

1

Medicinal Chemistry Approaches to Creating Targeted Medicines

Bruce D. Roth and Karen Lackey

1.1

Introduction

Personalized medicines are therapies that maximize the biological effectiveness of treatment by targeting the molecular drivers of the disease through a deep understanding of disease biology, identifying and treating the patients most likely to respond based on personal genomics, metabolomics, proteomics, and perhaps epigenomics. This ability to very selectively target appropriate patient populations has become the foundation of much of drug discovery in the past decade due to the remarkable advances in molecular biology and diagnostics that have enabled the understanding of many diseases at the genomic level. Personalized medicine has become even more important, as healthcare costs continue to soar, such that creating the ideal situation where patients would only receive a potent, safe, and efficacious drug that treats their specific disease at a dose that is titrated for their metabolism has become an ethical, a societal, and an economic imperative. The state of personalized medicine today finds different therapeutic areas at very different stages of development. For oncology most of the personalized medicine approaches reflect attempts to design drugs that very selectively target the drivers of a patient's specific cancer. In diseases of neuroscience, current personalized approaches attempt to treat these complex diseases through polypharmacy. For inflammatory diseases, personalized medicine requires strategies for subsetting patients to ensure that the medicine is treating the underlying causes of the disease. In all of these therapeutic areas, the role of medicinal chemistry is to create drugs with very specific properties and biological activities to achieve the objectives of personalization of medical care. The techniques and strategies needed by medicinal chemists ranging from identifying active compounds to optimizing chemical series for the intended patient population, delivery route, and combination therapy required to enable personalized medicine will be discussed in this book. This book will cover the meaning of personalized medicine, its importance, how it is implemented, and how medicinal chemistry has evolved to facilitate it. Since drug discovery research to achieve personalized health care is being

conducted in academia, biotechnology companies, pharmaceutical companies, and research institutes, we have tried to ensure representation from all of these institutions as chapter authors. Because therapeutic areas are in such different stages of achieving personalized medicine, we have dedicated sections of the book to cover the state of the art in oncology, neurosciences, and inflammation to demonstrate the diversity of approaches. Gleevec will be showcased in this introductory chapter as the groundbreaking example of personalized medicine, highlighting the key issues involved, including identification of the intended molecular target and target patient population, expanding the patient population by understanding the drug profile, and the need for alternatively designed drugs to combat resistance and nonresponsive patients. More recent advances in combination therapy and drug delivery will be discussed to show how medicinal chemistry can impact the effectiveness of individualized medicine. Drug repurposing of clinical candidates and marketed medicines can utilize the medicinal chemistry approaches to rapidly achieve personalized medicine goals and will also be covered. We have also included a chapter focused on diagnostics in oncology, an essential aspect of patient identification, highlighting the advanced state of science in this therapeutic area, as well as chapters on approaches to patient stratification in other therapeutic areas and a chapter on imaging as a new diagnostic frontier. The book will conclude with a future perspective on how medicinal chemistry will continue to be the driving force behind translating human genomic information into personalized medicines. Although targeted biologics are an essential part of the armamentarium of drug treatment and have been foundational in the development of personalized medicine, they are beyond the scope of this book and will only be mentioned briefly in this introduction. We will, however, in this introduction touch on the impact of biologics, most notably Herceptin, on the development of personalized medicines, as well as highlighting some of the topics not specifically covered by other authors, such as drug targeting through antibody–drug conjugates and nanoparticles.

1.2

Role of Medicinal Chemistry in Drug Discovery

Medicinal chemistry plays a critical role in the early research essential for lead identification and chemical tool generation, which enables the marrying of small molecules with important protein targets key to allow a deeper understanding of disease biology. Lead identification methods have different requirements for different target classes, gene families, mechanisms of actions, and currently available knowledge and have helped to drive the evolution of medicinal chemistry. For example, high-throughput screening is a well-established tool that has taken advantage of advances in automation technology and creative biological assay systems to evaluate compound libraries of 100 000 to a several million high-quality starting points. This has required medicinal chemists to become skilled in data analysis, hit evaluation, and prioritization of active compound series based on the

physicochemical properties needed for the specific biological target as well as “drug-likeness.”

Orthogonal screening approaches include fragment, virtual, and phenotypic screening. Fragment-based ligand discovery (FBLD) and fragment-based drug discovery (FBDD) have evolved fairly recently and involve screening small molecular weight compounds at high concentrations, usually employing biophysical techniques such as NMR or SPR, with the aid of protein crystallography. The aim of fragment-based discovery is to provide low molecular weight lead molecules that may provide better starting points for further functionalization. Alternatively, several differentially bound fragments can be connected in a way to rapidly increase ligand binding and potency. In some cases, specialized fragment sets can be created for particular target classes. For example, metal binding proteins make up a substantial number of potential drug targets, and fragment libraries can be designed that would preferentially bind to metals and pockets found in these proteins [1]. Virtual screening utilizes a variety of computational approaches (e.g., pharmacophore, shape, similarity searching) to identify potential active molecules for lower throughput assays or as a way to reduce assay screening costs by limiting the number of compounds evaluated. Success in these areas requires medicinal chemistry excellence in structure-based drug design, and the tools and skills to meet this need have evolved remarkably over the past two decades. Phenotypic screening is usually an efficacy assay of direct biological relevance to a disease, where the readout is the outcome desired for progression into *in vivo* assay systems. There is a resurgence of interest in this approach due to its historical success in translating early research to clinically useful drugs, albeit with the disadvantage of the difficulty in determining the precise mechanism of action in some cases [2]. The overall objectives of the lead identification techniques are to provide the medicinal chemist with options for starting points and tools for interrogating biologically important protein targets.

