Expert Opinion on Drug Discovery

ISSN: 1746-0441 (Print) 1746-045X (Online) Journal homepage: http://www.tandfonline.com/loi/iedc20

Combating mutations in genetic disease and drug resistance: understanding molecular mechanisms to guide drug design

Amanda T.S. Albanaz, Carlos H.M. Rodrigues, Douglas E.V. Pires & David B. Ascher

To cite this article: Amanda T.S. Albanaz, Carlos H.M. Rodrigues, Douglas E.V. Pires & David B. Ascher (2017) Combating mutations in genetic disease and drug resistance: understanding molecular mechanisms to guide drug design, Expert Opinion on Drug Discovery, 12:6, 553-563

To link to this article: http://dx.doi.org/10.1080/17460441.2017.1322579

Published online: 11 May 2017.

EXPERT OPINION ON DRUG DISCOVERY, 2017
VOL. 12, NO. 6, 553–563
https://doi.org/10.1080/17460441.2017.1322579

Taylor & Francis
Taylor & Francis Group

REVIEW

Combating mutations in genetic disease and drug resistance: understanding molecular mechanisms to guide drug design

Amanda T.S. Albanaz[a,b*], Carlos H.M. Rodrigues[a,b*], Douglas E.V. Pires[a*] and David B. Ascher [a,c,d]

[a]Centro de Pesquisas René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil; [b]Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [c]Department of Biochemistry, University of Cambridge, Cambridge, Cambridgeshire, UK; [d]Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Victoria, Australia

ABSTRACT

Introduction: Mutations introduce diversity into genomes, leading to selective changes and driving evolution. These changes have contributed to the emergence of many of the current major health concerns of the 21st century, from the development of genetic diseases and cancers to the rise and spread of drug resistance. The experimental systematic testing of all mutations in a system of interest is impractical and not cost-effective, which has created interest in the development of computational tools to understand the molecular consequences of mutations to aid and guide rational experimentation.

Areas covered: Here, the authors discuss the recent development of computational methods to understand the effects of coding mutations to protein function and interactions, particularly in the context of the 3D structure of the protein.

Expert opinion: While significant progress has been made in terms of innovative tools to understand and quantify the different range of effects in which a mutation or a set of mutations can give rise to a phenotype, a great gap still exists when integrating these predictions and drawing causality conclusions linking variants. This often requires a detailed understanding of the system being perturbed. However, as part of the drug development process it can be used preemptively in a similar fashion to pharmacokinetics predictions, to guide development of therapeutics to help guide the design and analysis of clinical trials, patient treatment and public health policy strategies.

ARTICLE HISTORY
Received 27 January 2017
Accepted 20 April 2017

KEYWORDS
Mutational analysis; genetic diseases; drug resistance; cancer; drug design; molecular mechanism; genotype-phenotype association

1. Introduction

Changes at the genetic level can result in drastic changes in cellular phenotypes and behavior. These changes can lead to disease, or provide selective advantages that promote the development of drug resistance. In particular, non-synonymous single-nucleotide polymorphisms (nsSNPs) within the protein coding regions of the genome have been strongly associated with occurrence and predisposition of human disease and drug resistance, sparking great interest from the research community.

The rapid developments in high-throughput sequencing, including dramatic drops in the cost, have created vast opportunities to understand the link between our genomes and phenotypes. This has opened up the promises of personalized medicines, targeted therapies, and targeted public health policies. In order to fully realize the potential of these developments, however, we still need to improve our understanding of what are the molecular consequences of a given mutation, and how do these lead to a given phenotype.

While considerable resources have been invested in the experimental evaluation of genomic mutations, characterizing mutation effects is a challenging task and impractical to systematically experimentally evaluate all possible mutations for a given protein of interest, even more considering the range of different mechanisms in which mutations can affect protein function and interactions. Traditional experimental approaches are also not efficient enough or do not achieve scalability required to provide real time guidance into patient treatment and public health policy. This has led to significant interest in the development of computational approaches to rapidly and accurately evaluate the effects of mutations. Figure 1 summarizes how *in silico* mutation analysis can be helpful in deconvoluting genotype-phenotype associations obtained from the wealth of genomic variation generated from sequencing efforts, including shedding light into disease predisposition and its mechanisms in a molecular level. Such methods can also be used to mutation prioritization for further experimental investigation, identification, and anticipation of resistant variants and resistance hotspots, knowledge that can be applied in the design of drugs less prone to resistance as well as to drive the development of public health policies and aid in establishing more appropriate and personalized treatments.

2. Analyzing the effects of mutations

The two most commonly used methods by clinical geneticists to look at the effects of coding nsSNP mutations in the human genome are SIFT [1] and Polyphen [2]. Other approaches

CONTACT David B. Ascher david.ascher@unimelb.edu.au
*These authors contributed equally for this work.

Article highlights

- Scalable and reliable structural based computational approaches are providing detailed insight into the molecular consequences of coding mutations.
- These have been used to guide patient treatment strategies for renal cell carcinoma and genetic diseases.
- Using these methods, drug resistance mutations can be identified and predicted.
- Used in a preemptive fashion, these can help guide drug development in the search for new therapeutics less likely to develop resistance.
- Mutations can give rise to a phenotype through different molecular mechanisms which can be assessed via integration of computational methods.

This box summarizes key points contained in the article.

include CADD [3] and MutationTaster [4]. These approaches use the protein sequence to evaluate whether a given mutation is likely to be pathogenic or not. However, they have been limited by the lack of mechanistic information they provide and their overestimation of mutations likely to be pathogenic [5]. Structural approaches can complement these analyses by providing detailed mechanistic information, but historically have involved a trade-off between scalability and molecular level mechanistic information, with molecular dynamics approaches providing greater atomic detail, but proving impractical for comprehensive analysis of a large number of different mutations.

In the 1990s, efforts to utilize the expanding structural information available for many proteins led to the development of SDM [6], the first method for predicting the effects

