REVIEW

Review: Precision medicine and driver mutations: Computational methods, functional assays and conformational principles for interpreting cancer drivers

Ruth Nussinov1,2*, Hyunbum Jang1, Chung-Jung Tsai1, Feixiong Cheng3,4

1 Computational Structural Biology Section, Basic Science Program, Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, Maryland, United States of America, 2 Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, 3 Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, United States of America, 4 Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, Ohio, United States of America

* NussinovR@mail.nih.gov

Abstract

At the root of the so-called precision medicine or precision oncology, which is our focus here, is the hypothesis that cancer treatment would be considerably better if therapies were guided by a tumor’s genomic alterations. This hypothesis has sparked major initiatives focusing on whole-genome and/or exome sequencing, creation of large databases, and developing tools for their statistical analyses—all aspiring to identify actionable alterations, and thus molecular targets, in a patient. At the center of the massive amount of collected sequence data is their interpretations that largely rest on statistical analysis and phenotypic observations. Statistics is vital, because it guides identification of cancer-driving alterations. However, statistics of mutations do not identify a change in protein conformation; therefore, it may not define sufficiently accurate actionable mutations, neglecting those that are rare. Among the many thematic overviews of precision oncology, this review innovates by further comprehensively including precision pharmacology, and within this framework, articulating its protein structural landscape and consequences to cellular signaling pathways. It provides the underlying physicochemical basis, thereby also opening the door to a broader community.

Introduction

What exactly is “precision oncology,” and how can we, as a community, contribute to it? Despite its captivating terminology, the concept is not new; after all, it has long been known that to be effective, specific ailments necessitate tailored treatments [1]. However, its current genomic basis distinguishes it from its outmoded cellular renditions [2, 3]. The term “precision medicine”, or “precision oncology,” as in our case here, is commonly used when the treatment strategies involve targeted therapies that are based on personal data from next-
generation sequencing [4–14]. That is, rather than select therapies based on the perceived type of cancer, for example, tissue or organ location, therapeutic selection is based on analysis of the individual’s genomic sequence and the specific identified mutational aberrations [15–18]. It is the mutation-guided therapeutics, rather than the traditional cancer type-dependence classification, such as that based on classical anatomy and histology, that has etched a new context into this terminology. This concept has compelled a paradigm shift. Now patients with *BRAF* V600E mutations would be prescribed the same drug regimen irrespective of their cancer type and location, for example, acute myeloid leukemia, breast cancer, or melanoma [19].

Precision oncology was not always based on the individual’s genomic sequence. Since its inception, exactly what precision oncology includes has been unclear. In 2015, Collins and Varmus proposed that blood-typing—based targeted therapies and immune therapy be included [20]. A 2017 analysis revealed that in the literature, “precision oncology” appears to have undergone an evolution [21]. Early on, therapies were disease and/or protein targeted, such as, for example, vascular endothelial growth factor (VEGF) inhibitors and Bcr-abl1 tyrosine kinase inhibitors (TKIs), bevacizumab (Avastin) and imatinib, respectively. Later, precision oncology treatments were referred to as selections of therapies based on analyses of biomarkers. Examples include crizotinib (Xalkori) in lung cancer with *EML4-ALK* rearrangements or adjuvant chemotherapy as in the Oncotype DX panel in breast cancer. The literature analysis observed that, only as of January 2016, precision oncology therapeutic selection appears to have been primarily based on next-generation sequencing data. As noted by Tsang and colleagues, various terms have been used to relate to precision genomic oncology, including simply precision oncology [22], genomics-driven oncology [23], genomic oncology, and personalized cancer medicine. All refer to high-throughput sequencing to inform clinical decisions at the point-of-care [24].

Even though the conceptual foundation of precision oncology is rational, thus stimulating broad enthusiasm, its current lack of demonstrated clear breakthroughs in clinical trials argues that in addition to more patient sequence data, critical components may be missing. Next-generation sequencing of patients with advanced cancers showed that less than 10% have actionable mutations [25, 26], and a randomized trial of precision medicine [27] did not observe improved outcomes with genome-based precision oncology. The biological complexity underlying target identification is challenging. The breakthrough of the next-generation sequencing delivered a new precision component: treatments might be tailored, not only to a certain illness but also to a specific person’s genetic make-up. This notion of prescribing “the right drug to the right person at the right time” [28–32] has stimulated considerable research efforts, which have been pushed to the fore by the Precision Medicine Initiative (also called All of Us Research Program). But to date, it seems to still fall short, and the magnitude of the task is daunting. The mutational landscape is highly heterogeneous and challenging to decipher. Data indicate that the least mutated cancers have on average 0.28 mutations per megabase, with most presenting 8.15 mutations per megabase [21, 33]. Whole-exome analysis of pancreatic cancer, which is considered only moderately mutated, indicates 2.64 mutations per megabase. Further disconcerting is the low consistency among mutated genes. For example, certain mutations are observed fairly rarely in pancreatic cancer and are observed in other tissues as well [34]. Additional factors tied to cancer and individual complexities cast long and formidable shadows as well.

