Polypharmacology in Precision Oncology: Current Applications and Future Prospects

Albert A. Antolin a,b,*, Paul Workman a, Jordi Mestres b and Bissan Al-Lazikani a

aCancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, London, UK; bSystems Pharmacology, Research Program on Biomedical Informatics (GRIB), IMIM Hospital del Mar Medical Research Institute and University Pompeu Fabra, Parc de Recerca Biomédica, Barcelona, Catalonia, Spain

Abstract: Over the past decade, a more comprehensive, large-scale approach to studying cancer genetics and biology has revealed the challenges of tumor heterogeneity, adaption, evolution and drug resistance, while systems-based pharmacology and chemical biology strategies have uncovered a much more complex interaction between drugs and the human proteome than was previously anticipated. In this mini-review we assess the progress and potential of drug polypharmacology in biomarker-driven precision oncology. Polypharmacology not only provides great opportunities for drug repurposing to exploit off-target effects in a new single-target indication but through simultaneous blockade of multiple targets or pathways offers exciting opportunities to slow, overcome or even prevent inherent or adaptive drug resistance. We highlight the many challenges associated with exploiting known or desired polypharmacology in drug design and development, and assess computational and experimental methods to uncover unknown polypharmacology. A comprehensive understanding of the intricate links between polypharmacology, efficacy and safety is urgently needed if we are to tackle the enduring challenge of cancer drug resistance and to fully exploit polypharmacology for the ultimate benefit of cancer patients.

Keywords: Polypharmacology, systems pharmacology, off-target, precision oncology, biomarker, target profiling, side-effects, multi-target drug design.

1. INTRODUCTION: PRECISION ONCOLOGY, POLYPHARMACOLOGY AND THE LIMITS OF THE SINGLE TARGET APPROACH

Despite advances in basic, translational and clinical research, cancer continues to represent a major global health burden. The lifetime risk of developing cancer by people living in developed countries is now approaching 50% and, worldwide cancer deaths are predicted to rise to 13 million per year within the next two decades [1]. These alarming statistics highlight the urgent need to accelerate the discovery of novel cancer therapeutics [1,2]. Our knowledge of oncogenesis and cancer progression has increased dramatically in recent years [2,3], enabling the progressive replacement of the one-size-fits-all cytotoxic chemotherapy drugs with more personalized, safer, targeted cancer therapeutics that exploit oncogene and nononcogene addiction as cancer vulnerabilities [4-6]. However, despite remarkable improvements in survival within certain types of cancer, responses to many single-agent targeted therapeutics are relatively short-lived [2]. Increasingly, molecular analysis and deep sequencing are uncovering extraordinary genetic complexity which goes a long way to explain why an overly simplistic, single targeted drug approach to cancer treatment has achieved relatively limited success in terms of prolonged survival [1,2]. Viewed from an evolutionary perspective, cancer is increasingly recognized as a complex and adaptive system, and strategies to overcome resistance to both chemotherapy and molecularly targeted therapeutics limiting disease control and cure are urgently needed [1].

Several strategies have been proposed to tackle the issue of cancer drug resistance. First, one could better exploit the full potential of the druggable cancer genome, as only 5% of the more than 500 cancer-causing proteins described to date are targeted by current therapeutics [7]. However, while this is important, a typical cancer harbors between two and eight pathogenic mutations per tumor [2] so targeting a single mutated protein may be suboptimal, particularly if the target concerned is confined to subclonal branches of the cancer’s evolutionary tree [1]. For this reason, any increase in the number of drugged cancer proteins must be accompanied by smarter ways to use the drugs concerned. An alternative and increasingly accepted solution to polygenic cancer drug resistance is rational combinatorial targeted therapy, that has already yielded several approved drug cocktails [8]. Unfortunately, the exponential number of possible drug combinations means that testing all possibilities is prohibitive and smart methods for evaluating and prioritizing combinations are urgently needed [8]. Moreover, emerging evidence suggests that a large number of cancer driver genes are mutated at very low frequencies [9]. Thus, developing a specific drug for each one might not be cost-effective, and the aim of drugging the entire cancer genome to maximize the potential benefits of combinatorial therapy is not necessarily within easy reach [10]. A third proposed solution is the development of network drugs that are capable of inhibiting more than one of the cellular signaling pathways hijacked in cancer, in order to overcome or prevent resistance [2]. Overall, since drug resistance is the biggest single factor limiting improvements in cancer treatment, a combination of these strategies, together with promising new treatments such as immunotherapies [11], are likely to be needed to achieve long term survival and cure.

In line with the development of a more comprehensive and systems-based approach to cancer research, our understanding of
the complex pharmacology and mechanisms-of-action of cancer drugs is increasing [12-16]. In the earlier days of modern drug discovery, therapeutic agents were developed using phenotypic assays and their mechanism of action remained largely elusive [17]. But advances in pharmacology and molecular biology ushered in a new paradigm of target-based drug discovery, whereby drugs were developed as ‘selective’ inhibitors of a single protein believed to be solely responsible for a disease phenotype. Moreover, the withdrawal of several drugs due to a severe side-effect caused by off-target binding to the potassium channel hERG supported the idea of selective drugs as inherently safer [18]. This new approach was termed ‘rational drug design’ – based on detailed knowledge of the drug target [19]. However, due to insufficient time and resources, as well modern screening technology being unavailable, the selectivity of these targeted drugs or ‘magic bullets’ was commonly evaluated only against a few potential off-target proteins, mainly those sharing significant sequence homology with the protein of interest or known to be promiscuous targets and responsible for serious side-effects (such as hERG or cytochrome P450) [20]. This earlier, limited understanding of drug selectivity started to be challenged in the late 2000s, when large scale profiling of drugs uncovered many new targets of drugs that had been considered previously as selective – highlighting the limitations of the classical approach to drug discovery and selectivity [12,21,22]. On the other hand, these newly revealed drug-target interactions illustrated that multi-target drugs could be as safe as single-target drugs and challenged the previous assumption that promiscuity was inherently linked to increased toxicity [18]. In addition, the increasing availability of protein-ligand interaction data in the public domain – going beyond safety pharmacology panels used to derisk lead compounds and drug candidates to include whole families exemplified by kinases - revealed promiscuous interactions between small-molecules and proteins both within and outside of their target protein’s family [23-25]. The term polypharmacology was coined to refer to the binding of a small-molecule to multiple targets and it rapidly became apparent that our understanding of the interactions between drugs and the proteome, though growing, was far from complete [20,24].

We could distinguish between two kinds of beneficial polypharmacology. The first type are cases in which the inhibition of the secondary target could be responsible for activity in another indication where inhibition of the primary target did not cause a relevant effect, and thus the drug could be repurposed in the new indication solely on the basis of the newly identified off-target. The second type is a more complex, more difficult-to-prove, and interesting case where the inhibition of two or more targets could act in combination or synergistically in the same indication. Overall, while the notion of drugs binding solely to one protein target is increasingly being challenged and the number of studies uncovering polypharmacology continue to accumulate [15,26-31], the extent to which precise understanding of the binding of drugs to their target protein(s) is actually clearly known to contribute to drug efficacy and safety in the clinic remains to a worrying extent unknown [32].