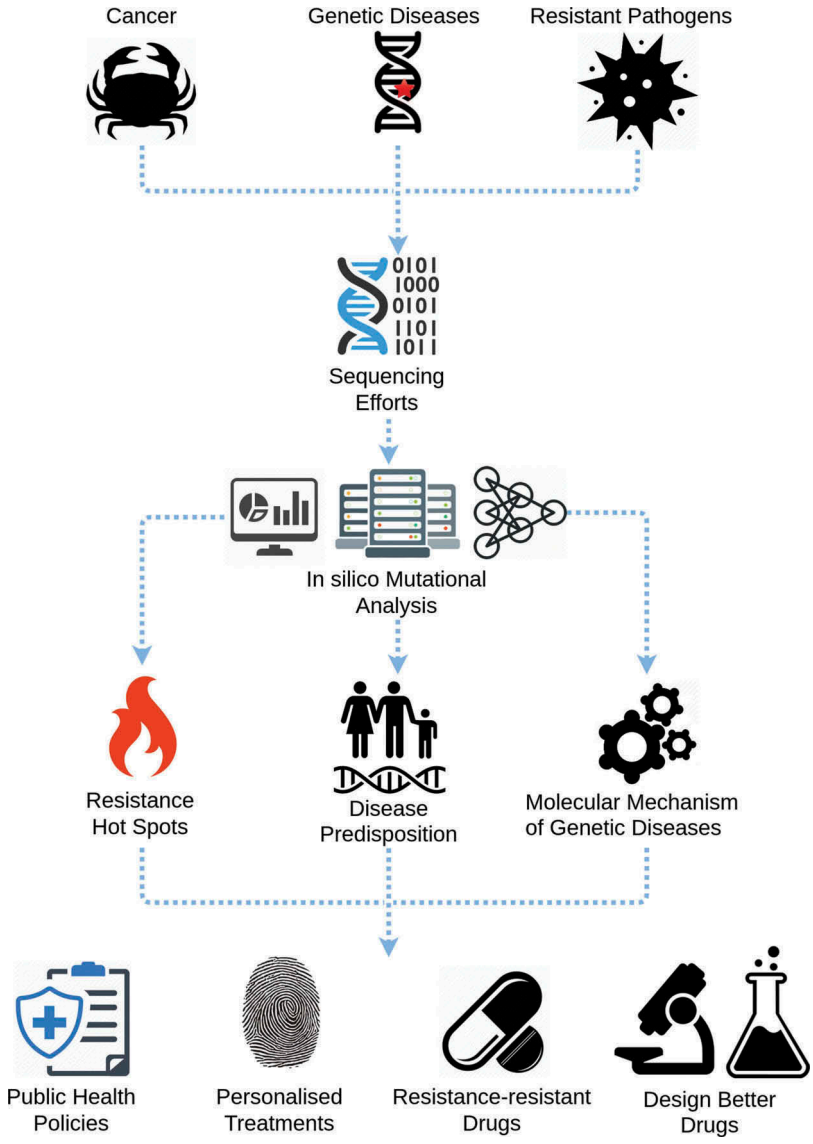

Figure 1. The use of in silico mutational analysis to tackle drug resistance and genetic diseases. Sequencing efforts generate a wealth of genomic variation. Computational mutation analysis can help deconvolute genotype-phenotype associations aiding in understanding the molecular mechanism of diseases and disease predisposition as well as in mutation prioritization for experimental validation, identification of resistant variants and resistance hot-spots, which can then fed into drug design pipelines as well drive the development of public health policies and choice of more appropriate and personalized treatments.

Table 1. Recent structure-based computational methods for analyzing the effects of coding mutations.

Method	Web server[a]	Publication year	Reference[b]
Effects of Mutations on Protein Stability and Folding			
SDM	http://www-cryst.bioc.cam.ac.uk/~sdm/sdm.php	2011	[23,24]
	http://structure.bioc.cam.ac.uk/sdm2	2017	
PoPMuSiC 2.1	http://babylone.ulb.ac.be/popmusic	2011	[25]
mCSM-Stability	http://structure.bioc.cam.ac.uk/mcsm/stability	2014	[13]
DUET	http://structure.bioc.cam.ac.uk/duet	2014	[12]
ENCoM	http://bcb.med.usherbrooke.ca/encom.php	2015	[26]
MAESTROweb	https://biwww.che.sbg.ac.at/maestro/web	2016	[27]
STRUM	http://zhanglab.ccmb.med.umich.edu/STRUM/	2016	[28]
ELASPIC	http://elaspic.kimlab.org	2016	[29]
Effects of Mutations on Protein-Protein Binding Affinity			
BeAtMuSiC	http://babylone.ulb.ac.be/beatmusic/	2013	[30]
mCSM-PPI	http://structure.bioc.cam.ac.uk/mcsm/protein_protein	2014	[13]
mCSM-AB	http://structure.bioc.cam.ac.uk/mcsm_ab	2016	[9]
MutaBind	https://www.ncbi.nlm.nih.gov/projects/mutabind	2016	[31]
Effects of Mutations on Protein-Nucleic Acid Interactions			
mCSM-NA	http://structure.bioc.cam.ac.uk/mcsm/protein_dna	2014	[13,11]
	http://structure.bioc.cam.ac.uk/mcsm_na	2017	
Effect of Mutations on Protein-Small Molecule Interactions			
mCSM-Lig	http://structure.bioc.cam.ac.uk/mcsm_lig	2016	[14]
CSM-Lig	http://structure.bioc.cam.ac.uk/csm_lig	2016	[10]

[a] The URLs link to the webserver to run the method. Links last accessed in April 2017.
[b]The primary reference describing the method, and which should be cited if used.

of mutations on protein folding and stability. Subsequent efforts by other groups led to a range of methods to predict the same effects, improving upon the accuracy but not considering the other potential structural effects mutations might lead to.

This was first addressed through the systematic application of cut-off scanning matrices [7,8] to quantitatively and scalably predict the effects of mutations on the binding affinities to other ligands, including other proteins, nucleic acids, small molecules, and metal ions [9–14]. Table 1 presents a summary of the main structure-based methods proposed over the past years to analyze the different effects of mutations on coding regions. While this started to allow the deconvolution of the individual molecular changes that might be occurring, the big question limiting their application, especially in a clinical setting, was how do these individual effects combine to lead to a phenotype? Recent efforts have started to integrate these structural effects in order to better understand phenotypes, and have been used to look at a number of different human health problems driven by mutations in protein coding regions [14–22].

3. Using mutation analysis to guide treatment: toward personalized treatments

3.1. Cancers

By analyzing the molecular effects of mutations in common renal cell carcinoma genes, including *p15* and *SDHA*, these have been correlated to a patient's risk of developing renal carcinoma. This was best demonstrated by recent studies looking at mutations in the von Hippel–Lindau protein (VHL) associated with the development of clear cell renal cell carcinoma (ccRCC) [15,16,32,33]. By assessing whether a mutation affected the stability of the protein, or disrupted interactions to Elongin or HIF-1α, a patient could be classified into high-, medium-, and low-risk groups that could help guide screening strategies and provide more focused genetic counseling. The available clinical data from over 100 patients was integrated with a saturation mutagenesis analysis of all possible mutations on VHL producing Symphony, a relational database mapping experimental and predicted risks of mutations to its molecular mechanism, aiding the characterization of newly discovered variants.

Understanding cancer genetics has been important for the diagnosis and treatment of a range of other cancers [34,35], with increasing interest in how the structural impacts of mutations can be used to interpret sequence information. This has led to recent efforts to map the COSMIC database onto protein structures.

3.2. Mendelian genetic diseases

Alkaptonuria (AKU), also known as ochronosis or black bone disease, is a rare recessive inherited genetic disease and first metabolic disorder firstly described over 100 years ago. AKU is caused by coding mutations that disrupt structure and function of the enzyme homogentisate 1,2-dioxygenase (HGD), related to phenylalanine and tyrosine metabolism. HGD gene product folds to form a homo-hexamer disposed as two stacked trimers, quaternary structure which is necessary for enzyme function.

Two comprehensive analysis on AKU causing mutations were carried out in an attempt to characterize the potential molecular mechanisms on which mutations could disruption enzyme activity [17,18].

Mutation effects on protein monomer stability as well as protein-protein and protein-ligand affinity were predicted with the DUET, mCSM-PPI and mCSM-Lig web servers respectively. Three mutation clusters emerged from this analysis, regarding the molecular mechanism for structure and function disruption: (a) mutations that greatly affected monomer stability,

therefore preventing oligomer formation; (b) mutations greatly reducing protein-protein affinity between the hexamer components, also preventing proper oligomer formation and (c) mutations with mild effects on both monomer stability and protein-protein affinity, which together caused functional impairment. The structural analysis of mutations in other Mendelian diseases, for example ornithine transcarbamylase deficiency [36], have identified that disease causing mutations lead to altered protein stability and interactions. Mutations with these molecular consequences occurred in roughly similar proportions to those observed in AKU.

These observations have been validated experimentally and expanded to examine all known disease causing mutations for inclusion in the HGD mutation database [37], which could hopefully guide the development of new, more effective and personalized drugs to treat this condition. For example, subsequent efforts have identified molecular stabilizers that reverse the effects of the destabilizing mutations, analogous to the recent successes on p53. They have also been used to classify patients in the SONIA2 clinical trial, as we know that the molecular mechanism of a mutation can alter how patients may respond to therapeutics [38].

Structural mutation analysis techniques have started to play important roles in the diagnosis of rare Mendelian genetic diseases. For example, establishing the genetic basis of epilepsy is a fundamental step for disease prognosis and choice of patient treatments [38]. Recently, these methods were used to not only identify the genetic cause of a previously undiagnosed or characterized human cohesinopathy but also characterize the molecular mechanism, subsequently experimentally validated [39]. The potential for the structural characterization of mutations to impact upon clinical practice will only continue to grow with the increasing availability of structural information, and routine use of exome sequencing in patient care.

