CHAPTER 1

Simple Drugs Do Not Cure Complex Diseases: The Need for Multi-Targeted Drugs

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1.1 Introduction

The contents of this book provide a comprehensive overview of the field of polypharmacology and modern approaches to identify drugs that hit multiple targets, including a number of case studies. But why do we actually need such multi-targeted drugs? This introductory chapter aims to answer that question. First, there is clearly a need for better and safer drugs in the clinic and also to improve output (productivity) of drug discovery and development in general. Second, many diseases with unmet medical needs are in essence complex and multi-factorial. I will discuss two disease areas, cancer and rheumatoid arthritis, to exemplify this complexity. Systems biology and network control analysis have shown that the systems underlying complex diseases are robust against perturbations and are always controlled by more than one biochemical process. Therefore, aiming to hit multiple targets is a better strategy than to hit a single target. Finally, though polypharmacology is naturally associated with toxicology and off-target side effects, it can be argued that multi-targeted drugs,

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when rationally designed, can actually have a larger therapeutic window than those hitting a single target and thus prove to be safer drugs.

1.2 The Need for Better and Safer Drugs

There are two main reasons why we need better and safer drugs.

Firstly, there is the unmet medical need in the clinic. Patients need safe cures and complex diseases are difficult to cure. Cancer survival rates, for example, are still lower than desired, roughly 50–65% in Europe and the US, making it a leading cause of death, responsible for almost 25% of all deaths in the US.[1,2] Other examples include autoimmune disorders, some cardiovascular diseases, diabetes and neurodegenerative diseases. The incidence of some of these diseases is expected to increase with the increasingly ageing population. Dementia, for instance, affects almost 1% of those at 60–64 years of age and that number doubles for each subsequent 5-year cohort to 25–33% of those $\geq$85 years of age.[3] Furthermore, the incidence of serious adverse drug reactions in hospitalized patients is so high that it ranks as the 4th–6th leading cause of death.[4]

Secondly, the low success rate of drug development calls for better and safer drugs. In the past few years, an average of only $\sim$20 new drugs were approved annually.[5,6] This is the result of the high attrition rates in clinical development: about 90% of all new drugs fail after first-in-human testing, varying from 80% for cardiovascular diseases to 95% for cancer.[7] The main underlying causes were identified to be lack of efficacy and poor safety (toxicology and clinical safety), each accounting for $\sim$30% of all failures.[7]

One may therefore argue that we need to hit better targets. However, going after novel targets has, in itself, not proven to be a particularly successful strategy for drug development. Attrition of candidates with a novel mechanism of action is higher than average.[8] In addition, many diseases with unmet medical needs are complex and multi-factorial. Therefore, an approach to hit multiple targets may be more successful. The next two sections of this chapter discuss the complexity of cancer and rheumatoid arthritis.

1.3 Cancer

Two decades ago, Fearon and Vogelstein proposed their genetic model for colorectal tumorigenesis.[9] From comparing cells from multiple stages of colorectal cancer, it became apparent that, at each stage, cells had acquired at least one additional mutation, compared to the previous stage. The fact that carcinogenesis is a multi-step process requiring multiple sequential mutations has since been confirmed many times, e.g. by similar models for other types of cancer and by the artificial creation of tumour cells by introducing defined genetic alterations.[10–13] Besides mutations that change the structure and function of a gene product, (epi)genetic alterations which influence gene expression also contribute to carcinogenesis, such as gene amplification, changes in DNA methylation and histone acetylation, and the functioning of micro-RNAs.[14–17] The list of genes which are causally implicated in cancer via

genetic alteration currently contains 436 genes.[18,19] Though some genes are frequently mutated in many cancer types, it is rather a combination of low frequency mutations that drive the cancer phenotype and they differ per cancer type.[20] This (epi)genetic heterogeneity of cancer presents a major challenge.[21] Disturbances of the signal transduction pathways in which most of these cancer genes function leads to the so-called 'hallmarks of cancer', including evasion of apoptosis and growth control, self-sufficiency in growth signals, induction of angiogenesis, the ability to metastasize, evade immune surveillance and, indirectly, stress phenotypes.[20,22–24] It is important to recognize that cancer is a multi-factorial disease already at its origin (the genetic level) and in its essence (the hallmarks). But this is merely where the complexity starts.

