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Personalized Network-Based Treatments in Oncology

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Network medicine aims at unraveling cell signaling networks to propose personalized treatments for patients suffering from complex diseases. In this short review, we show the relevance of network medicine to cancer treatment by outlining the potential convergence points of the most recent technological and scientific developments in both drug design and signaling network analysis.

For many years, cancer research has been focused on developing new drug treatments, especially personalized treatments, that aim to target a patient's cancer specifically. Personalized or targeted therapies have been proposed, based primarily on gene sequencing[1,2] or gene expression patterns.[3–5] A common significant limitation of these personalized therapies is that they are focused on identifying a single marker that will be measured to determine the optimal treatment. However, as demonstrated by Janes *et al.*[6] in a landmark article, this simplistic approach can lead to wrong decisions. They found that the protein Jun N-terminal kinase could have either a pro- or an antiapoptotic effect, depending on the state of the signaling network. This highlights a very important aspect of cellular decision processes: signaling follows rules of complex systems, in which the final outcome is dependent not only on the parameters of the system but also on the initial conditions (context dependency). The importance of signaling network architecture has direct implications on biomarker discovery, and several authors have advocated for a shift of the paradigm from measurements of one or a few parameters to evaluation of signaling networks.[7–12, 23, 25]

In the signaling network paradigm, which considers external cues to be processed by a series of protein–protein interactions and posttranslational modifications and results in phenotypic changes, the analysis of a disease is based on several signals or markers and the treatment is based on the assessment of the disease as a system. Suggested therapies take into account the capacity of the system to adapt to perturbation (e.g., drug resistance), and therefore the proposal of combination therapies that would be able to overcome the system's robustness, by anticipating its adaptation mechanisms, has been put forth.

Combination therapies have been around for decades,[13,14] and several combination therapies with agents that target different so-called "pathways" are in clinical trials. For example, in advanced melanoma, studies combining a BRAF inhibitor and a Pi3K inhibitor are currently recruiting patients (see ClinicalTrials.gov). Even though these combination therapies have been designed with the knowledge that cells can adapt to one perturbation,[15] they have not been designed following a systematic analysis of both the dynamics of cell signaling networks (i.e., how signals and networks themselves change over time) and the cross talk between these networks. A fundamental problem is the inherent incomplete, non-context-aware, and even incorrect descriptions of dynamic network states as simplistic "pathway" diagrams.[16]

Thus, other ways to design combination treatments have been proposed, ranging from time-staggered application[17] to the use of several potentially nonspecific drugs.[18] Lee and colleagues' use of the system's dynamics to improve the efficacy of drugs is a groundbreaking approach, and we predict that many more such studies will pave the way for new, more powerful treatment strategies, even for combinations of drugs that have previously been deemed ineffective. **Figure 1** highlights the main differences between classic targeting strategies, which hit a single target, and network-based strategies, which hit part or the whole of a signaling network.

From this perspective, we review the state of the art in network-based drugs and treatments and propose ways to provide more effective treatments. We show that a better understanding of signaling networks is a critical step toward such treatments.

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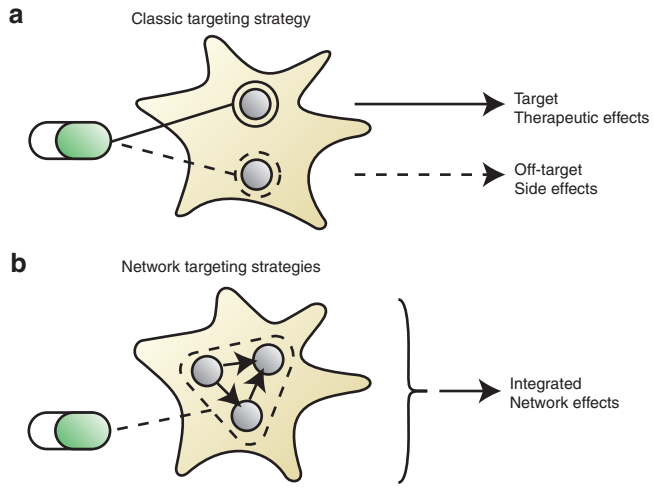

Figure 1 Classic vs. network view of drug action. In (**a**) the classic view, a drug has both on-target and off-target effects, which activate pathways of effectors to trigger therapeutic and side effects, respectively. In (**b**) the network or systemic view, multiple targets of a signaling network are perturbed, resulting in integrated therapeutic and side effects.

NETWORK TREATMENTS

A growing amount of experimental evidence shows the impact of network medicine on drug development. The initial work of Schoeberl *et al.*,[19] who built a computational model of the ErbB signaling network in order to detect the most effective ligands of ErbB, enabled the identification of ErbB3 and epidermal growth factor receptor as key nodes for ligand response and the design of an antibody to specifically target those nodes, which stopped the growth of tumors in xenograft mice. This research has been followed by clinical trials, and the MM-121 antibody is now in phase II clinical trial (see ClinicalTrials.gov).[20] Earlier, Huang *et al.*[21] determined that a combination treatment with c-Met kinase inhibitor and either an epidermal growth factor receptor kinase inhibitor or cisplatin resulted in an increased cytotoxicity. It also resulted in phase I and II clinical trials.[22] Other examples found in the literature[23] propose treatments attacking only a single or a very small number of nodes in these networks.