3.3. Screening for drug resistance in tuberculosis

The reduction of sequencing costs, and improvements in accuracy and sensitivity, have led to interest in using high-throughput sequencing to diagnose patients, and identify drug resistance mutations. For infectious diseases such as tuberculosis (TB), where the drug susceptibility screening is time consuming and costly, genomic sequencing opens up the possibility of being able to more rapidly identify the correct treatment strategies for a patient, but also to guide public health policy by following the spread of resistance. Experimental innovations have allowed researchers to sequence the TB genome based on a sample of the patient's sputum, and Public Health England is now sequencing all new TB cases in the UK.

Many resistance mutations in TB have been well characterized, but one of the limitations of these approaches is how to interpret novel mutations identified within the genome. Due to the lack of horizontal gene transfer, TB is an ideal pathogen to apply structural based mutational analysis approaches. Looking at mutations in rpoB and katG, which leads to rifampicin and isoniazid resistance, respectively, clear structural features were identified that correlated strongly with the resulting effectiveness of the drugs (MIC) [40]. A number of resistance mutations have also been observed across protein-protein interfaces, which raises the interesting hypothesis that similar to Mendelian disease mutations, those at interfaces might be prone to lead to disease and resistance because they have a lower fitness cost associated to them than those in the active site that completely disrupt activity [36,41,42].

While previous experimental and clinical knowledge about the effect of a given mutation in a given strain on drug susceptibility will always provide the gold standard for predicting and identifying drug resistance, structural based approaches complement this limited available information by providing the power to look at novel mutations.

4. Targeting resistance mutations: toward resistance-resistant therapies

4.1. HIV protease 1 inhibitors

HIV protease catalyzes the cleavage of the polypeptide precursors into mature enzymes and structural proteins, an essential step in the HIV-1 replication cycle. Inhibitors targeting the HIV protease have been in clinical use since 1995 and include darunavir, amprenavir, atazanavir, nelfinavir, indinavir, saquinavir, and lopinavir [43,44].

Due to the HIV's error prone replication, resistance mutations against these inhibitors have evolved rapidly and been widely observed clinically, limiting the effectiveness of these therapies. These include mutations in the active site (V32I, L33F, I54M, and I84V) that through changes in hydrogen bonding and Van der Waals interactions between the inhibitors and the catalytic site amino acids, can reduce their binding affinities [45,46].

A better understanding of the effects of mutations on inhibitor binding and their molecular mechanism giving rise to resistance are crucial for designing novel drugs, more effectively and less prone to failure. Computational structure-based methods play an important role in tackling this challenge. The mCSM suite was successfully used to predict the effect of the aforementioned mutations upon the binding affinities. Molecular dynamics simulations have also been used to elucidate the effects of the protease inhibitor resistance mutations D30N, I50V, I54M, and V82A, providing interesting mechanistic information on how these mutations alter binding affinities, including changes in the binding conformation (I50V), conformational changes (I54M) and large enthalpic changes reducing binding affinity (V82A) [47]. While genomic methods have proven unreliable for phenotypic characterization of HIV [48], this potentially offers a means to better leverage this information and suggests ways to guide new designs that avoid these common hotspots.

The last HIV protease inhibitor approved, darunavir, was designed with this in mind and is capable of inhibiting the replication of both wild-type and multidrug-resistant strains of HIV-1. While earlier inhibitors interacted with the side-chains of Asp-28 and Asp-30, darunavir contained a *bis*-tetrahydrofuranylurethane functional group that made close, tight interactions with the main chain of these residues, making only

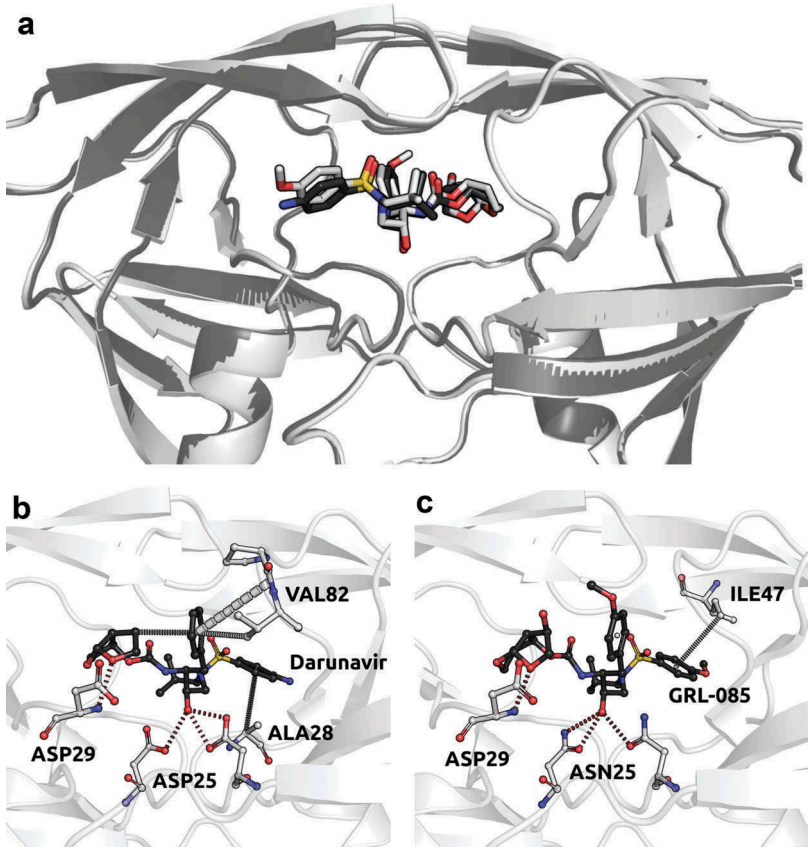

Figure 2. HIV-1 protease in complex with the non-peptidic inhibitor GRL-085 and darunavir (PDB: 5COO and 4HLA, respectively). (a) Shows the two aligned structures of HIV-1 protease in complex with GRL-085 (light gray) and darunavir (dark gray). (b) Depicts the main interactions between the key residues of the binding site of HIV-1 protease and darunavir. (c) Shows the interactions between GRL-085 and the wild-type protease, calculated by Arpeggio [50].

minimal interactions with the side chains [49]. This made darunavir less sensitive to substitutions in either of these positions. Figure 2(a) depicts an alignment between darunavir and a non-peptidic inhibitor GRL-085 and the interactions made by the inhibitors (Figure 2(b,c), respectively).

Many resistant strains against darunavir, however, have emerged. These mutations often lead to a change in the conformation of the active site residues, reducing affinity for darunavir, but also leading to a significant fitness cost [51]. In the effort to avoid these resistance mutations, current medicinal chemistry efforts have identified potent inhibitors that differ from the currently approved protease inhibitors by the number and proximity of contacts to the main chains of these catalytic amino acids [49]. These compounds will be hopefully even more effective therapeutics that are significantly less prone to develop resistance.

4.2. Influenza neuraminidase inhibitors

Influenza neuraminidase inhibitors (NAIs) are the major specific anti-influenza drugs used clinically, despite the emergence of resistance [52]. Currently, the NAIs oseltamivir, zanamivir, peramivir, and laninamivir (currently approved only in Japan) have been approved to prevent and treat influenza A and B [52–55]. Many governments have stockpiled resources of these drugs in the event of an Influenza outbreak. During the recent H1N1 and H7N9 influenza outbreaks, significant resources were focused on identifying and monitoring potential resistance mutations, primarily through genetic screening, with sporadic oseltamivir-resistant 2009 H1N1 virus infections identified. Thus, understanding the mechanisms of influenza NA drug resistance is crucial to develop drugs that can get around mutations and be more successful to fight the epidemics and pandemics [52].

