Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

How Physiological Targets Can Be Distinguished from Drug-binding Proteins

Kojo Mensa-Wilmot[1,2]

[1]Department of Molecular and Cellular Biology
Kennesaw State University
Kennesaw, Georgia 30144

[2]Center for Tropical and Emerging Global Diseases
University of Georgia
Athens, Georgia 30602

Corresponding author: kojo.mensawilmot@kennesaw.edu

Abstract

Some drugs in clinical trial owe their effectiveness to "off-target" activity. This and other observations raise a possibility that many studies to identify targets of drugs are incomplete. If "off-target" proteins are pharmacologically important it will be worthwhile to identify them early in the development process, for a better understanding of the molecular basis of drug action. Herein, we outline a multidisciplinary strategy for systematic identification of physiological targets of drugs in cells. A drug-binding protein whose genetic disruption yields very similar molecular effects as treatment of cells with the drug may be defined as a physiological target of the drug. For a drug developed with a "rational approach", it is desirable to verify experimentally that a protein used for hit optimization *in vitro* remains the sole polypeptide recognized by the drug in a cell.

Significance Statement:

A body of evidence indicates that inactivation of many drug-binding proteins may not cause the pharmacological effects triggered by the drugs. A multidisciplinary cell-based approach can be of great value in identifying the physiological targets of drugs, including those developed with target-based strategies.

Running title: Drug-Binging Proteins May Not Be Physiological Targets
Non-standard abbreviations: CEC, cytocidal effective concentration; PTD, physiological target of drug.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Phentotypic (*i.e.*, whole cell) screening (Chatelain and Ioset, 2018; Low et al., 2009; Swinney, 2013; Sykes and Avery, 2013) has made important contributions to discovery of hits and drugs for infectious diseases (Jacobs et al., 2011; Mesu et al., 2018)). As investigators turn their attention to possible mechanisms of action for the drugs, it is instructive to inform those studies with reports that therapeutic effects of several drugs in clinical trial are due to their "off-target" activity (Lin et al., 2019). Similarly, some drugs that were optimized *in vitro* against purified proteins have other targets in cells (Dai et al., 2008; Dolloff et al., 2011; Dunne et al., 2011; Hafner et al., 2019; Karaman et al., 2008; Lackey, 2006; Nathan G. Dollof, 2011). A possible explanation for these observations is that studies used to identify drug targets were incomplete. Rational drug development does not guarantee exclusive recognition of the *in vitro* target of a medicinal chemistry campaign in cells. Is there a way forward, amidst this uncertainty?

Many paths, all producing a list of candidates that need experimental validation, can be used to identify drug targets (reviewed in (Haanstra and Bakker, 2015; Manchado et al., 2016; Muller and Hemphill, 2016; Schenone et al., 2013; Zhu et al., 2015)). In the case of single cell eukaryote pathogens (*e.g.*, *Trypanosoma, Leishmania, Plasmodium* spp.), three general strategies, alone or in combination, are employed; (i) knockdown of the candidate gene to show that it is essential for cell viability; (ii) over-expression of the gene leading to reduced susceptibility of the cell to the drug (or selection of resistant lines); and (iii) detection of complexes between drugs and proteins. In all these strategies, it is not routine practice for the molecular effect resulting from a target gene's loss (or over-expression) to be compared directly to the molecular effects of drug treatment on cells: In the absence of this information, it may be difficult to conclude that identified genes are physiological targets of drugs in question.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Identification of Physiological Targets of Drugs

A "physiological target of a drug" (PTD) may be defined as a gene whose knockdown or over-expression yields very similar/identical molecular effects as treatment of cells with the drug. Cell death is not a good surrogate for molecular effects in this regard, since many pathways lead to that endpoint. Many drug-binding proteins may not be the physiological targets. For example, plasma proteins bind to drugs (Berezhkovskiy, 2008; Cho et al., 2010; Svennebring, 2016) but are not considered physiological targets of the small molecules.

Physiological changes in organisms originate in biochemical and molecular stimuli in cells. Thus, data from; (a) molecular genetics, (b) molecular cell biology/biochemistry, and (c) perturbation of cells, are relevant for identification of physiological targets of a drug. A central role of molecular cell biology in this effort is difficult to ignore. Although some drug-binding proteins are described as targets because knockdown of their gene products compromises cell viability, the conclusion could use further support from experiments that directly link the events being monitored at a molecular level. Drug binding to a protein needs to be directly linked to disruption of a molecular function of the target, since knockdown of a target by itself can compromise cell viability in absence of drug. For these reasons, it is important to functionally separate "drug-binding proteins" from physiological targets of drugs.

Concepts from the field of receptor signaling have relevance for attempts to discover physiological targets of drugs. Not all proteins that bind a ligand can transduce signals, qualifying for classification as receptors. Similarly, not all drug-binding proteins are physiological targets whose inhibition leads to the same molecular defects observed when the drug is added to cells. How can we distinguish between drug-binding proteins and physiological targets of the drugs?

Three stages may be described for this challenging task. First, drug-binding proteins can be identified using one of many strategies including affinity chromatography and photoaffinity labeling (Campos et al., 1996; Das, 2019; Muskens et al., 2019; Tulloch et al., 2017; Wang et al., 2012; Wang et

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

al., 2011; Xiao and Li, 2020). Second, molecular effects of a drug can be documented in molecular studies after perturbing cells with drug concentrations that are not toxic (CEC_{25} concentration, for example (Bachovchin et al., 2019)). Third, genes of drug-binding proteins can be knocked down, and the molecular effects in cells compared to those obtained from drug perturbation studies. The expectation is that when a physiological target is knocked down the molecular defects observed are very similar to those obtained when the drug is added to cells. In the case of polypharmacology (Anighoro et al., 2014; Proschak et al., 2019), more than one gene may need to be knocked down to recapitulate all the molecular defects obtained by adding the drug to cells. It is also conceivable that a physiological target does not directly bind the drug, because it interacts with a drug-binding protein.