For targets that do not yield lead matter using these more traditional techniques, alternative approaches have been adapted to the lead-finding process. For example, although DNA encoded library technology has been around for over 20 years, only recently has it added significant value to drug discovery [3]. This technology entails creating libraries with tens to hundreds of millions of small molecules that can be pooled together and screened against protein targets under multiple conditions to obtain active compounds based on target affinity. The assay hits are decoded based on the DNA “bar code” of bound compounds, which can be sequenced after using PCR technology. Different families of compounds with a variety of mechanisms of modulating the protein can be found using this technology. This technology has forced medicinal chemists to expand the chemistries available in solvents compatible with DNA (e.g., water), while developing the informatics tools required in dealing with massive, complex data sets.

Ultimately, it is medicinal chemists who must generate the clinical drug candidate during the lead optimization phase of a research project. This requires optimizing the ability to potently modulate the biological target of interest both *in vitro* and *in vivo*, while controlling the physicochemical properties that govern

absorption, distribution, metabolism, and excretion required for the intended route of administration. For an oral drug, medicinal chemists optimize small molecules to be swallowed, to be absorbed into the blood stream, to be carried to the site of the diseased tissue without negative biological effects along the way (i.e., toxicity), and to modulate the intended biological target to restore the tissue to the fully effective and normal state (cure), with an exit from the body that is safe and timely. Each of these components requires specialized design criteria or in many cases, formulation science working with an efficacious compound to modulate properties through salt forms, crystallization techniques, and additives. Later, drug delivery advances in personalized medicine will be discussed and ways for medicinal chemists to make an impact highlighted. Other routes of administration require different properties to be built into the drug candidates. For example, asthma drugs may need to be inhaled or acute care drugs may need to be given intravenously. The medicinal chemist needs to incorporate specific properties that create extreme potency in lung and low systemic exposure for the inhaled drugs, while an IV drug needs to be highly soluble for low injection volumes. The route and dosing can play a role in personalized medicine by delivering the medicine to the diseased tissue in the most expedient manner, and by avoiding exposure to organs where toxicity could present a potential issue for treatment.

1.3 Evolution of Molecular Design for Subsets of Patients

The complexity of disease biology and human systems biology makes it seem impossible to believe that one treatment approach or one drug could achieve a cure for all patients with a particular disease. A small-molecule medicine would need to be absorbed systemically across diverse groups of patients and demonstrate specificity for the diseased cell or aberrant target or tissue, without exerting significant side effects along the way. Within the diseased tissue, the medicine needs to have specificity for the mechanism of action needed to reverse the pathology or to stop the progression. Most likely, there are a combination of mutations or aberrations responsible for the cause or progression of the disease, all of which are affected by genetics, epigenetics, the microenvironment, and as will be discussed later even the microbiome.

Just two decades ago, most projects worked on by medicinal chemists in oncology were variants of chemotherapeutics, where toxicity to the patient was accepted as part of the therapy. The objective was to kill tumor cells at a greater ratio than normal cells. No one expected to achieve oncology treatments without very significant toxicity. For example, camptothecin was shown to be a powerful anticancer agent in preclinical studies, with a mechanism of action of topoisomerase I inhibition. It went into clinical trials based on its ability to achieve a greater ratio of killing tumor cells as compared to normal cells, but with the assumption that the treatment for patients would be inherently toxic [4]. Many drug discovery projects continued throughout the pharmaceutical industry to improve the drug

properties of camptothecin, such as solubility, metabolic stability, and improved therapeutic window. Topotecan and GG211 progressed into the clinic, with incrementally improved drug profiles, but still were designed to treat solid tumors of all patients by killing tumor and normal cells, with just an improved ratio of the former. During this time in the late 1980s and early 1990s, the inhibition of kinases was being debated as a viable way to treat subsets of cancer patients based on protein expression patterns. The transition in oncology drug discovery began with the development of targeted biological agents such as Herceptin [5]. The recognition of Herceptin's exceptional efficacy in the 35% of breast cancer patients who overexpressed the erbB2 receptor first demonstrated the power of targeting therapy to a diagnostically defined patient population based on the mechanism of action of the therapeutic agent.

The landmark discovery of Gleevec (Glivec, STI571, imatinib), first synthesized in the mid-1990s and approved for marketing by the FDA in 2001, ushered in an era of targeted small-molecule anticancer drugs aimed at capitalizing on advances in the understanding of oncogenes and the key drivers of cancer. This event transformed medicinal chemistry in oncology to focus on targeted anticancer drugs, with the potential to be highly selective and much less toxic by preferentially killing tumor cells by attacking targets overexpressed or amplified in cancer, but not in normal cells. Only the highlights of the discovery of Gleevec will be discussed here, while more in-depth information can be obtained in Refs [6–9]. Chronic myelogenous leukemia (CML) is a blood disorder with excessive proliferation of cells (myeloid lineage) associated with a specific genetic abnormality: a reciprocal translocation between chromosome 9 and 22 (the so-called Philadelphia chromosome). The protein product of the aberrant gene, a fusion of the *abl* proto-oncogene and the *bcr* gene called *bcr-abl*, possessed significantly increased tyrosine kinase activity that was subsequently proved to be essential to cell transforming activity. Gleevec was designed to selectively inhibit this kinase activity, revolutionizing treatment of CML. This new paradigm for drug discovery and development was facilitated by having all of the tools required for a drug discovery project available, effectively linking preclinical models with disease in a clinical setting. Thus, the elevated kinase activity could be measured in a catalytic enzyme assay. Cells overexpressing *bcr-abl* could be used for *in vitro* and *in vivo* models. Clinical trials could be designed based on inhibiting a specific mechanism of action in a subset of cancer patients. These tools, combined with the molecular and genetic understanding of this disease, allowed the very rapid development of this molecule from bench to market, including a remarkably short 3 years of clinical trials prior to approval by the FDA for treatment of CML in the US. The ability to demonstrate safety and efficacy in humans in such a short period of time demonstrated the power of this paradigm to rapidly unite patients with therapies effective for their disease.