Precision oncology involves in one definition to ‘coupling an established clinical-pathological index with state-of-the-art molecular profiling to enable diagnostic, prognostic and therapeutic strategies precisely tailored to each patient’s requirements’ – and thus requires a detailed understanding of the relationship between drug binding to one or more molecular targets and clinical effectiveness [33]. It is now widely accepted that the successful exploitation of molecularly targeted cancer therapeutics depends on the use of appropriate biomarkers [10]. We can distinguish between several types of biomarkers. Of note, pharmacodynamic (PD) biomarkers, used in parallel with corresponding pharmacokinetic (PK) data, are important to confirm target engagement and pathway modulation in a Pharmacological Audit Trail (PhAT) [34,35]. They provide valuable supportive evidence (although not definitive proof) that a drug is acting via a known mechanism and they can be used to identify the ideal dose for administration in follow-up clinical trials [34]. Predictive biomarkers are measurements associated with a response to, or lack of response to, a particular therapy and are used to identify the patient population that will respond to a given molecularly targeted drug [34]. Given impressive advances in cancer genomics, which have enabled patient sequencing, genomic biomarkers have great potential to transform clinical practice. Prior to exploitation in the clinic, all biomarker types must be thoroughly validated and related to the molecular target or the mechanism-of-action of the drug [34]. The development of precision oncology, which requires predictive biomarkers for patient selection, is already transforming clinical trial design and enabling new customized, adaptive, hypothesis-testing early trials that incorporate analytically validated and clinically qualified biomarkers. These trials accelerate the drug approval process, maximize the benefit to patients and enable the construction of a framework for rational decision-making in early clinical trials using the PhAT [34]. Furthermore, we can now envisage a future in which validated biomarkers are combined with longitudinal genome sequencing and other ‘omics’ technologies to inform adaptive combinatorial treatment – facilitated especially by plasma DNA sequencing [36]. Such an approach enables us to tackle genetic and phenotypic heterogeneity and overcome drug resistance, allowing a more nuanced, sophisticated and comprehensive approach to cancer treatment [8].

Against this background, in the remainder of this mini-review, we assess the current understanding of, and future prospects for, cancer drug polypharmacology in the context of genomic biomarkers. First, we discuss how drug polypharmacology is currently being exploited in precision oncology, using U.S. Food and Drug Administration (FDA) approved pharmacogenomic biomarkers as a means of establishing a link between drug binding and efficacy. Second, we assess the opportunities for exploiting known polypharmacology as we move towards a more comprehensive and systems-based approach to both pharmacology and drug discovery, especially to defeat drug resistance.

2. CURRENT CLINICAL APPLICATIONS OF DRUG POLYPHARMACOLOGY IN ONCOLOGY

The use of targeted cancer drugs coupled with accompanying biomarkers can potentially link the binding of a drug to its target with its efficacy. Accordingly, we have reviewed the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling [37] to shed light on the extent to which polypharmacology is actually exploited in precision oncology. Currently, there are 41 cancer drugs approved by the FDA with at least one pharmacogenomic biomarker (Supplementary Table S1). Of these, 22 are small-molecule targeted cancer drugs (54%), 7 are antibodies (17%), 2 are antibody-drug conjugates (5%), 9 are chemotherapeutics (22%), and 1 is a protein therapeutic (2%). Of the 22 small-molecule targeted cancer therapeutics, 16 bind directly to their cognate approved pharmacogenomic biomarker(s), enabling us to make a strong link between drug binding and efficacy. This link can be made unequivocal through the use of resistant drug alleles pre-clinically and the discovery of drug-resistant mutant proteins in the clinic. Imatinib and crizotinib are the only two of the drugs in the list that inhibit more than one approved biomarker/target, illustrating the limited extent to which polypharmacology is knowingly being exploited in precision oncology (Table 1).

However, when we reviewed the U.S. National Institutes of Health registry database of clinical studies [38] we identified at least six additional drugs that inhibit more than one biomarker being currently tested in clinical trials (Table 1). This indicates that the polypharmacology of oncology drugs is under investigation and its use is likely to increase in the near future [39]. Table 1 lists all eight of these polypharmacological drugs, together with the predictive biomarkers that they directly inhibit. Also shown is further information curated from the knowledgebase canSAR [40], including their median target binding affinities. This information can aid discussions about how to further exploit poly-
pharmacology in a prospective rather than serendipitous way in precision oncology. In the following sections we review the discovery of the multi-target drugs listed in Table 1 and their clinical development.

2.1. Case History of Imatinib

Imatinib was the first kinase inhibitor to be approved by the FDA in 2001 [2]. Given the identification of the Philadelphia chromosome and then breakpoint cluster region protein – tyrosine-protein kinase ABL1 (BCR-ABL) translocation as the key transformation event in chronic myelogenous leukaemia (CML), scientists at Ciba-Geigy (now Novartis) selected BCR-ABL as the target for a drug discovery project [41]. They subsequently evolved a lead compound from a screen against protein kinase C (PKC), eventually identifying a drug candidate devoid of PKC activity and with strong affinity for BCR-ABL [41]. The approval of imatinib transformed the treatment of CML to a manageable chronic condition with a six-year survival rate of above 80%. Moreover, the subsequent identification of BCR-ABL second mutations and also amplifications among patients that responded to imatinib initially but relapsed afterwards provides definitive clinical proof that imatinib’s efficacy in CML is driven through BCR-ABL inhibition [42]. Imatinib became the flagship for the development of molecularly targeted cancer therapeutics, although the extent to which the lessons learned are truly translatable are clearly now questionable, given that CML is a monoclonal disease [43].

Interestingly, imatinib is not only an inhibitor of BCR-ABL, but also strongly inhibits other kinases, including mast/stem cell growth factor receptor Kit (KIT) and platelet-derived growth factor receptor beta (PDGFRB) [41]. KIT was known to have a driver role in gastrointestinal stromal tumors (GIST). Accordingly, imatinib was tested and shown to be effective in GIST cancer cell lines and patients, finally gaining FDA approval for use in KIT-mutated GIST in 2002 [44]. Moreover, rearrangement of PDGFRB had been described in myelodysplastic/myeloproliferative (MDS/MPD) diseases [41]. Imatinib was also developed in clinical trials for MDS/MPD diseases with PDGFR gene re-arrangements and finally received FDA approval in 2006 [45]. Overall, although developed as a BCR-ABL kinase inhibitor, imatinib’s serendipitously discovered polypharmacology has been exploited in several cancer indications, due to its inhibition of four targets that are all now used as predictive biomarkers. However, the independent use of a single and different biomarker/molecular target for each of these indications suggests strongly that imatinib is always effective through a single target in each case. Mutations in KIT and PDGFR have been isolated in imatinib-resistant patients, providing clinical proof that these are indeed bona fide single targets of imatinib that are involved in efficacy [46]. Interestingly, at least one patient showed amplification of both KIT and PDGFR, providing evidence for a putative combinatorial or synergistic effect by dual inhibition of KIT and PDGFR in some GIST patients [46]. More recently, several additional targets of imatinib, both kinase and non-kinase, have been identified (mainly through chemical proteomics) but it is not yet known if they are involved in its mechanism of action [47].