Unbiased Strategies for Discovery of Drug-binding Proteins in Cells

Drugs are optimized to promote health by modulating aspects of human physiology. Since the smallest unit of human life is a cell; found in many differentiated states, and organized into tissues and organs, it stands to reason that drugs exert their primary effect on cells. From this view point the effects of drugs on cells that are associated with specific diseases deserves to be highlighted in molecular pharmacology studies.

Studies to identify physiological targets of drugs (PTDs) begin with discovering cellular macromolecules that bind the small molecule, even for drugs designed with rational approaches (Chintakrindi et al., 2012; Cozza, 2017; Milligan et al., 2013; Wang et al., 2018; Yang and Huang, 2006). While a host of methods are used to find drug-binding proteins, usually termed "targets" (Bunnage et al., 2015), confidence in data obtained using different techniques are not equivalent, so it is important to interpret the information cautiously.

Photoaffinity labeling of proteins using drugs functionalized with reactive moieties (i.e., "warheads") represents a powerful strategy for identifying drug-binding proteins, when combined with

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

proteome-wide analysis enabled by mass spectrometry (Fischer et al., 2010; Fischer et al., 2012; Hamouda et al., 2014; Hill and Robertson, 2018; Kawamura et al., 2014; Phizackerley et al., 2017; Thomas et al., 2017). On-target labeling of proteins using this approach can be detected by adding excess untagged drug to outcompete modification of the specific protein by probe (Parker et al., 2017). When more than one protein is labeled in this technique, approaches that rely on physical protein-drug interactions come in handy to verify true targets (see next paragraph).

Biochemical/chemical approaches, judiciously used, offer a direct path to finding drug-binding proteins, because the assays (*e.g.,* photo-affinity labeling) track physical interactions between drug and protein targets. Column affinity chromatography in which drugs are coupled to matrices have been used to identify drug-binding proteins (Jones et al., 2015; Katiyar et al., 2013; Mercer et al., 2011; Rylova et al., 2015; Schenone et al., 2013; Shi et al., 2012; Thomas et al., 2017). Here it is important to remember that proteins eluted from the column, even after rigid washing protocols (Amarasinghe and Jin, 2015; Mensa-Wilmot et al., 1995; Mishra, 2020), might not bind the drug that is coupled to the chromatography matrix; some eluted proteins may be present on the column due to interaction with other proteins that bound directly to the drug. For this reason, a second set of studies that measure direct interactions between drugs and proteins (*e.g.,* surface plasmon resonance (Douzi, 2017; Nikolovska-Coleska, 2015); microscale thermophoresis (Hellinen et al., 2020; Khavrutskii et al., 2013; Wienken et al., 2010); thermal shift assays (Alshareef et al., 2016; Dziekan et al., 2019; Lucet and Murphy, 2017; Martinez Molina et al., 2013); activity-based proteomic profiling (Cravatt et al., 2008; Golkowski et al., 2017) or drug inhibition of purified enzyme activity (Guyett et al., 2016; Labar et al., 2007; Simcic et al., 2014; Warrilow et al., 2016) is essential for confirming drug-binding properties of proteins identified with affinity chromatography techniques.

Selection of genes that confer resistance, or reduced susceptibility, to drugs offers an indirect approach for discovery of potential drug-binding proteins. In isolated cases, reduced cell susceptibility

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

to a drug against a metabolic enzyme occurs by mutations in the enzyme itself (Dicker et al., 1993; Wilson et al., 1992; Yun et al., 2008). However, the myriad of pathways employed by cells to counter susceptibility to drugs, including efflux and rewiring of metabolic pathways, is astounding (Aouida et al., 2004; Barrett et al., 2011; Chang et al., 2019; Chitanga et al., 2011; Claus et al., 2014; Dermawan et al., 2014; Gatti et al., 2015; Haider et al., 2020; Seyhan et al., 2012; Yobi et al., 2020; Zhang et al., 2019b). For these reasons, it is prudent to delay conclusions that genes identified by sequencing of genomes from drug-resistant cells are targets of the drugs until confirmatory data from other studies are obtained.

When crystal structures or appropriate homology models are available, computational docking studies can have a role in verifying drug targets (Abdolmaleki et al., 2017; Sharma et al., 2020; Zhang et al., 2019a). Understandably, the most convincing data from such predictions are supported by biochemical/biophysical studies using a library of small molecules to test predictions from docking studies. Unfortunately, validation of data from docking studies is rarely performed.

Targets Are Unknown for A Large Number of Drugs

For decades, metformin has been used as an antidiabetic drug, and now appears to be on track for "repurposing" to treat prostrate and breast cancer (Dowling et al., 2007; Wang et al., 2014). In cells, modes of action of metformin include inhibition of (a) mitochondrial glycerophosphate dehydrogenase (Madiraju et al., 2014), (b) thiamine transport by OCT1 (Chen et al., 2014), and (c) protein synthesis initiation (Dowling et al., 2007). It is highly unlikely that all these actions of metformin are mediated by a single protein target. Thus, although no evidence for direct binding of metformin to a specific protein is available, the range of its modes of cellular action point to the drug having multiple targets.

Aspirin is an analgesic with anti-inflammatory properties; it can inhibit cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) (Kaur et al., 2012), IKK-β kinase (Yin et al., 1998), and it prevents movement

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

of activating transcription factor 6 (ATF6) from the Golgi to the cell nucleus (Mugge and Silva, 2017). Such diverse cellular effects hint at a multiplicity of targets for the drug. Indeed, aspirin acetylates over 120 proteins (Wang et al., 2015), suggesting a unifying theme for pleiotropic actions for the drug; extensive post-translational acetylation of proteins. Further work is needed to identify physiological targets of aspirin in each of the cell types where the drug disrupts specific biological pathways.

