CANCER SYSTEMS BIOLOGY

# Harnessing off-target effects

The 'off-targets' of a drug are often poorly characterized yet could be harnessed in the treatment of complex diseases. A recent study used a small-molecule screening in non-small-cell lung cancer to repurpose an FDA-approved ALK/IGF1R inhibitor and uncover its mechanism of action.

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Two potential treatment strategies for attacking multiple targets are combination therapy, requiring the use of various synergistic drugs, and polypharmacology, in which a single compound binds to multiple specific targets. The former approach has been shown to increase the effectiveness of chemotherapy in cancer, though successful combinations are relatively rare[1,2]. Not only is identification of a drug combination that has a synergistic effect challenging, but the approach often suffers from problems such as increased toxicity and undesired drug–drug interactions. On the other hand, polypharmacology presents the difficulty of optimizing the inhibition of several targets simultaneously, thus limiting the number of cases in which rational design is fruitful. However, most small molecules are intrinsically polypharmacological because of their noncognate effects[3,4]. A careful inspection of these 'off-target' effects can widen the therapeutic applications of a drug. Kuenzi *et al.* combined phenotypic screening and quantitative phosphoproteomics to demonstrate that the US Food and Drug Administration (FDA)-approved ALK/IGF1R inhibitor ceritinib has an antiproliferative effect on non-small-cell lung cancer (NSCLC) cell lines lacking the ALK oncogene expression by simultaneously acting on multiple noncanonical targets[5].

Early chemotherapeutic agents were discovered following their inhibitory effect on the proliferation of rapidly dividing cells. Their high toxicity to healthy cells, which can cause severe side effects in patients, has led to a shift in the focus of cancer drug discovery toward developing highly selective compounds targeting a specific molecular aberration driving the disease, also known as the 'one-gene, one-drug, one-disease' paradigm[4]. However, as exciting as targeted therapies are, they are limited to indications presenting a strong driver, and they often fail in complex diseases like cancer. Indeed, many cancers either lack a strong driver or present a difficult-to-drug driver (for example, TP53 and MYC)[6]. Even when present, dominant drivers often require the cooperation of additional oncogenes or loss of tumor suppressor genes to achieve malignant transformation[7]. Moreover, targeted therapies typically result in the development of resistance mechanisms via rewiring of signaling networks[8]. The multistep nature of carcinogenesis and the robustness and dynamic structure of signaling networks underscore the importance of attacking aberrant signaling networks at multiple nodes. For example, the combinatorial use of ALK and IGF1R inhibitors has been proposed to improve efficacy in ALK-positive lung cancer[9]. In fact, ceritinib, which targets both ALK and IGF1R (albeit at a higher $IC_{50}$ dose), has been shown to outperform the selective

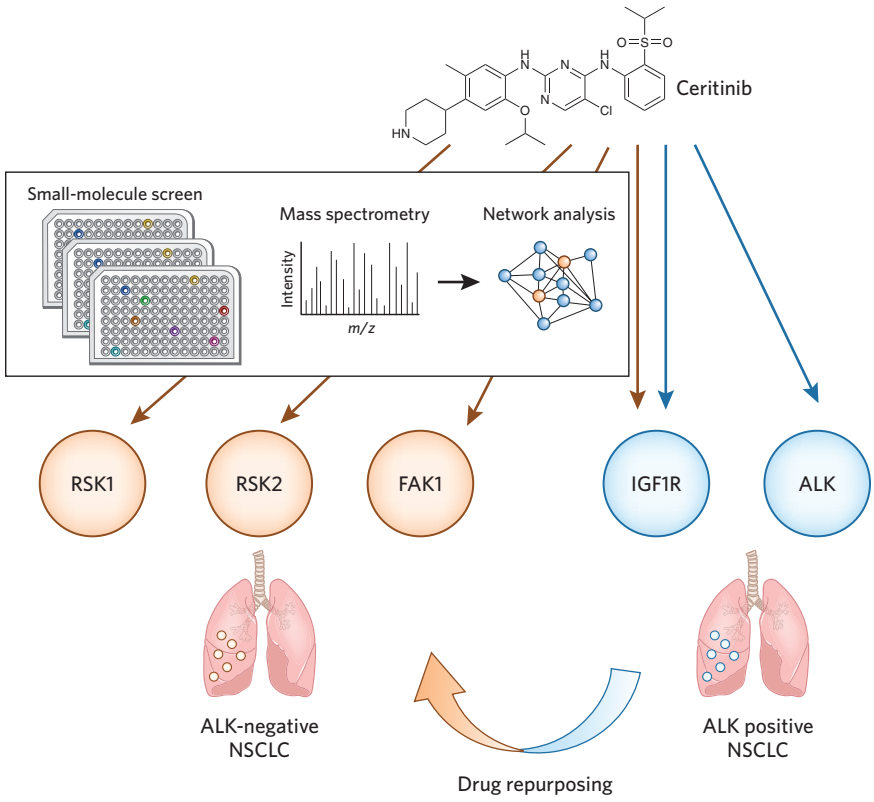


**Figure 1 |** Noncognate effects of the FDA-approved ALK/IGF1R inhibitor ceritinib lead to repurposing its use in ALK-negative non-small-cell lung cancer (NSCLC). Ceritinib, which is being used for the treatment of ALK-positive NSCLC patients, has been known to act on ALK, as well as on IGF1R (albeit at a higher $IC_{50}$ dose). Characterization of ceritinib's mechanism of action in ALK-negative NSCLC cell lines by phenotypic screening and mass-spectrometry-based phosphoproteomics revealed the downstream network of ceritinib, in which RSK1, RSK2, FAK1 and IGF1R were the main effectors, and simultaneous inhibition of these nodes was required for a significant effect on viability. The newly discovered 'off-target' network suggests that ceritinib's clinical use may potentially be expanded to patients with ALK-negative NSCLC.

