table 2). Thus, the “omic” technologies lead us away from the paradigm of one drug, one target and one disease.

If monosubstances have not only one, but multiple targets, a group of drugs or multicomponent mixtures are expected to have multiples of these targets. This multiplicity of targets and the expected subsequent unpredictable mode of action has over many years been the main argument against the use of phytoremedies. It was also expected that the adverse events (ADS) of single drugs would equally multiply in a combination therapy. However, the potential of synergistic positive effects was not really considered.

With respect to the multiplicity of targets, it has been observed that the number of active components of a plant extract does not necessarily influence the number of targets. We compared in a fibroblast model the modulation of genes by acetylsalicylic acid (ASA) and by the multiextract mixture Phytodolor\(^\text{TM}\) (PD) as well as its single extract components using the topic defined PIQUOR\(^\text{TM}\) Skinpatho Microarray which measures the modulation of inflammatory genes. The multiextract mixture PD is composed of three alcoholic extracts: *Populus tremula* (S1), *Solidago virgaurea* (S2) and *Fraxinus excelsior* (S3). S1 possesses a high content of salicin, salicylates\(^8\) and salicyl alcohols, but also phenolic components like flavonoids and catechins\(^{17}\). S2 has a high content of rutin and S3 a high content of fraxin. Each of the single extracts modulated a different number of genes based on the microarray assessment: S1 modulated 51 genes, S2 24 genes and S3 31 genes. The extract combination PD did not reveal an additive modulation of genes, it modulated “only” 40 genes and ASA 44 different genes. Thus, the number of active components in an extract does not necessarily determine the number of targets\(^{18}\).

It was further observed that the gene expression profiles of the single extracts S1, S2 and S3 did not allow a prediction of the gene expression profiles of their combination (S1+S2+S3)\(^{18}\). A comparable observation was made by Cheok *et al.*\(^{16}\) earlier. Gene microarrays (HG-U95A oligonucleotide microarray) were applied to investigate the mode of action of methotrexate (MTX) and mercaptopurin alone and in combination on human leukemia cells. It was demonstrated that the combination of both in human leukemia cells causes a different gene expression

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Table 2 — Monosubstance “Drugs” with multiple targets (modified according to Ulrich-Merzenich *et al.*\(^7\))

<table>
<thead>
<tr>
<th>Drug/monosubstance</th>
<th>Gene targets (examples)</th>
<th>Protein-targets (examples)</th>
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<tr>
<td>(\alpha)-Tocopherol</td>
<td>Immune response, phosphate metabolism, protein modification, lipid metabolism, inflammatory responses(^{15})</td>
<td>Protein kinase C, Phospholipase A2 5-Lipoxy genase (^3)</td>
</tr>
<tr>
<td>Daidzein</td>
<td>Androgenresponsive genes, IGF-1 Pathway, MAP kinase pathway genes(^{43})</td>
<td>Oestrogen-receptors, Peroxisome-Proliferator-Activated Receptors(^{59})</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Immune Response (Interleukines like IL-1, IL-6 or cytokines like TNF-(\alpha))(^3)</td>
<td>20 protein (e.g. heat shock proteins, annexins involved in apoptosis, metabolism, detoxification and gene regulation(^{60})</td>
</tr>
<tr>
<td>Epigallocatechin-3-gallate</td>
<td>Zinc finger protein 33A, Ubiquitin-activating enzyme E1C, basic fibroblast growth factor in human bronchial epithelial 21BES cells(^{62})</td>
<td>Mitogen-activated protein kinases, nuclear factor-(\kappaB) and others(^{61,62})</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>S 100 calcium binding protein A8 and A9; Leucocyte immunoglobulin receptor; densin, alpha 3, neutrophil specific(^{46})</td>
<td>Dihydrofolic acid, T-Cells, Livertransaminases (Folic acid synthesis inhibition)</td>
</tr>
<tr>
<td>Mercaptopurin</td>
<td>Splicing factor proline/glutamine rich; nucleoside diphosphatase kinase type 6, CDC28 protein kinase 2, polymerase (DNA directed), gamma 2(^{16})</td>
<td>Adenylosuccinate-synthase, Phosphoribosylpyrophosphat-amidotransferase, (DNA- and RNA-synthesisinhibition)</td>
</tr>
</tbody>
</table>