A strong correlation has been observed between mutations that affect the slow binding and dissociation of these NAIs, and the association with resistance [56]. Resistance mutations that have been observed to residues E119 and I222 of Influenza A lead to high and slight resistance to oseltamivir and zanamivir, respectively [57]. Figure 3(a,b) highlight these resistance hotspots on the solved complex of the neuraminidase with oseltamivir and the interactions established on the wild-type protein. Mutations on E119, include substitutions to Gly, Asp, Ala, Ile, and Val, lead to the loss of a salt bridge to the inhibitors [58], with zanamivir showing less susceptibility due to the presence of the 4-guanidino group that maintains typical interactions [52].

Mutations at I222 alter the hydrophobic drug-binding pocket. While I222R leads to a reduction in oseltamivir, peramivir, and zanamivir effectiveness [53,59,60], the I222L mutation, which is also found in Influenza B, has been reported to not lead to significant drug resistance [52]. The other common mutation in N2 is R292K, which leads to resistance against

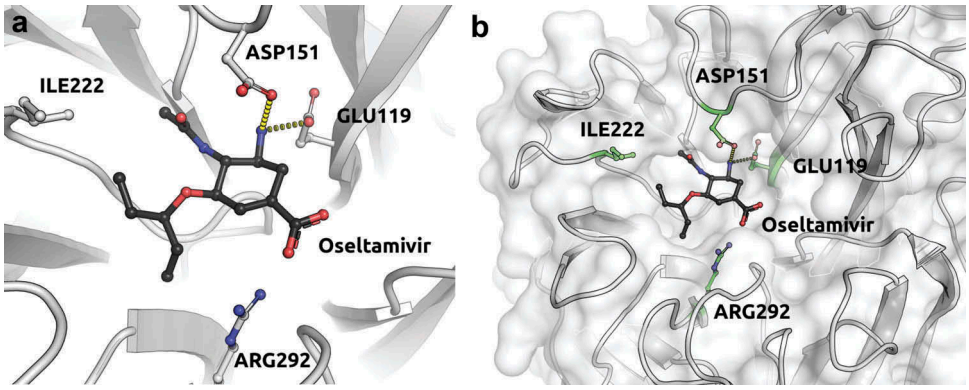

Figure 3. Neuraminidase subtype 2 of Influenza A in complex with Oseltamivir (PDB: 4GZP). (a) Shows the main resistance hot-spot residues Glu119, Asp151 and Ile222 shown as sticks. The two negatively charged residues interact with Oseltamivir via ionic interactions shown as dashes, as calculated by Arpeggio [50]. Arg292, another important binding residue is also shown. (b) Shows the four aforementioned residues and the oseltamivir molecule in a surface perspective.

oseltamivir and peramivir and a slight reduction of zanamivir and laninamivir effectiveness [53].

Following treatment with oseltamivir, the N1 subtype-specific substitution H274Y has also been observed, leading to resistance to this drug and also peramivir, but not to zanamivir and laninamivir [61,62]. The change in volume of the side chains upon this mutation causes the carbonyl group of E276 to be shifted into the binding site of the enzyme, disturbing the hydrophobic pocket that would accommodate the pentyloxy group of oseltamivir [62].

Therefore in efforts to overcome some of these resistance problems, the guanidino group of zanamivir and the hydrophobic pentyloxy group of oseltamivir were merged [61]. The guanidino group was capable of inhibiting the spread of Influenza A with the hydrogen bond interactions between the guanidino group and neuraminidase binding site crucial for the inhibition of the enzyme and virus replication [62,63]. However, the inhibition profile of MS-257 and zanamivir was comparable against the E119V and I222L mutant strains [52].

The sequence database compiled by the WHO containing lists of amino acid substitutions in the neuraminidase has been widely used to identify key mutations and regions, guiding genomic analysis of resistance and proving invaluable for testing new compounds targeting inhibition of neuraminidase [64,65]. It has also facilitated the use of next-generation sequencing to detect resistance markers in the NA gene and predict the effect of drug treatment [66], which have been complemented by the use of structural-based approaches to identify likely resistance mutations.

4.3. Kinase drug development

4.3.1. Kinase inhibition

Abnormal regulation of kinases through occurrence of mutations is responsible for many human diseases, including metabolic disorders and certain types of cancer [67]. The development of small-molecule kinase inhibitors has therefore been seen as an attractive treatment option [68]. Unlike conventional chemotherapy (cytotoxic), molecular targeted therapies using kinase inhibitors are designed to act at specific biological points that are essential for development of tumor cells [69].

The design of kinase inhibitors has great impact on their efficacy and sensitivity to resistance. The first kinase inhibitors developed targeted the ATP-binding site via competitive binding. As resistance to these inhibitors was identified, other strategies including allosteric and covalently bound inhibitors were used to avoid these common resistance mutations [68].

4.3.2. ATP-competitive inhibitors –first generation

ATP-competitive kinase inhibitors inhibit ATP binding in the catalytic site of the target kinase, or bind at alternative sites to induce conformational molecular changes that inhibit the activity of the enzyme [69]. Imatinib was the first kinase small-molecule inhibitor clinically approved by the US Food and Drug Administration (FDA) for treatment of chronic myeloid leukemia [70]. Imatinib binds to the active site of the target enzyme preventing other substrates from phosphorylation and consequently inhibiting kinase activity. Figure 4(a) shows the Abelson tyrosine-protein kinase 2 (ABL2) in complex with imatinib. The inhibitor only binds to the enzyme when it is in inactive conformation. Another example of an inhibitor with a mechanism similar to imatinib is gefitinib which is used for treatment of non-small-cell lung cancer through inhibition of the epidermal growth factor receptor (EGFR).

Despite the success of imatinib, studies have shown that patients can develop resistance and relapse after initial response to therapy. The effect of mutations linked to imatinib resistance were analyzed by mCSM-Lig [14], which could correctly identify resistance mutations located even quite distal from the active site. mCSM-Lig quantitatively predicts the effect of mutations on small molecule affinity. Resistance mutations of competitive inhibitor, however, can exist by shifting the preference of the protein toward the natural ligand (ATP), not necessarily by dramatically reducing the affinity of the protein to the drug. Interestingly, using a fold-ratio between the predicted affinity effect on the natural ligand and the drug, mCSM-Lig was successful in identifying the majority of the imatinib resistance mutations.

Several mechanisms of resistance have been observed, including mutations in the BCR-ABL kinase domain, with the most common resistant observed the gatekeeper mutant T315I [71]. This amino acid substitution eliminates a critical