2.2. Case History of Crizotinib

Non-small-cell lung cancer (NSCLC) accounts for around 85% of lung cancer cases. Historically, NSCLC was a leading cause of cancer deaths worldwide, often diagnosed at a late stage, and with poor prognosis. Drug treatment involved one-size-fits-all chemotherapy with significant side effects from which only around 10% of patients responded.

This changed when specific subgroups of patients with exon 19 deletions or exon 21 (L858R) substitution mutations in the epidermal growth factor receptor (EGFR) were found to respond to the EGFR kinase inhibitors gefitinib and erlotinib. These two drugs were subsequently approved for this patient population, concomitantly improving prognosis despite the emergence of drug resistance [2,49]. These results prompted research into new driver mutations in NSCLC. In 2007, an echinoderm microtubule associated protein like 4 – anaplastic lymphoma receptor tyrosine kinase (EML4-ALK) rearrangement was identified in another subgroup of NSCLC patients and was shown to have transforming activity. At that time, the kinase inhibitor crizotinib was being developed as a hepatocyte growth factor receptor (MET) inhibitor and was thought to be ‘selective’ against 90% of kinases tested in a 120-kinase panel [49,50]. ALK was among the 13 kinases more potently inhibited by crizotinib, below 100-fold selectivity from the intended target MET [51]. The discovery of the EML4-ALK rearrangement as a driver genetic abnormality in NSCLC prompted an investigation into the use of crizotinib in this cancer indication [49]. Because of this, crizotinib rapidly received accelerated approval by the FDA in 2011 for ALK-positive NSCLC patients. Soon afterwards, second-site mutations and overexpression of ALK were identified among relapsed patients proving that the efficacy of crizotinib in this patient population was driven through ALK inhibition [52]. Hence, crizotinib was first approved for use against a protein which was not the intended target of the drug discovery program, but an accidental off-target.

Interestingly, crizotinib has just received approval for use in another subgroup of NSCLC patients based on its effect on another off-target protein. The proto-oncogene tyrosine-protein kinase ROS (ROS1) was not present in the first panel used to determine crizotinib selectivity and thus it was not identified as an off-target until a cancer cell line screen was performed in 2012 [53,54]. An initial clinical case report showed preliminary evidence of efficacy and this was rapidly translated into clinical trials, leading to Breakthrough Therapy Designation, priority review, and finally FDA approval for crizotinib in ROS1-altered NSCLC patients in March 2016 [53]. The first second-site ROS1 mutation in relapsed patients, proving that ROS1 inhibition is likely driving crizotinib efficacy in this patient population, has also been recently reported [55]. To our knowledge, there is no reported evidence of patients with resistant aberrations in both ROS1 and ALK, supporting a distinct and unique target driving crizotinib’s efficacy in each respective NSCLC patient population. In the meantime, crizotinib is still under investigation in clinical trials for cancers harboring alterations of its initially intended target MET [56].

2.3. Polypharmacology Biomarkers in Clinical Trials

The exploitation of polypharmacology in precision oncology is likely to increase in the near future since at least six approved kinase inhibitors are under clinical investigation in new indications as a result of their activity against additional biomarker targets (Table 1). In this section we examine the status of this research.

In 2013, the FDA approved the irreversible EGFR and receptor tyrosine-protein kinase erbB-2 (ERBB2) inhibitor afatinib, as a first-line treatment for patients with metastatic NSCLC harboring deletions in EGFR exon 19 or the L858R mutation in exon 20 [57]. Today, afatinib is in clinical trials for several other cancer types, including several harboring alterations in ERBB2. Afatinib showed early promise in pre-clinical and clinical studies looking at the treatment of ERBB2-positive metastatic breast cancer but recently failed to show improved efficacy in a Phase 2 clinical trial [58,59]. Despite this, there are several other cancer indications for which treatment with afatinib has potential, based on reports from its use in the clinic. These include urothelial carcinoma, which is the subject of a recent Phase 2 clinical trial with afatinib, and in which receptor tyrosine-protein kinase erbB-3 (ERBB3) has also shown promise as another predictive biomarker [60]. Both EGFR mutations and ERBB2 amplifications have been identified as resistance mechanisms to afatinib treatment in human samples [61]. More interestingly, they appear to be mutually exclusive and pre-clinical evidence suggests a major role of ERBB2 in mediating drug
sensitivity, supporting a beneficial effect of simultaneous inhibition of EGFR and ERBB2 [61].

In 2014, the FDA granted accelerated approval and Breakthrough Designation to the kinase inhibitor ceritinib for the treatment of patients with ALK-positive metastatic NSCLC with disease progression or who are resistant to crizotinib. This approval was based on the impressive efficacy of ceritinib in overcoming resistance to crizotinib [62]. These drugs have a markedly different

