Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials?

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Personalized medicine – defined as customized medical care for each patient's unique condition – in the broader context of personalized health, will make significant strides forward when a systems approach is implemented to achieve the ultimate in disease phenotyping and to create novel therapeutics that address system-wide molecular perturbations caused by disease processes. Combination drug therapies with individualized optimization are likely to become a major focus. Metabolomics incorporates the most advanced approaches to molecular phenotype system readout and provides the ideal theranostic technology platform for the discovery of biomarker patterns associated with healthy and diseased states, for use in personalized health monitoring programs, and for the design of individualized interventions.

Author Proof

The present era in life sciences is characterized by fundamental changes in our views of health and disease that are driven by recent advances in bioanalytical and bioinformatic technologies and the novel insights into human biology that are emerging through the application of these technologies. It is inevitable that future paths for research and development in biomedical, pharmaceutical and nutritional domains will converge into a unified activity. These new discoveries and new insights are stimulating discussions of a paradigm shift in healthcare and consequently are highlighting the challenges that we face in the immediate future.

Currently, experimental studies can not keep pace with the new concepts that are presented in the numerous 'opinion pieces' or review papers focused on personalized medicine. Examples of the substantial and thoughtful publications in this general area are: the '2029 Report' [101] and the publication 'Life Sciences: A Changing Prescription' [1]. Personalized medicine is linked to almost all the key future developments mentioned in those reports.

From a commercial perspective, the pharmaceutical industry is struggling with a reliance on blockbuster drugs, a continuing decrease in new product launches, the increasing drug development costs and the withdrawals of drugs from the market due to unexpected safety issues. In such an environment, it is evident that the blockbuster approach is not viable in the longer term and that moving to 'niche busters' becomes an attractive option [2] with potential advantages, such as reduced development time, smaller focused clinical trials, better safety and attractive profitability. Given the uniqueness of every human being, the best possible solution for personalized health is obviously personalized medicine that could be based on a 'niche buster' pharmacopeia, but which migrates iteratively to truly personalized treatment strategies (discussed later).

The concept of personalized health is not actually new, as it has been the basis of non-Western medical practices for centuries, such as in traditional Chinese medicine and Ayurveda, in which personalized treatments have largely been the sole approach to medicine. A unique opportunity therefore exists to improve both diagnosis and treatment of human diseases by generating a unified view on biology and medicine through the integration of Western and Eastern knowledge [2,3].

Directly related to the shift in our thinking about healthcare generally is a parallel scientific shift toward a systems approach to biology and medicine, using system molecular biomarker profiles as an important tool for optimizing the drug discovery and drug development process – encompassing a better molecular understanding of disease processes (system pharmacology), drug safety profiles (systems toxicology) and drug efficacy (systems pharmacology) [4]. In addition, stratifying patients on molecular biomarker profiles is a key step towards treatment responder/nonresponder differentiation. Furthermore, the interspecies comparison of molecular systems characteristics is a key tool for translational activities in drug development that depend upon the relevance of preclinical models to the human clinical situation [5].

Keywords: integrative medicine, metabolomics, personalized medicine, phenotyping, systems biology

future medicine

A molecular systems approach and the application of metabolomics technologies have been identified as a unique bridge between different cultural perspectives on personalized healthcare [3] and will definitely play an important role in the advancement of personalized medicine [6], as recognized by the US FDA [7]. However, despite the opportunities for this highly-relevant molecular phenotyping technology to impact personalized medicine, to date high expectations have been tempered by disappointing achievements [8].

The integration of gene transcript, protein and metabolite information into a systems biology perspective for molecular phenotyping has already been described in detail for animal studies [9–11]. Additional reports relevant to personalized medicine have been made for diabetic nephropathy [12] and rheumatoid arthritis [13]. Furthermore, the power of metabolite profiling in case of silent genotypes [14] was demonstrated and discussed in relation to studies on yeast [15].

In this short review, we will describe the current status of metabolomics from a systems perspective and provide a provocative view of the future. The working definitions used in this review are:

- Metabolome: the entire complement of all the low-molecular-weight molecules (metabolites in cells, bodyfluids, tissues and so on)
- Metabolomics: the comprehensive quantitative and qualitative analysis of all small molecules in a system (in samples of cells, body fluids, tissues and so on)

We view personalized medicine as part of a larger, personalized health concept. Although the discussion in this paper is limited to personalized medicine, we assert that the integration of systems-based molecular phenotyping with nutrition, psychology and environmental aspects into a total lifestyle 'package' is a prerequisite for revolutionizing healthcare. The area of nutrition metabolomics in the context of personalized health, with respect to defining the nutritional phenotype, is currently also an important topic [16,17].