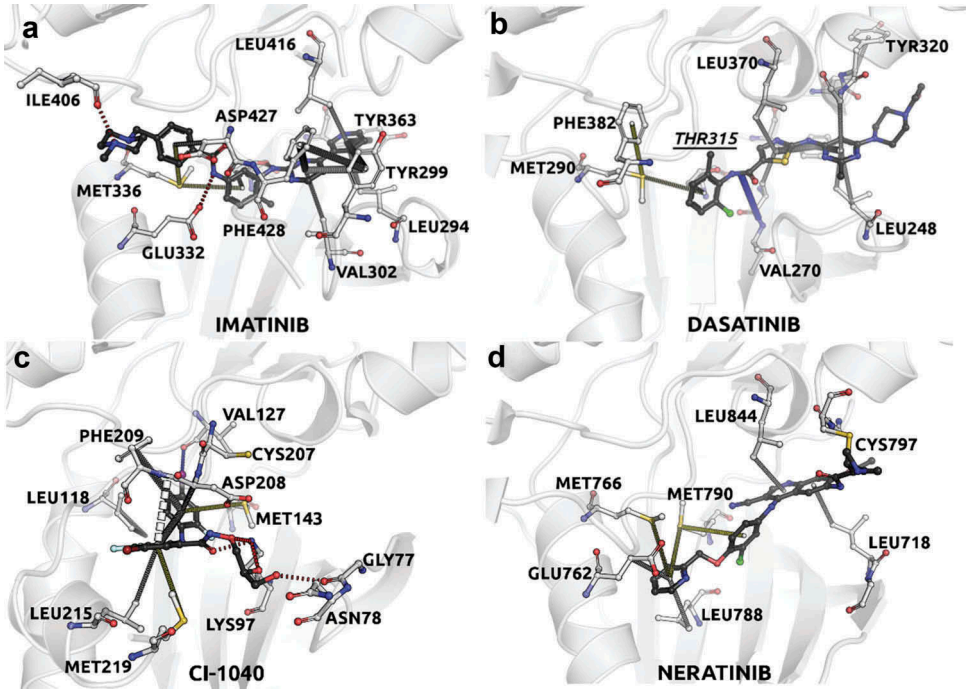

Figure 4. Four generations of kinase inhibitors. (a) Shows ABL2 in complex with first generation kinase inhibitor Imatinib (PDB: 3GVU). Imatinib binds to the active site of the enzyme preventing other substrates from phosphorylation only when the ABL2 is in inactive conformation. (b) Shows ABL1 in complex with second generation inhibitor Dasatinib (PDB: 2GQG). Dasatinib is a multitargeted tyrosine kinase inhibitor more potent than Imatinib due to its capability of binding to the enzyme in inactive imatinib-bound conformation, also effective against several imatinib-resistant mutations, except for T315I gatekeeper mutation as a result of a crucial hydrogen bond with T315 (underlined) for the stabilization of the complex. (c) Shows MEK1 in complex with CI-1040 allosteric kinase inhibitor adjacent to the ATP binding site of the enzyme (PDB: 1S9J). The third generation of kinase inhibitors can bind either to the kinase domain or to other sites giving them clear advantage over ATP-competitive in first and second generation. (d) Shows EGFR mutant T790M/L858R in complex with fourth generation kinase inhibitor Neratinib (PDB: 3W2Q). Unlike first and second generation inhibitors, this fourth generation inhibitor binds covalently to the kinase active site, blocking ATP binding.

oxygen molecule needed for hydrogen bonding between imatinib and the ABL kinase, and also introduces a steric clash preventing drug binding. The gatekeeper residue determines the relative accessibility of a hydrophobic pocket located adjacent to the ATP-binding site, which is important for imatinib binding given that hydrophobic interactions are crucial for inhibitor binding affinity [68,72,73]. In fact, mutations in gatekeeper residues have also been studied for other kinases in different types of cancer, such as the Threonine 790 of EGFR in Lung cancer that mutates to a methionine (T790M) increasing the affinity for ATP and making it difficult for the gefitinib to compete for the binding site [74–76]. Such mechanisms of resistance have contributed to the development of more sophisticated generations of inhibitors with mechanisms to overcome resistances conferred by these gatekeeper mutations.

4.3.3. ATP-competitive inhibitors –second generation

The second generation of small-molecule kinase inhibitors preferentially binds to regions outside the ATP-binding site, for example, to the inactive conformation, also known as DFG-out, of the protein kinase. The transition from the active conformation to DFG-out conformation exposes additional hydrophobic pockets adjacent to the ATP site that can be used by the inhibitors to stabilize the kinase in its inactive conformation [77], preventing ATP binding.

Dasatinib is a multitargeted tyrosine kinase inhibitor that targets oncogenic pathways and is a more potent inhibitor than imatinib that binds only when the ABL enzyme is in its inactive conformation. Dasatinib is also effective against several imatinib-resistant ABL mutations that occur in regions that are in contact with imatinib or mutations involved in stabilization of specific inactive imatinib-bound conformation of the enzyme. However, the T315I gatekeeper mutation is also resistant to dasatinib due crucial hydrogen bond with the T315 side chain [78]. Figure 4(b) shows ABL1 in complex with dasatinib. The main residues involved in the binding of the drug are highlighted, including T315.

4.3.4. Allosteric inhibitors – third generation

These inhibitors regulate the kinase activity in an allosteric manner, exhibiting a higher degree of selectivity due the exploitation of binding sites and regulatory mechanisms that are specific to a particular kinase [68]. Figure 4(c) shows the allosteric inhibitor CI-1040 binding MEK1 immediately adjacent to the ATP binding site.

This class of inhibitors can bind either to the kinase domain (or close to the ATP binding site) or to sites outside the kinase domain. These range of options for inhibiting the catalytic activity of kinases represent clear advantages over the ATP-competitive inhibitors [79,80]. However, the lack of methods to identify such inactive conformations or binding modes in kinases to drive the development of this type of inhibitor still remains a challenge [81]. Inhibitors that disrupt formation of the higher order oligomers, which play an important role in achieving high signal-to-noise throughout the signal

transduction process, have also proven to be effective kinase inhibitors that avoid the common ATP resistance mutations [82–84].

ABL001, also known as Asciminib, is a potent and selective third generation kinase inhibitor with activity against chronic myeloid leukemia and Philadelphia chromosome-positive (Ph +) acute lymphoblastic leukemia. ABL001 binds to the myristoyl pocket of ABL1 kinase leading to a formation of an inactive kinase conformation [85]. Recent studies have shown that treatment with ABL001 combined with ATP-competitive inhibitors can help prevent resistance in chronic myeloid leukemia [86,87].

4.3.5. Covalent inhibitors – fourth generation

Recent studies [88,89] described a fourth class of kinase inhibitors that are capable of forming covalent bonds to the kinase active site, most frequently by reacting with a nucleophilic cysteine residue. Unlike first- and second-generation inhibitors, the fourth generation blocks the binding of ATP irreversibly preventing the kinase from being activated. Figure 4(d) shows the fourth-generation inhibitor Neratinib (HKI-272) in complex with EGFR kinase T790M mutant, making a covalent bond to Cysteine 797.

4.3.6. Tackling kinase inhibitor resistance

Much of the effort to target and avoid resistance against common kinase inhibitors has focused on the development of inhibitors with different modes of action. This has in part been driven by the lack of selectivity of the early inhibitors that targeted the ATP-binding site – which is highly conserved among many proteins. Structural methods such as mCSM-lig and molecular dynamics approaches have been able to correctly identify and predict likely resistance mutations, which could also potentially facilitate the design of new inhibitors avoiding these resistance hotspots, similar to the efforts in antiviral inhibitor design. However, more practically, as sequencing of cancers is becoming more routine, these methods offer the opportunity to help guide the selection of the most effective therapeutics- facilitating the widespread implementation of personalized medicine.

The advent of fast and precise computational methods to predict effect of mutations can be leveraged to assist and guide the development of new drugs. Since resistance can emerge from different molecular mechanisms, current predictors can be integrated in novel drug resistance identification methods that can then be used in large-scale screening to identify better protein targets, identify and avoid potential resistance hotspots as well as optimize ligand affinity and selectivity, driving the experimental design of better, more potent and efficacious drugs.

5. Expert opinion

While significant progress has been made in terms of innovative tools to understand and quantify the different range of effects in which a mutation or a set of mutations can give rise to a phenotype, a great gap still exists when integrating these predictions and drawing causality conclusions linking variants, compounded by the need for detailed information regarding the system/protein. The availability of scalable, effective computational methods to assess mutation effects creates new opportunities of development of such integrated approaches and decipher complex genomic background patterns, shedding light into their role in the emergence of a given phenotype and molecular mechanisms of action. This capability can then be used to systematically study, for instance, how drug resistance emerges on specific drug targets, aiding the drug development process. Initial efforts on that matter have focused on preparing predictors and databases for specific diseases and proteins; however, greater effort needs to be invested in making these predictors user friendly, integrated, and accessible to geneticists. This is particularly important considering that most structural information is a snapshot of a protein conformation, but how mutations affect the equilibrium between different states can play a very important role in disease and drug resistance [90]. A complementary and important effort refers to the collection and curation of experimental data regarding mutation effects linked to phenotype in comprehensive databases. This information forms the evidence set necessary for the proposal of novel computational methods as well as the improvement of current approaches. Initiatives like the Platinum database [91], the first curated online database linking effects of mutations on protein-small-molecule affinity for complexes with known structures, are fundamental.

