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Is there a case for selectively promiscuous anticancer drugs?

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We critically survey the paradigms of molecularly targeted cancer therapy in light of systems-level observations on tumor robustness and resilience. Multipronged attacks covering various clinical fronts such as angiogenesis, apoptosis and cancer progression, among others appear to be favored approaches at present, yet the enhancement of side effects has become a serious concern. In this regard, we argue that a departure from the yet untested single-target paradigm in favor of the notion of 'selective nonselectivity' may be necessary, but will ultimately require a rational control of specificity to curb side effects. This control may be achieved by drug redesign guided by known selectivity-promoting molecular features.

Is there a real case for single-target drugs?

As it turns into a mature discipline, systems biology, the multiscale integration of the functional components of organismic complexity, is expected to contribute significantly to the drug discovery endeavor [1,2]. Thus, the rational modulation of clinical targets in living systems remains a central challenge of drug-based therapy and systems biology is entrusted with providing meaningful approaches to face this challenge [3]. This issue is perceived to be worthy of attention mainly because of the disappointing outcome in the discovery of new drugs [2–5]. This endeavor is still ingrained on the premise that successful therapeutic agents will result from targeting single disease-causing molecules. Thus, it seems reasonable to critically revisit the single-target paradigm in an attempt to assess the therapeutic advantage of multitarget or even promiscuous drugs [6–8].

In this opinion piece we shall survey this issue focusing on molecular anticancer therapy and propose translational approaches to overcome the problems that are likely to have brought drug discovery to a near-stalemate. Particular attention will be paid to small-molecule kinase inhibitors, the well-known therapeutic agents widely adopted in cancer treatment to modulate clinically relevant targets along signaling pathways that control cell fate or proliferation [9–11].

Tumors are perceived to be complex systems whose survival and propagation is inherently a consequence of their resilience to exogenous perturbations [3]. In our context of interest, these perturbations materialize as drug-based therapeutic interventions. Thus, when facing a drug-induced node failure arising from the inhibition of a signal transducer in the cell, the tumor is expected to resort to basic robustness-enhancing possibilities to ensure its survival. These options arise either from the tumor microenvironment [12], the resilient architecture of the signaling network or from the inherent genetic instability of the tumor and its resulting heterogeneity [13]. The features conferring robustness [14] include: (a) network modularity to hinder the propagation of effects arising from drug-induced node failure; (b) network redundancy to maintain a biochemical flux in the face of a drug-induced node failure [15]; (c) feedback adaptive controls to ensure the habilitation of alternative pathways as one pathway is blocked by drug action; (d) heterogeneity in the tumor cell population, promoted by genetic instability and ensuring the adaptive survival of a cell subpopulation which defines a drug-resistant trait; and (e) obstacles to the therapeutic access to the tumor, to drug metabolism and to drug efficacy introduced by the tumor microenvironment [12]. This latter aspect involves stromal fibroblasts, inflammatory cells, vasculature components, normal epithelia and the extracellular matrix, and interacts with tumor cells through the release of cytokines, proteases and growth factors. Thus, tumor growth, vascular development, immune-system evasion, invasion and metastasis are tightly regulated within the tumor microenvironment [12].

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GLOSSARY

Abl Abelson murine leukemia viral oncogene
ACC Acetyl-coenzyme A carboxylase
AMPK 5′-AMP (adenosine monophosphate)-activated protein kinase
ATP Adenosine triphosphate
BAD Bcl2 (B-cell CLL/lymphoma 2) antagonist of cell death
BAX Bcl2-associated X protein
Bcr Breakpoint cluster region
CAMK1G Calcium/calmodulin-dependent protein kinase type 1 gamma
CML Chronic myeloid leukemia
CSF1R Macrophage colony-stimulating factor 1 receptor
Cyt c Cytochrome c
EEF2 Eukaryotic elongation factor-2
EGFR Epidermal growth factor receptor
EPHA Ephrin type-A receptor
ERBB2 Erythroblastic leukemia viral oncogene homolog 2
FAK Focal adhesion kinase
FDA Food and drug administration
FLT3 FMS (fenpropimorph resistance multicopy suppressor)-related tyrosine kinase 3
GISTs Gastrointestinal stromal tumors
KIT Mast/stem cell growth factor receptor
mTOR Mammalian target of rapamycin
PDGFR Platelet-derived growth factor receptor
SRC Proto-oncogene tyrosine-protein kinase Src (sarcoma)
RET Proto-oncogene receptor ret (rearranged during transfection)
RSK Ribosomal protein S6 kinase
VEGFA Vascular endothelial growth factor A
VEGFR Vascular endothelial growth factor receptor