| Drug | Target | IC50 | Dose | Indication | Approval | Biomarker | References | Population |
|------|--------|------|------|------------|----------|-----------|------------|------------|
| Imatinib | ABL1 | 61 nM | 400-600 mg/day | CML, ALL | 2001 | BCR-ABL translocation | FDA label | 100%, N/A |
| | KIT | 100 nM | 100-400 mg/day | GIST, ASM | 2002 | KIT+, without D816V | FDA label | 85%, N/A |
| | PDGFRA | 50 nM | 400 mg/day | MDS/MPD | 2006 | PDGFR rearrangements | FDA label | N/A |
| | PDGFRB | 50 nM | | | | | |
| Crizotinib | ALK | 183 nM | 200-250 mg BID | NSCLC | 2011 | ALK positive | FDA label | 3-7% |
| | ROS1 | 4.1 nM | 250 mg BID | NSCLC | 2016 | ROS1 positive | NCT02499614 | 2% |
| | MET | 2.25 nM | 250 mg BID | NSCLC | Phase 2 | MET-arrangements | NCT02499614, etc. | 2-4% |
| Afatinib | EGFR | 0.22 nM | 40 mg/day | NSCLC | 2013 | EGFR ex.19 del. or ex.21 L858R | FDA label | 5-17% |
| | HER2 | 5 nM | 40 mg/day | NSCLC, etc. | Phase 2 | HER2 positive /overexpression | NCT02274012, etc. | N/A |
| Ceritinib | ALK | 14.1 nM | 750 mg/day | NSCLC | 2014 | ALK positive | FDA label | 3-7% |
| | ROS1 | 141.8 nM | 750 mg/day | several | Phase 2 | ROS1 mutation | NCT02186821 | 2% |
| Dasatinib | ABL1 | 0.71 nM | 140 mg/day | CML, ALL | 2006 | BCR-ABL translocation | FDA label | 100%, N/A |
| | DDR2 | 3.2 nM | 140 mg/day | NSCLC | Phase 2 | DDR2 mutation | NCT01514864 | 2-4% |
| | SRC | 0.6 nM | 100 mg/day | HNSCC, NSCLC | Phase 1 | SRC modulation | NCT00779389 | N/A, N/A |
| Erlotinib | EGFR | 19.3 nM | 100-150 mg/day | NSCLC, PACA | 2004 | EGFR ex.19 del. or ex.21 L858R | FDA label | 5-17% |
| | JAK2(V617F) | N/A | 150 mg/day | PV | Phase 2 | JAK2 V617F | NCT01038856 | N/A |
| | HER2 | 360 nM | 100-150 mg/day | PACA | Phase 2 | HER2 expression | NCT00674973 | N/A |
| | HER3 | 1100 nM | N/A (100-150 mg/day) | PACA | Phase 2 | HER3 expression | NCT00674973 | N/A |
| Nilotinib | ABL1 | 18 nM | 300-400 mg BID | CML | 2007 | BCR-ABL translocation | FDA label | 100% |
| | KIT | 98 nM | 400 mg BID | SKCM | Phase 2 | KIT aberration | NCT01099514 | 2-8% |
| Ponatinib | ABL1 | 1.7 nM | 45 mg/day | CML, ALL | 2012 | BCR-ABL translocation | FDA label | 100%, N/A |
| | FLT3 | 0.3 nM | 45 mg/day | AML | Phase 2 | FLT3-ITD mutant | NCT02428543 | 24.30% |
| | FGFR2 | N/A | N/A | AML | Phase 2 | FGFR2 fusion | NCT02265341 | N/A |
| | RET | N/A | 30 mg/day | NSCLC | Phase 2 | RET translocation | NCT01813734 | 1.30% |
that has been difficult to translate into new predictive biomarker-driven indications [85]. First, erlotinib was shown to effectively inhibit the activity of V617F-mutated tyrosine-protein kinase JAK2 (JAK2) in pre-clinical models of polycythemia vera, but it later failed to show efficacy in the clinic in this setting [86,87]. Second, ERBB2 and ERBB3 have also been difficult to validate as predictive biomarkers in clinical trials such as a recent Phase 2 trial in advanced pancreatic carcinoma [88]. Overall, it is clear that having a broad polypharmacology does not guarantee an increased number of approved clinical uses and that polypharmacology-based repurposing can be very challenging to exploit in the clinic, even when sound pre-clinical evidence is available.

In summary, these examples of kinase inhibitors illustrate that polypharmacology-based repurposing is already being exploited clinically in precision oncology. The pathfinder capacity of imatinib and crizotinib for inhibiting several targets that harbor driving aberrations in different types of cancer has led to the biomarker-driven approval of these drugs in more than one cancer indication without increased side-effects or toxicity. Moreover, the large number of ongoing clinical trials testing new biomarker-driven drug indications based on polypharmacology suggests that more cases are likely to be approved in the near future. The use of drug resistant alleles in pre-clinical studies and the discovery of mutated or over-expressed proteins in the clinic provides proof that these drugs are achieving efficacy via different targets and suggests that they generally act via a unique target in each of the indications as opposed to having a synergistic or combinatorial effect. However, preclinical and clinical evidence suggests that imatinib and afatinib could be benefiting from combinatorial or synergistic polypharmacology in some of their indications. Finally, several clinical failures illustrate that there are also many challenges ahead.

3. TOWARDS FULLY EXPLOITING POLYPHARMACOLOGY IN PRECISION ONCOLOGY

Expanding the use of drugs through polypharmacology has the potential to accelerate access to additional precision treatments for cancer patients and to overcome or prevent drug resistance. In this section, we review the challenges associated with prospectively exploiting polypharmacology, assess available experimental and computational methods to identify new targets of drugs, and discuss recent advancements in the emerging fields of systems pharmacology and multi-target drug design.

3.1. Exploiting Known Polypharmacology

The increasing number of reports detailing new targets of approved drugs and the increasing availability of data in public online repositories provides new opportunities for drug repurposing [89,90]. To illustrate the information available, we have constructed a drug-target network for the drugs listed in Table I using information available via our knowledgebase canSAR (Fig. 1) [40]. As shown in Table I, for the aforementioned cases where a drug is repurposed in more than one indication due to the binding to more than one target (as supported by biomarker use and drug resistance), the drug tends to bind to each of the targets with similar affinity. It is worth mentioning that these are mainly cases where a single target is believed to be the responsible of efficacy in each of the indications. Accordingly, the network shown in Fig. (1) includes only those target interactions within a conservative 10-fold selectivity range of the most- potent interaction validated with a biomarker [40]. As shown in the Figure, our knowledge of the targets of the eight approved drugs shown goes well beyond the 16 targets currently approved or in clinical development as predictive biomarkers (Table I). There are 64 targets in the network that are inhibited within the 10-fold selectivity range (Fig. 1). Unsurprisingly, the majority of these targets are other kinases, as the promiscuity of this target family is widely documented [21]. But interestingly, imatinib and nilotinib bind very strongly to several carbonic anhydrases (CAs), a totally distinct family of enzymes form kinases [91], illus-
There are many challenges and considerations that need to be taken into account when repurposing a drug on the basis of a new drug-protein interaction. When the drug was designed through target-based drug discovery it is unlikely that the newly identified off-target is more potent than the intended one. Accordingly, target selectivity and side-effects resulting from the interaction with the primary target need to be carefully considered [89]. Another key point is the need to demonstrate clear involvement of the new target in a disease that represents a highly unmet medical need, without difficult competition from other drugs. In this respect, more rigorous target validation efforts are very important, especially given the published reports of lack of data reproducibility in the scientific literature [89]. In the context of precision oncology, the association of the new target with a biomarker for patient selection is also a key and often challenging step [10]. Finally, the issue of intellectual property space needs to be also taken carefully into consideration. The publication of many new drug-target interactions in the public domain certainly helps pre-competitive and open source research but challenges the commercial exploitation of these new interactions, as they may represent ‘prior art’ and potentially block any new patent indication of the drug [89]. Given the challenges associated with drug repurposing in the public domain, including re-sourcing costly clinical trials and negotiating public initiatives to ease the process such as the UK Off-Patent Drugs Bill [93] it is paramount for both patient as well as commercial benefit that new drug-target interactions are protected before publication if their further development is believed to be therapeutically relevant. Overall, there are many opportunities for drug repurposing with already known polypharmacology but lack of target validation, biomarker identification and its disclosure prior patenting seriously challenge their exploitation.

The second and distinct case of proactive identification of beneficial polypharmacology through combination or synergistic effects within an individual patient and its exploitation for patient benefit – distinct from repurposing – is exciting in terms of potential for overcoming drug resistance but probably far more challenging to achieve. We have already mentioned some evidence of potential combinatorial effects on the targets inhibited by imatinib and afatinib (see above), but this evidence is far from conclusive. A commonly used example to illustrate beneficial polypharmacology through effects of a single drug on more than one target in a particular cancer is sunitinib [18]. Sunitinib is a ‘multi-targeted’ kinase inhibitor that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly inhibited kinase that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly targeted drug on cancer patients in precision oncology? 