As the molecular targets of Gleevec became better understood, alternative indications were uncovered. Thus, two additional kinases potentially inhibited by Gleevec are c-Kit, a member of the type III group of receptor kinases, and the

PDGF receptor tyrosine kinase. Based on the work of Hirota *et al.* [10], which identified gain-of-function mutations in c-Kit in gastrointestinal tumors (GIST), clinical trials of Gleevec were initiated in these patients and such profound efficacy was demonstrated that it was approved by the FDA in 2002 based on phase II data [8]. Given the nature of tumor progression, a multitude of mutations have been identified, requiring second and third generation bcr-abl drug candidates along with drugs with unique mechanisms of action to treat CML patients [11].

Subsequent to the discovery of Gleevec, studies of aberrant cell signaling over the past two decades have demonstrated key roles for numerous protein kinases in proliferation, migration, apoptosis, and survival. It is common now to examine tumor tissue for overexpression, mutation, and constitutive activation of a driver-kinase protein, looking for correlation of the kinase activity and disease outcome. Relatively selective kinase inhibitors have been brought to the clinic and many have been approved for use as medicines, providing clear benefit to patients [12]. However, as mentioned earlier, drug resistance typically emerges with prolonged treatment.

1.4

Combinations for Effective Therapies

Targeted therapies also include the concept of combinations, but based on a deep understanding of biology. The most common combinations are where the treatment plan includes separate, selective drugs taken at prescribed intervals, allowing some flexibility in dosage for each medication. There can also be a single drug molecule with a built-in combination profile, where the modulation of more than one protein target makes the treatment more effective than a selective modulator. With diagnostics readily available, a personalized fixed-dose combination could also be possible with snap-together pills.

The identification of optimal drug combinations depends on many factors; however, deep understanding of disease biology is required to fully exploit available drugs in combinations to achieve personalized therapies. Medicinal chemistry has made great strides in creating molecularly targeted drugs with impressive selectivity. Treatments for individual cancer patients need to be designed for their tumors' complex signaling network, with consideration of feedback and compensation phenomena when driver pathways are inhibited. By way of example, Iadevaia *et al.* developed computational approaches for predicting effective combinations using IGF-1-stimulated breast cancer cells (MDA-MB231) as their model system [13]. Without going into the complexity of the modeling and experimental data, we will focus on how a medicinal chemist can use the approach to designing more effective medicines. Much of the signaling data in the literature is difficult to compare due to the effect of the diversity of experimental procedures on the quantitative values and the often, qualitative nature of the information. These authors chose the IGFR network to create a computational model because there exists a large body of data that can be analyzed to create and test a consensus

network, and with clinical candidates progressing in cancer trials, the outcome of the work is highly relevant to patients. The trained model was used to predict the effect of new perturbations in the signaling network and then tested experimentally to validate the model. They wanted to identify the most influential proteins responsible for the aberrant cell signaling to determine the best combinations of inhibitors and siRNAs. While the tool compounds they used were not selective for the protein targets studied, the model could be used for evaluating the next generation of signaling inhibitors, with more advanced designs.

The IGF1R signaling network in the MDA-MB231 cell line included node points, activating and inactivating proteins, and the protein interactions. To illustrate the complexity, their formulation included 77 chemical reactions to describe the consensus IGF1R network. A simplified subset of 41 reactions was used in the model based on inclusion of the most relevant interaction mechanisms in the network. Results of the model suggested that targeting one protein in the signal cascade at a time might activate nontargeted proteins, thus making ultrasensitive drugs or the use of single signaling inhibitors insufficient to block aberrant signaling. In order to determine the right combinations of target molecules, perturbing all molecules in the network simultaneously would help identify the optimal combinations needed to effectively block proliferation signaling. Their research conclusion was that optimal inhibition could be achieved by inhibition of both MAPK and PI3K pathways by correlating it to decreased cell viability. In an important contrast, nonoptimal combinations led to inadequate inhibition of the network and increased cell viability. The computational procedure is one example of many emerging algorithms and data analysis tools, rapidly advancing the field of personalized medicine. The goal is to have tools available to rapidly generate experimentally testable drug intervention strategies, allowing patients to receive optimized combination therapies and to discover novel signaling targets for medicinal chemists to design effective candidate drugs for future more effective combinations.

A cautionary example to counter the apparent success of the IGF1 signaling computational analysis outcome are the lessons for designing combination therapy with dasatinib reported by Park *et al.* [14]. Dasatinib is an oral, small-molecule src/abl tyrosine kinase inhibitor that received FDA approval in 2006 for CML patients who developed resistance to Gleevec. Disappointingly, phase II clinical trials with dasatinib as monotherapy were not encouraging, although preclinical studies with diverse agents suggested dasatinib combinations would be synergistic, although there appeared to be no clear rationale for the synergism. Park *et al.* concluded that molecularly targeted agents like dasatinib should be effective in combinations, but the trial designs and combination therapies may remain empiric. For medicinal chemistry, creating effective mechanism-based components of the therapeutic options remains a high priority, but the sheer number of empiric possibilities to be investigated by translational medicine experts is daunting. Deeper biological understanding and better *in silico* methods for cost-effective, timely, and predictive combinations for personalized medicine, taking into account the genetic heterogeneity and

plasticity of tumors, are urgently needed. These investigators felt that the critical hubs of tumorigenesis were likely to be determined, but felt that modeling of the compensatory pathways or genetic instability was too difficult with the current state of the art.