This analysis leaves us with a conundrum: if the tumor is capable of displaying such a plethora of drug-resistant adaptations and of introducing manifold obstacles to therapy, what would be the advantage of a single-target therapeutic agent? It surely seems that a single drug-induced node failure can be readily overcome by merely resorting to the adaptive resilience of the network itself, unless the node happens to coincide with a point of network fragility. By contrast, if the latter happens to be the case, the tumor can ultimately resort to its biological heterogeneity as an adaptive property. Thus, the genetic instability of the tumor is likely to promote a heterogeneous cellular population whereby the specific subpopulation that expresses drug-resistant kinase mutants or genetic variants are likely to survive drug treatment [3].

The current disappointing output of the drug discovery endeavor is often attributed to the prevalence of the single-target or 'magic bullet' paradigm [2]. Few success stories seem to support this view at present. One such case arises with the kinase inhibitor imatinib (Gleevec®, Novartis), which targets the sole causal agent in chronic myeloid leukemia (CML), the constitutively active or deregulated chimera Bcr-Abl kinase [16]. This protein is expressed in CML as a result of chromosomal translocation and happens to be a point of fragility in the signaling network of CML cells. Ultimately, the tumor robustness manifests itself through genetic instability as mutations, such as T315I in the kinase domain of Bcr-Abl, confer resistance to imatinib treatment [17].

Not only are there few successful therapeutic agents in support of the magic bullet paradigm but also this paradigm remains largely untested. With the advent of high-throughput *ex vivo* screening techniques, it has become apparent that there is no single-target kinase inhibitor currently available or under development [18].

Is multitarget therapy the right paradigm shift in anticancer drug treatment?

Systems biology has led to the perception that an effective drug should target biochemical fluxes embedded in networks rather than specific molecules [19]. However valid this may be, it is operationally unfeasible from the rational perspective of structure-based drug design. Furthermore, abandoning the single-target paradigm plunges us into the daunting challenges of controlling side effects. This is especially true for kinase inhibitors because kinases play different signal-transduction roles in different contexts and inhibition in one cellular network may prove fatal in another [20]. Thus, imatinib may be effective in treating CML through its direct inhibition of the Bcr-Abl kinase, but this activity has also been shown to cause toxicity in cardiomyocytes [20]. In these off-target cells the inhibition of the wild-type Abl kinase initiates a signaling cascade that promotes mitochondrial depolarization and ATP depletion, posing a risk to the heart [20].

Furthermore, this multiplicity of context-dependent roles for a target protein poses a danger of side effects *even* in highly selective drugs, such as the monoclonal antibodies trastuzumab (Herceptin®, Genentech) [21] and bevacizumab (Avastin®, Genentech/Roche) [22]. These therapeutic agents targeting, respectively, ERBB2 and VEGFA kinases are cardiotoxic and exhibit additional side effects like neutropenia, hemorrhage, hypertension and thromboembolism [20–22].

In light of these disappointing outcomes, much effort is now directed at reassessing the therapeutic value of promiscuity. Several examples of the clinical relevance of highly cross-reactive drugs have arisen in anticancer therapy: multitarget kinase inhibitors such as sunitinib (Sutent®, Pfizer) and sorafenib (Nexavar®, Bayer) have recently received FDA approval with important caveats highlighting their side effects [6,7,20]. The rationale for such therapeutic alternatives stems from the need for a multipronged attack to the tumor, covering several clinical fronts such as angiogenesis (VEGFR and PDGFR), apoptosis (Abl and KIT), cancer metastasis (FAK and SRC), among others.

As suggested by the systems biology assessment of robustness, a simultaneous modulation of multiple targets is probably necessary to alter a clinical phenotype because biological redundancies and alternative pathways can often bypass the inhibition of a single target [8]. Thus, in spite of the issues pertaining to the control of side effects, a 'magic-shotgun' targeting multiple proteins may, in some instances, possess a higher therapeutic index than a more specific agent [6].