Moreover, only drug-resistant KIT mutations have been identified accompanied by validated biomarker(s) for precision oncology. Although sunitinib has been authorized in RCC, GIST and pancreatic neuroendocrine tumors (pNET), its approval has not been achieved. We have already mentioned some evidence of potential polypharmacology but lack of target validation, biomarker identification and its disclosure prior patenting seriously challenge their exploitation.

The second and distinct case of proactive identification of beneficial polypharmacology through combination or synergistic effects within an individual patient and its exploitation for patient benefit – distinct from repurposing – is exciting in terms of potential for overcoming drug resistance but probably far more challenging to achieve. We have already mentioned some evidence of potential combinatorial effects on the targets inhibited by imatinib and afatinib (see above), but this evidence is far from conclusive. A commonly used example to illustrate beneficial polypharmacology through effects of a single drug on more than one target in a particular cancer is sunitinib [18]. Sunitinib is a ‘multi-targeted’ kinase inhibitor that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly inhibited kinase that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly targeted drug on cancer patients in precision oncology? 

processed that distant polypharmacology can also lead to very potent interactions [92]. The number of targets per drug, within this 10-fold selectivity range, varies considerably. Apart from afatinib, all the drugs have at least one very potent interaction with a target that is not currently under investigation in the clinic. Of these drugs, dasatinib is the most promiscuous drug with 38 targets strongly inhibited. Could these interactions, or any of the known or yet to be discovered off-target interactions of other drugs, be used to repurpose these drugs in other cancer indications? Can we identify combinatorial or synergistic polypharmacology and use this information to better tailor drugs to cancer patients in precision oncology?

There are many challenges and considerations that need to be taken into account when repurposing a drug on the basis of a new drug-protein interaction. When the drug was designed through target-based drug discovery it is unlikely that the newly identified off-target is more potent than the intended one. Accordingly, target selectivity and side-effects resulting from the interaction with the primary target need to be carefully considered [89]. Another key point is the need to demonstrate clear involvement of the new target in a disease that represents a highly unmet medical need, without difficult competition from other drugs. In this respect, more rigorous target validation efforts are very important, especially given the published reports of lack of data reproducibility in the scientific literature [89]. In the context of precision oncology, the association of the new target with a biomarker for patient selection is also a key and often challenging step [10]. Finally, the issue of intellectual property space needs to be also taken carefully into consideration. The publication of many new drug-target interactions in the public domain certainly helps pre-competitive and open source research but challenges the commercial exploitation of these new interactions, as they may represent ‘prior art’ and potentially block any new patent indication of the drug [89]. Given the challenges associated with drug repurposing in the public domain, including re-sourcing costly clinical trials and negotiating public initiatives to ease the process such as the UK Off-Patent Drugs Bill [93] it is paramount for both patient as well as commercial benefit that new drug-target interactions are protected before publication if their further development is believed to be therapeutically relevant. Overall, there are many opportunities for drug repurposing with already known polypharmacology but lack of target validation, biomarker identification and its disclosure prior patenting seriously challenge their exploitation.

The second and distinct case of proactive identification of beneficial polypharmacology through combination or synergistic effects within an individual patient and its exploitation for patient benefit – distinct from repurposing – is exciting in terms of potential for overcoming drug resistance but probably far more challenging to achieve. We have already mentioned some evidence of potential combinatorial effects on the targets inhibited by imatinib and afatinib (see above), but this evidence is far from conclusive. A commonly used example to illustrate beneficial polypharmacology through effects of a single drug on more than one target in a particular cancer is sunitinib [18]. Sunitinib is a ‘multi-targeted’ kinase inhibitor that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly inhibited kinase that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly targeted drug on cancer patients in precision oncology? 

processed that distant polypharmacology can also lead to very potent interactions [92]. The number of targets per drug, within this 10-fold selectivity range, varies considerably. Apart from afatinib, all the drugs have at least one very potent interaction with a target that is not currently under investigation in the clinic. Of these drugs, dasatinib is the most promiscuous drug with 38 targets strongly inhibited. Could these interactions, or any of the known or yet to be discovered off-target interactions of other drugs, be used to repurpose these drugs in other cancer indications? Can we identify combinatorial or synergistic polypharmacology and use this information to better tailor drugs to cancer patients in precision oncology?

There are many challenges and considerations that need to be taken into account when repurposing a drug on the basis of a new drug-protein interaction. When the drug was designed through target-based drug discovery it is unlikely that the newly identified off-target is more potent than the intended one. Accordingly, target selectivity and side-effects resulting from the interaction with the primary target need to be carefully considered [89]. Another key point is the need to demonstrate clear involvement of the new target in a disease that represents a highly unmet medical need, without difficult competition from other drugs. In this respect, more rigorous target validation efforts are very important, especially given the published reports of lack of data reproducibility in the scientific literature [89]. In the context of precision oncology, the association of the new target with a biomarker for patient selection is also a key and often challenging step [10]. Finally, the issue of intellectual property space needs to be also taken carefully into consideration. The publication of many new drug-target interactions in the public domain certainly helps pre-competitive and open source research but challenges the commercial exploitation of these new interactions, as they may represent ‘prior art’ and potentially block any new patent indication of the drug [89]. Given the challenges associated with drug repurposing in the public domain, including re-sourcing costly clinical trials and negotiating public initiatives to ease the process such as the UK Off-Patent Drugs Bill [93] it is paramount for both patient as well as commercial benefit that new drug-target interactions are protected before publication if their further development is believed to be therapeutically relevant. Overall, there are many opportunities for drug repurposing with already known polypharmacology but lack of target validation, biomarker identification and its disclosure prior patenting seriously challenge their exploitation.

The second and distinct case of proactive identification of beneficial polypharmacology through combination or synergistic effects within an individual patient and its exploitation for patient benefit – distinct from repurposing – is exciting in terms of potential for overcoming drug resistance but probably far more challenging to achieve. We have already mentioned some evidence of potential combinatorial effects on the targets inhibited by imatinib and afatinib (see above), but this evidence is far from conclusive. A commonly used example to illustrate beneficial polypharmacology through effects of a single drug on more than one target in a particular cancer is sunitinib [18]. Sunitinib is a ‘multi-targeted’ kinase inhibitor that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly inhibited kinase that was initially approved by the FDA for renal cell carcinoma (RCC) and imatinib-resistant GIST in 2006. It is a broadly targeted drug on cancer patients in precision oncology? 

processed that distant polypharmacology can also lead to very potent interactions [92]. The number of targets per drug, within this 10-fold selectivity range, varies considerably. Apart from afatinib, all the drugs have at least one very potent interaction with a target that is not currently under investigation in the clinic. Of these drugs, dasatinib is the most promiscuous drug with 38 targets strongly inhibited. Could these interactions, or any of the known or yet to be discovered off-target interactions of other drugs, be used to repurpose these drugs in other cancer indications? Can we identify combinatorial or synergistic polypharmacology and use this information to better tailor drugs to cancer patients in precision oncology?
in GIST and its mechanisms of resistance in GIST or other cancers do not provide unequivocal proof of the combinatorial benefit of its polypharmacology in individual cancers [95-97]. More recently, pan-RAF inhibitors that also target Src family kinases (SFKs) have been shown to prevent paradoxical pathway activation in pre-clinical models of BRAF-mutant melanoma, a common resistant mechanism to BRAF and dual specificity mitogen-activated protein kinase kinase 1 (MEK) inhibitors, thus illustrating how polypharmacology can effectively prevent drug resistance [98]. The deliberate development of such agents is therefore gaining greater interest.