Despite this limitation, these methodologies have already provided invaluable insights into many diseases. Current genomic analyses are dependent upon preexisting information; either extensive genomic or biochemical analyses. This limits the insight and information that can be drawn regarding novel mutations. As these structural methods become more widely used, they will complement traditional analyses methods to provide much greater power from genomic analysis.

In the shorter term, the ability of these methods to predict likely resistance mutations before they arise offers enormous potential throughout the drug development process. Peter Coleman first suggested that the design of inhibitors that resemble transition state analogs should be more resilient to the development of resistance. Out of this, Zanamivir was developed, the first successful structure guided drug development, but as we have seen over the intervening years resistance against Relenza has been widely reported, although it has been less prone to resistance than Oseltamivir.

During the development of a recent class of *Mycobacterium tuberculosis* IMPDH inhibitors, structural-guided mutational prediction was used to identify likely resistance mutations, defined in this case as point mutations that disrupted inhibitor binding, but did not affect NAD binding, protein solubility or formation of the active tetramer. One mutation in particular, Y487C, was highlighted, and subsequently confirmed to be one of the few mutations to arise during resistance screening [92]. Subsequent drug development attempts avoided this resistance hotspot and were active against the Y487C mutant [93]. This also enables the analysis of multiple mutations, some of which have been characterized to facilitate the development of resistance. In many cases, these seem to increase protein stability or natural ligand binding, which can be decreased due to the primary resistance mutation.

While current medicinal chemistry efforts are currently normally retroactive – we observe which mutations arise in the lab or clinic and then design new generations of inhibitors to target or avoid them – the power of computational mutational analysis enables us to preemptively identify likely resistance hotspots, and to take this information under consideration when optimizing candidate molecules. In a similar fashion to how experimental structures [94–98] and pharmacokinetic predictors are now widely used to guide medicinal chemistry efforts [99], playing a role in dramatically reducing failure rates of clinical trials due to these problems. The use of *in silico* mutational analysis in the development of new therapeutics will hopefully avoid likely resistance mutations. While the evolutionary forces and the constant selective battle makes the development of resistance somewhat inevitable, this will hopefully aid in the development of the next generation of therapeutics that are more resistant to the development of resistance.

Funding

This work was funded by the Jack Brockhoff Foundation (JBF 4186, 2016) and a Newton Fund RCUK-CONFAP Grant awarded by The Medical Research Council (MRC) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (MR/M026302/1). This research was supported by the Victorian Life Sciences Computation Initiative (VLSCI), an initiative of the Victorian Government, Australia, on its Facility hosted at the University of Melbourne (UOM0017). DEV Pires receives support from the René Rachou Research Center (CPqRR/FIOCRUZ Minas), Brazil. DB Ascher is supported by a C. J. Martin Research Fellowship from the National Health and Medical Research Council of Australia (APP1072476), and the Department of Biochemistry, University of Melbourne.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

ORCID

David B. Ascher http://orcid.org/0000-0003-2948-2413

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073–1081.
2. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010 Apr;7(4):248–249.
3. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014 Mar;46(3):310–315.
4. Schwarz JM, Rodelsperger C, Schuelke M, et al. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010 Aug;7(8):575–576.
5. Rethink the links between genes and disease. Nature. 2016 Oct 13;538(7624):140.
6. Topham CM, Srinivasan N, Blundell TL. Prediction of the stability of protein mutants based on structural environment-dependent amino acid substitution and propensity tables. Protein Eng. 1997 Jan;10(1):7–21.
7. Pires DE, De Melo-Minardi RC, Dos Santos MA, et al. Cutoff scanning matrix (CSM): structural classification and function prediction by protein inter-residue distance patterns. BMC Genomics. 2011 Dec 22;12(Suppl 4):S12.
8. Pires DE, De Melo-Minardi RC, Da Silveira CH, et al. aCSM: noise-free graph-based signatures to large-scale receptor-based ligand prediction. Bioinformatics. 2013 Apr 01;29(7):855–861.
9. Pires DE, Ascher DB. mCSM-AB: a web server for predicting antibody-antigen affinity changes upon mutation with graph-based signatures. Nucleic Acids Res. 2016 Jul 08; 44(W1):W469–73.
• **A high-throughput and accurate method to predict the effects of mutations on antibody-antigen binding affinity. Used for antibody maturation and to predict likely antibody escape mutations.**
10. Pires DE, Ascher DB. CSM-lig: a web server for assessing and comparing protein-small molecule affinities. Nucleic Acids Res. 2016 Jul 08;44(W1):W557–61.
11. Pires DE, Ascher DB. mCSM-NA: predicting the effects of mutations on protein-nucleic acids interactions. Nucleic Acids Res. DOI: 10.1093/nar/gkx236.
• **Optimized method to predict the effect of mutations on protein-nucleic acid binding.**
12. Pires DE, Ascher DB, Blundell TL. DUET: a server for predicting effects of mutations on protein stability using an integrated computational approach. Nucleic Acids Res. 2014 Jul;42(Web Server issue):W314–9.
• **An integrated structural method to predict effects of mutations on protein stability.**
13. Pires DE, Ascher DB, Blundell TL. mCSM: predicting the effects of mutations in proteins using graph-based signatures. Bioinformatics. 2014 Feb 01; 30(3):335–342.
•• **Comprehensive platform for analysis of the effects of mutations on protein structure and function, including the first published methods to assess the affects of mutations on protein-protein and protein-nucleic acid binding affinity.**
14. Pires DE, Blundell TL, Ascher DB. mCSM-lig: quantifying the effects of mutations on protein-small molecule affinity in genetic disease and emergence of drug resistance. Sci Rep. 2016 Jul 07;6:29575.
•• **The first scalable and accurate method to predict the effects of single-point mutations on protein-small-molecule interactions. This was capable of identifying and anticipating drug resistance mutations.**
15. Andrews KA, Vialard L, Ascher DB, et al. Tumour risks and genotype–phenotype–proteotype analysis of patients with germline mutations in the succinate dehydrogenase subunit genes SDHB, SDHC, and SDHD. Lancet. 2016 Feb 25;387:S19.
16. Gossage L, Pires DE, Olivera-Nappa A, et al. An integrated computational approach can classify VHL missense mutations according to risk of clear cell renal carcinoma. Hum Mol Genet. 2014 Nov 15;23(22):5976–5988.
17. Nemethova M, Radvanszky J, Kadasi L, et al. Twelve novel HGD gene variants identified in 99 alkaptonuria patients: focus on 'black bone disease' in Italy. Eur J Hum Genet. 2016 Jan;24(1):66–72.
18. Usher JL, Ascher DB, Pires DE, et al. Analysis of HGD gene mutations in patients with alkaptonuria from the United Kingdom: identification of novel mutations. JIMD Rep. 2015 Feb;15(24):3–11.
19. Kano FS, Souza-Silva FA, Torres LM, et al. The presence, persistence and functional properties of plasmodium vivax duffy binding protein II antibodies are Influenced by HLA class II allelic variants. Plos Negl Trop Dis. 2016 Dec;10(12):e0005177.
20. Silvino AC, Costa GL, Araujo FC, et al. Variation in human cytochrome P-450 drug-metabolism genes: a gateway to the understanding of plasmodium vivax relapses. Plos One. 2016;11(7):e0160172.
21. White RR, Ponsford AH, Weekes MP, et al. Ubiquitin-dependent modification of skeletal muscle by the parasitic nematode, trichinella spiralis. Plos Pathog. 2016 Nov;12(11):e1005977.