Multitarget drugs may be more resilient against drug-resistant mutations [23], providing another motivation to exploit promiscuity. This assertion readily follows from the fact that cross-reactive drugs typically make interactions with evolutionarily conserved residues and fewer interactions with residues that need to mutate to confer drug resistance [23].

For all their current appeal in light of systems biology arguments, promiscuous drugs probably entail a higher risk of promoting severe side effects than more-specific compounds simply because they target more kinases. For instance, the more-promiscuous anticancer kinase

inhibitor sunitinib is suspected to be more cardiotoxic than the more-specific imatinib [20]. Yet, to counterbalance the argument, the highly specific anti-VEGFA bevacizumab introduces multiple and severe toxicities (hemorrhage, hypertension and cardiotoxicity) comparable to sunitinib [20].

Side effects resulting from kinase inhibition in off-target cells led to the suspicion that promiscuous drug treatments are likely to entail higher uncertainty in clinical outcome. For example, the highly specific EGFR-inhibitor lapatinib (Tykerb®, Glaxo-Smith-Kline) imports fewer side effects than the less selective EGFR inhibitors: erlotinib (Tarceva®, Genentech/OSI Pharmaceuticals/Roche) or gefitinib (Iressa®, AstraZeneca/Teva) [20]. Hence, it is risky to welcome promiscuous compounds without a rational strategy to control their specificity. Such control may be achieved through rational drug redesign, by identifying unique features of the target structure and effectively introducing chemical modifications in the drug that promote interactions with such unique features.

Toward a controlled multitarget therapy

In light of the previous discussion, it becomes obvious that controlling specificity in molecular cancer therapy is a major challenge to be faced by those who advocate promiscuity. Hence, a basic goal that arises from the paradigm shift is to reduce toxic side effects by structure-based drug redesign exploiting our current understanding of the molecular basis of specificity [24].

Nearly all drug-targeted therapies directed against a constitutively deregulated kinase or a target downstream of such a kinase present side effects. This is probably caused by the inhibition of 'bystander' targets, not essential for cancer cell killing, or primary targets that may be essential to kill the cancer cell but play crucial roles associated with cell survival in other contexts [20,24]. Thus, the multitargeted kinase inhibitor sunitinib appears to carry unfathomable therapeutic value against renal cell carcinoma, gastrointestinal stromal tumors (GISTs) and probably breast cancer [25]; but, precisely because of its target multiplicity, sunitinib's clinical outcome entails dangerous side effects, especially cardiotoxicity [20,26,27].

An innovation is on its way in terms of rationally redesigning kinase inhibitors to reduce their toxicity: we can control specificity to an unprecedented degree and hence contribute to test the very limits of their therapeutic efficacy.

Cross-reactivities of kinase inhibitors arise because of the structural similarity of evolutionarily related (paralog) kinases. Yet, while paralogs share a similar structure, they are packed differently. If we compare the microenvironments of intramolecular hydrogen bonds aligned across paralog structures, we notice that they are different: some hydrogen bonds are exposed to solvent in one kinase and completely shielded in another. Taking into account such local differences in packing quality [24], we are able to redesign a kinase inhibitor because deficiently packed hydrogen bonds – the so-called dehydrons [28] – not only distinguish paralogs but they are sticky, hence targetable. Dehydrons are sticky because they promote their dehydration to enhance the underlying amide-carbonyl electrostatic attraction [28].

Thus, a design strategy to achieve higher specificity emerges as we redesign inhibitors to turn them into protectors (wrappers) of dehydrons [24,29–32]. Such wrapping designs enable paralog discrimination and hence lead to drugs with controlled specificity [24,29–32]. For example, the wrapping concept has been recently adopted to tackle the challenge of 'cleaning' staurosporine, the most promiscuous kinase inhibitor known [32]. Our wrapping-modified staurosporine, targeting an EGFR dehydron with low level of conservation, binds only to 12% of the 220 kinases experimentally assayed by high-throughput screening [18]. This percentage of hits reveals a massive enhancement in selectivity, when compared with 90% for staurosporine. Strikingly, our cross-reactivity prediction based on wrapping differences across targets is 95% accurate, when contrasted with the experimental results [32].