Moreover, polypharmacology has also been shown to enable the synergy in danusertib and bosutinib combination in pre-clinical models of imatinib-resistant CML [99]. A recent computational pan-cancer genomics analysis linking cancer driver identification with in silico drug prescription showed that around 11% of cancer patients harbor genomic alterations – that are predicted as cancer drivers – in more than one protein, and which could potentially be inhibited with a single drug [9]. There is growing evidence of patient populations that could be tested for – and potentially obtain benefit from – combinatorial polypharmacology but the lack of widespread adoption of patient sequencing in routine healthcare systems (although common in clinical trials), of biomarker validation and of repeated sampling for drug-resistant mutations all currently limits this approach. We expect that the ongoing implementation of longitudinal genome sequencing and other omics technologies, facilitated by use of plasma DNA, should enable us to better understand, and assess the value of, combinatorial polypharmacology in the near future.

3.2. Identifying New Targets of Known Drugs

Although there are many options for exploiting known polypharmacology, it is essential to comprehensively uncover all of the interactions between drugs and biomolecules in order to maximize the therapeutic potential arising from drug discovery efforts. Accordingly, it is necessary to exploit currently available methods for target profiling – as well as develop completely new ones – if we are to fully characterize drug-protein interactions and exploit them for patient benefit. In this section we briefly discuss some of the available experimental and computational methods.

The first methods that were used to identify polypharmacology were experimental. Advances in recombinant DNA technology, protein production and robotics enabled the development of a number of miniaturized biochemical activity and binding assays to test an increasing number of targets. Initially, these assays were developed for members of the same protein family, as illustrated by the early work on kinases and G protein-coupled receptors (GPCRs) that initially led to the identification of polypharmacology [22,100]. As the use and breadth of these screening panels increased, involving broad safety panels and larger family coverage, new targets of known drugs were identified and many of the panels became commercially available through contract research organizations (CROs) [21,101]. Today, these CROs continue to increase the scope of their target panels, with the largest panels now covering approximately 80% of the human kinome [102,103]. As CROs work to include new members of well-characterized families, and to add new families, research using these panels will continue to be a source of identifying new targets of drugs, which may be unexpected and surprising - as nicely illustrated by the recent discovery of strong off-target effects on bromodomains among some clinical kinase inhibitors [27]. A second widely-used experimental method for target profiling is chemical proteomics [104]. This was a pioneering method used to uncover new targets of BCR-ABL inhibitors, including the non-kinase oxidoeductase NQO2 [47]. Today, it continues to be used to identify totally unexpected off-targets, such as the recent identification of the nudix family phosphohydrolase MTH1 as an off-target of the (S)-enantiomer of the kinase inhibitor drug crizotinib [105]. This approach is employed increasingly for target deconvolution in phenotypic screening [106]. Exciting new experimental methods are continually being developed, such as the recent cellular thermal shift assay (CETSA) that enables measurement of target engagement in living cells and which has already been used to identify unknown off-target affinity for thymidylate synthase among some known drugs [107,108]. Overall, innovative experimental technologies continue to be a major source of identifying new targets of drugs.

Computational methods are becoming increasingly important as a means of identifying potential new targets of drugs, especially since they are increasing in accuracy due to the much greater volume of high-quality publicly available data and their cost-effectiveness compared to experimental technologies [109,110]. Historically, we can distinguish between ligand- and structure-based computational methods. Ligand-based methods rely on annotated chemical libraries that connect small molecules with target proteins to facilitate creation of ligand-based protein models. Several strategies have been successfully implemented to develop computational models, from Bayesian statistics to neural networks and machine learning [110]. Among these, and worth highlighting, are methods that rely on chemical similarity and use fingerprints or feature-based distribution descriptors, as they have now been widely used to successfully identify new targets of drugs [28,111,112]. As an example, serotonin and norepinephrine transporters were predicted as putative targets of cyclobenzaprine and subsequently validated in vitro, providing a plausible explanation for its association with the serotonin syndrome [113]. Structure-based methods, in contrast, use information on protein structure and methods such as docking or binding site similarity to identify new drug-target interactions [112,114]. They have also been successfully used to identify new targets of drugs, such as in the identification of carbonic anhydrase as a nanomolar off-target of celecoxib [115]. More recently, methods that use both ligand and structural information have also been developed, as well as methods that rely on network biology and text mining, among others [13].

A final group of methods that often mix characteristics of computational and experimental methods are also being developed, often using cluster analysis of omics data to infer new targets of known drugs under the hypothesis that clusters should share the same target(s). Several types of omics data have already been successfully used to identify polypharmacology. These include use of gene expression data (either alone or coupled with network analysis) [116,117], cancer cell line profiling coupled with omics data [118] and ex vivo screening of patient cells coupled with genomics that recently enabled the identification of BCR-ABL T315I as a nanomolar off-target of the VEGFR-kinase inhibitor axitinib [26]. Overall, both experimental and computational methods are increasingly robust and complement each other in overcoming the limitations associated with any one method. Accordingly, all a range of both types of method will play their role as we advance towards a comprehensive understanding of how drugs interact with the whole human proteome.

3.3. Towards Systems Pharmacology and Multi-Target Drug Design

The advance of omics technologies has revolutionized our approach to studying biology and disease, progressing over the last several years from initial successes and approvals with single-agent targeted therapies to the more recent recognition of the need to address the challenges of greater genetic and biochemical complexity, heterogeneity and drug resistance, requiring a more network or systems-based approach to pharmacology and drug design. We are still far from a comprehensive understanding of the effects of drugs in the human body at both a detailed and multi-scale level, but new methods are certainly starting to advance the field towards this goal [13]. Computational methods to predict toxicity and side-effects are also increasingly being reported, enabling a better understanding of how the binding to each target may contribute to side-effects
Unfortunately, lead-to-drug optimization of multi-target drug candidates – involving enhancement of potency and selectivity toward two or more desired targets, is still technically challenging, especially where the chemical starting points do not serendipitously provide potent multi-targeting. Thus polypharmacological drug discovery has not been fully embraced in industry [18]. However, we are starting to witness attempts to rationally design multi-target drugs, particularly in academia [120]. There have been several attempts to rationally design dual inhibitors of different protein families, including the construction of dual tyrosine-phosphoamino kinase inhibitors and of HSP90-kinase inhibitors [121-123]. There are also several examples of combination of similar pharmacophores into a single compound or dissimilar pharmacophores being connected by linkers, such as the dual HDAC-PI3K kinase inhibitor CUDC-907 [18,124]. Beyond dual inhibition, a computational method to rationally design ligands against profiles of multiple drug targets has also been described and applied to GPCR targeted polypharmacology [120]. However, identifying the ideal polypharmacological profile to reverse a given disease phenotype is a general limitation, particularly given our incomplete understanding of the function of many proteins and our limited capacity to predict combinatorial polypharmacology [18]. Interestingly, the recent return to phenotypic drug discovery offers new opportunities to facilitate multi-target drug design, as nicely illustrated by the recent use of a fruit fly cancer model to identify the optimal multi-kinase profile to achieve maximal efficacy and minimal toxicity [125]. Overall, a more comprehensive approach to pharmacology and drug discovery is underway which is set to benefit from our increasing appreciation of the complex and rich polypharmacology of small-molecule drugs and the potential to exploit this for therapeutic benefit.