The information flow through signalling pathways that ultimately constitute these hallmarks is highly complex. The many sequential steps in a pathway encompass many different biochemical processes, such as protein binding (e.g. recruitment of pathway components by scaffolds, binding of ligand to receptor, receptor dimerization, transcription factor binding) and enzymatic reactions (e.g. phosphorylation, methylation, acetylation, ubiquitination). The output of a signalling pathway does not follow the input in a linear fashion. In part, this is caused by non-linear kinetics of biochemical reactions and different expression levels of pathway components. Signalling is also subject to spatio-temporal control and macromolecular crowding, since pathway components can be physically separated or highly concentrated locally.[25–27] Moreover, about a decade ago, the concept that signal transduction pathways have a linear architecture had to be abandoned. First, the topology of regulatory circuitry, such as negative feedback loops, appeared to be a recurrent theme in signalling networks.[28] Second, pathways are so highly interconnected by direct interactions (e.g. via phosphorylation), indirect regulation (e.g. via gene expression) or by sharing pathway components, that they form complex signalling networks rather than linear pathways.[29–32] This network structure itself can give rise to new 'emergent properties' or 'systems behaviour'.[33–36]

Beyond the multitude of contributing factors on the genetic level and in signalling networks, the complexity extends on the supra-cellular level. The population of cancer cells in a tumour is heterogenic, e.g. with respect to their state of differentiation.[37] Cells communicate with each other either by direct interaction or by stimulating each other in paracrine growth factor loops. Targeting those loops is a strategy for treatment of certain cancers.[38,39] And within the microenvironment, complex interactions between tumour cells and stromal cells, like paracrine growth factor networks, stimulate tumour progression.[40,41] Then, there is the interaction with the host immune system. On the one hand, tumour cells can secrete cytokines which trigger macrophages to differentiate to a subtype that actually promotes tumour progression.[42] On the other hand, immune cells can clean up tumour cells, which makes the therapeutic strategy to stimulate the host immune system to launch an immunological attack on the tumour very promising.[43] A further example of an important supra-cellular interaction is the activation of angiogenesis.[44] Under hypoxic conditions, tumour cells secrete pro-angiogenic growth factors,

thereby stimulating the proliferation of endothelial cells. This leads to new blood vessel formation and provides the tumour cells with access to more oxygen. If tumours do not trigger this 'angiogenic switch', they will not be able to grow and will remain dormant.[45]

In summary, it can be concluded that a combination of multiple genetic alterations that affect multiple processes give rise to the development of cancer.

1.4 Rheumatoid Arthritis

A similar conclusion can be drawn from the biology of many autoimmune disorders, such as rheumatoid arthritis (RA). This systemic disease is characterized by joint inflammation and subsequent cartilage and bone destruction. The development of the disease occurs roughly in three steps: (i) the onset of a modest local inflammation in the synovium by the innate immune system; (ii) triggering of an adaptive immune response by the recruitment and activation of immune cells, leading to full-blown inflammation; and (iii) the invasion of the cartilage and increased bone resorption leading to bone destruction.[46]

Many different cell types are involved during this process. Mast cells produce pro-inflammatory mediators, such as cytokines, and also tissue destructive proteases. They contribute to the initiation of the adaptive immune response by recruiting T-cells, influencing T-cell skewing and antigen presentation.[47] Also dendritic cells, in their function as antigen-presenting cells, are thought to play a role in initiation of the adaptive immune response.[48] Macrophages produce a large variety of cytokines and chemokines. Their abundance and activation in the synovium correlates with the severity of RA.[49] Abnormal lymphocyte function is thought to be a central part of RA pathology. T-cells mediate both the recruitment and the activation of other immune cells by direct cell–cell contact and cytokine production.[50] B-cells produce self-reactive antibodies, function as antigen-presenting cells and secrete cytokines.[51] Fibroblast-like synoviocytes in the synovial intimal lining also play a key role by producing cytokines that perpetuate inflammation and proteases that contribute to cartilage destruction. They develop a unique aggressive phenotype that increases invasiveness into the extracellular matrix and further exacerbates joint damage.[52] Another important cell type for bone destruction are osteoclasts.[53] Joint degeneration is ultimately mediated by a disturbance in bone homeostasis, which is normally kept by balancing bone formation (by osteoblasts) and bone resorption (by osteoclasts). In RA, bone loss is due to excess bone resorption by osteoclasts, which are activated by inflammatory factors, and simultaneously, bone formation by osteoblasts is also impaired at erosion sites.