Current state of metabolomics in personalized medicine

Since the initiation, in the early 1980s, of studies on metabolite fingerprinting of body fluids using pattern recognition to study gender differences [18], the sensitivity of these methodologies for revealing phenotype in terms of metabolite patterns has been recognized. Metabolomic studies have generated important findings, ranging from gender-specific differences to ethnic, nutritional, environmental, microbial, psychological and disease aspects [19–25]. From a technology perspective modern metabolomics strategies involve a metabolomics platform typically composed of various approaches using different techniques such as nuclear magnetic resonance (NMR), gas chromatography mass spectroscopy (GC-MS) and liquid chromatography mass spectroscopy (LC-MS) to achieve a high coverage of metabolites. Phenotypic information is present across the whole concentration range and, because metabolite coverage is key for success, mass spectrometry is especially important to reveal the metabolite patterns at low concentrations. Moreover, integration of this information into a systems perspective [4] is mandatory to obtain a better understanding. An example of an advanced systems biology platform is given in **Figure 1**, metabolomics-related aspects have been discussed previously [26].

Different molecular subphenotypes in human disease were reported using plasma metabolomics analysis by NMR of samples from patients with coronary heart disease [27], but a recent publication could not reproduce the effects and it was argued that the original work did not adequately incorporate gender and medicine used in the statistical evaluation [28].

Over the past few years, studies have appeared that illustrate disease-specific metabolite profiles, but no study has yet demonstrated the next step of fine tuning a therapeutic intervention based on this information. Such an approach would be fundamentally out-of-phase with approaches in the pharmaceutical industry where there is an overwhelming emphasis on 'mechanism'. It might be worth contrasting the current interest in, for example, the Omega-3-Index as a new cardiovascular risk factor with the lack of wholesale acceptance of multivariate approaches. A recent study on metabolite urine profiling of rats, in which the metabolite pattern has been claimed to contain predictive power for liver injury following paracetamol administration, is the closest published so far in demonstrating the power of metabolite profiles in predicting drug response, but the statistical basis is not yet solid enough and more work using larger groups and more significant multivariate statistics is required [28].

It is important to note that animal studies under strictly controlled conditions are still far away from the human situation characterized by its diversity of states. In addition, even in controlled animal experiments, it has been found that differences in microflora [29] strongly influence the metabolite fingerprint of the individual animals.

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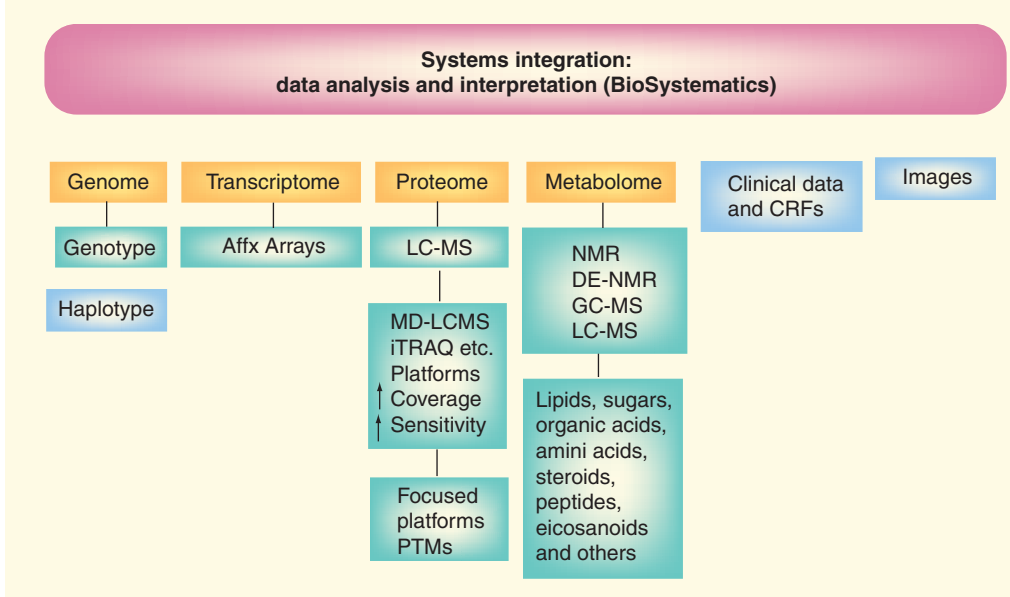
Figure 1. Metabolomics as part of the systems biology platform.