Achieving personalized medicine in autoimmune and inflammatory diseases is an emerging field of science that holds great promise, but the identification of mechanism-based, diagnostically identified subtypes of patient populations to increase the likelihood of individual response to treatments is still developing. Virgin and Todd recently reported on the concept of understanding disease metagenomics, defined as the sum of the genetic elements of the patient (host) plus all of the genetic elements in all of the microorganisms (bacteria, viruses, and parasites) that live in or on the host [15]. The relationship between genotype and phenotype in complex, chronic diseases such as type 1 diabetes and inflammatory bowel disease were shown to be determined by host gene–microbe interactions and the immune system damaged tissues. Information from genome-wide association studies (GWAS) and analysis of the microbiome can help define mechanisms for inflammatory diseases. The genes (and gene products) identified in the analyses of genotype–phenotype relationships, which lead to pathogenesis should provide validated biomarkers and druggable pathways for medicinal chemistry to discover tool compounds and ultimately drugs for specific subsets of patients.

“A diagnosis may be “clinically” precise but “mechanistically” imprecise Over many decades, pathologists have lumped patients with similar but non-identical clinical and pathological signs and symptoms into diagnostic categories that predict outcomes and complications. Indeed, this has enormous value clinically, but it emphasizes similarities between patients in outcome rather than the differences in pathways that lead to a common endpoint” [15]. The key learnings making an analysis metagenetic, and not just genetic, are the disease diagnostics, the sum of multiple mechanism subsets, and the interactions of individual microorganisms and their genomes with specific host genes and pathways, all critical for understanding the genotype–phenotype relationships in complex diseases. For the medicinal chemist, this approach of subsetting patients by pathways and/or mechanisms of action, despite the complexity of many diseases, aids in the development of selective medicines or combinations. Moving away from diseases as a single pathological mechanism to diseases as multiple mechanism-based subtypes may require the chemist to work across normally separated therapeutic areas (e.g., antibacterial agents and immunomodulation). Since the microbiome, and thus the metagenomics, varies from person to person and affects the development of the immune system, understanding the host gene–microbe interactions is essential to improve drug outcomes. To devise a patient stratification strategy and uncover novel therapeutic opportunities, Virgin and Todd [15] proposed an iterative process of evaluating candidate pathways followed by mechanistic studies in animal models and microbial genetic studies to define the mechanism-based disease subtype with inherent biomarkers that distinguish between patients based on mechanism. The medicinal chemist can play a key role in the iterative cycle by designing drug candidates that target the subtypes.

1.5

Biomarkers in Targeting Patients

Biomarkers, as defined by the NIH, are “a characteristic objectively measured and evaluated as an indicator of normal biological and pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Biomarkers can be divided into two types: diagnostic biomarkers used in patient identification and stratification and pharmacodynamic (PD) biomarkers used to measure therapeutic response. These can be the same or different. For drug discovery and development, the best situation is continuity in the PD biomarker used preclinically to discover and optimize the drug for maximum efficacy and in therapeutic safety margins and the biomarker used clinically to evaluate response in patients. Diagnostic biomarkers are more involved because they are directly linked to the disease pathology and/or progression, and typically distinguish between normal and diseased tissues and patients. The promise for patients is that only those identified as having that diagnostic biomarker will receive the treatment and only responding patients will continue receiving the medicine. Many of the kinase inhibitors that have been successfully launched as drugs were designed to treat specific, diagnostically identified patients and were facilitated by codeveloped drug efficacy (PD) biomarkers that were used preclinically to validate the target and clinically to assess initial clinical response.

Drug efficacy (PD) biomarkers are important for a variety of reasons, but are most critical in developing the relationship between drug exposure and pharmacologic response. The availability of PD biomarkers in early clinical development ensures that only drugs that engage the target adequately are advanced into efficacy trials, greatly increasing the potential for success and reducing the cost of clinical trials, especially when combined with a diagnostic biomarker that has identified the patients most likely to respond. As an example, in the development of the dual erbB1 and erbB2 tyrosine kinase inhibitor (Tykerb), measuring the inhibition of autophosphorylation of the protein in the tumor tissue of patients allowed the early assessment of pharmacological activity. During clinical trials, the biologically effective dose was determined (rather than the maximally tolerated dose) based on target engagement using as the PD biomarker pathway inhibition as measured by the reduction in phosphorylated erbB2, or a downstream protein such as MAPK [16].

1.6

Emerging Field of Epigenetics

Epigenetics is an emerging field, still in the early stages of medicinal chemistry input, but worthy of mention in personalized medicine approaches. Budiman *et al.* recently reported studies on DNA methylation in personalized medicine [17]. To understand how the signature of DNA methylation can inform patient care, we will provide a brief background in epigenetics. While the human genetic code is

relatively static, epigenetics involves heritable changes that affect gene expression and phenotypes. Unfortunately, there is no known single baseline reference for the epigenome to make comparisons between normal and diseased tissues. The epigenome can vary among healthy, normal cellular populations as well as in disease cellular contexts. The way these changes in gene expression, and thus their related protein production, occur is through molecular modifications of histone proteins and the effect these marks have on cooperating partners. In perhaps an oversimplified model of epigenetics, modifications such as methylation, acetylation, ubiquitination, and the reverse (e.g., deacetylation) cause genes to be turned on or off, thus changing the cellular processes. These changes can be positively adaptive (i.e., they are good outcomes of gene expression changes) or they can cause aberrations that lead to disease.

For epigenetics to impact personalized medicine, the pattern of the histone or DNA modifications would need a diagnostic biomarker that meets the NIH definition. Budiman makes the case that patterns of loss of DNA methylation as well as acquired methylation can play a role in an individual's response to therapy and susceptibility to age-related diseases. It is fair to say that these are still very early days in the role of epigenetics in personalized medicine; however, biomarker development is technically feasible as long as the signatures of DNA methylation can be decoded. Once the field matures, it should be possible for a medicinal chemist to create drug candidates that modulate the epigenetic signature. There are several global public-private partnerships involved in the precompetitive research space, working on creating chemical probes and biological reagents to fully annotate the epigenome (e.g., <http://www.thesgc.org/scientists/epigenetics>). Ideally, a few prominent modifications will be linked to disease progression, similar to the computational algorithms being developed for complex signaling networks, and patients will be treated with personalized combinations that reverse the epigenetic modifications to restore healthy cellular processes.