Lapatinib (Tykerb) is an Erb1/2 (EGFR/Her2) kinase inhibitor used for treatment of breast cancer (Lackey, 2006). Lapatinib binds three other human protein kinases (Karaman et al., 2008), it inhibits drug efflux by ABCG1 and ABCB2 transporters (Chun et al., 2015; Kuang et al., 2010), and activates JNK/c-Jun signaling through unknown effectors (Dolloff et al., 2011). In the single cell eukaryote *Trypanosoma brucei* which lacks EGFR/Her2 , lapatinib forms complexes with three protein kinases (Behera et al., 2014; Katiyar et al., 2013), disrupts assembly of the paraflagellar rod, and inhibits endocytosis of transferrin (Guyett et al., 2017). Human cell resistance to lapatinib treatment is associated with PAK2 (Chang et al., 2018), NF-kappaβ signaling (Wetterskog et al., 2013), and ABC transporters (Dai et al., 2008; Kuang et al., 2010; Sodani et al., 2012). Recent acceptance of kinase-independent pro-survival functions of EGFR (Cossu-Rocca et al., 2016; Refaat et al., 2015; Tan et al., 2015; Thomas and Weihua, 2019) suggest a need to determine physiological targets of lapatinib in human and trypanosome cells in order to understand the drug's mechanisms of action.

Molecular Outcomes of Target Engagement by Drugs can Be Validated *In Vivo*

We will use two studies to illustrate feasibility of identifying physiological targets of drugs, although it was not the intention of the authors of selected publications to address issues raised in this publication.

Kuppeveld and colleagues studied Itraconazole (ITZ) inhibition of enterovirus EV71 replication in human HeLa R19 cells (Strating et al., 2015). The authors found, using microscale thermophoresis, that ITZ bound human oxysterol-binding protein (OSBP) and OSBP-related protein 4 (Wienken et al.,

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

2010). Genetic knockdown of OSBP enhanced susceptibility of virus replication to a reduced concentration of ITZ. In addition, OSW-1 an antagonist of OSBP (Burgett et al., 2011), exerted stronger inhibition of EV71 replication under these circumstances. Finally, overexpression of OSBP restored replication of EV71 in the presence of ITZ, confirming that inhibition of viral replication by ITZ depended on OSBP, as a physiological target of the drug.

Studying the eukaryotic microorganism *Trypanosoma brucei,* Guyett and colleagues showed that the drug TWS119 (Ding et al., 2003) killed trypanosomes, and prevented endocytosis of transferrin (Tf), as a mode of action. Employing a combination of genetic, chemical perturbation, and molecular cell biology tools they validated trypanosome glycogen synthase kinase-3β (TbGSK3β) as a physiological target of TWS119 (Guyett et al., 2016). Evidence presented was as follows. First, TWS119 inhibited Tf endocytosis in *T. brucei*. Second, TWS119 inhibited enzyme activity of purified TbGSK3β, demonstrating that TWS119 binds to the protein. Third, genetic knockdown of TbGSK3β reduced Tf endocytosis, whereas overexpression of TbGSK3β increased Tf uptake, firmly establishing a positive role of TbGSK3β in endocytosis of Tf. Finally, TWS119 blocked the increase in Tf endocytosis caused by overexpression of TbGSK3β, consistent with TWS119 inhibition of TbGSK3β activity *in vivo*.

The two studies summarized in this section illustrate how a judicious choice of molecular modes of action studies can be used to validate the outcome of drug engagement of a physiological target in a cell.

The Value of Knowing Physiological Targets of a Drug

There are multiple benefits of knowing physiological targets of drugs. First, the concept makes it easier to accept a possibility that "off-target" proteins may be biological effectors. By definition, drugs are foreign to cells, which contain over twenty-thousand proteins (Venter et al., 2001; Venter et al., 2015). One expects a drug to bind multiple proteins in a cell (Davis et al., 2011; Fabian et al., 2005; Katiyar et

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

al., 2013): None of those binding-proteins should be termed "off-target" even if that protein is not our preferred one.

Second, a focus on physiological targets enables investigators to make a direct link between protein binding of drug and molecular changes elicited by target engagement (see last section). Re-evaluation of targets for drugs optimization *in vitro* against purified proteins is appropriate, given these considerations. One cannot assume that the target for medicinal chemistry optimization *in vitro* is the sole binding-protein in a cell for the drug. Human cells contain tens of thousands of proteins (Venter et al., 2001; Venter et al., 2015), so an analog of a hit that emerges from optimization as the drug may have new cellular targets. Experiments to determine cellular specificity of new analogs will enable medicinal chemists to know whether optimized hits remain on-target during drug development (Lepovitz et al., 2020).

Third, overexpression of a protein may reduce a cell's susceptibility to a drug. However, knockdown of the protein could produce a different molecular change than that obtained by perturbing cells with the drug. It is important to obtain both sets of data before conclusions about physiological drug targets are made when a protein overexpression strategy is used for target identification.

For the pharmaceutical industry these ideas may be viewed from several perspectives. Ability to distinguish true physiological targets from drug-binding proteins could foresee issues that arise late in drug development or, more likely, in the post-marketing phase. For a new therapeutic agent alternative targets that produce unanticipated pharmacological effects could be identified, and that information could inform repurposing of the drug for other indications. Nevertheless, due to additional costs involved the multi-disciplinary studies described here are unlikely to be applied to every hit in a medicinal chemistry project. However, it will be feasible to perform these studies for clinical candidates, since physiological targets of drugs are likely to include "off-target" proteins whose inhibition might be the basis of drug action (Lin et al., 2019). Further, these studies are likely to reveal targets whose

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

inhibition may cause selective toxicity, and lead to cost saving from attrition of some candidate drugs (Choi et al., 2014; Flynn et al., 2017; von Kleist et al., 2016; Zuhl et al., 2016).

Authorship contributions:

Performed data analysis: Kojo Mensa-Wilmot.
Wrote or contributed to the writing of the manuscript: Kojo Mensa-Wilmot.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

LITERATURE CITED

Abdolmaleki A, Ghasemi JB and Ghasemi F (2017) Computer Aided Drug Design for Multi-Target Drug Design: SAR /QSAR, Molecular Docking and Pharmacophore Methods. *Curr Drug Targets* **18**(5): 556-575.