Therefore, even though it is broadly believed that precision oncology can improve treatments and prognosis, and that precision data are essential for precision oncology, the consensus is that this may not be enough [35]. Current clinical results do not question the sequence-based hypothesis and strategies, but they do emphasize the need for considering their completeness [36]. The literature thrives with proposed additional clinical considerations, and vital
statistical and network tools are also being developed [37, 38]. One powerful element that we believe is missing is the energy landscape; biomolecules are not static sculptures but interconvert between structures with varying energies [39]. Therefore, on its own, genomic sequence data may not provide the entire information to the oncologist in target selection. If the mutations are in protein coding regions, which is our focus here, then they translate to dynamic protein conformational ensembles and interactions, which in principle, we, as a community, can exploit to accomplish more accurate prognosis.

The low consistency among mutated genes in cancer argues that only translating sequence alterations to heterogeneous ensembles may not be sufficient, and new concepts should be brought to bear. One of these [40] is the notion that we should not only rely on classical categories and definitions of passenger mutations (appearing to confer no survival advantage) and driver actionable mutations (which propel cancer initiation and progression) [41–49]. Passenger mutations whose effects on their own appear insignificant may transform into driver mutations when acting in certain combinations. We dubbed such mutations latent drivers [49]. The low consistency of mutations among genes also argues that focusing on single proteins may be lacking. Additionally, mutations can take place during cancer evolution within the same or different tissues [50].

Cancer disrupts normal physiological tissue homeostasis due to loss of function or gain of function, which can take place in multiple ways [51–82]. Even though the underlying tenet of precision oncology largely rests on the notion that reversing one target will halt cancer, parallel signaling pathways leading to the same cellular outcome, compensatory mutations, and more are not overlooked. These are taken up through combinatorial drug regimens.

The excellent literature reviews and research papers in this area cover clinical and social benefits, as well as genomic sequence analysis, pattern identification, and approaches and/or corroboration of target discovery. This latter category produces vital software tools [83–96]. Recently, functional advances were reviewed as well [97]. Below, following a background, we relate to precision oncology from a different standpoint. Our premise is the sensitivity of the free energy landscape to its environment [98–104]. We view actionable mutations within this framework, as well as their consequences for cell-specific signaling networks, and finally, we comment on how our community can help.

**Definition: “Protein space” and “protein conformation”**

The theme of the paper is the change of population of states caused by various factors, including mutations. Within this framework, the general terms “protein space” and “protein conformation” refer to “energy states.” Protein space and conformations are discussed as having components as energy, conformation, and dynamics.

**Precision pharmacology**

Is precision medicine, or its precision pharmacology abettor, a “hype” or is it real? And if it is, is it attainable? First off, the precision medicine initiative is not aimed at developing “personalized drugs,” which is infeasible. Instead, pharmacogenomics studies are carried out on groups of patients, and the drugs are tested in clinical trials, which inherently involve patients. Therefore, personalized drug development is not a priori for an individual; instead, the underlying premise is that it could be incorporated into cancer treatments fitting certain individuals, with the expectation that within that population, individual responses will vary. Because certain genetic variants in individuals can interfere with drug processing, the Food and Drug Administration (FDA) recommends genetic testing before giving a chemotherapy drug, as in the case of mercaptopurine (Purinethol) in acute lymphoblastic leukemia. Most drugs developed
within this framework are in oncology. Still, only approximately 13% of the oncology drugs approved since Herceptin in 1998 have an FDA-approved personalized Companion Diagnostic, that is, laboratory analysis and clinical evaluation [105]; however, since 2016, 29 out of 30 with FDA-approved personalized companion diagnostics assays are in oncology. A major reason that most drugs that have been developed are in oncology is that the assays that are involved largely reflect single point mutations, as in the case of KRAS [106–131]. This makes the clinical result easier to interpret, as compared to more complex diseases involving multiple disease-associated genes.

Pharmacogenomics aims to develop effective and safe drugs for patients bearing specific genetic signatures. It integrates pharmacology and genomics, with the premise that a person’s response to a drug will depend on their genetic makeup. Whereas next-generation sequencing aims to identify driver mutations and molecular targets, pharmacogenomics aims to forecast how a person’s genetic makeup will affect their response to the drugs. Classically, drugs were developed for diseases with the assumption that a drug works similarly for all patients. By contrast, precision pharmacology countered the “one size fits all” notion, and because all physician treatments are personal, the personalized medicine concept has been renamed precision medicine [132–134].

One example of precision pharmacology is the breast cancer drug trastuzumab (Herceptin) discussed above, which works only for tumors involving human epidermal growth factor receptor 2 (HER2)-overexpression and/or -amplification (Fig 1A). A second example is the chemotherapy drug mercaptopurine (Purinethol) against acute lymphoblastic leukemia (Fig 1B). Patients with a genetic variant that impedes processing of this drug can suffer from severe side effects. Chemotherapy drugs gefitinib (Iressa) and erlotinib (Tarceva) show improved outcome in patients with certain lung cancer genetic change. In contrast, chemotherapy drugs cetuximab (Erbitux) and panitumumab (Vectibix) (Fig 1C and 1D) do not present good efficacy in 40% of colon cancer patients with certain genomic alteration. To be successful, pharmacogenomics data need not only be extensive but also go beyond one gene for one disease with one technology platform at a time. Precision pharmacology implies that rather than screening for compounds with broad action against a disease, genomic information should guide drug development for subgroups of patients with specific genetic profiles.