These many different cell types (and their subtypes) all contribute to RA and they communicate with each other by direct cellular contact and by an extensive extracellular network of cytokines and chemokines.[46] Factors like TNFα, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-15, IL-17, IL-18, IL-21, IL-22, IL-23,

GM-CSF, MMPs, RANKL and RANTES function in autocrine and paracrine networks that mediate the onset and propagation of the inflammation, as well as bone destruction.[54] The central role of cytokines in RA pathology is clearly evidenced by the relatively successful application of biologics that block cytokine function to treat the disease.[55]

Zooming in on the individual cell types presents a further level of complexity: the structure and function of signal transduction pathways mediating the inflammatory response and regulating cell proliferation, survival and differentiation, and expression of aforementioned extracellular factors. The activation of lymphocytes often requires two signals.[56] T-cell activation occurs after engagement of the T-cell receptor (TCR) with its cognate peptide–major histocompatibility complex (signal 1) and subsequent engagement of co-stimulatory molecules (signal 2). This 'second signal' contributes to T-cell activation by promoting proliferation, survival and effector function. A multitude of factors are involved in processing the signal from the TCR and activating downstream signalling pathways.[57,58] Similarly, B-cell activation upon stimulation of the B-cell receptor (BCR) requires co-stimulation for the development of complete effector function, and a complex cascade is involved in propagation of the signal.[56,59] The key downstream signalling pathways in inflammation are similar to, overlap with or are often even the same as the signalling pathways involved in cancer, e.g. JAK-STAT, NF-κB, MAP kinase pathways.[60–62] As discussed above in Section 1.3, these pathways are highly complex with respect to the large number of signalling molecules, the different nature of the reactions they are involved in, the structure of the pathways, with cross-talk and feedback loops, etc. Many kinases in these pathways are currently being pursued as drug targets for treatment of RA.[60,63–66]

In conclusion, RA is a complex disease, which depends on the combined action of many cell types and multiple factors at the supra- and intra-cellular levels.

1.5 Control of Complex Biological Systems

As complex diseases, such as cancer and rheumatoid arthritis, are indeed dependent on so many factors, it is a major challenge to produce successful treatment strategies. Systems biology is needed to integrate the available knowledge and develop comprehensive understanding of how systems behaviour depends on the components of the system and their interactions.[67–69] Substantial advances in understanding (and prediction) have been achieved by integrating computational modelling with quantitative experimental data on molecular and cellular networks, in particular in the field of cancer systems biology.[70,71] On the basis of network models, it has been proposed that partial inhibition of multiple drug targets is more effective than full inhibition of a single target.[72] This can be explained by the intrinsic robustness (the ability to maintain homeostasis) of living systems.[73] Biological networks are built up with a high degree of redundancy and a small fraction of essential

components.[74] In order to modulate the phenotype of a complex and robust disease system, several perturbations of non-essential components must be combined. Network analysis can then further aid to identify successful combinations of drug targets.[71,75–79] It is essential to recognize that the robust nature of complex diseases is caused by their network properties and it is therefore the network that should be targeted, rather than isolated parts.[79,80]

A similar lesson can be drawn from the application of metabolic control analysis (MCA). MCA quantifies the extent to which individual reactions or network parts control the entire reaction network, such as the control of a kinase on downstream phosphorylation, the control of a signalling pathway on cell proliferation, or the control of a particular cell type on disease pathology. In that way, MCA aids in the selection of drug targets, based on the magnitude of their control.[81,82] Applying MCA to computational models of signal transduction indeed allowed for rank-ordering individual reactions: some reactions exert more control than others. Perhaps more surprisingly, it was also found that control tends to be distributed over more than a single reaction.[83–85] In other words, there was no single rate-limiting step. One can thus argue that multi-targeted drugs will be more effective than mono-targeted drugs.

1.6 Safety of Multi-Targeted Drugs

As mentioned above, where ~90% of all drug development projects fail after first-in-human testing, ~30% do so because of toxicology or clinical safety criteria.[7] Since many animal toxicity studies are done in pre-clinical development, the attrition related to safety/toxicology issues of development candidates produced by drug discovery research is even higher. Serious adverse drug reactions leading to attrition and drug withdrawal manifest themselves in a variety of different ways and organs, in particular in cardiovascular side effects and in liver toxicity.[86] Because of its association with toxicity (often referred to as side effects), polypharmacology has long been considered undesired. However, there are some arguments that polypharmacology in itself does not necessarily have to be associated with toxicity and that multi-targeted drugs can actually have a better efficacy/safety ratio than mono-targeted drugs. The underlying causes for safety/toxicology-related issues can roughly be grouped into three categories.

1.6.1 Target-Related Toxicity

Target-related toxicity (also often referred to as mechanism-based toxicity or as exaggerated primary pharmacology), is caused by hitting the intended pharmacological target.

An example of target-related toxicity is the proarrhythmic effect of class III antiarrhythmic drugs, such as dofetilide. These drugs modulate the activity of cardiac potassium channels, such as hERG, which leads to prolongation of the refractory period in the myocardium, and reduction of arrhythmias.

But paradoxically, this prolongation of the so-called QT interval also increases the risk for proarrythmia in predisposed individuals, in particular torsade de pointes, and sudden death.[87–89] This makes the risk-benefit assessment of this drug class very important.[90]

Intuitively one could argue that hitting more targets implies a higher risk for target-related toxicity. However, polypharmacology can also be used to reduce target-related toxicity. By rationally selecting multiple drug targets, such that the therapeutic effects of those targets overlap, but the detrimental effects do not overlap, one automatically designs in a therapeutic window based on target choice.