Alshareef A, Zhang HF, Huang YH, Wu C, Zhang JD, Wang P, El-Sehemy A, Fares M and Lai R (2016) The use of cellular thermal shift assay (CETSA) to study Crizotinib resistance in ALK-expressing human cancers. *Sci Rep* **6**: 33710.

Amarasinghe C and Jin JP (2015) The Use of Affinity Tags to Overcome Obstacles in Recombinant Protein Expression and Purification. *Protein Pept Lett* **22**(10): 885-892.

Anighoro A, Bajorath J and Rastelli G (2014) Polypharmacology: challenges and opportunities in drug discovery. *J Med Chem* **57**(19): 7874-7887.

Aouida M, Page N, Leduc A, Peter M and Ramotar D (2004) A genome-wide screen in Saccharomyces cerevisiae reveals altered transport as a mechanism of resistance to the anticancer drug bleomycin. *Cancer Res* **64**(3): 1102-1109.

Bachovchin KA, Sharma A, Bag S, Klug DM, Schneider KM, Singh B, Jalani HB, Buskes MJ, Mehta N, Tanghe S, Momper JD, Sciotti RJ, Rodriguez A, Mensa-Wilmot K, Pollastri MP and Ferrins L (2019) Improvement of Aqueous Solubility of Lapatinib-Derived Analogues: Identification of a Quinolinimine Lead for Human African Trypanosomiasis Drug Development. *Journal of Medicinal Chemistry* **62**(2): 665-687.

Barrett MP, Vincent IM, Burchmore RJ, Kazibwe AJ and Matovu E (2011) Drug resistance in human African trypanosomiasis. *Future microbiology* **6**(9): 1037-1047.

Behera R, Thomas SM and Mensa-Wilmot K (2014) New Chemical Scaffolds for Human African Trypanosomiasis Lead Discovery from a Screen of Tyrosine Kinase Inhibitor Drugs. *Antimicrob Agents Chemother*.

Berezhkovskiy LM (2008) Some features of the kinetics and equilibrium of drug binding to plasma proteins. *Expert opinion on drug metabolism & toxicology* **4**(12): 1479-1498.

Bunnage ME, Gilbert AM, Jones LH and Hett EC (2015) Know your target, know your molecule. *Nat Chem Biol* **11**(6): 368-372.

Burgett AW, Poulsen TB, Wangkanont K, Anderson DR, Kikuchi C, Shimada K, Okubo S, Fortner KC, Mimaki Y, Kuroda M, Murphy JP, Schwalb DJ, Petrella EC, Cornella-Taracido I, Schirle M, Tallarico JA and Shair MD (2011) Natural products reveal cancer cell dependence on oxysterol-binding proteins. *Nat Chem Biol* **7**(9): 639-647.

Campos M, Fadden P, Alms G, Qian Z and Haystead TA (1996) Identification of protein phosphatase-1-binding proteins by microcystin-biotin affinity chromatography. *J Biol Chem* **271**(45): 28478-28484.

Chang Y, Park KH, Lee JE and Han KC (2018) Phosphoproteomic analysis reveals PAK2 as a therapeutic target for lapatinib resistance in HER2-positive breast cancer cells. *Biochem Biophys Res Commun* **505**(1): 187-193.

Chang Z, Yadav V, Lee SC and Heitman J (2019) Epigenetic mechanisms of drug resistance in fungi. *Fungal Genet Biol* **132**: 103253.

Chatelain E and Ioset JR (2018) Phenotypic screening approaches for Chagas disease drug discovery. *Expert opinion on drug discovery* **13**(2): 141-153.

Chen L, Shu Y, Liang X, Chen EC, Yee SW, Zur AA, Li S, Xu L, Keshari KR, Lin MJ, Chien HC, Zhang Y, Morrissey KM, Liu J, Ostrem J, Younger NS, Kurhanewicz J, Shokat KM, Ashrafi K and Giacomini KM (2014) OCT1 is a high-capacity thiamine transporter that regulates hepatic steatosis and is a target of metformin. *Proc Natl Acad Sci U S A* **111**(27): 9983-9988.

Chintakrindi A, D'Souza C and Kanyalkar M (2012) Rational development of neuraminidase inhibitor as novel anti-flu drug. *Mini Rev Med Chem* **12**(12): 1273-1281.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Chitanga S, Marcotty T, Namangala B, Van den Bossche P, Van Den Abbeele J and Delespaux V (2011) High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. *PLoS Negl Trop Dis* **5**(12): e1454.

Cho SY, Ohnuma T, Silverman LR, Holland JF and Roboz J (2010) Discontinuous drug binding to proteins: binding of an antineoplastic benzyl styryl sulfone to albumin and enzymes in vitro and in phase I clinical trials. *Drug metabolism and disposition: the biological fate of chemicals* **38**(9): 1480-1485.

Choi YJ, Seo JH and Shin KJ (2014) Successful reduction of off-target hERG toxicity by structural modification of a T-type calcium channel blocker. *Bioorg Med Chem Lett* **24**(3): 880-883.

Chun SY, Kwon YS, Nam KS and Kim S (2015) Lapatinib enhances the cytotoxic effects of doxorubicin in MCF-7 tumorspheres by inhibiting the drug efflux function of ABC transporters. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **72**: 37-43.

Claus J, Patel G, Ng T and Parker PJ (2014) A role for the pseudokinase HER3 in the acquired resistance against EGFR- and HER2-directed targeted therapy. *Biochem Soc Trans* **42**(4): 831-836.

Cossu-Rocca P, Muroni MR, Sanges F, Sotgiu G, Asunis A, Tanca L, Onnis D, Pira G, Manca A, Dore S, Uras MG, Ena S and De Miglio MR (2016) EGFR kinase-dependent and kinase-independent roles in clear cell renal cell carcinoma. *Am J Cancer Res* **6**(1): 71-83.

Cozza G (2017) The Development of CK2 Inhibitors: From Traditional Pharmacology to in Silico Rational Drug Design. *Pharmaceuticals (Basel)* **10**(1).

Cravatt BF, Wright AT and Kozarich JW (2008) Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. *Annu Rev Biochem* **77**: 383-414.