Metabolomics as part of the systems biology platform enables integration of different levels of biological information and interpretation in terms of connectivity and communication within a system; the key for phenotyping and personalized medicine.
CRF: Case report forms; DE-NMR: Diffusion-edited-nuclear magnetic resonance; GC-MS: Gas chromatography mass spectroscopy; LC-MS: Liquid chromatography mass spectroscopy; MD-LCMS: Multidimensional liquid chromatography mass spectroscopy; NMR: Nuclear magnetic resonance; PTM: Post-translational modification.

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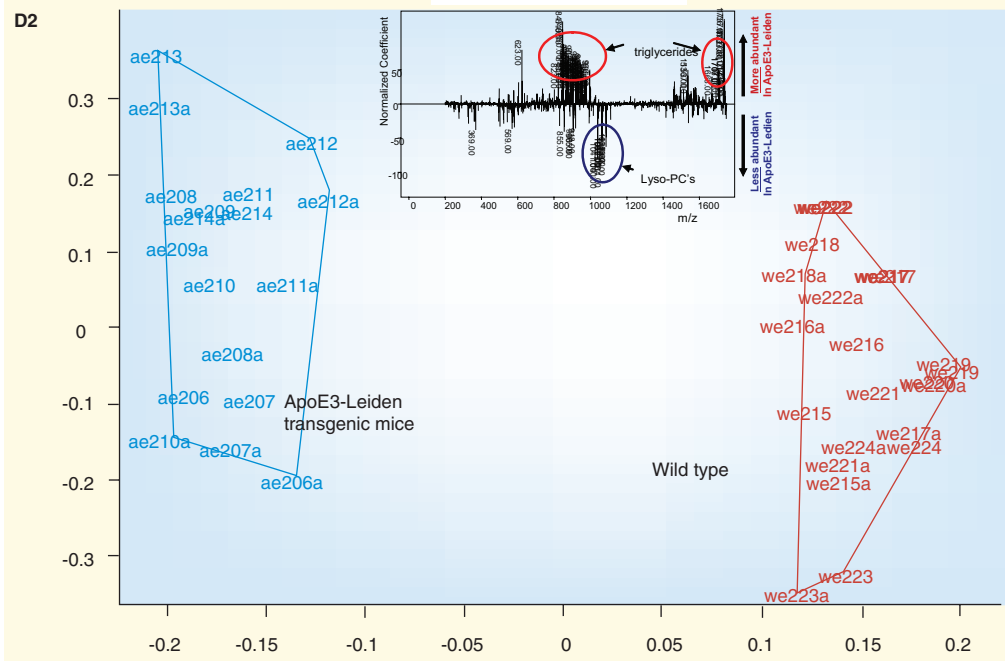
However, as reported at the recent 2nd International Meeting of the Metabolomics Society, convincing pharmacometabolomics data is on the horizon demonstrating segregation of patients according to drug responder/nonresponder groups in validated efficacy studies or according to adverse effect sensitivity in safety studies [31,32]. Finally, in schizophrenia, a study that employed lipid-focused metabolomics correlated the effects of olanzapine, risperidone and aripiprazole to a metabolomics phenotype profile [31].

Expert commentary

"What are the challenges that must be overcome to realize the potential of metabolomics in the field of personalized medicine?"

Obviously, the phenotype molecular profile is extremely complex and studying this complexity necessitates considerable efforts in time and budget. Fortunately, metabolomics can capture this phenotype very effectively. Moreover, phenotypic differentiation of the unique profile of each individual needs to be guided by clear goals and therapeutic interventions options. While pharmaco '-omics' strategies can, in principle, be used to stratify patients for given drugs, currently-available drugs have typically not been developed for specific subphenotypes and consequently likely have a 'cross-subphenotype' activity profile. Some recent exceptions involve anticancer drugs, such as Herceptin® (trastuzumab), Gleevec® (imatinib mesylate) and Iressa® (gefitinib). Heterogeneity in patient response to cancer chemotherapy is a major issue with many probable causes, including those directly related to systems toxicology and interindividual differences in drug disposition or pharmacokinetics. In some cases, drug effects have been linked to polymorphisms in genes encoding for drug-metabolizing enzymes [33]. Such links might be caused by the intense nature of the system perturbations caused by certain drugs, which makes the enzymatic principles – poor, extended and hypermetabolizers – surface because drug metabolism is an important bottleneck under such toxicological conditions. In combination therapy, the most important molecular drivers of drug synergies are largely not understood in detail. Development of novel therapies based on such molecular-systems-based approaches are very appealing, but still in their infancy due to limited accessibility of robust and affordable molecular systems biology platforms.