\(^{15}\) salicylates: esters of salicin like salicortin, tremulacin or 2’-O-acetylsalicortin. These prodrugs are metabolised like salicin to the active drug salicyclic acid.

[^43]: "...
[^15]: "...
[^60]: "...
[^16]: "...
[^61]: "...
[^62]: "...
[^46]: "...
profile than each single applied drug. The expression profile of the combination shared only 14% of genes with the ones of the single application. Thus, 86% of the previous modulated genes did not respond. This demonstrates that drug combinations can lead to the activation of more or less entirely different sets of genes compared to those activated by the single agents. Thus, the application of drug combinations including phytopharmaceuticals does not necessarily mean the addition or multiplication of targets. It can lead to new modes of actions. The consequences of these findings specifically for phytopharmaceuticals are: the mode of action of a medication is most reliably assessed if all applied single drugs are taken into consideration since a new mode of action may arise through the combination of drugs. Therefore, the demand to demonstrate the mode of action of each single component in a phytopharmaceutical may not be obligatory any more.

The “omic”-approach as methodology for the development of plant drugs

Standardization of single and multicomponent plant extracts—Prerequisite of the use of phytopharmaceuticals is their standardisation. This starts with the proof of the authenticity and quality of the plant material, which has for long relied on morphological and chemical methods. Metabolomic fingerprinting by GC-MS, HPLC-MS or NMR-spectroscopy are increasingly used with the aim to develop metabolomic profiles. The principle component analysis (PCA), which is used to reduce the complexity of spectral data has simplified the visualisation (and recognition) of profiles from the multiple data sets. Daniel et al. proposed to use PCA besides standard analytic methods for the characterisation and identification of plants and multicomponent plant extracts. DNA-based assays have also been introduced for complementing the morphological and chemical methods with the aim to generate molecular “bar codes” for the correct identification of medicinal plants. Demands are increasing to establish open access data banks in a multidisciplinary effort for medicinal plant research and to include e.g. the obtained data (profiles) into the European Pharmacopoeia in order to develop a common standard for the characterisation of plants.

Another level of standardisation lays on the assessment of a reproducible mode of action of plant ingredients or plant extract irrespective of a certain degree of variation in their composition. Here it will be essential to demonstrate that single plant ingredients or plant extracts yield reproducible and specific expression profiles (signatures) by using the array technology. Figure 2 shows the gene expression profile of the willow bark extract STW-33-1 in human chondrocyte cultures. In four different microarrays (Agilent human whole genome microarray), a specific profile of the willow bark extract in comparison to other tested substances could be recognized. In the given example four arrays with two different concentrations of the willow bark extract were investigated. For standardisation, however, the use of

![Fig. 2](image-url)
more than four microarrays in different concentration would be more reliable to obtain the specific and reproducible core set of genes, which is activated by a plant extract. Lines 7 and 8 show the gene expression profile of the reference substances diclofenac and acetylsalicyclic acid (ASA). The comparison of expression profiles of complex mixtures with known reference substances (monosubstances) is useful, since this will simplify the identification of the essential pathways which finally lead to the observed clinical or pharmacological effects. Figure 3 shows the hierachical clustering of genes which are simultaneously up- or down regulated. Thereby common pathways are identified. In order to cope with the flood of bioinformatic data, it will be advisable not to start measurements with too complex combinations of extract mixtures.