1.7 Systems Chemical Biology

All of the examples given thus far have been reliant on analyzing a subset of data in the context of a single target or pathway, or taking things that were discovered in single pathways and combining them for the desired effect. David Wild *et al.* have defined systems chemical biology as the integration of chemistry, biology, and computation to generate an understanding about the way small molecules affect biological systems as a whole [18]. Chemical genomics builds models based on effects of compounds on multiple biological targets and pathways by studying relationships between chemical compounds and genes and their protein products. Systems chemical biology involves a broader view of analyzing networks of many kinds of data, including compounds, targets, genes, diseases, side effects, clinical data, metabolic data, and more. Thus, these are heterogeneous data sets that are very difficult to integrate, but for the future of personalized medicines, it is critical

that the scientific community taps into all of the information that is being generated in separate public data sources (combined with proprietary databases) to create knowledge about the entire biological system and how the components are differentially affected by treatments. The phrase semantic web refers to “a shared understanding of meaning and accessibility to tools across the data sets” [18]. Thus, a semantically integrated network of data would allow searches using common terminology across multiple databases with a single framework, and would allow the discovery of relationships that go across multiple data sets. The authors discuss a pathfinding algorithm that links drugs and side effects. The algorithm determined that a drug undergoing biological evaluation interacted with genes that had previously been found linked with older drugs with known, specific side effects (all with the gene in common). This information provides a testable hypothesis for a potential side effect. By analogy, one can discover potential risk factors for new drugs and uncover potential mechanisms causing side effects. Medicinal chemists can use this information as an opportunity to design out the side effect by adding the gene target as a selectivity assay in their lead optimization campaign. The World Wide Web Consortium (W3C) is responsible for making recommendations for components of the semantic web. For scientists, the desire is for a straightforward way to integrate heterogeneous data sets between organizations or data silos. This effort is important for the future of medicinal chemistry, since public databases, open access to clinical trial data, and proprietary databases need to be accessible for optimally determining drug efficacy and patient benefit, side effect profiles, stratification of patients, drug differentiation, appropriate combination therapies, unmet medical needs, and potential disease associations for new compounds. The ultimate objective in realizing systems chemical biology is in integrating diverse data resources, building knowledge and using existing computational approaches like homology modeling, QSAR, and virtual screening to enhance our drug design capabilities. The underlying question in this entire approach will be the quality and relevance of the data.

The ability to take a systems biology approach may allow the treatment of complex diseases, such as traumatic brain injury (TBI), where there have been over 200 clinical drug trials, but no successes and thus no FDA approved drugs [19]. For systems biology, as a mimic of complex disease, to result in personalized medicines, it must be integrated with diagnostic or biomarker-based codiscovery. TBI causes physical and chemical perturbations of brain cells, which activate certain targets and signaling pathways resulting in cell injury. As in systems chemical biology described in this section, Zhang *et al.* [19] state that to successfully treat a patient's brain damage and functional deficit, a holistic approach utilizing and integrating diverse databases is necessary – proteomics, genomics, interactome, literature, text mining, experimental data, and more. The goals of systems biology in TBI is to better understand the mechanisms of disease to uncover targets, biomarkers, and diagnostic tools, and to create models to predict the interrelated functions of the system to discover the proteins that regulate cellular decisions. With the dearth of available treatments, TBI seems like a rich area for medicinal chemistry impact. The authors list over a dozen general, relevant public

databases that could be mined for a theranostic approach with an aim to create a TBI medicine that combines the diagnosis, treatment, and monitoring of patient response in one entity. Because nonbiomarker-based trials have resulted in failure, it is highly unlikely that future investment in the area will be supported without them.

Systems biology combined with structural bioinformatics equals systems medicine. Systems biology combines and analyzes diverse data sets to predict the outcomes of system perturbations, using network models. Structural bioinformatics has made significant progress in enabling the science of identifying protein–drug off-targets based on analyzing ligand binding sites to either predict potential toxicities, polypharmacy, or repurposing opportunities. To this end, Chang *et al.* have developed a novel *in silico* drug testing approach for systems medicine with the aim to maximize benefits to patients with treatment and identify risk factors (off-target mechanisms or genetic polymorphisms) that may preclude treatment [20]. Chang *et al.* used their integrative computational approach on predictions for the failed clinical candidate torcetrapib – a cholesteryl ester transfer protein (CETP) inhibitor. This drug candidate was designed to treat cardiovascular diseases by raising high-density lipoprotein cholesterol, but failed due to an increase in mortality in the torcetrapib-treated patients. Because one side effect observed in patients receiving torcetrapib was hypertension, the authors performed context-specific kidney metabolic modeling. The complexity of this approach is such that the authors used 336 explicitly predicted active metabolic genes, 1587 active reactions in the model, and 333 active reactions to develop a submodel for the pathways in the specified renal objectives. They also found different binding affinities for off-targets, using their structural analysis of the three CETP inhibitors that have reached clinical trials (torcetrapib, anacetrapib, and dalcetrapib), suggesting that there will likely be differences in the drug response phenotypes, especially with regards to side effects. Of course, there are limitations to the models because of the subsetting of complex data and the need to test the *in silico* predictions with real clinical data. However, it is important for a medicinal chemist embarking on a drug discovery project to understand potential off-targets to avoid as well as the design features needed to maximize the effectiveness of the drug.