Data point to a need for enhancing the theory underlying the precision oncology framework. The elucidation of the human genome sequence [135] and identification of alterations that drive distinct cancers take place at an increasingly rapid pace, revealing additional genomic variants and more complex scenarios [132, 136]. Unlike the earlier imatinib and trastuzumab, the targeted therapies that were discovered recently are not as specific, suggesting drugs that might target such complexity are becoming available. This inspired the “actionable mutation” terminology, unraveling additional candidate targets [61, 90].

Despite the success of imatinib in chronic myeloid leukemia [137] (Fig 2A), and subsequently in gastrointestinal stromal tumors [138], which propelled optimism (OSI Pharmaceuticals 2002 Annual Report), progress is slow and stymied by failures. The concept of precision medicine is attractive; but the challenges are daunting. Among these are the multiple genes that can be involved, as well as the observations that tumors evolve to accumulate additional genomic alterations, and that targeting a specific actionable mutation may only be moderately effective. Data science aims to uncover these mutations through broad sequencing of samples, and statistical analyses correlate these with clinical observations. Within this framework, identifying predominant conformational states of the actionable driver mutations may only partially alleviate the dilemma; detecting which mutations, or combinations of mutations, that do not fall into the statistically actionable definition can also shift the landscape from the inactive to the active states (or vice versa) is a laudable aim.
The complex frameworks of precision oncology

Cancer genomes are complex. Precision oncology tailors ways to surmount this complexity and achieve increased precision \cite{139}. The key question it strives to surmount is how. The
Fig 2. Examples of chemotherapy drugs. Molecular structures of (A) imatinib, (B) vemurafenib, and (C) dabrafenib. Imatinib is used for chronic myelogenous leukemia and acute lymphocytic leukemia. Both vemurafenib and dabrafenib are drugs for the treatment of melanoma targeting the B-Raf^{V600E} mutant. In the structure, C, N, and O atoms are denoted as white, blue, and red spheres, respectively. Hydrogen atom is represented as an edge of stick. Other heavy atoms, S, Cl, and Fe atoms, are marked on the sphere. Molecular topologies with the coordinates are generated by using Avogadro software [412].

https://doi.org/10.1371/journal.pcbi.1006658.g002
clinical view is that laid-out frameworks should contend with validation. It advocates increasingly complex diagnostic tests, innovative methods for evaluating efficacy, and review of what evidence should be considered adequate, especially for patients with genomic abnormalities whose clinical implications are unclear. The clinics endorse and urge large-scale profiling of advanced-stage tumors to recognize genomic alterations and large cohorts of patients to support therapeutic strategies, despite this contradicting the basic patient-tailored tenet of precision medicine. An additional element that the clinics contend should be rectified is the absence of databases classifying the risks and possible advantages of a specific plan for the patient. The clinics also recognize that a tissue-specific diagnosis requires understanding of cancer biology, clearly defined driver alterations, and drug combinations. In those examples above of successful clinical trials (for example, trastuzumab in HER2-positive breast cancer [140, 141] (Fig 1A), erlotinib and gefitinib in non-small-cell lung cancer with epidermal growth factor receptor (EGFR) mutations [142, 143] (Fig 1B), imatinib in Bcr-abl1-fusion-positive chronic myeloid leukaemia [144–146] (Fig 1B), and vemurafenib and dabrafenib in advanced-stage melanoma with B-RafV600E mutation [147] (Fig 2B and 2C)), there was no conflict between the essence of the trials and the definition of precision oncology. The genomic alterations were diagnosed in many patients with these cancers, along with the markers and companion diagnostic tests.

Because tumors are heterogeneous, include genetically divergent subclones, and with time acquire drug resistance, frameworks that target a single mutation may not work long term, which argues for combinatorial drug regimes. This however has its own concerns, because drug combinations increase toxicity, including that arising from drug—drug interactions, which may require dose modulation, potentially limiting their effectiveness [148, 149]. The possible presence of subclones, especially in advanced stages, as well as tumor evolution that may have been influenced by prior treatments, also argue for genomic sequencing of multiple biopsy samples. In line with this, only a minority of treated patients show clinical improvement, and in those cases, it is only for a short time.

A framework for precision oncology would also need to account for cancer cell metabolism. Oncogenic drivers and cancer-cell metabolism have been linked, suggesting that cancer cells may be sensitive to metabolic interventions. Oncogenes rewire cellular metabolism, and this rewiring of the metabolic interactions among tumor cells and between tumor and nontumor cells in the tumor microenvironment has been deemed druggable. For example, KRAS mutations drive metabolic alterations in colorectal cancer contributing to cell adaptation to glutamine depletion by up-regulation of asparagine synthetase [150]. Targeting nutrient transport and utilization can critically affect the tumor cell energetic needs [151]. However, the intricacy of the tumor microenvironment and the dynamic circadian metabolism question the precision of targeting cancer metabolism. Nonetheless, recent progress has raised hope that the tumor microenvironment may be precisely targeted [152].