The finding that reproducible gene expression profiles can be obtained in cell culture models for plant extracts is supported by recent findings of Pakalapati \textit{et al}.\textsuperscript{21}. Rats were treated with a \textit{Trifolium pratense} extract rich in isoflavones. Whole genome microarray (Affymetrix Rae 230_2) measurements showed a reproducibly modulated core set of genes. The microarray data were combined with proteom studies. Those revealed that the modulation of several genes of the lipid metabolism was converted onto the protein level.

The reproducibility of metabolomic expression profiles induced by multicomponent mixtures is already supported by several studies in the field of nutrigenomics, e. g. Wang \textit{et al}.\textsuperscript{22} have demonstrated with an $^1$H NMR spectroscopy-based study in conjunction with chemometric methods differences

![Hierarchical clustering of genes](image)

\textbf{Fig. 3}—The hierarchically clustered map of genes expressed in blood cells of rats after treating the animals with different fractions of the aqueous willow bark extract (STW-33-1). Each line represents the blood cell expression profile of one animal. \textit{(Line 1-3: controls; line 4-6: ethanol fraction; line 7-9: ethylacetat fraction; line 10, 11: watery fraction; line 12: Total extract (TS)). The figure shows only a small part of the clustered gene map as illustration. A red colour indicates the up-regulation of a gene (names of the genes are indicated on the right side of the map), the green colour indicates the down-regulation of a gene. (Ulrich-Merzenich \textit{et al}.\textsuperscript{3})}
between the metabolome pattern induced by chamomile tea in urine before and after tea ingestions. The effect of a dietary intervention of soy isoflavones in humans was equally detectable by NMR\textsuperscript{23, 24}.

For a complete understanding of the mode of action, the integration and correlation of data sets from the different “omic” areas and from functional tests is essential, but on the other hand tremendously challenging. For example, not all expressed mRNAs are converted into proteins, the mRNA-splicing process needs to be considered. Also the differential timings of these events is a challenging factor. Bilello\textsuperscript{25} proposed for the understanding of pathophysiological processes an integrated system based approach involving modelling and simulating the complex dynamic interactions between genes, transcripts, proteins, metabolites and cells, encompassing many of the “omic” technologies and using computational and mathematical models to analyse and simulate networks and pathways. This could also take into account spatial and temporal relationships that give rise to cause and effect in biological systems\textsuperscript{25}. Data of such integrated approaches have so far not been published in the field of phytomedicine. However, an integrated approach based on “omic” technologies was already used e.g. by Kleno \textit{et al.}\textsuperscript{26}, who identified potential biomarkers (related to the glucose and lipid metabolism and to oxidative stress) for hepatotoxicity in rats. Rochfort\textsuperscript{24} showed that correlations from liver mRNA, liver proteome, and metabolome analysis of serum corresponded to changes in glucose, lipid metabolism and oxidative stress responses. Mayr \textit{et al.}\textsuperscript{27} combined proteomic and metabolomic studies in the cardiovascular system and described the results that “the simultaneous assessment of protein and metabolite changes translated purely descriptive proteomic and metabolomic profiles into a functional context and provided important insights into pathophysiological mechanisms that would not have been obtained by other techniques.”

For standardisation of plant extracts, the primary focus will be the reproducibility of profiles with the different “omic”-techniques resulting most likely in a tremendously improved insight into the pharmacological mode of action. Issues like the permitted magnitude of variation for each technology, however, still need to be defined. An overview about the currently available biological databases for annotations of genes and proteins such as GenBank, UCSC Genome Browser, Esembl. and non-sequence centric databases like the Protein Data Bank (PDB) has been given by Baxevanis\textsuperscript{28}.

\textbf{Pharmacokinetics and bioavailability of plant extracts and their combinations—}Pharmacokinetic and bioavailability studies are an essential need to determine the exact pharmacological action of phytopharmaceuticals, but still insufficient data do exist. Due to the high number of components in herbal drugs, their variable absorption and their complex biotransformation, assessments with complete coverage have been practically impossible by conventional methods. The new high-throughput technologies make these kinds of assessments possible and will improve the speed and yield. But again, even after the identification of the available plant components and their metabolites in plasma, functional studies are essential for determining the mode of action. This includes toxicity testing – the latter being the best developed field in the context of “omic”-technologies so far.