1.8

Theranostics and Designing Drug Delivery Systems

An extensive review of the concept and state of the art of theranostics, materials that integrate therapy and diagnostic imaging, was reported recently by Kelkar and Reineke [21]. While much of the details are beyond the scope of this medicinal chemistry perspective, a good understanding of the aims, the components that a medicinal chemist could impact and the current limitations of theranostics are critical for considering its application to personalizing medicine. Kelkar and Reineke state, “the ultimate goal of the theranostic field is to gain the ability to image and monitor the disease tissue, delivery kinetics, and drug efficacy with the

long term hope of gaining the ability to tune the therapy and dose with heretofore unattainable control” [21]. It is also possible that theranostic agents could impact all stages of drug discovery and development because they help to develop biomarkers of diseases both preclinically and clinically, greatly assisting in target validation, fine-tuning drug efficacy, and determining the final construction of the medicine. One clear limitation is the understanding or even the ability to optimally image and dose drug simultaneously (i.e., stoichiometry and issues with drug mechanism not interfering with imaging). In Section 1.9, two types of theranostics will be described to show areas of potential medicinal chemistry involvement.

Several examples in the recent literature demonstrate the concept of using a delivery system to construct an integrated system for personalizing medicines. Nanoparticles have some unique advantages beyond the design of conjugates, carrier materials, and payloads. For example, nanoparticles are not cleared by kidney, thus they could theoretically attain longer circulating blood levels. In addition, due to tumor tissue characteristics, nanoparticles selectively accumulate near tumors. Dual targeted nanoparticles with the potential to act as both a diagnostic and a therapeutic are particularly advantageous, as diagnostics and targeted therapies could benefit from effective and specific delivery to the site of disease tissue. Nanotechnology, via nanoparticles, could offer drug delivery methods that meet these requirements. Kluza *et al.* reported on a highly functionalized system, whereby they attached two ligands to a liposomal layer, surrounding a nanoparticle carrier with a diagnostic contrast agent [22]. While this sounds complicated, a medicinal chemist may be able to impact the optimization of such systems to create personalized medicines. The concept that these researchers pursued took advantage of the differential expression of specific molecules in the endothelium of newly formed versus normal vasculature. Thus, potentially a medicine could image blood vessels, while treating tumors via an antiangiogenic mechanism. Furthermore, the liposomal nanoparticles were considered bimodal because they were detectable via magnetic resonance imaging (MRI) as well as by fluorescence. MRI contrast agents are used to differentially image normal and diseased tissue; like therapeutic drugs, they must possess the desired properties of high contrast, stability, and acceptable pharmacokinetic properties. The two angiogenic biomarkers that Kluza *et al.* used were based on two receptors: $\alpha_v\beta_3$ integrin and galectin-1. The cyclic peptide cRGD (extensively studied $\alpha_v\beta_3$ inhibitor) and a designer 33-mer peptide Anginex (galectin-1 inhibitor) were conjugated to the bimodal liposomes [23,24]. The dual targeted liposomes were compared with single targeted liposomes, the peptides alone, the liposomes alone, and controls. The investigators found that all types of targeted liposomes were internalized and efficacy was observed for each of the single targeted liposomes. When they were mixed (i.e., like fixed-dose combinations) and examined for cellular uptake and cell cycle analysis, additive effects were observed. However, the dual targeted liposomes demonstrated synergistic effects. This outcome seems particularly important, where the mechanism of the disease has multiple pathways and numerous angiogenic factors to compensate with when a single pathway is blocked. A medicinal chemist could make an impact in this type of system by

optimizing the peptides as targeting agents attached to the nanoparticle carrier. Also, a medicinal chemist could use small-molecule inhibitors instead of peptides or target mechanisms beyond angiogenesis. In all of these targeting strategies, the connection to the nanoparticle carrier needs to be optimized through medicinal chemistry (further explained in the next example).

Liao *et al.* described a nanoscale platform to take an effective, but toxic drug like Doxorubicin and deliver it with greater tissue specificity in combination with an MRI agent [25]. The concept of a cancer therapy nanocarrier is to create a drug delivery system to reduce side effects by encapsulating the anticancer drug until it reaches the tumor and releases the cytotoxic agent. These researchers designed a hydrophobic core with high loading capacity, using a polymer of lactide and glycolide termed PLGA for poly(DL-lactide-co-glycolide). The ratio of the monomers was adjusted to vary the drug release rate and to avoid drug leakage in route to the tumor. A hydrophilic PEGylated lipid shell, similar to the one described earlier, was made paramagnetic by chelating diethylenetriamine pentaacetic acid–gadolinium [Gd(DTPA)(H₂O)]₂⁻ and targeted by linking to folic acid. The idea combines multimodal imaging, simultaneous diagnosis and therapy, specific targeting, and controlled release of therapeutics. Medicinal chemistry changes to the system could include folate replacement for alternative targeting or for creating dual target enhancement by linking folate plus an additional targeting agent. The release rates of the drug may need to be modified for specific tumor types and the payload could be two synergistic drugs that block cell signaling rather than a cytotoxin. Of course, a strong partnership between a medicinal chemist and a materials science expert would be needed to ensure that the nanoparticle morphology, stability, size distribution, and pharmacokinetic properties were optimized along with the target potency and efficacy.

A prodrug strategy can be employed to take effective, nonpersonalized medicines with toxicities or dose-limiting side effects and convert them to targeted medicines with fewer side effects and greater efficacy if properly targeted. Examples include monoclonal antibody–drug conjugates, aptamers, receptor agonists and antagonists, peptide hormones, and vitamins to name a few. The definition of a prodrug is a biologically inactive form of a drug that can be converted into the active parent molecule before or at the site of action. Focusing on ligand-targeted prodrug therapeutics, Kularatne *et al.* reported a method for targeting highly potent cytotoxic agents to prostate cancer tumors via PSMA (prostate-specific membrane antigen)-targeted prodrugs [26]. These researchers started with the design of the targeting agent to be attached to the cytotoxic drug; (dicarboxypropyl)ureidopentanedioic acid (DUPA) binds to cell surface glycoprotein PSMA and enters via endocytosis. The medicinal chemist's role is synthesis, design of the linker, optimization of the targeting agent, improving binding affinity, ensuring appropriate stability, and water solubility. Kularatne surveyed several cytotoxic agents and found eight suitable candidates for prodrug attachment with IC₅₀ values as nontargeted agents in the LNCaP (prostate) cancer cell line less than 10 nM, a threshold determined from their experience in the field. These cytotoxic agents were modified with linkers that preserved their cytotoxicity and terminated in a

moiety to allow facile disulfide linkage to DUPA. Thus, the design could be described as warhead – linker – DUPA.