Dai CL, Tiwari AK, Wu CP, Su XD, Wang SR, Liu DG, Ashby CR, Jr., Huang Y, Robey RW, Liang YJ, Chen LM, Shi CJ, Ambudkar SV, Chen ZS and Fu LW (2008) Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. *Cancer Res* **68**(19): 7905-7914.

Das J (2019) Identification of alcohol-binding site(s) in proteins using diazirine-based photoaffinity labeling and mass spectrometry. *Chemical biology & drug design* **93**(6): 1158-1165.

Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, Hocker M, Treiber DK and Zarrinkar PP (2011) Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol* **29**(11): 1046-1051.

Dermawan JK, Gurova K, Pink J, Dowlati A, De S, Narla G, Sharma N and Stark GR (2014) Quinacrine Overcomes Resistance to Erlotinib by Inhibiting FACT, NF-kappaB, and Cell-Cycle Progression in Non-Small Cell Lung Cancer. *Mol Cancer Ther* **13**(9): 2203-2214.

Dicker AP, Waltham MC, Volkenandt M, Schweitzer BI, Otter GM, Schmid FA, Sirotnak FM and Bertino JR (1993) Methotrexate resistance in an in vivo mouse tumor due to a non-active-site dihydrofolate reductase mutation. *Proc Natl Acad Sci U S A* **90**(24): 11797-11801.

Ding S, Wu TY, Brinker A, Peters EC, Hur W, Gray NS and Schultz PG (2003) Synthetic small molecules that control stem cell fate. *Proc Natl Acad Sci U S A* **100**(13): 7632-7637.

Dolloff NG, Mayes PA, Hart LS, Dicker DT, Humphreys R and El-Deiry WS (2011) Off-target lapatinib activity sensitizes colon cancer cells through TRAIL death receptor up-regulation. *Science translational medicine* **3**(86): 86ra50.

Douzi B (2017) Protein-Protein Interactions: Surface Plasmon Resonance. *Methods Mol Biol* **1615**: 257-275.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Dowling RJ, Zakikhani M, Fantus IG, Pollak M and Sonenberg N (2007) Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* **67**(22): 10804-10812.

Dunne G, Breen L, Collins DM, Roche S, Clynes M and O'Connor R (2011) Modulation of P-gp expression by lapatinib. *Investigational new drugs* **29**(6): 1284-1293.

Dziekan JM, Yu H, Chen D, Dai L, Wirjanata G, Larsson A, Prabhu N, Sobota RM, Bozdech Z and Nordlund P (2019) Identifying purine nucleoside phosphorylase as the target of quinine using cellular thermal shift assay. *Science translational medicine* **11**(473).

Fabian MA, Biggs WH, 3rd, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias JM, Mehta SA, Milanov ZV, Velasco AM, Wodicka LM, Patel HK, Zarrinkar PP and Lockhart DJ (2005) A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* **23**(3): 329-336.

Fischer JJ, Graebner Baessler OY, Dalhoff C, Michaelis S, Schrey AK, Ungewiss J, Andrich K, Jeske D, Kroll F, Glinski M, Sefkow M, Dreger M and Koester H (2010) Comprehensive identification of staurosporine-binding kinases in the hepatocyte cell line HepG2 using Capture Compound Mass Spectrometry (CCMS). *J Proteome Res* **9**(2): 806-817.

Fischer JJ, Graebner Nee Baessler OY and Dreger M (2012) Proteome-wide identification of staurosporine-binding kinases using capture compound mass spectrometry. *Methods Mol Biol* **795**: 135-147.

Flynn M, Heale KA and Alisaraie L (2017) Mechanism of Off-Target Interactions and Toxicity of Tamoxifen and Its Metabolites. *Chemical research in toxicology* **30**(7): 1492-1507.

Gatti L, Cassinelli G, Zaffaroni N, Lanzi C and Perego P (2015) New mechanisms for old drugs: Insights into DNA-unrelated effects of platinum compounds and drug resistance determinants. *Drug Resist Updat* **20**: 1-11.

Golkowski M, Maly DJ and Ong SE (2017) Proteomic Profiling of Protein Kinase Inhibitor Targets by Mass Spectrometry. *Methods Mol Biol* **1636**: 105-117.

Guyett PJ, Behera R, Ogata Y, Pollastri M and Mensa-Wilmot K (2017) Novel Effects of Lapatinib Revealed in the African Trypanosome by Using Hypothesis-Generating Proteomics and Chemical Biology Strategies. *Antimicrob Agents Chemother* **61**(2).

Guyett PJ, Xia S, Swinney DC, Pollastri MP and Mensa-Wilmot K (2016) Glycogen Synthase Kinase 3beta Promotes the Endocytosis of Transferrin in the African Trypanosome. *ACS Infect Dis* **2**(7): 518-528.

Haanstra JR and Bakker BM (2015) Drug target identification through systems biology. *Drug discovery today Technologies* **15**: 17-22.

Hafner M, Mills CE, Subramanian K, Chen C, Chung M, Boswell SA, Everley RA, Liu C, Walmsley CS, Juric D and Sorger PK (2019) Multiomics Profiling Establishes the Polypharmacology of FDA-Approved CDK4/6 Inhibitors and the Potential for Differential Clinical Activity. *Cell Chem Biol* **26**(8): 1067-1080 e1068.

Haider T, Pandey V, Banjare N, Gupta PN and Soni V (2020) Drug resistance in cancer: mechanisms and tackling strategies. *Pharmacol Rep* **72**(5): 1125-1151.

Hamouda AK, Jayakar SS, Chiara DC and Cohen JB (2014) Photoaffinity labeling of nicotinic receptors: diversity of drug binding sites! *J Mol Neurosci* **53**(3): 480-486.

Hellinen L, Bahrpeyma S, Rimpela AK, Hagstrom M, Reinisalo M and Urtti A (2020) Microscale Thermophoresis as a Screening Tool to Predict Melanin Binding of Drugs. *Pharmaceutics* **12**(6).