\textbf{Assessment of the toxicity of plant extracts—}It is generally expected that the use of gene expression data is more sensitive than traditional toxicological endpoints\textsuperscript{29}. Searfoss \textit{et al.}\textsuperscript{29} identified the following general goals for the new field of Toxicogenomics equally applicable to the development of synthetic drugs or phytopharmaceuticals: a) understand mechanisms of toxicity; b) predict toxicity; c) develop in vivo and in vitro surrogate models and screens; and d) develop toxicity biomarkers. These should lead to an improvement of safety, to the shortening of the drug development and a cost reduction. Recent reviews on the issue have been published by Searfoss \textit{et al.}\textsuperscript{29}, Suter \textit{et al.}\textsuperscript{30} and Stork \textit{et al.}\textsuperscript{31}.

There is already a collaborative effort between the European Molecular Biology Laboratory-European Bioinformatics Institute ArrayExpress, the International Life Sciences Institute Health and Environmental Science Institute, and the National Institute of Environments Health Sciences National Centre for Toxigenomics Chemical Effects in Biological Systems knowledge base to establish a public infrastructure on an international scale and examine other developments aimed at establishing toxicogenomics data-bases. An overview of the major toxicogenomics database efforts in the public sector has been given by Mattes \textit{et al.}\textsuperscript{32}. These data bases are under development and offer on one hand information (gene expression profiles) on the toxicity
endpoints dependant on organs as well as on different
diseases. For example, it has been estimated that the
liver has 10-20 distinct toxic phenotypes, including
steatosis, multifocal necrosis and hypertrophy. On the
other hand they accept submissions from investigators to create and increase these data bases. The Chemical Effects in Biological Systems
Knowldege Base (CEBS) data base (http://www.niehs.nih.gov/ncr/cebs) is under
development. Specific objectives have been given by
Mattes et al. as follows: a) to compare
toxicogenomic effects of chemicals/stressors across
species – yielding signatures of altered molecular
expression; b) to phenotypically anchor these changes
with conventional toxicology data – classifying
biological effects as well as disease phenotypes; and
c) to delineate global changes as adaptive,
pharmacologic or toxic outcomes – defining early
biomarkers, the sequence of key events and
mechanism of toxicant actions. CEBS is a dynamic
concept for integrating large volumes of
transcriptomic, proteomic, metabonomic, and
toxicological knowledge in a framework that serves as
a continually changing «heuristic» engine. For
further information and internet adress lists see Mattes
et al.

Some predictive toxicology products have already emerged by different biotechnology firms (Table 3). The development of these microarrays is based on the
above mentioned type of investigations of gene
expression signatures or “fingerprints” which are
highly predictive for toxicity endpoints (phenotypes) by testing toxic (test) compounds. These products can be readily used for phytopharmaceuticals to screen for so called off- and on-target toxicities early in the drug
development. Different tissues or cells can be exposed to the selected plant extracts and then screened for
mRNA or metabolite modulation relevant for toxicity. Off-target toxicities are those caused by actions unrelated to the targets such as non-specific immune or
inflammatory reactions or hepatotoxicities due to drug
metabolism effects. On-target toxicities are additional unintentional effects due to the interaction
of target and drug. Besides these already existing
products (Table 3), it will be necessary to develop
additional gene- or proteinchips specifically suited to
the toxicology demands of phytopharmaceuticals.

Synergism, signal cascades and trigger points
of the metabolism—Synergism can occur through
multitargeting. But the identification of multiple
targets, which will be simplified through the use of
“omic” techniques, will be only one part of the puzzle
to understand synergistic actions. The type of target,
which can be as diverse as enzymes, receptors,
antibodies or signalcascades is equally important.
Signalcascades are highly interesting targets for drug
development since their activation can lead to an
amplification of the original signal by a million times.
The investigation of such signal cascades may
elucidate our understanding of synergistic, but also
antagonistic effects.