The advantages of this approach are therapeutic flexibility, potential diagnostic value, and improved cell permeability. Therapeutic flexibility was demonstrated by taking an optimized targeting agent DUPA, attaching eight different cytotoxic agents via a common linker connection, and obtaining enhanced efficacy and cellular selectivity. Presumably, a similar approach could be taken to target any drug to pathological tissue with an appropriate linkage plus targeting ligand. As with the nanoparticle technology described in this section, a diagnostic agent could be cognate to the targeting agent to more easily identify responders to the new drug entity. Receptor-mediated endocytosis could be utilized for enhanced cellular uptake for the targeted agents, thus potentially improving cell permeability. All of these advantages also convey challenges. For example, many diseases do not have sufficiently potent compounds to fill the warhead role. The medicinal chemistry needed to create a linker of sufficient stability to reach the site of action, yet labile enough to release the drug, can be a challenge. A disadvantage of the whole approach is both the small quantity of molecules that enter the cell via endocytosis and the requirement of drug release into the cytosol once inside the cell. There are many components to the system that need to be optimized for it to work for personalized medicine. However, considerable success has been achieved with the antibody–drug conjugate approach as evidenced by the recent approval of T-DM1 for treatment of Her2+ breast cancer [27].

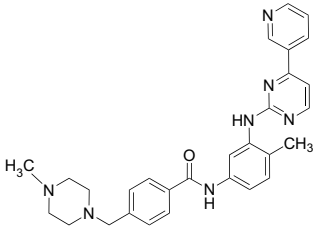
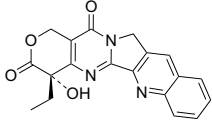
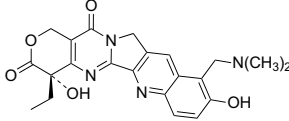
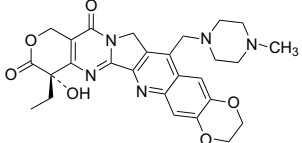
1.9

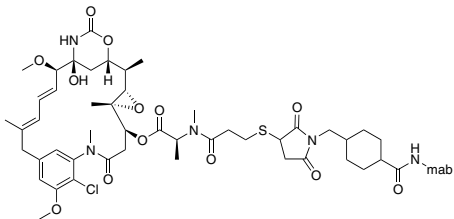
Rapid Progress in Further Personalizing Medicine Expected

While the science of drug delivery and antibody–drug conjugates is beyond the scope of this book, it is tantalizing to believe that biomarker-induced drug release or tissue-specific distribution (chronobiology) could make personalized medicine safer and more efficacious within the next decade. With smart phones to collect clinical trial data and adaptive clinical trial design progressing, we are rapidly approaching a world of electronic information allowing incremental adjustments in dosing and combination. You can imagine a patient ingesting a multisegmented pill that had the ability to disperse the correct amount of each drug, titrated at a level for maximum patient benefit. Polypharmacy controlled by a patient and doctor's understanding of their physical well-being would be a huge advance in medicine. For example, Parkinson's disease and rheumatoid arthritis can be present in the same elderly patient. Finding devices or triggers based on patients' real-time data could allow optimized dopamine release and anti-inflammatory cotreatment. For these combinations to be effective, the medicinal chemist would need to design exquisitely selective compounds, with little or no drug–drug interactions. Fixed-dose combinations are probably more feasible in the near future, but the ideal would be to reach a state where pharmacogenomic/proteomic

feedback drives dosing. This need is especially true where tolerance, resistance, and comorbidity exist in patients.

Achieving true personalized medicine will necessarily require personalized administration. The traditional definition of drug delivery involves optimized devices and formulations. However, Florence and Lee reported “personalized medicine involves the correct diagnosis, the correct choice of drugs, the choice of optimal dose, the calculation of the dose for specific individuals, and drug administration at the appropriate time and, as with intravenous medication and implanted pumps, the proper rate” [28]. The authors describe the current standards in healthcare, where patients with chronic diseases typically have more than one diagnosis, and patients over 65 years take multiple medications (the authors quote an average of 13 per patient!). Patient compliance issues are significant with the complexity of multiple medications and different coexisting chronic diseases. Individualized dosage forms are needed for key patient parameters such as tissue distribution, metabolism, and avoiding drug–drug interactions. Advances in biotechnology, genomics, proteomics, and pharmacology have positioned medicinal chemists to design and create remarkable lifesaving medicines that will continue to push the frontiers of personalized drug therapy.