When analysing signalling pathways with MCA, it was found that activation of one of the pathway components, for example one which occurs in the event of an oncogenic mutation, reduces the control exerted by that component.[91] It is a given that total control of all pathway components (i.e. the sum of the quantified control for each individual pathway component) on signal transduction always sums to a constant value.[92,93] Therefore, a change in control by one pathway component will always be compensated for by a change in control of other pathway components. As a result, the distribution of control will be different in diseased versus healthy cells.[76,91] It should therefore be possible to hit multiple targets which, in combination, exert more control on the diseased system than on the healthy system. This would provide an inherent therapeutic window, and a smaller risk for target-related toxicity.

1.6.2 Off-Target Toxicity

Off-target toxicity is caused by hitting one or more targets other than the intended pharmacological target.

Polypharmacology has in particular been associated with toxicity or side effects caused by hitting off-targets. Though there are many, a good example is again the inhibition of hERG, which via prolongation of the QT interval leads to cardiac side effects in many drugs that exert their therapeutic effect via other targets.[88] However, such type of polypharmacology-related toxicity is caused by the compound hitting *unintended* targets, and such targets were often not identified until later compound profiling efforts. In contrast, a modern polypharmacology approach focuses on the design of drugs aimed at multiple *intended* targets. Moreover, polypharmacology can nowadays be predicted using *in silico* approaches and monitored carefully by *in vitro* profiling on a large set of targets in early stages of drug discovery (discussed elsewhere in this book). It is perhaps noteworthy that unintended targets can also contribute to efficacy. Sorafenib, for example, was originally developed as a Raf kinase inhibitor to treat malignant Raf-dependent melanoma, but it turned out to be a multi-kinase inhibitor that, because of its effect on receptor tyrosine kinases such as the VEGF receptor, is now approved for the treatment of renal cell carcinoma and hepatocellular carcinoma.[94,95]

1.6.3 Chemistry-Related Toxicity

Chemistry-related toxicity is caused by the chemical properties of the compound.

Examples of compound chemistry-related toxicity include oxidative damage, where reactive oxygen species cause oxidation of DNA, proteins or lipid membranes, and covalent binding to macromolecules by reactive metabolites.[96–98] The occurrence of those, often idiosyncratic, toxicities is clearly related to the daily dose: drugs dosed at 10 mg/day or less rarely cause toxicity.[96,97] Recently, this was further refined with the introduction of a zone classification system, which captures the risk for idiosyncratic toxicity in varying zones, based on daily dose and reactivity in human hepatocytes.[99] As mentioned in Section 1.5, it has been proposed that if multiple targets are hit simultaneously, partial inhibition of those targets may be sufficient in order to perturb the robust disease system, and will be more effective than complete inhibition of a single target.[72] That implies, with the assumption that target-binding kinetics are comparable, that the free drug concentration required to obtain a therapeutic effect is lower for a multi-targeted drug than for a mono-targeted drug. At lower dose levels, the risk for idiosyncratic chemistry-related toxicity is reduced.

In summary, toxicity has long been considered inherent to drugs that hit more than a single target. However, it is important to recognize that modern polypharmacology is actually an opportunity to produce safer drugs, by combining rational selection of target combinations with rational design of compounds that hit those targets.

1.7 Concluding Remarks

In clinical practice, combination therapy is a well-established concept. Numerous examples exist in which multi-factorial diseases can be treated more successfully either with a mixture of drugs or with a multi-targeted drug, including the treatment of cancer with multi-targeted kinase inhibitors and RA with methotrexate and other agents.[100–106] The approach to hit multiple targets has, however, not always been part of the initial strategy for their discovery (one gene, one drug, one disease). If we appreciate the underlying complexity of multi-factorial diseases, rational design of multi-targeted drugs becomes essential: Simple drugs do not cure complex diseases.

Many diseases with high medical need are multi-factorial. Here, I have highlighted that cancer and RA are diseases that originate and develop because of the combined action of multiple factors. A single mutation does not cause cancer; a single immune cell type does not sustain inflammation in RA. In both disease areas, highly complex genetic and signal transduction networks are affected; many different cell types are involved. Systems biology and network analysis have shown that the multi-factorial nature of complex diseases and the intrinsic robustness of the complex disease networks require perturbation of multiple targets simultaneously. Control of such networks is

always dispersed over multiple steps. Multi-targeted drugs may not only be more efficacious, they may also be safer and reduce toxicity-related attrition. It is precisely these drugs that we need in the pharmaceutical pipelines and that patients need in the clinic.

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