Recently G-protein-coupled receptors (GPCRs)
have gained increasing importance as targets for drug
development. GPCRs constitute a large family of
receptors which initiate various signal cascades. Such
cascades may be called trigger points of the
metabolism, not only because they amplify a signal,

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<tr>
<th>Provider</th>
<th>Product</th>
<th>Description of Product/approach</th>
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<tbody>
<tr>
<td>Iconix.Pharmaceuticals</td>
<td>DrugMatrix™ Chemogenomics</td>
<td>Global expression profiling in vivo+in vitro &gt;600 compounds chemogenomic database, +200 validated gene signature sets ToxExpress™ reference data base predictive system with in vivo+in vitro models. Extensive liver toxicity database. DATAS™ screening technology reveals the presence of alternatively spliced mRNAs, and thus protein isoforms linked with toxicity Determination of classical toxic endpoint genes relating to apoptosis, DNA-damage + repair, inflammation, ox-stress 850 mRNA transcripts and EST clusters selected from UNIGene database (Build 34) Forensic Toxicology Data Base of 277 compounds</td>
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but they often can divert the signal into opposing directions. One example of such a trigger point is the mitogen activated protein kinase (MAPkinase) cascade. Several molecules like H₂O₂, vitamin C or the cytokine TNF-α\textsuperscript{35,36} can activate this cascade. The result of activation can be twofold—apoptosis or proliferation. E.g. the stimulation with a low dose of H₂O₂ over a short time leads to the activation of the MAPkinases ERK-1 and 2 and mounts into proliferation. Contrary, a long time exposure of H₂O₂ in low dosage leads to apoptosis. A high dosage shortly given or applied for a long time leads also to apoptosis\textsuperscript{3,36,37}. Thus, the activation of the same signal cascade can lead to opposite effects depending on time and dosage of the trigger. Higher dosages of H₂O₂ activate besides ERK-1 and 2 also the so called “stress induced” MAPkinases p38 and c-jun. Their additional activation leads to apoptosis instead of proliferation\textsuperscript{36,37}.

Such dosage dependent reversal effects have been observed for several herbal drugs like Ginkgo biloba\textsuperscript{38}, Hypericum perforatum\textsuperscript{39} or Curcuma longa\textsuperscript{40}. One possible explanation for reversal, but also synergistic effects could be such an activation of signal cascades. In case of reversal effects, the signal cascades antagonize each other in certain dosage ranges; in case of synergism signal cascades interact and agonize each other.

Indeed, the ultimate result of the activation of a signal cascade can be due to signal amplification much greater than originally calculated by the summation of the single effects. Immig et al.\textsuperscript{12} called these effects vaguely “rippels” in drug action. The “omic”-technologies can support the analysis of the simultaneous up- or downregulation of signal cascade molecules and thereby may factually describe these “rippels”. Gene microarray-analysis identify via hierarchical clustering genes which are commonly up- or down regulated (Fig. 2). Thereby pathways are identified. Synergism may be the result of the activation of central trigger points of such pathways and may be an expression of a nonlinear correlation of molecular interactions (of such pathways) and clinical effects. Such trigger points could be taken as new drug target or as targets to be protected to safeguard a normal metabolism. As already mentioned the GPCRs have gained recently an extremely high interest as drug targets\textsuperscript{34}. Several plant constituents like genistein\textsuperscript{41} or flavonoids\textsuperscript{42} like naringin and cyanidin-3-glucosides\textsuperscript{43} were already shown to interact with them. The potential of curcumin to modulate signalling pathways leading to cell cycle regulation or directly altering cell cycle regulatory molecules in cancer therapy is presently under intensive investigation\textsuperscript{44}. Synergism as the result of the coordinated activation of signal cascades may be a highly rewarding area for drug development. Synergism as result of an activation of transport proteins to increase the bioavailability of certain drugs will be described in a future review.