Figure 2. Differentiation of ApoE3-Leiden transgenic mice and wild type at an early stage of development.



Using standard anatomical or histopathological phenotyping no significant differences could be detected. Using broad lipid-focused metabolomics the differentiation between the mouse groups is very significant. The inset shows the differences of various important phenotype-related lipids between the mouse groups. In total, 20 mice were included in the analysis (10/group, duplicate samples from each mouse).
ApoE3: Apolipoprotein E3.

The ability of metabolomics to trace 'subtle' differences at the genotype level is demonstrated in **Figure 2**, which was generated from the datasets of a study on the early diagnosis of atherosclerosis in ApoE3-Leiden transgenic mice. Under the conditions of the experiment no anatomical or histopathological phenotypical differences can be detected using standard techniques, but strong differences are displayed in the plasma metabolite profile as shown for plasma lipids. In this figure, the inset shows the differential plasma lipid profile comprised of components that have different concentrations in the transgenic mice compared with the wild-type mice. In addition, correlation networks can be generated from the datasets and these reveal the interrelationship between transcripts, proteins and metabolites in an integrated systems biology perspective [9–11], representing the ultimate in molecular systems biology phenotyping, including insights into the patterns of relationships.

Outlook

Future role of metabolomics in systems-based personalized medicine

The technological developments in metabolomics will be significant in the near future, directed toward improving coverage, sensitivity, miniaturization of sample size, throughput and cost level. Fully automated systems can be expected with the necessary robustness to measure thousands of metabolites in plasma samples with volumes of 5–10 µl.

Based on such technologies, new options for molecular phenotyping will become available, such as those presented in **Figure 3**. Three major routes are discussed for molecular phenotyping. Route A is based on the current rapid developments in stem cell research and tissue bioengineering [101] that will enable the creation of a personalized cell screening platform for most general tissues related to efficacy and safety in medical practice. Of course, a collection of different (personalized) cell types is ideal to perform rapid and multiple readouts; however, such an approach will not generate systems-level molecular phenotype information, and certainly not any psychology-related phenotypic information. Route B derives metabolite profiles from a steady-state system viewpoint, while route C uses a 'challenge test' to enable measurements of a dynamic systems response profile (SRP). We believe that the latter

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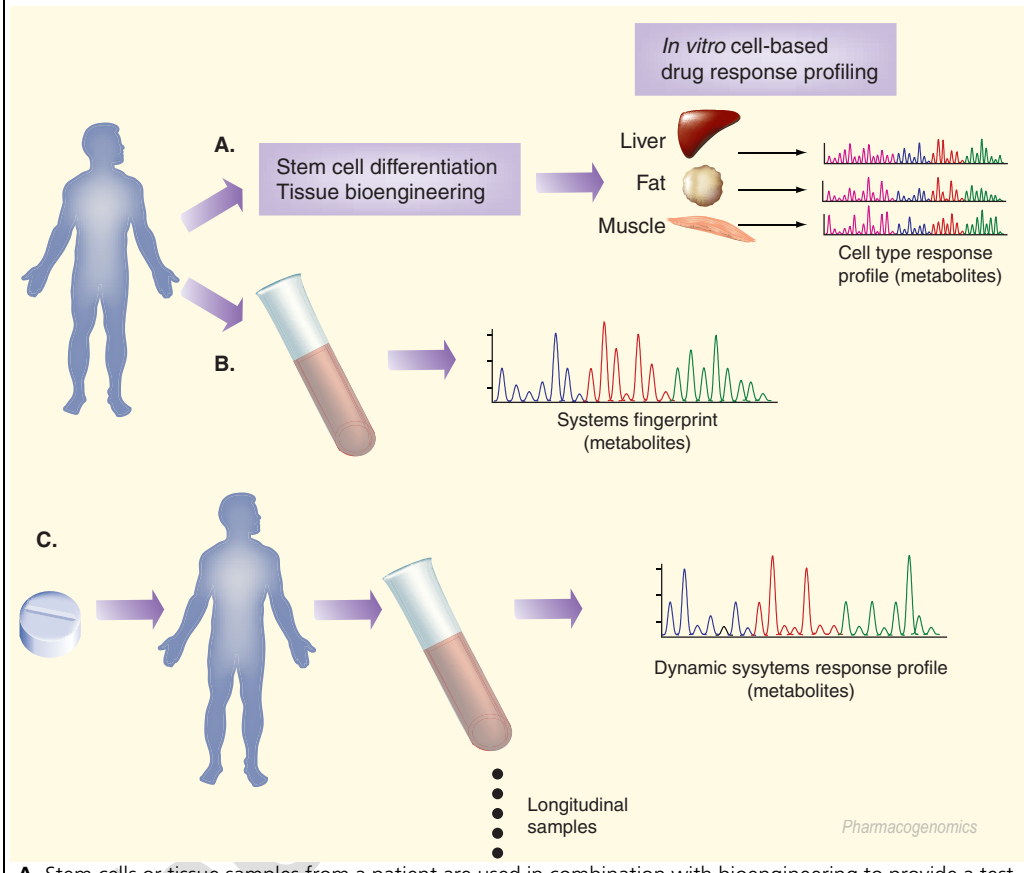
Figure 3. Different routes for phenotyping in personalized medicine.