The overall complexity challenges the clinics; it also challenges our community as to how to devise reasonable frameworks to make a difference. In one example of this complexity, lung adenocarcinoma histopathology was classified by metastatic potential and survival; however, in the absence of molecular profiles, the underlying explanation has been unclear. Subtypes mutational screening of the EGFR, KRAS, and BRAF genes revealed that KRAS and BRAF mutants are more abundant in higher grades, but EGFR driver mutations appear in all subtypes pointing to the difficulties in prognostically identifying key mutations [51, 153]. Genome-wide gene copy number aberrations and somatic mutation statistics suggest that cancer types emerged through distinct pathways. Therefore, identification of aberrations that impact key signaling pathways in cancer cells is a necessary component in development of frameworks for targeted therapeutics. Furthermore, the oncogene addiction concept,
supported by observations on human cancer cell lines, mouse tumor models, and clinical cancer studies [154, 155], argues that signaling that emerges because of single constitutively activated protein can be adaptively rewired, which suggests that a single transformed oncogene can have disastrous consequences on the cancer cell.

The broad range of potential driver mutations, their diverse molecular environments, and the multiple possible targets reinforce the perception that charting a path forward is fraught with doubts. The first step should enable converting batches of raw sequencing data into interpretable results; in its absence, substantial collected data, which may be related to the clinical or research question [3, 156] may not be considered. Even though clinical oncology—related bioinformatics methods and software are being developed [157], the dynamic multifactorial environment, coupled with the need for rapid, highly reproducible and robust procedures make a comprehensive bioinformatics toolkit difficult to achieve. A workflow of a molecular tumor board, which includes bioinformatics tools ranging from the analysis of profiling data to automated formulation of clinical report, has been described and implemented in several clinical trials, helping in the treatment decision-making. Even though such software support provides treatment recommendations, and bioinformatics tools and data platforms are essential to complement advances in precision oncology, their underlying foundation is restricted and fall short of what is required. We believe that one key missing component is a better understanding of the biology. Insight into the underlying biological mechanisms will help in better defining “actionability” for molecular targets [158]. The complexity of the framework is further amplified by intercellular signaling and cellular heterogeneity. A comprehensive framework of precision oncology requires an integrative, holistic approach (Fig 3). Within this fabric, multiscale approaches to systems biology may help address the competing and reciprocal nature of processes underlying cancer.

The concept of driver mutations

Effective medical care requires accurate information. Current treatment is mostly based on identification of the patient’s driver mutations [51, 85]. Classically, a mutation is catalogued as either a driver or a passenger. Passenger mutations are taken as unrelated to the disease and thus are not considered actionable; driver mutations are. A major question has been how to distinguish between a passenger mutation and a driver mutation. This question is particularly challenging given the heterogeneity of somatic mutations in tumor cells, as well as practical considerations related to sample collection, which include populations of normal and tumor cells [69, 159]. The clonal theory of cancer [160] postulates that all cells in the tumor evolved from a single stem cell and that the driver mutation arose in that cell and propagated in successive clonal expansions. Three strategies have been proposed to define driver mutations in genomic data: (i) detection of frequent mutations through sequence analysis; (ii) predicting the functional influence of the mutation (i.e., activation and/or inactivation); and (iii) the impact of the mutation and combinations of driver mutations on signaling pathways and statistical correlations. The output may depend on how the background mutation rate (BMR) is computed and analyzed. The BMR varies not only across the genome of a given patient but also across patients and tumor types [161]. Because computing somatic mutational frequencies (normalized by that expected by chance) appears the most straightforward as compared to functional and cellular network impact assessments, most studies adopt this strategy to distinguish driver mutations from passenger mutations. In the simplest approach, the BMR of a gene is assumed as known, and the probability of passenger mutations is then estimated [162, 163]. However, BMR and mutational frequencies estimates are inaccurate and error prone. Therefore, the resulting inaccurate classification of the mutations can lead to false positives.
Fig 3. A flowchart representing the comprehensive framework to precision oncology. Here, our focus is the process of the interpretation of the driver mutation using the databases in cancer biology, cancer pathways, and the distribution of the ensembles of protein conformation. The next process to match the driver mutations against drug databases and known drug-target interactions is not discussed in this paper.