Because signaling networks can and will rewire themselves after being attacked,[11] network drugs should be constituted of compounds with significant coverage of the network. A classic way to investigate this strategy is to perform a systematic, genome-wide screen[24] with known compounds. This can highlight potential synthetic drugs; however, it does not make use of any information about the network connectivity or dynamics. A major breakthrough in the field has been achieved by Yaffe's group,[17] which performed a detailed study of the network changes after time-staggered inhibition of epidermal growth factor receptor, which made the cancer cells more sensitive to DNA-damaging drugs. This effect was highly time-dependent, and simple coadministration of the drugs resulted in a radically decreased effect. The combination of the two approaches—i.e., time-staggered, genome-wide RNA interference (RNAi) screens—results in a very large number of putative combinations. Advanced algorithms need to be developed to make this approach feasible, and a better understanding of the regulation networks is a critical step toward that end.[12]

Despite the accumulating evidence of its potential impact, network medicine is still in its infancy. However, network biology and personalized approaches are clearly expected to play significant roles in the development of novel and more sustainable treatments in the future.

PERSONALIZED MEDICINE

Beyond being a buzzword, personalized medicine is the development of therapies that are targeted at the specific tumor affecting a patient.[10,25] With the scientific and technological advances discussed above, it becomes feasible to integrate sequencing, mass spectrometry, and genome-wide screening data into predictive computer models. We refer to the ability to predict how cells will respond to input cues or treatments from their current state of their signaling networks as "biological forecasting." Similar to weather forecasts, supercomputing facilities are required to model the complex networks of interactions and their effects on cellular phenotype. In the case of a cellular model, large-memory systems must hold the whole data set in memory because integrative and nonlinear effects make it impossible to split the problem into smaller subproblems. Such models should integrate genetic (sequencing), expression (proteomics, not messenger RNA expression), signaling (e.g., phosphoproteomics), and phenotypic (e.g., screening and imaging) data. Early integrative network biology studies were undertaken by Janes *et al.*,[6] Linding *et al.*,[26] Jørgensen *et al.*,[27] and Bakal *et al.*,[28,29] who modeled genetic and phosphoproteomic data with neural networks and other algorithms to derive information on the signaling network architecture. More advanced models of this type can then be used to predict which treatment will be most effective against a specific cancer.[10]

Practically, several network-targeting strategies are available; the most important ones are shown in **Figure 2**. A network drug can target a single, central node of a signaling network (**Figure 2a**). But more complex, multitarget therapies can be developed as well, with either a single multitargeted drug hitting several nodes (**Figure 2b**) or each of several specific drugs hitting a different node of the signaling network (**Figure 2c**). Alternatively, multiple multitarget kinase inhibitors (**Figure 2d**) may result in a specific overall impact on the tumor cells' signaling networks that can be beneficial from the therapeutic point of view.

However, the limited number of drugs currently approved by the US Food and Drug Administration reduces the choice of potential targets. In 2006, Overington *et al.*[30] estimated that the pharmacopoeia available at this time contained 324 distinct molecular drug targets. This relatively small number of targets must be mitigated by the relative lack of specificity of many drugs, also termed "promiscuity," meaning that most drugs actually hit several targets.[31,32] Kinase inhibitors in particular have been shown not to be very selective.[33–35] Some researchers, including Andrew Hopkins, have argued that promiscuity is an aspect of a drug's efficacy and that one

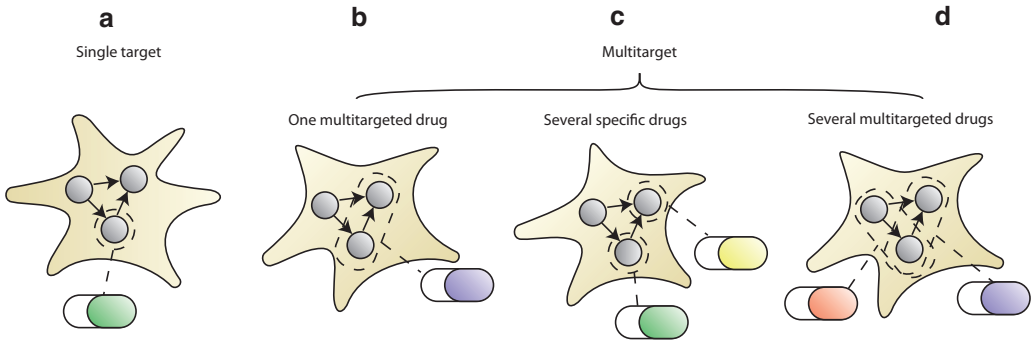

Figure 2 The three most important network-targeting strategies: (**a**) a single drug can be used to target a single node of the network, (**b**) multiple nodes can be targeted with one multitargeted drug, or multiple nodes can be targeted with several (**c**) specific or (**d**) multitargeted drugs.