A. Stem cells or tissue samples from a patient are used in combination with bioengineering to provide a test platform of different cell types with metabolomics as readout for efficacy and safety evaluations. **B.** Biofluid measurements are used to predict system responses in pharmacology and toxicology or to provide a measure of health. **C.** A 'challenge test' is used to generate dynamic system response profiles indicative of the system phenotype, typically this approach is attractive for assessment of early stages of disease or as a measure of health.

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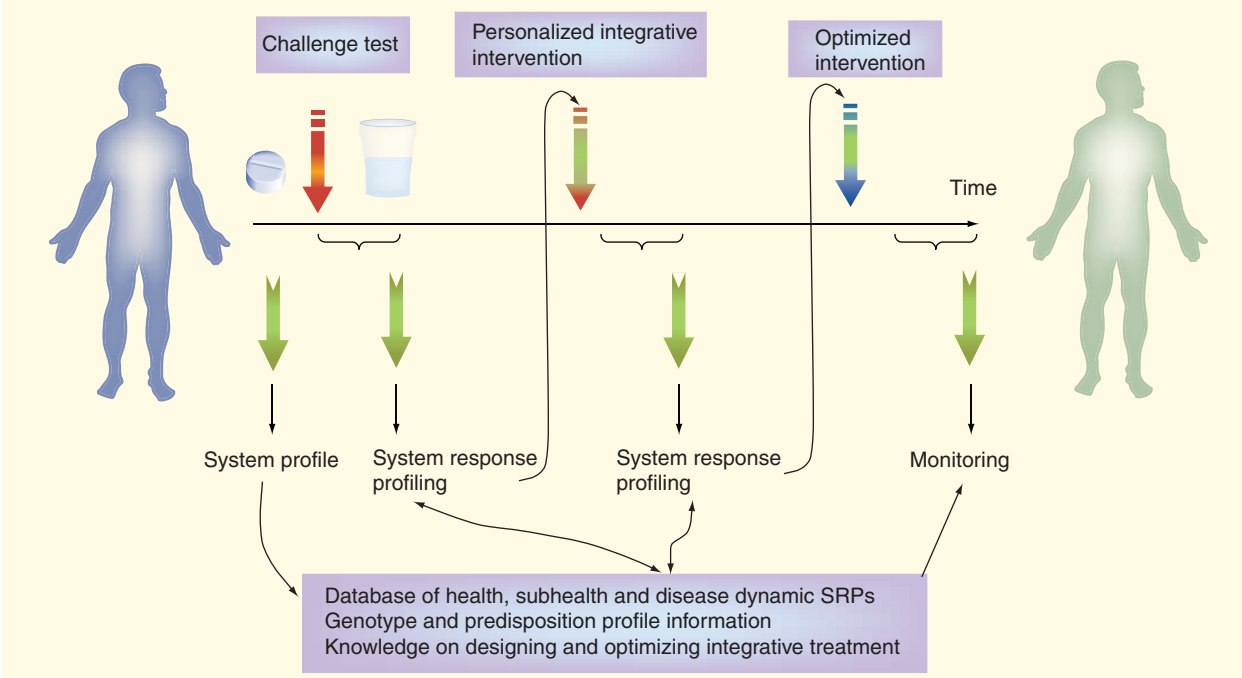
approach will provide a more sensitive and informative readout at the early onset of disease or loss of homeostasis [34], and that this type of information will be crucial for prevention-oriented approaches. A common example of this approach is the oral glucose tolerance test (OGTT) which can reveal early onset of type 2 diabetes. A more specialized example would be an acetylator phenotype test using caffeine [35]. An exercise 'challenge test' has also recently been reported to be an effective approach to reveal molecular biomarkers of myocardial ischemia [36].