ALK inhibitor crizotinib in the treatment of ALK-positive NSCLC patients[10].

Interestingly, the phenotypic screen conducted by Kuenzi *et al.* revealed that ceritinib was also active in ALK-negative NSCLC cell lines. To identify the ALK-independent targets, the authors chemically modified ceritinib and detected binding to multiple proteins, including FAK1, RSK1 and RSK2, as well as ceritinib's known 'off-target' IGF1R. To glean a deeper understanding of ceritinib's ALK-independent mechanism of action, the authors constructed the effector network of ceritinib using genetic information pertaining to the mutational profile of NSCLC cell lines responding to ceritinib treatment. Analysis of large-scale data sets is akin to looking for a needle in a haystack, yet the authors aptly made use of predictor analysis tools ReKINect and NetworKIN to identify RSK1, RSK2, FAK1 and IGF1R as the most critical nodes in mediating ceritinib-induced signaling (**Fig. 1**). They also showed that inhibition of these nodes individually had little effect on cell viability, confirming the polypharmacological action of the drug. Demonstrating the strength of their integrated approach, the authors went on to use the ceritinib effector network modules to predict and illustrate the synergistic effect of ceritinib with the microtubule-targeting inhibitor paclitaxel. This synergy was mediated by FAK1, whose phosphorylation was discovered to be a potential clinical biomarker predictive of sensitivity to the synergistic drug combination.

This study demonstrates that a deeper understanding of how drugs affect signaling networks can be used to increase therapeutic potential and applications. The success of targeting multiple proteins should not be surprising, given that oncologists regularly utilize drug cocktails to treat patients[1]. By ignoring clinical practices and the complexity of biological systems the progress of drug discovery is hampered. Providing an integrative framework between the genetic and environmental cues that a cell receives and the cellular phenotypic outcome, signaling networks constitute a road map to drug discovery. With the help of state-of-the-art predictor analysis tools, deep characterization of network states could pave the way for the rational design of multitarget treatment strategies, decreasing the need for massive screens. Understanding the effects of drugs on a cell's signaling network state could lead to the repurposing of a drug in the clinic via rational modification of the drug's polypharmacological profile or design of synergistic combinations to overcome the system's robustness and drug resistance. The pharmaceutical industry and patients would both benefit from repurposing a drug in this way, which would most likely contribute to decreased bench-to-bedside time and costs. In view of the slow but increasing interest in systems biology among cancer researchers, moving forward, we expect network-based pharmacology to become a key element in next-generation drug discovery. Providing a good example of both polypharmacology and combination therapy, the study by Kuenzi *et al.* demonstrates how understanding the noncanonical effects of a compound can offer an opportunity for rational multitarget treatment strategy design. ■

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## Competing financial interests

The authors declare no competing financial interests.

GENETIC CODE EXPANSION

# Synthetases pick up the PACE

Phage-assisted evolution can rapidly improve the efficiency and substrate specificity of orthogonal aminoacyl-tRNA synthetases. Furthermore, the crystal structure of the pyrrolysyl-tRNA synthetase N-terminal domain reveals the basis for these improvements and provides a structural rationale for orthogonality.

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In genetic code expansion, researchers utilize orthogonal aminoacyl-tRNA synthetase (aaRS)–tRNA pairs to incorporate noncanonical amino acids (ncAAs) in response to redefined codons in living organisms. A major bottleneck for this field arises from the need to evolve new, selective aaRS mutants to incorporate each new amino acid. Traditional methods of aaRS evolution involve sequential rounds of alternating positive and negative selection using an aaRS library with random mutations in the amino acid binding pocket[1]. However, this process is time consuming, setting a practical limit for selection to only 3–5 rounds. In addition, these selections limit the search for beneficial mutations to just a handful of residues in the aaRS enzyme. For these reasons, aaRS mutants with suboptimal efficiency and selectivity are often obtained[2]. Phage-assisted evolution is a directed evolution strategy that couples the propagation of bacteriophages to an evolvable trait (for example, enzyme activity)[3]. In this issue, two papers employ phage-assisted evolution to identify aaRS mutants with enhanced activity and substrate selectivity for use in genetic code expansion.

In one example of this approach, Bryson *et al.* coupled stop codon suppression efficiency to the expression of pIII, an essential phage capsid protein (**Fig. 1**)[4]. First, the gene encoding an aaRS facilitating stop codon suppression was introduced into the phage genome, creating the essential link between genotype and phenotype. Next, the phages were grown in a specialized