New combinations of plant components based on traditional systems of medicine

Traditional systems of medicine like the Chinese or Ayurvedic systems have detailed description of methods to prepare their medicines. Even though tremendous efforts have been made e.g. in India over the past 40 years supported by the Council of Research in Ayurveda, Siddha and Unani to standardize these methods and at the same time to justify the different procedures, both goals are difficult to achieve due to the often high number of plants in a single medication and due to the high number of steps involved in the preparation. As already mentioned the “omic”-technologies allow the assessment of complex mixtures and could be used to monitor the different steps of the preparation in order to validate or dismiss the original recipes and to look into the mode of action of Ayurvedic preparations which contain sometimes up to 70 different plants\textsuperscript{45}.

Chinese medicines are primarily prepared as watery decoctions. It is recognized since long that this type of preparation is difficult to standardize. The Chinese Pharmacopeia is based on the analyses of alcohol extracts. The results, however, may not mirror the contents of the decoctions. Figure 2 shows that the application of different preparations of plant extracts in rats lead to clearly distinguishable patterns of gene expressions in blood cells. These differences are likely to be translated onto the protein and functional level, too. Thus, “omic”-technologies can be used to support the selection and development of the optimal extract form or preparation for Chinese or Ayurvedic medications in an acceptable time frame. However, the following premises and recommendations may be considered to obtain reliable evidence-based “new” phytopharmaceuticals:

1. The basic material should be quality assured in form of a standardization according to conventional methods (fingerprint and if available fixed content of biological active substances).
2. A toxicology screening of the “phytopharmaceutical” should be performed with one of the presently already available screening tools (Table 3). In this context specific toxicology assays and data banks for critical plant components need to be developed.

3. An evaluation of the possibility to identify “surrogate” plant components which represent the activity of the plant extract should follow and could be used for standardization.

4. Standardisation of the plant extracts according to an expression profile taking into consideration relevant concentrations and the bioavailability. Matching of plant signatures with signatures of the mode of action in different surrogate models should be attempted.

5. Determination of the down-stream effects influenced by phytopharmaceuticals and bioactive plant components via identification of molecular pathways including the identification of relevant mechanisms of action for the different plant components through comparison with reference-listed drugs.

6. Integration of the profiles of single plant components and complex extract mixtures into the international freely accessible data banks of genetic, proteomic and metabolomic screens.

7. Exploring the connectivity of biological pathways by a systematic combination screening and thereby determining the mode of action (agonistic, additive, agonistic=synergistic) of single components and extract mixtures in comparison to reference-listed drugs. Complex mode of actions of combination therapies (Table 2) should be investigated.

8. Based on plant and disease profiles relevant clinical applications for phytopharmaceuticals should be identified.

**Outlook and conclusions**

In future drug development from natural products will not necessarily rely only on the discovery and analysis of new structures from nature’s extremely rich biodiversity, but can systematically explore combinatory drug regimes. The introduction of the high throughput-technologies makes this possible. Their use has already changed our conceptual frame work of drug targets, drug action and our understanding of the pathology of diseases (in favour of using phytomedicine). The systematic application of these technologies in the assessment of (a) the authenticity; (b) the quality; (c) the toxicology; and (d) in the analysis of the mode of action of plants and multieextract mixtures, should create in a long term perspective evidence-based plant medications and should lead to the discovery of effective phytomedical interventions in the prevention and treatment of different diseases in form of new single treatments or new combinatory drug regimes while exploiting synergy-effects. However, presently we are far ahead with data collection, but still without a thorough conceptual framework for the integration of the tremendous amount of data. System biology is evolving as a key discipline to address this task, which is a multidisciplinary challenge still based on experimental biology.

**References**