Integration of comprehensive phenotyping information into an innovative personalized health system including diagnosis, intervention and monitoring, which will occur in the future, is depicted in Figure 4. In this scheme, patients are phenotyped via a direct molecular systems profiling or via a 'challenge test' approach. The resulting information is the input to an algorithm, which also draws upon a large database of information on environmental factors, genotype/phenotype information related to health and disease, and so on, to generate its output – an initial proposal for therapeutic intervention. This intervention will be a personalized version of a more generic niche busters combination therapy, in which different systems characteristics for a given disease domain allow adjustment of the combination intervention, which might include nutrition, lifestyle and psychological recommendations [37]. The result of this first intervention is monitored via the patient's molecular phenotype and the intervention is optimized in an iterative fashion until a satisfactory treatment is achieved. This approach would effectively be an 'n = 1 clinical trial'.

Figure 4. Schematic representation of future personalized healthcare system, an 'n = 1 clinical trial'.



Via steady-state molecular profiling or by molecular profiling in the context of a challenge test, information is obtained and combined with a database on phenotype-related health and disease information, as well as intervention options. This generates an initial therapeutic intervention proposal that is checked and adjusted until a satisfactory treatment is obtained in an iterative process that is monitored through the patient's molecular phenotype. Interventions are based on integrative medicine with lifestyle, nutrition and psychology as important components.
SRP: System response profile.

In such a case, diagnosis and intervention go hand in hand, and the newly developed, personalized, drug combinations will have evolved from better understood molecular phenotypes. The provocative concept presented here is in line with the proposal that a molecular systems approach is mandatory to advance personalized healthcare. Such an approach will require large, metabolomics-based screening programs to obtain appropriate information across ethnicities, different environmental conditions, health/disease states, age, gender, psychological aspects and so on. Surprisingly, this 'futuristic' scheme has already existed for a long time in Eastern medicine, which has been personalized since its origin and is heavily focused on prevention. There is a lot to be gained through the integration of the different medical practices across different cultures, and the personalized diagnostic approaches and the personalized treatment strategies of different cultures could be a great example of capturing value on a global basis to advance healthcare worldwide. The focus on preventive strategies cannot be emphasized enough, and is an additional important factor to be incorporated in future personalized healthcare practices [38]. Metabolomics-based systems analysis and phenotyping will be key and, as outlined above, the 'challenge test' is likely to provide early disease diagnosis.

Perhaps the large clinical trial paradigm will fade away along with blockbuster drugs. Niche busters will become the most common output of the pharmaceutical industry and the scheme shown in **Figure 4** representing an 'n = 1 clinical trial' will become the daily practice of physicians, with multiple, molecular-phenotype-guided and -monitored studies on a single patient. If this vision of future healthcare is to be realized it will be essential to begin planning soon for stepwise implementation in the coming decennia.

Obviously, the present healthcare systems need to be changed radically in order to introduce this new paradigm. However, as has been elegantly analyzed and described based on a complex system analysis [39], for cost and error reduction in drug prescribing and healthcare delivery in the USA, such a strategy at the right operational scale might be a perfect solution.

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Highlights

- A phenotype is comprised of numerous underlying molecular processes that are interdependent, interconnected, multi-leveled and nonlinear; a molecular systems approach is mandatory for understanding and defining the phenotype.
- Metabolomics is the bio-analytical platform that is currently capable of describing the phenotype most comprehensively.
- Personalized medicine depends on an understanding of systems biology and on systems-based developed interventions; monotherapy will fade away and combination therapy will grow in importance.
- Personalized medicine, as part of a personalized health encompassing lifestyle, nutrition, psychology, environmental issues and medicine, is the ultimate opportunity to improve healthcare.
- Biomarkers as health indicators are urgently needed to move the personalized medicine field forward.

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