could take advantage of this lack of specificity to design drugs that hit several targets at once and thus maximize the clinical effect.[18,36] This is supported by the approval of Gleevec and many other drugs for secondary indications and by extensive off-label usage.[37] A potentially interesting advantage of designing network-based treatment strategies could be the possibility of using lower doses of each drug to maintain, or improve, efficacy while reducing side effects, as proposed by Lötsch and Geisslinger.[38]

Unfortunately, the pharmaceutical industry is still essentially focusing on developing drugs that hit a given target as specifically as possible. We question this strategy, which, although useful in identifying the best drug for a given node, is not the best treatment strategy, nor does it aim at the best target. Such knowledge must be derived through systematic biological studies of the signaling networks associated with the disease or cancer type. A notable exception is Merrimack Pharmaceuticals (Cambridge, MA), which has been developing multitargeted, network-attacking antibodies named MM-111,[39] MM-141, and MM-151.[20]

New strategies for drug development are being developed that will eventually provide a larger catalog of potential drugs to choose from and combine. Initiatives to establish this catalog are ongoing,[40] with the DrugBank,[41] CANsar,[42] and ChEMBL[43] databases providing mapping between drugs and their targets. A new class of drugs that, we predict, will soon expand this catalog comprises small interfering RNA (siRNA) molecules that suppress the expression of a gene. After entering the cell, the siRNA molecule triggers the assembly of the RNA-induced silencing complex (RISC) and binds with the target messenger RNA, which will be cleaved. The main barrier to the design of siRNA-based drugs is the delivery of a charged molecule to the target tissue. This challenge is addressed with the development of a large variety of groundbreaking carrier nanoparticles.[17,44] The first clinical trials are under way[45] and will hopefully soon deliver a genome-wide catalog of potential treatments and treatment combinations from which a personalized treatment can be chosen. Furthermore, research is ongoing to develop the delivery of various combinations of siRNA with other drugs.[46]

PERSPECTIVES

Currently, even with the relatively low number of drugs approved by the Food and Drug Administration, it would be impractical to screen all possible combinations across all cell types and secondary or higher-order alternative input cues.[6] If we include all potential candidate drugs, the number of combinations scales to an unfeasible level. A better understanding of the signaling networks, together with biological forecasting models, will be needed to guide the relevant questions and hypotheses to be tested in drug screens.[10] Such models can also help explore parts of the networks' state spaces otherwise not reachable through screening.[10] Lee *et al.*[17] demonstrated the complexity of signaling networks and provided a proof of concept that complex treatment strategies can deal with this complexity. However, approaches that are more systematic and based on signaling network models are required to efficiently drive the development of more network therapies.

Several important issues remain to be solved. What data can we reasonably collect from the patient? Although it seems realistic to obtain a tissue sample from the primary tumor that can be genotyped and analyzed by mass spectrometry for (phospho)proteomic profiling, it could be impractical to collect such data in certain cases, for instance, from metastatic sites where the cells are spread through the patient's body and often undetectable. In some cases, this can be counteracted by single-cell technology, for example, by using the CyTOF single-cell mass cytometer.[47] Research is ongoing to exploit tumor cells and DNA markers circulating in blood to gain additional information regarding the tumor[48] such as initial or acquired mutations, drug sensitivity, and early relapse detection.[49] Then again, there remains the question of how we can verify whether the treatment is having an effect. The most elaborate approach to monitoring cancer treatment response is imaging using positron emission tomography (PET), computed tomography (CT), or diffusion-weighted magnetic resonance imaging to estimate tumor size, shape, texture, structure, and dynamics.[50] These techniques can also be used to detect therapeutic effects on tumor metabolism (^{18}F-fluorodeoxyglucose uptake) and cell proliferation (^{18}F-fluorothymidine incorporation).[51] However, metastatic sites are often missed owing to noncomprehensive data analysis or tumor cell colonies below the detection limit surviving treatment.

An interesting alternative to patient sample collection is being developed at the University of Texas MD Anderson Cancer

Center that uses immunocompromised mice as xenograft models. In this approach, code-named the "T9 project," a small biopsy of patient tumor is transplanted into mice, which are then treated with various drugs in order to establish which induces the strongest effect.[25] Such *in vivo* testing could complement *in vitro* and *in silico* models and eventually lead to better and more personalized drug treatments.

CONCLUSION

Network medicine holds the promise of delivering more personalized and efficient treatments for cancers. With the larger sets of candidate drugs that will be available in the near future, network-based analysis could be the guide that will point out which treatment—a single drug vs. a combination and time-staggered vs. another complex treatment—will most efficiently treat a given patient. This will also pave the way for sustainable medicine in the future.

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CONFLICT OF INTEREST

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