22. Pires DE, Chen J, Blundell TL, et al. In silico functional dissection of saturation mutagenesis: interpreting the relationship between phenotypes and changes in protein stability, interactions and activity. Sci Rep. 2016 Jan 22;6:19848.
•• **An integrated pipeline using changes in protein structure and function to elucidate the relationship between genotype and phenotype.**
23. Worth CL, Preissner R, Blundell TL. SDM–a server for predicting effects of mutations on protein stability and malfunction. Nucleic Acids Res. 2011 Jul;39(Web Server issue):W215–22.
24. Pandurangan AP, Ochoa-Montaño B, Ascher DB, et al. SDM: a server for predicting effects of mutations on protein stability and malfunction. Nucleic Acids Res. Fourth coming.
25. Dehouck Y, Kwasigroch JM, Gilis D, et al. PoPMuSiC 2.1: a web server for the estimation of protein stability changes upon mutation and sequence optimality. BMC Bioinformatics. 2011 May 13;12:151.
26. Frappier V, Chartier M, Najmanovich RJ. ENCoM server: exploring protein conformational space and the effect of mutations on protein function and stability. Nucleic Acids Res. 2015 Jul 01;43(W1): W395–400.
27. Laimer J, Hiebl-Flach J, Lengauer D, et al. MAESTROweb: a web server for structure-based protein stability prediction. Bioinformatics. 2016 May 01;32(9):1414–1416.
28. Quan L, Lv Q, Zhang Y. STRUM: structure-based prediction of protein stability changes upon single-point mutation. Bioinformatics. 2016 Oct 01;32(19):2936–2946.
29. Witvliet DK, Strokach A, Giraldo-Forero AF, et al. ELASPIC webserver: proteome-wide structure-based prediction of mutation effects on protein stability and binding affinity. Bioinformatics. 2016 May 15;32(10):1589–1591.
30. Dehouck Y, Kwasigroch JM, Rooman M, et al. BeAtMuSiC: prediction of changes in protein-protein binding affinity on mutations. Nucleic Acids Res. 2013 Jul;41(Web Server issue): W333–9.
31. Li M, Simonetti FL, Goncearenco A, et al. MutaBind estimates and interprets the effects of sequence variants on protein-protein interactions. Nucleic Acids Res. 2016 Jul 08;44(W1):W494–501.
32. Jafri M, Wake NC, Ascher DB, et al. Germline mutations in the CDKN2B tumor suppressor gene predispose to renal cell carcinoma. Cancer Discov. 2015 Jul;5(7):723–729.
33. Casey RT, Ascher DB, Rattenberry E, et al. SDHA related tumorigenesis: a new case series and literature review for variant interpretation and pathogenicity. Mol Genet Genomic Med. DOI: 10.1002/mgg3.279.
34. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004 Jun 04;304(5676):1497–1500.
35. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006 Apr 15;66(8):3992–3995.
36. Jubb HC, Pandurangan AP, Turner MA, et al. Mutations at protein-protein interfaces: small changes over big surfaces have large impacts on human health. Prog Biophys Mol Biol. DOI: 10.1016/j.pbiomolbio.2016.10.002.
37. Zatkova A, Sedlackova T, Radvansky J, et al. Identification of 11 novel homogentisate 1,2 dioxygenase variants in alkaptonuria patients and establishment of a novel LOVD-based HGD mutation database. JIMD Rep. 2012;4:55–65.
38. Poduri A, Sheidley BR, Shostak S, et al. Genetic testing in the epilepsies-developments and dilemmas. Nat Rev Neurol. 2014 May;10(5):293–299.
39. Soardi FC, Machado-Silva A, Linhares ND, et al. Familial STAG2 germline mutation defines a new human cohesinopathy. Npj Genomic Medicine. 2017;2(1):7.
40. Phelan J, Coll F, McNerney R, et al. Mycobacterium tuberculosis whole genome sequencing and protein structure modelling provides insights into anti-tuberculosis drug resistance. BMC Med. 2016 Mar 23;14(1):31.
• **Structural characterization of *Mycobacterium Tuberculosis* drug resistance mutations, highlighting the strong correlation between MIC and structural effects.**
41. Ascher DB, Jubb HC, Pires DE, et al. Protein-protein interactions: structures and druggability. In: Scapin G, Patel D, Arnold E eds. Multifaceted roles of crystallography in modern drug discovery. Netherlands: Springer; 2015. p. 141–163.
42. Pandurangan AP, Ascher DB, Thomas SE, et al. Genomes, structural biology, and drug discovery: combating the impacts of mutations in genetic disease and antibiotic resistance. Biochem Soc Trans. 2017;45(2):303–311. DOI: 10.1042/BST20160422
43. Park JH, Sayer JM, Aniana A, et al. Binding of clinical inhibitors to a model precursor of a rationally selected multidrug resistant HIV-1 protease is significantly weaker than that to the released mature enzyme. Biochemistry. 2016 Apr 26;55(16):2390–2400.
44. Zhang H, Wang YF, Shen CH, et al. Novel P2 tris-tetrahydrofuran group in antiviral compound 1 (GRL-0519) fills the S2 binding pocket of selected mutants of HIV-1 protease. J Med Chem. 2013 Feb 14;56(3):1074–1083.
45. Koh Y, Amano M, Towata T, et al. In vitro selection of highly darunavir-resistant and replication-competent HIV-1 variants by using a mixture of clinical HIV-1 isolates resistant to multiple conventional protease inhibitors. J Virol. 2010 Nov;84(22):11961–11969.
46. Hosseini A, Alibes A, Noguera-Julian M, et al. Computational prediction of HIV-1 resistance to protease inhibitors. J Chem Inf Model. 2016 May 23;56(5):915–923.
47. Hu G, Ma A, Dou X, et al. Computational studies of a mechanism for binding and drug resistance in the wild type and four mutations of HIV-1 protease with a GRL-0519 inhibitor. Int J Mol Sci. 2016 May 27;17(6).
48. Hanna GJ, D'Aquila RT. Clinical use of genotypic and phenotypic drug resistance testing to monitor antiretroviral chemotherapy. Clin Infect Dis. 2001 Mar 01;32(5):774–782.
49. Koh Y, Nakata H, Maeda K, et al. Novel bis-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus in vitro. Antimicrob Agents Chemother. 2003 Oct;47(10):3123–3129.
50. Jubb HC, Higueruelo AP, Ochoa-Montano B, et al. Arpeggio: a web server for calculating and visualising interatomic interactions in protein structures. J Mol Biol. 2017 Feb 03;429(3):365–371.
•• **Novel method for calculating and displaying all possible intra- and inter-molecular interactions.**
51. Yoshimura K, Kato R, Kavlick MF, et al. A potent human immunodeficiency virus type 1 protease inhibitor, UIC-94003 (TMC-126), and selection of a novel (A28S) mutation in the protease active site. J Virol. 2002 Feb;76(3):1349–1358.
52. Wu Y, Gao F, Qi J, et al. Resistance to mutant group 2 influenza virus neuraminidases of an oseltamivir-zanamivir hybrid inhibitor. J Virol. 2016 Dec 01;90(23):10693–10700.
53. Yen HL. Current and novel antiviral strategies for influenza infection. Curr Opin Virol. 2016 Jun;18:126–134.
54. Hata A, Akashi-Ueda R, Takamatsu K, et al. Safety and efficacy of peramivir for influenza treatment. Drug Des Devel Ther. 2014;8:2017–2038.
55. Moscona A. Neuraminidase inhibitors for influenza. N Engl J Med. 2005 Sep 29;353(13):1363–1373.
56. McKimm-Breschkin JL, Barrett S. Neuraminidase mutations conferring resistance to laninamivir lead to faster drug binding and dissociation. Antiviral Res. 2015 Feb;114:62–66.
57. Richard M, Ferraris O, Erny A, et al. Combinatorial effect of two framework mutations (E119V and I222L) in the neuraminidase active site of H3N2 influenza virus on resistance to oseltamivir. Antimicrob Agents Chemother. 2011 Jun;55(6):2942–2952.
58. Okomo-Adhiambo M, Demmler-Harrison GJ, Deyde VM, et al. Detection of E119V and E119I mutations in influenza A (H3N2) viruses isolated from an immunocompromised patient: challenges in diagnosis of oseltamivir resistance. Antimicrob Agents Chemother. 2010 May;54(5):1834–1841.