https://doi.org/10.1371/journal.pcbi.1006658.g003
Heuristic approaches based on protein sequences, i.e., the so-called 20/20 rule [46] classifies a gene as an oncogene if over 20% of its mutations are at a certain residue [164]. Clustering of mutations not only in protein sequences [165] but also in structures [166] have also been proposed, as well as other driver gene identification approaches [167]. Protein structural features included in the analysis can consist of predicted amino acid propensities for secondary structure, solvent accessibility, backbone flexibility, and more [45]. As examples of methods using genomic sequences and structures, cancer-specific high-throughput annotation of somatic mutations (CHASM) exploits supervised machine learning to prioritize somatic missense mutations detected in tumor sequencing [168]. CHASM trains a random forest classifier on driver mutations from the catalogue of somatic mutations in cancer (COSMIC) databases. The nucleotide substitutions that are detected in the tumor are used to identify passenger mutations. A nonsynonymous mutation is described by several quantitative features and physicochemical properties, scores obtained from multiple sequence alignments (of the protein or the DNA), residue composition in the sequence window where it falls, local structural characteristics, and more. Following the ranking of the predicted driver mutations, based on vectors of features, CHASM can predict how the mutation acts in tumorigenesis. The subsequently developed cancer-related analysis of variants toolkit (CRAVAT) is a suite of tools to interpret nonsynonymous mutations, including their mapping, annotation, impact, interpretation, and possible structural consequences [169]. Some software tools, such as Quaternary Protein Amino acid Clustering (QuartPAC) [170], identify statistically significant mutational clustering in 3D protein space, i.e., energy states, through combining available protein structures and mutational data in the COSMIC database. Recently, ParsSNP, an unsupervised functional impact predictor that is guided by parsimony was introduced [171]. ParsSNP identified many known and likely driver mutations that were missed by other methods. A detailed list of currently available tools for personalized cancer medicine is provided in Table 1.

Analysis of clusters, or spatial proximity of rare mutations, in 10,000 tumor exomes detected over 3,000 rare mutations and catalogued them as potentially driver mutations [172]. Among them, those in \textit{RAC1} and \textit{MAP2K1} were validated. These not only confirm the paradigm that statistics can identify driver mutations but also that driver mutations often cluster. This tendency for proximal associations can suggest cooperativity, enhancing their effects, similar to the observation of a tendency of residue hot spots to cluster into “hot regions” [173, 174]. This comprehensive study analyzed over a million somatic missense mutations in 11,119 human tumors in 32,445 protein structures from 7,390 genes. Well-established drivers such as KRas\textsuperscript{G12D} were observed, but most residues in the clusters are rare mutations, such as KRas\textsuperscript{D33E} (defined as observed in <0.1% of the sample) [175]. Many are oncoprotein such as KRas (KRAS), B-Raf (BRAF), and p53 (\textit{TP53}). All are cataloged and available. The authors note that in their analysis that most of these rare mutations appear to be passenger mutations, but some may be hitherto unknown drivers [175]. Cluster analysis in protein structures also revealed rare missense driver mutations in tumor suppressors including \textit{PTEN}, \textit{CDH1}, and \textit{KEAP1}, which may inactivate the proteins. \textit{PTEN} tumor suppressor yielded 15 clusters totaling 48 residues (with 2 established drivers and 46 rare mutations). All are around the phosphatase catalytic core motif [176]. Because the respective experimental structures are available, these can serve as a benchmark for testing methods for predictions of rare driver mutations, or latent driver mutations, exploiting conformational sampling and analysis, including allosteric.

When mutated, hundreds of genes can drive cancer development. However, a surprisingly small number of driver mutations can already suffice. For lung and colorectal cancers, mathematical modeling has estimated the number to be three [177]. However, other estimates suggest 1 to 10 mutations with the number of mutations driving cancer varying considerably across different cancer types; four mutations per patient on average drive liver cancers,
Table 1. Computational and bioinformatics tools for personalized cancer medicine.