CONCLUSION AND OUTLOOK

In summary, a more comprehensive, systematic and unbiased approach to studying the genetics, biology and pharmacology of cancer is uncovering the complexity of this set of diseases and the evolutionary nature of cancer while at the same time new technological advances are also enhancing our understanding of the complex interaction between cancer (and other) drugs and the proteome. In this mini-review, we have shown that polypharmacology has so far been exploited to a very limited extent within the paradigm of precision oncology. Moreover, the case histories reviewed in detail here – those of imatinib and crizotinib – illustrate that polypharmacology is mainly being used to repurpose drugs to new cancer indications where only one of the drugs’ targets is suspected to be responsible for therapeutic efficacy in each different indication. While several other cancer drugs are currently in biomarker-driven clinical trials to extend their uses through the binding to new protein targets, these again largely represent single-target repurposing strategies. Our analysis shows that the number of true polypharmacology approaches that are under investigation for precision oncology – whereby the aim is to hit more than one target simultaneously to achieve a given anticancer effect – represent a very low proportion of the already known polypharmacology. Limitations in target validation, biomarker development and patenting, as well the challenges of multi-target drug design and lead optimization, are currently preventing us from exploiting our knowledge of polypharmacology for the benefit of cancer patients. The application of polypharmacology is however likely to increase since pharmacological control of two or more targets or pathways, even amounting to network or systems pharmacological perturbation, can be seen as representing an important approach to overcoming the major clinical challenge of drug resistance due to adaptive response or clonal evolution. With respect to clinical evaluation of polypharmacology drugs, a wider adoption of longitudinal genome sequencing and other omics technologies in the clinic is urgently needed to identify cases in which we can exploit polypharmacology to identify beneficial effects of inhibiting several targets simultaneously in the same indication and maximize therapeutic potential from drug discovery efforts. We must also exploit currently available experimental and computational methods in the drug discovery phase, as well as develop exciting new methods, to uncover all the targets of currently available drugs in order to better understand the complex relationship between the binding of drugs to their target protein(s) and their efficacy and safety in the clinic – which despite the progress made remains to a worrying extent unknown. The increased understanding of the molecular mechanism-of-action of cancer drugs is paramount if we are to advance to a more systems-based approach to cancer drug discovery in order to overcome or prevent the key clinical challenge of cancer drug resistance.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

CONFLICT OF INTEREST

Paul Workman is an advisor to Astex Therapeutics, Nuevolution, Nextech Invest and Chroma Therapeutics. Jordi Mestres is affiliated with Chemotargets SL.

ACKNOWLEDGEMENTS

Albert A. Antolin is funded by the People Programme (Marie Curie Actions) of the 7th Framework Programme of the European Union (FP7/2007-2013) under REA grant agreement no. 600388 (TECNIOspring programme), and from the Agency of Business Competitiveness of the Government of Catalonia, ACCIO. Bissan Al-Lazikani and Paul Workman are funded by Cancer Research UK Programme Grant No. C309/A8725. They are also supported by funding from the Cancer Research UK Centre and the NIHR Biomedical Research Centre at The Institute of Cancer Research, London (ICR) and the Royal Marsden. Professor Workman is also acknowledges support from the ICR and is a Cancer Research UK Life Fellow. Jordi Mestres is funded by the Spanish Ministerio de Economía y Competitividad (project BIO2014-54404-R). We thank Nicky Evans for editorial assistance.

REFERENCES

[1] Greaves M. Evolutionary determinants of cancer. Cancer Discov 2015; 5(8): 806-21.
[2] Workman P, Al-Lazikani B, Clarke PA. Genome-based cancer therapeutics: targets, kinase drug resistance and future strategies for precision oncology. Curr Opin Pharmacol 2013; 13(4): 486-96.
[3] Workman P, Al-Lazikani B. Drugging cancer genomes. Nat Rev Drug Discov 2013; 12(12): 889-90.
[4] Chabner BA, Roberts TG. Timeline: Chemotherapy and the war on cancer. Nat Rev Cancer 2005; 5: 65-72.
[5] Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 2009; 136(5): 823-37.
[6] Weinstein IB. Addiction to oncogenes — the Achilles heel of cancer. Science 2002; 297: 63-4.
[7] Patel MN, Halling-Brown MD, Tym JE, Workman P, Al-Lazikani B. Objective assessment of cancer genes for drug discovery. Nat Rev Drug Discov 2013; 12: 35-50.
[8] Al-Lazikani B, Banerji U, Workman P, Al-Lazikani B. Personalized cancer medicine: molecular diagnostics, predictive biomarkers, and drug resistance. Clin Pharmacol Ther 2013; 93(3): 252-9.
[9] Willyard C. Cancer therapy: an evolved approach. Nature 2016; 532(7598): 166-8.
[10] Kushner D. Playing dirty. IEEE Spectr 2007; 44(12): 32-7.
alatipin in tumors with high levels of phospho-Src. Oncotarget 2016; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27105527

[85] Dowell J, Dowell J, Minna JD, Minna JD, Kirkpatrick P, Kirkpatrick P. Fresh from the Pipeline: Erolitinib hydrochloride. Nat Rev Drug Discov 2005; 4(4): 71-7.

[86] Li Z, Xu M, Xing S, et al. Erolitinib effectively inhibits JAK2V617F activity and polyclinematic vera cell growth. J Biol Chem 2007; 282(6): 3428-32.

[87] Cherry M, Khawandana M, Zhao ZJ, et al. Erolitinib is not effective in patients with JAK2V617F-positive polyclinematic vera. Ann Hematol 2014; 94(4): 717-9.

[88] Propper D, Davidenko I, Bridgewater J, et al. Phase II, randomized, biomarker identification trial (MARK) for erlotinib in patients with advanced pancreatic carcinoma. Ann Oncol 2014; 25(7): 1384-90.

[89] Oprea TI, Mestres J. Drug repurposing: far beyond new targets for old drugs. AAPS J 2012; 14(4): 759-63.

[90] Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology—patient and health systems opportunities. Nat Rev Clin Oncol 2015; 12(12): 732-42.

[91] Parkkila S, Innocenti A, Kallio H, Hilvo M, Szczotkawa A, Supuran CT. The protein tyrosine kinase inhibitors imatinib and nilotinib strongly inhibit several mammalian alpha-carbonic anhydrase isoforms. Biosci Med Chem Lett 2009; 19(15): 4102-6.