59. Van Der Vries E, Stelma FF, Boucher CA. Emergence of a multidrug-resistant pandemic influenza A (H1N1) virus. N Engl J Med. 2010 Sep 30;363(14):1381–1382.
60. McKimm-Breschkin JL. Influenza neuraminidase inhibitors: antiviral action and mechanisms of resistance. Influenza Other Respir Viruses. 2013 Jan;7(Suppl 1):25–36.
61. Kerry PS, Mohan S, Russell RJ, et al. Structural basis for a class of nanomolar influenza A neuraminidase inhibitors. Sci Rep. 2013 Oct;16(3):2871.
62. Collins PJ, Haire LF, Lin YP, et al. Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature. 2008 Jun 26;453(7199):1258–1261.
63. Niikura M, Bance N, Mohan S, et al. Replication inhibition activity of carbocycles related to oseltamivir on influenza A virus in vitro. Antiviral Res. 2011 Jun;90(3):160–163.
64. Meijer A, Rebelo-de-Andrade H, Correia V, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2012-2013. Antiviral Res. 2014;110:31–41.
65. Hurt AC, Besselaar TG, Daniels RS, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014-2015. Antiviral Res. 2016;132:178–185.
66. Parker J, Chen J. Application of next generation sequencing for the detection of human viral pathogens in clinical specimens. J Clin Virol. 2017 Jan;86:20–26.
67. Lahiry P, Torkamani A, Schork NJ, et al. Kinase mutations in human disease: interpreting genotype-phenotype relationships. Nat Rev Genet. 2010 Jan;11(1):60–74.
68. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009 Jan;9(1):28–39.
69. Gharwan H, Groninger H. Kinase inhibitors and monoclonal antibodies in oncology: clinical implications. Nat Rev Clin Oncol. 2016 Apr;13(4):209–227.
70. Agafonov RV, Wilson C, Kern D. Evolution and intelligent design in drug development. Front Mol Biosci. 2015;2:27.
71. Weisberg E, Manley P, Mestan J, et al. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. Br J Cancer. 2006 Jun 19;94 (12):1765–1769.
72. Azam M, Seeliger MA, Gray NS, et al. Activation of tyrosine kinases by mutation of the gatekeeper threonine. Nat Struct Mol Biol. 2008 Oct;15(10):1109–1118.
73. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res. 2008 May 15;14 (10):2895–2899.
74. Tetsu O, Hangauer MJ, Phuchareon J, et al. Drug resistance to EGFR inhibitors in lung cancer. Chemotherapy. 2016;61(5):223–235.
75. Klebl BM, Muller G. Second-generation kinase inhibitors. Expert Opin Ther Targets. 2005 Oct;9(5):975–993.
76. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A. 2008 Feb 12;105 (6):2070–2075.
77. Cowan-Jacob SW, Jahnke W, Knapp S. Novel approaches for targeting kinases: allosteric inhibition, allosteric activation and pseudokinases. Future Med Chem. 2014 Apr;6(5):541–561.
78. Tokarski JS, Newitt JA, Chang CY, et al. The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. Cancer Res. 2006 Jun 01;66(11):5790–5797.
79. Fasano M, Della Corte CM, Califano R, et al. Type III or allosteric kinase inhibitors for the treatment of non-small cell lung cancer. Expert Opin Investig Drugs. 2014 Jun;23(6):809–821.
80. Wu P, Clausen MH, Nielsen TE. Allosteric small-molecule kinase inhibitors. Pharmacol Ther. 2015 Dec;156:59–68.
81. Muller S, Chaikuad A, Gray NS, et al. The ins and outs of selective kinase inhibitor development. Nat Chem Biol. 2015 Nov;11(11):818–821.
82. Blaszczyk M, Harmer NJ, Chirgadze DY, et al. Achieving high signal-to-noise in cell regulatory systems: spatial organization of multi-protein transmembrane assemblies of FGFR and MET receptors. Prog Biophys Mol Biol. 2015 Sep;118(3):103–111.
83. Liang S, Esswein SR, Ochi T, et al. Achieving selectivity in space and time with DNA double-strand-break response and repair: molecular stages and scaffolds come with strings attached. Struct Chem. 2016;28(1):161–171.
84. Sibanda BL, Chirgadze DY, Ascher DB, et al. DNA-PKcs structure suggests an allosteric mechanism modulating DNA double-strand break repair. Science. 2017 Feb 03;355(6324):520–524.
85. Wylie AA, Schoepfer J, Jahnke W, et al. The allosteric inhibitor ABL001 enables dual targeting of BCR-ABL1. Nature. 2017 Mar 30;543(7647):733–737.
86. Wylie A, Schoepfer J, Berellini G, et al. ABL001, a potent allosteric inhibitor of BCR-ABL, prevents emergence of resistant disease when administered in combination with nilotinib in an in vivo murine model of chronic myeloid leukemia. Blood. 2014;124(21):398–398.
87. Eide CA, Savage SL, Heinrich MC, et al. Combining the allosteric ABL1 tyrosine kinase inhibitor ABL001 with ATP-competitive inhibitors to suppress resistance in chronic myeloid leukemia. Blood. 2016;128(22):2747–2747.
88. Tan L, Wang J, Tanizaki J, et al. Development of covalent inhibitors that can overcome resistance to first-generation FGFR kinase inhibitors. Proc Natl Acad Sci U S A. 2014 Nov 11;111(45):E4869–77.
89. Zou Y, Xiao J, Tu Z, et al. Structure-based discovery of novel 4,5,6-trisubstituted pyrimidines as potent covalent Bruton's tyrosine kinase inhibitors. Bioorg Med Chem Lett. 2016 Jul 01;26(13):3052–3059.
90. Ascher DB, Wielens J, Nero TL, et al. Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. Sci Rep. 2014 Apr 23;4:4765.
91. Pires DE, Blundell TL, Ascher DB. Platinum: a database of experimentally measured effects of mutations on structurally defined protein-ligand complexes. Nucleic Acids Res. 2015 Jan;43 (Database issue):D387–91.
92. Singh V, Donini S, Pacitto A, et al. The inosine monophosphate dehydrogenase, GuaB2, is a vulnerable new bactericidal drug target for tuberculosis. ACS Infect Dis. 2017 Jan 13;3(1):5–17.
93. Park Y, Pacitto A, Bayliss T, et al. Essential but not vulnerable: indazole sulfonamides targeting inosine monophosphate dehydrogenase as potential leads against mycobacterium tuberculosis. ACS Infect Dis. 2017 Jan 13;3(1):18–33.
94. Ascher DB, Cromer BA, Morton CJ, et al. Regulation of insulin-regulated membrane aminopeptidase activity by its C-terminal domain. Biochemistry. 2011 Apr 05;50(13):2611–2622.
95. Hermans SJ, Ascher DB, Hancock NC, et al. Crystal structure of human insulin-regulated aminopeptidase with specificity for cyclic peptides. Protein Sci. 2015 Feb;24(2):190–199.
96. Chai SY, Yeatman HR, Parker MW, et al. Development of cognitive enhancers based on inhibition of insulin-regulated aminopeptidase. BMC Neurosci. 2008 Dec 03;9(Suppl 2):S14.
97. Albiston AL, Morton CJ, Ng HL, et al. Identification and characterization of a new cognitive enhancer based on inhibition of insulin-regulated aminopeptidase. Faseb J. 2008 Dec;22(12):4209–4217.
98. Sigurdardottir AG, Winter A, Sobkowicz A, et al. Exploring the chemical space of the lysine-binding pocket of the first kringle domain of hepatocyte growth factor/scatter factor (HGF/SF) yields a new class of inhibitors of HGF/SF-MET binding. Chem Sci. 2015;6 (11):6147–6157.
99. Pires DE, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem. 2015 May 14;58(9):4066–4072.