Hill JR and Robertson AAB (2018) Fishing for Drug Targets: A Focus on Diazirine Photoaffinity Probe Synthesis. *J Med Chem* **61**(16): 6945-6963.

Jacobs RT, Nare B, Wring SA, Orr MD, Chen D, Sligar JM, Jenks MX, Noe RA, Bowling TS, Mercer LT, Rewerts C, Gaukel E, Owens J, Parham R, Randolph R, Beaudet B, Bacchi CJ, Yarlett N, Plattner JJ, Freund Y, Ding C, Akama T, Zhang YK, Brun R, Kaiser M,

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Scandale I and Don R (2011) SCYX-7158, an Orally-Active Benzoxaborole for the Treatment of Stage 2 Human African Trypanosomiasis. *PLoS Negl Trop Dis* **5**(6): e1151.

Jones DC, Foth BJ, Urbaniak MD, Patterson S, Ong HB, Berriman M and Fairlamb AH (2015) Genomic and Proteomic Studies on the Mode of Action of Oxaboroles against the African Trypanosome. *PLoS Negl Trop Dis* **9**(12): e0004299.

Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhart DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM and Zarrinkar PP (2008) A quantitative analysis of kinase inhibitor selectivity. *Nat Biotech* **26**(1): 127-132.

Katiyar S, Kufareva I, Behera R, Thomas SM, Ogata Y, Pollastri M, Abagyan R and Mensa-Wilmot K (2013) Lapatinib-binding protein kinases in the african trypanosome: identification of cellular targets for kinase-directed chemical scaffolds. *PLoS One* **8**(2): e56150.

Kaur J, Bhardwaj A, Huang Z and Knaus EE (2012) Aspirin analogues as dual cyclooxygenase-2/5-lipoxygenase inhibitors: synthesis, nitric oxide release, molecular modeling, and biological evaluation as anti-inflammatory agents. *ChemMedChem* **7**(1): 144-150.

Kawamura S, Unno Y, Asai A, Arisawa M and Shuto S (2014) Development of a new class of proteasome inhibitors with an epoxyketone warhead: Rational hybridization of non-peptidic belactosin derivatives and peptide epoxyketones. *Bioorg Med Chem* **22**(12): 3091-3095.

Khavrutskii L, Yeh J, Timofeeva O, Tarasov SG, Pritt S, Stefanisko K and Tarasova N (2013) Protein purification-free method of binding affinity determination by microscale thermophoresis. *Journal of visualized experiments : JoVE*(78).

Kuang YH, Shen T, Chen X, Sodani K, Hopper-Borge E, Tiwari AK, Lee JW, Fu LW and Chen ZS (2010) Lapatinib and erlotinib are potent reversal agents for MRP7 (ABCC10)-mediated multidrug resistance. *Biochem Pharmacol* **79**(2): 154-161.

Labar G, Bauvois C, Muccioli GG, Wouters J and Lambert DM (2007) Disulfiram is an inhibitor of human purified monoacylglycerol lipase, the enzyme regulating 2-arachidonoylglycerol signaling. *Chembiochem* **8**(11): 1293-1297.

Lackey KE (2006) Lessons from the drug discovery of lapatinib, a dual ErbB1/2 tyrosine kinase inhibitor. *Curr Top Med Chem* **6**(5): 435-460.

Lepovitz LT, Meis AR, Thomas SM, Wiedeman J, Pham A, Mensa-Wilmot K and Martin SF (2020) Design, Synthesis, and Evaluation of Novel Anti-Trypanosomal Compounds. *Tetrahedron* **76**(16).

Lin A, Giuliano CJ, Palladino A, John KM, Abramowicz C, Yuan ML, Sausville EL, Lukow DA, Liu L, Chait AR, Galluzzo ZC, Tucker C and Sheltzer JM (2019) Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. *Science translational medicine* **11**(509).

Low J, Chakravartty A, Blosser W, Dowless M, Chalfant C, Bragger P and Stancato L (2009) Phenotypic fingerprinting of small molecule cell cycle kinase inhibitors for drug discovery. *Current chemical genomics* **3**: 13-21.

Lucet IS and Murphy JM (2017) Characterization of Ligand Binding to Pseudokinases Using a Thermal Shift Assay. *Methods Mol Biol* **1636**: 91-104.

Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ, Camporez JP, Lee HY, Cline GW, Samuel VT, Kibbey RG and Shulman GI (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510**(7506): 542-546.

Manchado E, Huang CH, Tasdemir N, Tschaharganeh DF, Wilkinson JE and Lowe SW (2016) A Pipeline for Drug Target Identification and Validation. *Cold Spring Harb Symp Quant Biol* **81**: 257-267.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Martinez Molina D, Jafari R, Ignatushchenko M, Seki T, Larsson EA, Dan C, Sreekumar L, Cao Y and Nordlund P (2013) Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science* **341**(6141): 84-87.

Mensa-Wilmot K, Morris JC, al-Qahtani A and Englund PT (1995) Purification and use of recombinant glycosylphosphatidylinositol-phospholipase C. *Methods Enzymol* **250**: 641-655.

Mercer L, Bowling T, Perales J, Freeman J, Nguyen T, Bacchi C, Yarlett N, Don R, Jacobs R and Nare B (2011) 2,4-Diaminopyrimidines as potent inhibitors of Trypanosoma brucei and identification of molecular targets by a chemical proteomics approach. *PLoS Negl Trop Dis* **5**(2): e956.

Mesu V, Kalonji WM, Bardonneau C, Mordt OV, Blesson S, Simon F, Delhomme S, Bernhard S, Kuziena W, Lubaki JF, Vuvu SL, Ngima PN, Mbembo HM, Ilunga M, Bonama AK, Heradi JA, Solomo JLL, Mandula G, Badibabi LK, Dama FR, Lukula PK, Tete DN, Lumbala C, Scherrer B, Strub-Wourgaft N and Tarral A (2018) Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. *Lancet* **391**(10116): 144-154.