| Names                          | Description                                                                 | Website                                                                 | References |
|--------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|------------|
| **Somatic mutation callers**   |                                                                             |                                                                         |            |
| MuTect                         | Sensitive detection of somatic point mutations from exome and genome sequencing data. | https://github.com/broadinstitute/mutect                              | [413]      |
| GATK                           | Detection of variants from high-throughput sequencing data.                  | https://software.broadinstitute.org/gatk/                               | [414]      |
| VarScan                        | Somatic mutation and copy number alteration detection from exome sequencing data. | http://varscan.sourceforge.net/                                        | [415]      |
| Strelka                        | Somatic small-variant detection from sequenced tumor-normal sample pairs.   | https://github.com/Illumina/strelka                                      | [416]      |
| **Sequence-based predicting functional consequences of genomic variants**   |                                                                             |                                                                         |            |
| PolyPhen-2                     | A popular tool for predicting the functional impacts of protein sequence variants based on naive Bayes classifiers. | http://genetics.bwh.harvard.edu/pph2/                                    | [417]      |
| SIFT                           | A popular tool for predicting the biological effect of missense variations using protein sequence homology. | http://sift.bii.a-star.edu.sg/                                            | [418]      |
| CHASM & SNVbox                 | Python and C programs for predicting cancer-related mutations based on their tumorigenic impact. | http://wiki.chasmsoftware.org/                                           | [419]      |
| MutationAssessor               | Predicting functional impact scores based on evolutionary conservation patterns. | http://mutationassessor.org/r3/                                          | [93]       |
| CADD                           | A tool for scoring the deleteriousness of single nucleotide variants as well as insertion and/or deletions variants. | http://cadd.gs.washington.edu/                                           | [217, 420]|
| **Mutational Clustering tools on Protein Structures**                        |                                                                             |                                                                         |            |
| CLUMPS                         | Assess the significance of mutational clustering in a given 3D structure.    | NA                                                                      | [421]      |
| HotSpot3D                      | Detect mutation—mutation and mutation—drug clusters using 3D protein structures. | https://github.com/ding-lab/hotspot3d                                   | [422]      |
| SGDriver                       | Detect mutation clustering on protein—ligand binding site residues using a Bayes inference statistical framework. | NA                                                                      | [423]      |
| AlloDriver                     | Detect mutation clustering that alters dysregulation of protein allosteric sites. | NA                                                                      | [186]      |
| KNMPx                          | Detect mutation clustering that rewire phosphorylation-related signaling networks and drug sensitivity and/or resistance. | NA                                                                      | [193]      |
| ReKINect                       | Detect network-attacking mutations in phosphorylation-based signaling networks. | http://rkeinect.science/                                                | [226]      |
| Cancer3D                       | Identify potential cancer drivers or pharmacogenomic biomarkers using protein structure information. | http://cancer3d.org/                                                     | [424]      |
| **Sequence-based predicting cancer driver genes**                            |                                                                             |                                                                         |            |
| MutSig & MutSigCV              | Prediction of significantly mutated genes from heterogenous tumor exome sequencing data. | http://software.broadinstitute.org/cancer/software/genepattern/modules/docs/MutSigCV | [425]      |
| MSEA                           | Prediction of hotspots using mutation set enrichment analysis.              | NA                                                                      | [426]      |
| 20/20 rule                     | Evaluating the proportion of inactivating mutation and recurrent missense mutations in a gene. | NA                                                                      | [46]       |
| 20/20+                         | A machine-learning based, 20/20 rule by integrating multiple features of positive selection. | NA                                                                      | [84]       |
| ActiveDriver                   | Finding cancer driver proteins with enriched mutations in post-translational modification sites. | https://cran.r-project.org/web/packages/ActiveDriver/                   | [427]      |
| OncodriverFM & OncodriverFML   | Identifying driver genes based on functional impact bias.                  | http://bg.upf.edu/oncodriver                                            | [428]      |
| OncodriverClust                | Identify driver genes with a significant bias towards mutation clustering within the protein sequence. | http://bg.upf.edu/oncodriverclust                                       | [429]      |
| MuSiC                          | A driver gene detection framework by quantifying correlation of clinical data with mutation sites, affected genes and pathways. | http://gmt.genome.wustl.edu/                                             | [430]      |
| **Network-/pathway-based prediction of cancer driver genes**                 |                                                                             |                                                                         |            |
| HotNet2                        | A tool for detecting mutated subnetworks in cancer using an insulated heat diffusing algorithm. | http://compbio.cs.brown.edu/projects/hotnet2/                            | [431]      |

(Continued)
whereas colorectal cancers typically require about 10 [178]. Furthermore, the genes vary in the proportion of drivers versus passengers.

**Interpreting variants in precision genomic oncology**

To date, interpretation of the mutational landscape is essentially based on software packages. Among these is mapping genetic variations to 3D protein structures [179]. Ultimately, accurate translation of personal genomics to precision medicine requires factual interpretation of numerous genetic individual variants. Even though the structures of proteins with nonsynonymous mutations can be predicted, the functional implications may or may not be clear. The consequences of mutations in enzyme active sites or at protein—protein interfaces are more straightforward to interpret. In one example, mapping glioblastoma missense mutations on the human structural protein interactome observed that even though some missense mutations overstabilize protein complexes, most of them are destabilizing. In particular, mutations on interfaces indicated larger changes of amino acid physico-chemical properties than those occurring elsewhere. This allowed identification of potential driver genes and additional possible cancer [180]. This, however, is often not the case, and allosteric effects are often at play [99, 181–189]. Millions of genetic variants have been catalogued in genomic databases, and the Protein Data Bank (PDB) documents the structures of thousands. If a homologous structure is available, it is now straightforward to model the others. This strategy permits mapping the mutations, testing how these mutations affect protein stability or its interactions, and thereby guide drug discovery.

Recently, a comprehensive and critical overview and resource compilation for interpreting variants in precision genomic oncology applications has been published [24]. Small variants are defined as single nucleotide variants or short (up to approximately 20 base pairs) insertions and/or deletions. The authors outline a precision genomic oncology application scheme to high-throughput sequencing [190]. Their aim is to facilitate bedside decisions, while at the same time emphasizing the challenge embedded in the interpretation of individual variants. The essential question they aim to address is how to integrate statistical identification of variants with biological data. Toward this, they compile and develop variant catalogs, databases of actionable mutational variants, and software and tools for variant annotation. They also assemble databases for predicting the impact on protein function. They detail how to exploit this collection, either in individual cases or integrated, to be used for interpreting clinical results and acting on them at the bedside. Drug—target interaction resources have also been compiled into a so-called Cancer Targetome along with assessment of supporting evidence [191].