Another illustration of the power of the wrapping-based approach involved the redesign of the promiscuous inhibitor EKB-569 (Wyeth-Ayerst) that binds to 50 kinases [32]. The affinity screening of the dehydron-wrapping redesign agrees with the profile predicted based on dehydron conservation (95% of accuracy). Significantly, the redesigned compound is more selective than the parental compound: out of the 220 kinases screened [18] it binds to 5 kinases, whereas EKB-569 binds to 19 [32]. Thus, these experimentally validated examples suggest that wrapping redesign can be further exploited to obtain 'selectively nonselective' inhibitors. The operational aspects of this strategy are described in detail as we focus on a problem of immediate clinical relevance: the need for the removal of

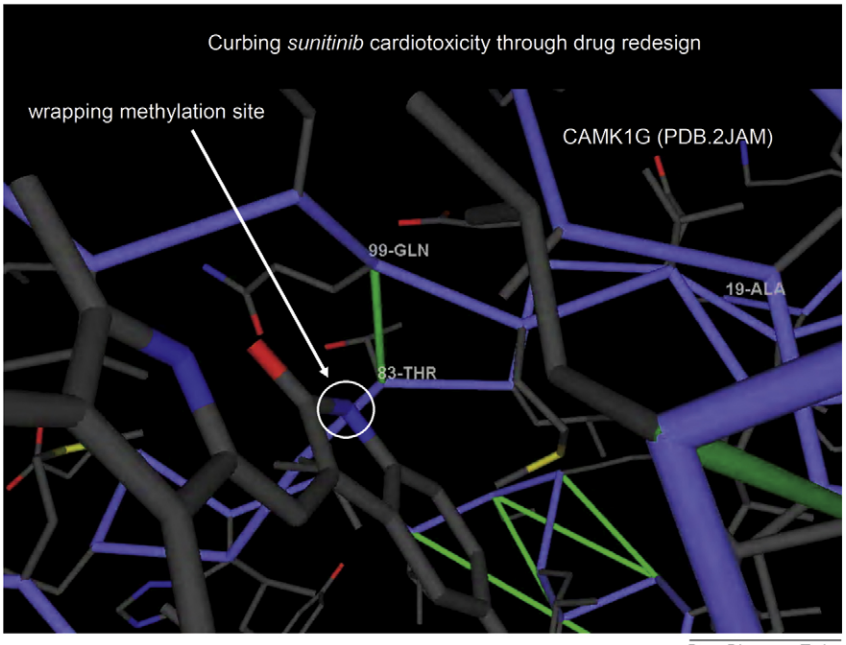

FIGURE 1

Structure of the CAMK1G kinase in complex with sunitinib (PDB.2JAM). The T83-Q99 dehydron (green virtual bond joining α-carbons) is conserved in all high-affinity targets relevant to angiogenesis and cancer-cell proliferation such as CAMK1D, CSF1R, EPHA2-3, KIT, FAK, SRC and VEGFR2 kinases. It aligns with the well-wrapped hydrogen bonds K78-E94 and T116-D132 in AMPK2 and RSK, respectively. This crucial packing difference suggests the modification to sunitinib needed to remove its cardiotoxicity: methylation at the N1 indol position (circle) would turn the drug into a wrapper of CAMK1G dehydron T83-Q99. This drug is likely to bind selectively to all clinically relevant targets, but will produce a steric clash upon association with AMPK2 and RSK.

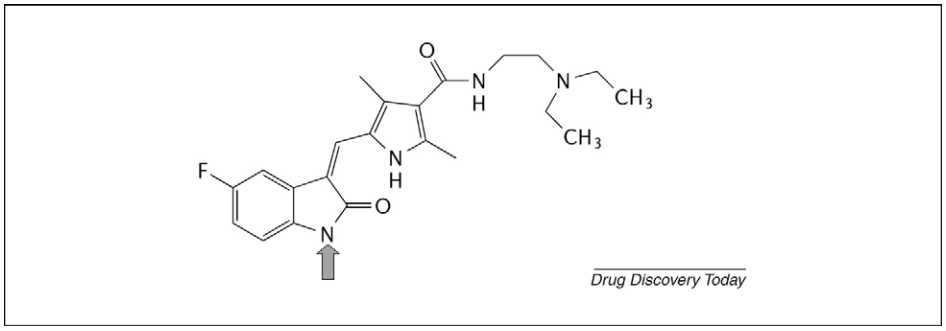

FIGURE 2

Schematic representation of the chemical structure of sunitinib. The wrapping modification involves the methylation at the indol position (arrow).

sunitinib cardiotoxicity through a redesign of the parental compound.