Milligan PA, Brown MJ, Marchant B, Martin SW, van der Graaf PH, Benson N, Nucci G, Nichols DJ, Boyd RA, Mandema JW, Krishnaswami S, Zwillich S, Gruben D, Anziano RJ, Stock TC and Lalonde RL (2013) Model-based drug development: a rational approach to efficiently accelerate drug development. *Clinical pharmacology and therapeutics* **93**(6): 502-514.

Mishra V (2020) Affinity Tags for Protein Purification. *Curr Protein Pept Sci* **21**(8): 821-830.

Mugge FLB and Silva AM (2017) Aspirin metabolite sodium salicylate selectively inhibits transcriptional activity of ATF6alpha and downstream target genes. *Sci Rep* **7**(1): 9190.

Muller J and Hemphill A (2016) Drug target identification in protozoan parasites. *Expert opinion on drug discovery* **11**(8): 815-824.

Muskens FM, Ward RJ, Herkt D, van de Langemheen H, Tobin AB, Liskamp RMJ and Milligan G (2019) Design, Synthesis, and Evaluation of a Diazirine Photoaffinity Probe for Ligand-Based Receptor Capture Targeting G Protein-Coupled Receptors. *Mol Pharmacol* **95**(2): 196-209.

Nathan G. Dollof PAM, Lori S. Hart, David T. Dicker, Robin Humphreys, and Wafik S. El-Deiry (2011) Off-target lapatinib activity sensitizes colon cancer cells through TRAIL death receptor up-regulation. *Science translational medicine* **3**(86).

Nikolovska-Coleska Z (2015) Studying protein-protein interactions using surface plasmon resonance. *Methods Mol Biol* **1278**: 109-138.

Parker CG, Kuttruff CA, Galmozzi A, Jorgensen L, Yeh CH, Hermanson DJ, Wang Y, Artola M, McKerrall SJ, Josyln CM, Norremark B, Dunstl G, Felding J, Saez E, Baran PS and Cravatt BF (2017) Chemical Proteomics Identifies SLC25A20 as a Functional Target of the Ingenol Class of Actinic Keratosis Drugs. *ACS Cent Sci* **3**(12): 1276-1285.

Phizackerley KM, Jumaa M, Lopalco A, Wolfe BH, Ablan CD and Stella VJ (2017) Mechanism of Degradation of an alpha-Keto-Epoxide, a Model for the Warhead for Various Proteasome Inhibitor Anticancer Agents. *Journal of pharmaceutical sciences* **106**(4): 1051-1061.

Proschak E, Stark H and Merk D (2019) Polypharmacology by Design: A Medicinal Chemist's Perspective on Multitargeting Compounds. *J Med Chem* **62**(2): 420-444.

Refaat A, Aminullah, Zhou Y, Kawanishi M, Tomaru R, Abdelhamed S, Shin MS, Koizumi K, Yokoyama S, Saiki I and Sakurai H (2015) Role of tyrosine kinase-independent phosphorylation of EGFR with activating mutation in cisplatin-treated lung cancer cells. *Biochem Biophys Res Commun* **458**(4): 856-861.

Rylova G, Ozdian T, Varanasi L, Soural M, Hlavac J, Holub D, Dzubak P and Hajduch M (2015) Affinity-based methods in drug-target discovery. *Curr Drug Targets* **16**(1): 60-76.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Schenone M, Dancik V, Wagner BK and Clemons PA (2013) Target identification and mechanism of action in chemical biology and drug discovery. *Nat Chem Biol* **9**(4): 232-240.

Seyhan AA, Varadarajan U, Choe S, Liu W and Ryan TE (2012) A genome-wide RNAi screen identifies novel targets of neratinib resistance leading to identification of potential drug resistant genetic markers. *Mol Biosyst* **8**(5): 1553-1570.

Sharma KK, Singh B, Mujwar S and Bisen PS (2020) Molecular Docking Based Analysis to Elucidate the DNA Topoisomerase IIbeta as the Potential Target for the Ganoderic Acid; A Natural Therapeutic Agent in Cancer Therapy. *Curr Comput Aided Drug Des* **16**(2): 176-189.

Shi H, Zhang CJ, Chen GY and Yao SQ (2012) Cell-based proteome profiling of potential dasatinib targets by use of affinity-based probes. *J Am Chem Soc* **134**(6): 3001-3014.

Simcic M, Pureber K, Kristan K, Urleb U, Kocjan D and Grdadolnik SG (2014) A novel 2-oxoindolinylidene inhibitor of bacterial MurD ligase: Enzyme kinetics, protein-inhibitor binding by NMR and a molecular dynamics study. *Eur J Med Chem* **83**: 92-101.

Sodani K, Tiwari AK, Singh S, Patel A, Xiao ZJ, Chen JJ, Sun YL, Talele TT and Chen ZS (2012) GW583340 and GW2974, human EGFR and HER-2 inhibitors, reverse ABCG2- and ABCB1-mediated drug resistance. *Biochem Pharmacol* **83**(12): 1613-1622.

Strating JR, van der Linden L, Albulescu L, Bigay J, Arita M, Delang L, Leyssen P, van der Schaar HM, Lanke KH, Thibaut HJ, Ulferts R, Drin G, Schlinck N, Wubbolts RW, Sever N, Head SA, Liu JO, Beachy PA, De Matteis MA, Shair MD, Olkkonen VM, Neyts J and van Kuppeveld FJ (2015) Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein. *Cell reports* **10**(4): 600-615.

Svennebring AM (2016) The impact of the concentration of drug binding plasma proteins on drug distribution according to Oie-Tozer's model. *Xenobiotica; the fate of foreign compounds in biological systems* **46**(4): 307-314.

Swinney DC (2013) The contribution of mechanistic understanding to phenotypic screening for first-in-class medicines. *J Biomol Screen* **18**(10): 1186-1192.

Sykes ML and Avery VM (2013) Approaches to Protozoan Drug Discovery: Phenotypic Screening. *J Med Chem*.

Tan X, Thapa N, Sun Y and Anderson RA (2015) A kinase-independent role for EGF receptor in autophagy initiation. *Cell* **160**(1-2): 145-160.