[92] Antonil AA, Mestres J. Distant Polypharmacology Among MLP Chemical Probes. ACS Chem Biol 2015; 10(2): 395-400.

[93] Off-patent Drugs Bill 2015-16. Available from: http://services.parliament.uk/bills/2015-16/offpatentdrugs.html.

[94] Fairev S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov 2007; 6(9): 734-45.

[95] Joosten SC, Hamming L, Soetekouw PM, et al. Resistance to sunitinib in renal cell carcinoma: From molecular mechanisms to predictive markers and future perspectives. Biochim Biophys Acta - Rev Cancer 2015; 1855(1): 1-16.

[96] Stewart GD, O’Mahony FC, Laird A, et al. Sunitinib treatment exacerbates intratumoral heterogeneity in metastatic renal cancer. Clin Cancer Res 2015; 21(18): 4212-23.

[97] Guo T, Hajdu M, Agaram NP, et al. Mechanisms of sunitinib resistance in gastrointestinal stromal tumors harboring KIT A5802-S3 mutation: An in vitro mutagenesis screen for drug resistance. Clin Cancer Res 2009; 15(22): 6862-70.

[98] Girotti MR, Lopes F, Preece N, et al. Paradox-Breaking RAF Inhibitors that Also Target SRC Are Effective in Drug-Resistant BRAF Mutant Melanoma. Cancer Cell 2015; 27(1): 85-96.

[99] Winter GE, Rux U, Carlson SM, et al. Systems-pharmacology dissection of a drug synergy in inatiatin-resistant CML. Nat Chem Biol 2012; 8(11): 905-12.

[100] Hopkins AL. Drug discovery: Predicting promiscuity. Nature 2009; 462(7270): 167-8.

[101] Krejsa CM, Horvath D, Rogalski SL, et al. Predicting ADME properties and side effects: the BioPrint approach. Curr Opin Drug Discov Dev 2003; 6(4): 470-80.

[102] Jacoby QE, Tresadern G, Bembenek S, et al. Extending kinase coverage by analysis of kinase inhibitor broad profiling data. Drug Discov Today 2015; 20(6): 652-58.

[103] Knapp S, Arruda P, Blagg J, et al. A public-private partnership to unlock the untargeted kinase. Nat Chem Biol 2014; 10(7): 55-7.

[104] Bhatia B, Gupta A, Pan H, et al. Extending kinome coverage by analysis of kinase inhibitor broad profiling data. Drug Discov Today 2015; 20(6): 652-58.

[105] Kuhn M, Brandenburg H, Kuhn M, et al. Functionalization of cellulose: a novel therapeutic target in squamous cell lung cancer. Cancer Discov 2011; 1(1): 78-89.

[106] Bristol-Myers Squibb. Trial of Dasatinib in Patients With Advanced Cancers Harboring DDR2 Mutation or Inactivating B-RAF Mutation. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US) 2000-2016. Available from: URL of the record NLM Identifier: NCT01813734.

[107] Radich J. Structure, function, and resistance in chronic myeloid leukemia. Cancer Cell 2014; 26(3): 305-8.

[108] Butun SA, Chen H, Worthmann A, et al. The N550K/H mutations in FGFR2 confer differential resistance to PD173074, dovitinib, and ponatinib ATP-competitive inhibitors. Neoplasia 2013; 15(8): 975-88.

[109] Kantarjian H, Jabbour E, Grimley J, Kirkpatrick P. Dasatinib. Nat Rev Drug Discov 2006; 5(9): 717-8.

[110] Hammaner PS, Soo ML, Ramos AH, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Cancer Discov 2011; 1(1): 78-89.

[111] Pitini V, Arrigo C, Di Mirto C, Mondello P, Altavilla G. Response of sarcomatous liver metastatic colorectal carcinoma to ox,
Polypharmacology in Precision Oncology: Current Applications and Future Prospects

[110] Koutsoukas A, Simms B, Kirchmair J, et al. From in silico target prediction to multi-target drug design: Current databases, methods and applications. J Proteomics 2011; 74(12): 2554-74.

[111] Keiser MJ, Setola V, Irwin JJ, et al. Predicting new molecular targets for known drugs. Nature 2009; 462(7270): 175-81.

[112] Ekins S, Mestres J, Testa B. In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. Br J Pharmacol 2007; 152(12): 9-20.

[113] Fonslow BR, Stein BD, Webb KJ, et al. Linking Pharmacology to Clinical Reports: Cyclobenzaprine and Its Possible Association With Serotonin Syndrome. Clin Pharmacol Ther 2013; 10(1): 54-6.

[114] Jalencas X, Mestres J. Chemoisosterism in the proteome. J Chem Inf Model 2013; 53(2): 279-92.

[115] Weber A, Casini A, Heine A, et al. Unexpected nanomolar inhibition of carbonic anhydrase by COX-2-selective celecoxib: new pharmacological opportunities due to related binding site recognition. J Med Chem 2004; 47(3): 550-7.

[116] Iorio F, Bosotti R, Scacheri E, et al. Discovery of drug mode of action and drug repositioning from transcriptional responses. Proc Natl Acad Sci U S A 2010; 107(33): 14621-6.

[117] Woo JH, Shimoni Y, Yang WS, et al. Elucidating compound mechanism of action by network perturbation analysis. Cell 2015; 162: 441-51.

[118] Seashore-Ludlow B, Rees MG, Cheah JH, et al. Harnessing connectivity in a large-scale small-molecule sensitivity dataset. Cancer Discov 2015; 5(11): 1210-23.

[119] Remez N, Garcia-serma R, Vidal D, Mestres J. The in vitro pharmacological profile of drugs as a proxy indicator of potential in vivo organ toxicities. Chem Res Toxicol 2016; 29(4): 637-48.

[120] Besnard J, Ruda GF, Setola V, et al. Automated design of ligands to polypharmacological profiles. Nature 2012; 492(7428): 215-20.

[121] Apsel B, Blair JA, Gonzalez B, et al. Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. Nat Chem Biol 2008; 4(11): 691-9.

[122] Wu L, Yu J, Chen R, et al. Dual inhibition of Bcr-Abl and Hsp90 by C086 potently Inhibits the proliferation of imatinib-resistant CML cells. Clin Cancer Res 2015; 21: 833-43.

[123] Meng T, Zhang D, Xie Z, et al. Discovery and optimization of 4,5-diarylisoxazoles as potent dual inhibitors of pyruvate dehydrogenase kinase and heat shock protein 90. J Med Chem Chem 2014; 57(23): 9832-43.

[124] Qian C, Lai CJ, Bao R, et al. Cancer network disruption by a single molecule inhibitor targeting both histone deacetylase activity and phosphatidylinositol 3-kinase signaling. Clin Cancer Res 2012; 18(15): 4104-13.

[125] Dar AC, Das TK, Shokat KM, Cagan RL. Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. Nature 2012; 486(7401): 80-4.