Sunitinib action illustrates the power of multitargeted kinase inhibitors with a dual pharmacological impact covering tumor-angiogenesis targets VEGFR1-3 and PDGFRα/β, and tumor-proliferation/survival targets KIT, CSF1R, FLT3 and RET. This level of promiscuity obviously entails a greater risk of cardiotoxicity owing to the likelihood of inducing cardiomyocyte apoptosis through the activation of pathways that compromise ATP replenishment or promote mitochondrial dysfunction [20,26,27]. Intriguingly, treated patients with advanced GIST and metastatic renal carcinoma often reveal significant reduction in left ventricular ejection fraction, a condition enhanced by other sunitinib-induced comorbidities [20,26,27].

A systems assessment of bystander sunitinib targets points to AMPK and RSK inhibition as culprit for cardiotoxicity [20,26,27]. Thus, RSK inhibition releases the proapoptotic factor BAD with the activation of BAX and cytochrome *c* release, in turn promoting apoptosis and ATP depletion. The inhibition of AMPK promotes ATP depletion by preventing downstream inhibition of EEF2, mTOR and ACC, which are normally inhibited by AMPK in energy-compromising settings [20]. In addition, AMPK inhibition affects cardiomyocyte survival if hypoxia occurs, because AMPK inhibits the energy-consuming processes of protein translation and lipid biosynthesis. Finally, the exacerbated activity of mTOR and EEF2 leads to hypertrophy [20,26,27].

Guided by the wrapping concept [24,29–32], we may approach the reengineering of sunitinib to enhance its selectivity toward therapeutic targets while avoiding inhibiting AMPK and RSK. A comparison of the local packing of several sunitinib targets through structure alignment led us to focus on a particular dehydron: the deficiently packed backbone hydrogen bond T83-Q99 in CAMK1G kinase (Fig. 1). This dehydron is conserved in all high-affinity targets relevant to angiogenesis and cancer-cell proliferation. Thus, it aligns with a dehydron in CAMK1D, CSF1R, EPHA2-3, KIT, FAK, SRC and VEGFR2 kinases. By contrast, it aligns with the well packed hydrogen bond K78-E94 in AMPK2 and with the well packed hydrogen bond T116-D132 in RSK. That is, the differences in the packing of the region that aligns with dehydron T83-Q99 in CAMK1G (Fig. 1) enable discrimination between clinically relevant and cardiotoxicity-promoting targets [24,29–32].

This crucial packing difference suggests the modification to sunitinib needed to remove its cardiotoxicity [24,29–32]: the examination of the sunitinib/CAMK1G PDB-complex (Fig. 1) reveals that methylation at N1 in sunitinib's indol (Fig. 2) would turn the drug into a wrapper of dehydron T83-Q99. This drug is likely to bind selectively to all clinically relevant targets because the latter contain a dehydron at the position that aligns with T83-Q99 in CAMK1G. By contrast, this wrapping modification will produce a steric clash upon association with kinases RSK and AMPK2.

This example illustrates the potentialities of rational drug redesign guided by selectivity filters to develop safer multitarget drugs.

Concluding remarks

In designing and implementing an anticancer therapy, the essential biological properties that contribute to tumor robustness must be properly assessed. Thus, single-target drug treatments are currently under critical scrutiny because it is felt that they may be inadequate to overcome the network resilience and tumor heterogeneities caused by genetic instability. By contrast, with the advent of high-throughput screening technologies it has become apparent that the potential of single-target drugs remains basically untested simply because unexpected cross reactivities have been detected even in the most specific compounds.

Novel systems biology insights are leading to the belief that multitarget or even promiscuous drugs would be more effective in dealing with complex diseases because dynamical objects like biochemical fluxes and not molecules are the appropriate targets. The greater danger of side effects arising in multitarget therapies, however, clearly counterbalances the recent faith bestowed upon them. Thus, we propose the use of selectivity filters to guide the rational control of specificity toward targets of clinical relevance within the systems biology frame of inference that dictated the use of multitarget drugs in the first place. It is clear that tumors may be more efficiently tackled by opening several therapeutic fronts but only a selectively promiscuous drug would be operative in such a scenario.

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