Thomas JR, Brittain SM, Lipps J, Llamas L, Jain RK and Schirle M (2017) A Photoaffinity Labeling-Based Chemoproteomics Strategy for Unbiased Target Deconvolution of Small Molecule Drug Candidates. *Methods Mol Biol* **1647**: 1-18.

Thomas R and Weihua Z (2019) Rethink of EGFR in Cancer With Its Kinase Independent Function on Board. *Front Oncol* **9**: 800.

Tulloch LB, Menzies SK, Fraser AL, Gould ER, King EF, Zacharova MK, Florence GJ and Smith TK (2017) Photo-affinity labelling and biochemical analyses identify the target of trypanocidal simplified natural product analogues. *PLoS Negl Trop Dis* **11**(9): e0005886.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B,

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A and Zhu X (2001) The sequence of the human genome. *Science* **291**(5507): 1304-1351.

Venter JC, Smith HO and Adams MD (2015) The Sequence of the Human Genome. *Clin Chem* **61**(9): 1207-1208.

von Kleist L, Michaelis S, Bartho K, Graebner O, Schlief M, Dreger M, Schrey AK, Sefkow M, Kroll F, Koester H and Luo Y (2016) Identification of Potential Off-target Toxicity Liabilities of Catechol-O-methyltransferase Inhibitors by Differential Competition Capture Compound Mass Spectrometry. *J Med Chem* **59**(10): 4664-4675.

Wang ECE, Dai Z and Christiano AM (2018) Novel therapies for alopecia areata: The era of rational drug development. *J Allergy Clin Immunol* **141**(2): 499-504.

Wang J, Zhang CJ, Zhang J, He Y, Lee YM, Chen S, Lim TK, Ng S, Shen HM and Lin Q (2015) Mapping sites of aspirin-induced acetylations in live cells by quantitative acid-cleavable activity-based protein profiling (QA-ABPP). *Sci Rep* **5**: 7896.

Wang K, Yang T, Wu Q, Zhao X, Nice EC and Huang C (2012) Chemistry-based functional proteomics for drug target deconvolution. *Expert review of proteomics* **9**(3): 293-310.

Wang RE, Hunt CR, Chen J and Taylor JS (2011) Biotinylated quercetin as an intrinsic photoaffinity proteomics probe for the identification of quercetin target proteins. *Bioorg Med Chem* **19**(16): 4710-4720.

Wang Y, Yao B, Wang Y, Zhang M, Fu S, Gao H, Peng R, Zhang L and Tang J (2014) Increased FoxM1 expression is a target for metformin in the suppression of EMT in prostate cancer. *International journal of molecular medicine* **33**(6): 1514-1522.

Warrilow AG, Parker JE, Price CL, Nes WD, Garvey EP, Hoekstra WJ, Schotzinger RJ, Kelly DE and Kelly SL (2016) The Investigational Drug VT-1129 Is a Highly Potent Inhibitor of Cryptococcus Species CYP51 but Only Weakly Inhibits the Human Enzyme. *Antimicrob Agents Chemother* **60**(8): 4530-4538.

Wetterskog D, Shiu KK, Chong I, Meijer T, Mackay A, Lambros M, Cunningham D, Reis-Filho JS, Lord CJ and Ashworth A (2013) Identification of novel determinants of resistance to lapatinib in ERBB2-amplified cancers. *Oncogene*.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.

Molecular Pharmacology Fast Forward. Published on May 3, 2021 as DOI: 10.1124/molpharm.120.000186
This article has not been copyedited and formatted. The final version may differ from this version.

Wienken CJ, Baaske P, Rothbauer U, Braun D and Duhr S (2010) Protein-binding assays in biological liquids using microscale thermophoresis. *Nat Commun* **1**: 100.

Wilson K, Beverley SM and Ullman B (1992) Stable amplification of a linear extrachromosomal DNA in mycophenolic acid-resistant Leishmania donovani. *MolBiochemParasitol* **55**: 197-206.

Xiao X and Li BX (2020) Identification of lamins as the molecular targets of LBL1 using a clickable photoaffinity probe. *Methods Enzymol* **633**: 185-201.

Yang GF and Huang X (2006) Development of quantitative structure-activity relationships and its application in rational drug design. *Curr Pharm Des* **12**(35): 4601-4611.

Yin MJ, Yamamoto Y and Gaynor RB (1998) The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* **396**(6706): 77-80.

Yobi DM, Kayiba NK, Mvumbi DM, Boreux R, Bontems S, Kabututu PZ, De Mol P, Speybroeck N, Mvumbi GL and Hayette MP (2020) The lack of K13-propeller mutations associated with artemisinin resistance in Plasmodium falciparum in Democratic Republic of Congo (DRC). *PLoS One* **15**(8): e0237791.

Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, Meyerson M and Eck MJ (2008) The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* **105**(6): 2070-2075.

Zhang H, Pan J, Wu X, Zuo AR, Wei Y and Ji ZL (2019a) Large-Scale Target Identification of Herbal Medicine Using a Reverse Docking Approach. *ACS Omega* **4**(6): 9710-9719.

Zhang J, Gu Y and Chen B (2019b) Mechanisms of drug resistance in acute myeloid leukemia. *Onco Targets Ther* **12**: 1937-1945.

Zhu Y, Xiao T, Lei S, Zhou F and Wang MW (2015) Application of chemical biology in target identification and drug discovery. *Arch Pharm Res* **38**(9): 1642-1650.

Zuhl AM, Nolan CE, Brodney MA, Niessen S, Atchison K, Houle C, Karanian DA, Ambroise C, Brulet JW, Beck EM, Doran SD, O'Neill BT, Am Ende CW, Chang C, Geoghegan KF, West GM, Judkins JC, Hou X, Riddell DR and Johnson DS (2016) Chemoproteomic profiling reveals that cathepsin D off-target activity drives ocular toxicity of beta-secretase inhibitors. *Nat Commun* **7**: 13042.

This work was supported by the National Institute of Allergy and Infectious Diseases [R01AI126311]. Authors declare no conflict of interest.