**Table 1. (Continued)**

| Names   | Description                                                                 | Website                                                                 | References |
|---------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|------------|
| NBS     | A somatic mutation network-based approach for stratifying tumor mutations. | http://chianti.ucsd.edu/~mhofree/NBS/                                   | [432]      |
| DawnRank| Prediction of cancer genes in a single patient based on the PageRank         | http://bioen-compbio.bioen.illinois.edu/DawnRank/                       | [433]      |
| VarWalker| Identifying putative cancer genes using personalized mutation network       | https://bioinfo.uth.edu/VarWalker.html                                   | [434]      |
| DriverNet| Predicting driver mutations by estimating their effect on mRNA expression   | http://compbio.bccrc.ca/software/drivernet/                               | [435]      |
| TieDIE  | Predicting cancer mutated subnetworks using a network-based diffusion       | https://sybiodata.soed.ucsc.edu/tiedie                                    | [436]      |

https://doi.org/10.1371/journal.pcbi.1006658.t001
Molecular alterations that act as drivers of cancer can also be ranked by integrating genomic and transcriptomic profiles. One example is HIT’nDRIVE. Its goal is to identify a set of patient-specific, sequence-altered genes that, when combined, can alter dysregulated transcripts [192]. When applied to 2,200 tumors from four types of cancer, it discovered potentially actionable driver genes and predicted drug efficacy. In a different approach, KNMPx identifies oncogenic alterations that rewire phosphorylation-related signaling networks [193]. This method considers the functional consequences of somatic missense mutations at phosphorylation sites observed in pancancer analysis. By integrating 746,631 missense mutations in approximately 5,000 tumor samples from 16 cancer types, KNMPx obtained 170,000 nonredundant phosphorylation sites in 18,610 proteins with 47 enriched with missense mutations at their phosphorylation sites. The mutations identified by KNMPx in tissue-specific kinase-substrate interaction modules were observed to relate to survival and sensitivity and/or resistance of inhibitors targeting epidermal growth factor (EGF), mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), mammalian target of rapamycin (mTOR), and Wnt signaling pathways. Integrating genomic and transcriptomic analyses benefits precision oncology [5], as does the integration of cancer genomics and clinical oncology [194]. To date, there are several computational approaches based on such integration to detect drivers and potential genetically suspect regions (e.g., [14, 195, 196]). Driver mutations affecting CBL ubiquitin ligase activation were identified through a new approach [197] and a method, MutaBind, for estimating and interpreting the effects of sequence variants on protein—protein interactions was developed as well as cellular networks [198].

Genomic analysis can identify patients with high tumor mutational burden as well as recurrent regulatory mutations, such as in the case of mismatch repair endonuclease PMS2 [199]. Personal genomics can also help in classification and thereby precision treatment. One recent example is pediatric oncology [200]. Pediatric high-grade gliomas (HGGs) have been treated following adult regimens with minimal clinical benefit [201, 202]. Even though phenotypically indistinguishable, next-generation sequencing unraveled alterations different from those of adults [203], including somatic mutations in histone genes H3F3A (replication-independent histone 3 variant H3.3) and histone 3 [204].

Altogether, interpreting gene copy number, transcriptome and proteome levels, epigenetic alterations, somatic mutational statistics, all within the framework of cancer types, as well the effects of drugs, are key hurdles challenging the investigator.

**Software tools are important but insufficient**

The need to predict and annotate mutational variants in individual genomes to accurately identify molecular targets is pressing. The surging tide of big data, machine learning, and data analytics has spawned the sweeping impression that efficient and capable programs can resolve most questions, and it may not be necessary to go deeper into the underlying biology [205]. Recent surveys vindicate the increasing appreciation and passion for powerful software development. Among the computational needs are high-performance computing, bioinformatics, workflows, and up-to-date analysis codes and/or servers and data integration with the belief that data science components and skills are necessary for in-depth understanding of processes within and between cells [206]. Machine learning can be a shortcut to discover correlations between processes at different scales. However, in biology and medicine approaches based solely on big data forsaking a conceptual account can fail [205]. Regardless of their sophistication, know-how, and cleverness, eventually, artificial intelligence methods work by fitting. If the data is “big,” they can be statistically reliable. However, when trying to capture and predict rare events, they may fail. Biological knowledge and conceptual insight should integrate with
big data—based methods to obtain more accurate and dependable conclusions on complex systems, like those needed here.

Software can analyze genomic, proteomic, microarray, and clinical data [206–210]. If the data is massive, it is possible to correlate the results with experimental observations [211, 212]; however, results based wholly on statistics cannot explain why the correlations are observed. A statistical result is the outcome of the many parameters that go into the training of the software. Even though strategies like bootstrapping and leave-one-out cross-validation can help in pinpointing the key variables, the multiple parameters still cloud the interpretation, diminishing the accuracy of predictive precision medicine [213]. However, powerful genomics, transcriptomics, and proteomics software can couple with computational, theoretical, and experimental structural biology. This compelling combination can more effectively unravel the underlying mechanisms of alterations in protein coding regions, and their interactions with other proteins, lipids, DNA and RNA, and drugs. If resources are available, computations can reach mechanistic details that are currently challenging for experiments to deliver. Integrative multiscale approaches can increasingly be applied across a range of scales to offer and test theories and concepts, and notably, the predictions and observations that they make are quantified.