Drug	Structure	Target
Gleevec, STI571, imatinib		bcr-abl, PDGFR, c-Kit
Camptothecin, CPT		Topoisomerase I
Topotecan		Topoisomerase I
GG211		Topoisomerase I

Drug	Structure	Target
TDM-1, trastuzumab emtansine		HER2

References

- Johnson, S., Barile, E., Farina, B., Purves, A., Wei, J., Chen, L.-H. *et al.* (2011) Targeting metalloproteins by fragment-based lead discovery. *Chemical Biology and Drug Design*, **78** (2), 211–223.
- Swinney, D.C. and Anthony, J. (2011) How were new medicines discovered? *Nature Reviews. Drug Discovery*, **10** (7), 507–519.
- Clark, M.A. (2010) Selecting chemicals: the emerging utility of DNA-encoded libraries. *Current Opinion in Chemical Biology*, **14**, 396–403.
- Wall, M.E. and Wani, M.C. (1995) Camptothecin and taxol: discovery to clinic: thirteenth Bruce F. Cain Memorial Award Lecture. *Cancer Research*, **55** (4), 753–760.
- Chang, J.C. (2007) HER2 inhibition: from discovery to clinical practice. *Clinical Cancer Research*, **13** (1), 1–3.
- Buchdunger, E. and Capdeville, R. (2006) Glivec (Gleevec, imatinib, STI571): a targeted therapy for chronic myelogenous leukemia, in *Protein Tyrosine Kinases* (eds D. Fabbro and F. McCormick), Humana Press, pp. 145–160.
- Zimmerman, J. (2002) Glivec: a new treatment modality for CML: a case history. *Chimia*, **7–8**, 428–431.
- Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Reviews. Drug Discovery*, **1** (7), 493–502.
- Sherbenou, D.W. and Druker, B.J. (2007) Applying the discovery of the Philadelphia chromosome. *Journal of Clinical Investigation*, **117** (8), 2067–2074.
- Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Tunio, G.M., Matsuzawa, Y., Kanakura, Y., Shinomura, Y., and Kitamura, Y. (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*, **279**, 577–580.
- Daley, G.Q. (2003) Gleevec resistance: lessons learned for target-directed drug development. *Cell Cycle*, **2** (3), 190–191.
- Cohen, P. and Alessi, D.R. (2013) Kinase drug discovery: what's next in the field? *ACS Chemical Biology*, **8**, 96–104.
- Iadevaia, S., Lu, Y., Morales, F.C., Mills, G.B., and Ram, P.T. (2011) Identification of optimal drug combinations targeting cellular networks: integrating phosphoproteomics and computational network analysis. *Cancer Research*, **70** (17), 6704–6714.
- Park, B.J., Whichard, Z.L., and Corey, S.J. (2012) Dasatinib synergizes with both cytotoxic and signal transduction inhibitors in heterogeneous breast cancer cell lines: lessons for design of combination targeted therapy. *Cancer Letters*, **320**, 104–110.
- Virgin, H.W. and Todd, J.A. (2011) Metagenomics and personalized medicine. *Cell*, **147**, 44–56.

- 16 Lackey, K.E. (2006) Lessons from the drug discovery of lapatinib, a dual ErbB1/2 tyrosine kinase inhibitor. *Current Topics in Medicinal Chemistry*, **6** (5), 435–460.
- 17 Budiman, M.A., Smith, S.W., and Ordway, J.M. (2011) DNA methylation in personalized medicine. *Personalized Medicine*, **8** (1), 35–43.
- 18 Wild, D.J., Ding, Y., Sheth, A.P., Harland, L., Gifford, E.M., and Lajiness, M.S. (2012) Systems chemical biology and the semantic web: what they mean for the future of drug discovery. *Drug Discovery Today*, **17** (9/10), 469–474.
- 19 Zhang, Z., Larner, S.F., Kobeissy, F., Hayes, R.L., and Wang, K.K.W. (2010) Systems biology and theranostic approach to drug discovery and development to treat traumatic brain injury, in *Systems Biology in Drug Discovery and Development: Methods and Protocols*, Methods in Molecular Biology, vol. 662 (ed. Q. Yan), Springer Science and Business Media, pp. 317–329, ISBN 978-1-60761-800-3.
- 20 Chang, R.L., Xie, L., Xie, L., Bourne, P.E., and Palsson, B.O. (2010) Drug off-target effects predicted using structural analysis in the context of a metabolic network model. *PLoS Computational Biology*, **6** (9), e1000938.
- 21 Kelkar, S.S. and Reineke, T.M. (2011) Theranostics: combining imaging and therapy. *Bioconjugate Chemistry*, **22**, 1879–1903.
- 22 Kluza, E., van derSchaft, D.W.J., Hautvast, P.A.I., Mulder, W.J.M., Mayo, K.H., Griffioen, A.W., Strijkers, G.J., and Nicolay, K. (2010) Synergistic targeting of $\alpha_v\beta_3$ integrin and galectin-1 with heteromultivalent paramagnetic liposomes for combined MR imaging and treatment of angiogenesis. *Nano Letters*, **10**, 52–58.
- 23 Ruoslahti, E. and Pierschbacher, M.D. (1986) Arg-Gly-Asp: a versatile cell recognition signal. *Cell*, **44**, 517–518.
- 24 Wang, J.B., Wang, M.D., Li, E.X., and Dong, D.F. (2012) Advances and prospects of anginex as a promising anti-angiogenesis and anti-tumor agent. *Peptides*, **38** (2), 457–462.
- 25 Liao, Z., Wang, H., Wang, X., Zhao, P., Wang, S., Su, W., and Chang, J. (2011) Multifunctional nanoparticles composed of a poly(DL-lactide-coglycolide) core and a paramagnetic liposome shell for simultaneous magnetic resonance imaging and targeted therapeutics. *Advanced Functional Materials*, **21**, 1179–1186.
- 26 Kularatne, S.A., Venkatesh, C., Santhapuram, H.-K.R., Wang, K., Vaitilingam, B., Henne, W.A., and Low, P.S. (2010) Synthesis and biological analysis of prostate-specific membrane antigen-targeted anticancer prodrugs. *Journal of Medicinal Chemistry*, **53**, 7767–7777.
- 27 Rush University Medical Center. (2013) New more effective treatment option for breast cancer patients approved by FDA. *Science Daily*, February 22, 2013.
- 28 Florence, A.T. and Lee, V.H.L. (2011) Personalised medicines: more tailored drugs, more tailored delivery. *International Journal of Pharmaceutics*, **415**, 29–33.