Precision oncology would benefit from merging genetic data and analysis with the underlying structural foundation. Structures not only help understand genetic aberrations, but they can also innovate hypotheses and offer mechanisms, helping in more accurate tailored treatments. Methods that predict the effects of mutations on protein stability, the hydrogen bond network, pH dependence, conformational dynamics, and protein function have been usefully compiled [214]. Below, we discuss their potential effects on the free energy landscape of the proteins, how driver mutations can affect them as compared to passenger mutations, and introduce the concept of “latent drivers.” Annotation and prediction methods based on structural and biophysical information have also been compiled [215].

**Functional assays of actionable variants: Tip of the iceberg**

Bioinformatics analysis and computational strategies offer important information for identifying hot spot mutations and significantly mutated genes in cancer. However, these analyses cannot provide definitive proof of causal mechanisms to permit transfer of basic discoveries directly to the point of care. An experimental measure of functional consequences of mutations or cancer genes is crucial to decipher the tumorigenic mechanisms and to identify novel therapeutic targets. Here, we briefly describe several experimental strategies to explore the functional consequences of mutations altering tumorigenesis, disease progression, and drug responses.

**Protein expression altered by somatic mutations**

Several antibody-based pipelines have been used to measure protein expression profiles altered by somatic mutations in cancer. Reverse-phase protein assay (RPPA) technology is a common protein microarray that uses antibodies to measure the relative expression levels of proteins in tissues or cells (Fig 4A). Ng and colleagues developed a moderate-throughput functional pipeline to inspect the functional impact of over 1,000 genomic alterations, including gene amplifications, point mutations, indels, and gene fusions [216]. They used two growth factor—dependent cell models and functional proteomic signaling profiles (RPPA) for selected alterations. They showed that this functional assay approach can be used to identify weak drivers. However, most RPPA datasets commonly involve approximately 200 antibodies that measure the total proteins or specific protein post-translational modifications, such as phosphorylation.
Alvarez and colleagues reported a network-based inference algorithm, a virtual inference of protein activity by enriched regulon analysis (VIPER), for functional characterization of somatic mutations in cancer [217]. They applied VIPER to investigate the functional relevance of genetic alterations in regulatory proteins in The Cancer Genome Atlas (TCGA) samples. Combining in vitro assays, they demonstrated that VIPER outperformed traditional mutational analysis in evaluating the sensitivity of targeted cancer agents by inferring protein activity. Inferring protein expression (activity) following genomic alterations is increasingly emerging as an optimal biomarker for identifying therapeutic targets in cancer. However, mRNA expression cannot reliably predict protein abundance differences among tumors [218]. Proteogenomics, which integrates proteomics, genomics, and transcriptomics, offer novel tools for understanding cancer biology [219]. Zhang and colleagues reported the first proteogenomic analysis of 95 TCGA colon tumor samples using liquid chromatography—tandem mass spectrometry; RPPA, reverse-phase protein assay.

https://doi.org/10.1371/journal.pcbi.1006658.g004

Fig 4. Examples of experimental strategies to explore the functional consequences of mutations altering tumorigenesis, disease progression, and drug responses. These include (A) RPPA technology, (B) integrated proteogenomic analysis, (C) high-throughput gateway-compatible enhanced yeast two-hybrid, and (D) CRISPR-Cas9 genome editing. LC-MS/MS, liquid chromatography—tandem mass spectrometry; RPPA, reverse-phase protein assay.
mass spectrometry (LC-MS/MS)-based shotgun proteomic approach [218]. Via integration of sequencing, transcriptomic, and methylation data available from TCGA, they showed that in colon cancer, proteogenomic analysis enabled finding novel candidate driver genes compared to a sequencing-only approach [218]. Subsequent proteogenomic analysis helps elucidate the functional consequences of somatic alterations and predicts clinical outcomes in multiple cancer types, including breast cancer [220] and high-grade serous ovarian cancer [221]. In summary, integrated proteogenomic analysis (Fig 4B) offers novel insights into genomic alterations, including novel biomarkers and therapeutic targets for understanding cancer biology and treating cancer, such as that shown by the Clinical Proteomic Tumor Analysis Consortium (CPTAC) project [218, 220, 221].

**Cellular networks can be modified by somatic alterations**

Genomic alterations can change cellular network perturbations [222, 223], such as protein—protein interactions (PPIs), protein—DNA interactions, and protein—metabolite interactions [224]. Several large-scale functional assay platforms have been used to elucidate these interaction changes. For example, Sahni and colleagues showed that most disease mutations do not impair protein folding or stability using a luminescence-based mammalian interactome mapping (LUMIER) assay [225]. However, using the high-throughput gateway-compatible enhanced yeast two-hybrid (HT-eY2H) (Fig 4C), orthogonal in vivo Gaussia princeps luciferase protein complementation (GPC), and the enhanced yeast one-hybrid (eY1H) assays, they found surprisingly widespread disease-specific perturbations of PPIs or protein—DNA interactions [225]. For example, using the integrated assays, they observed that the EGFR L858R mutant can interact with heat shock protein 90 (HSP90) [225]. In addition to PPIs, protein post-translational-modification signaling network (i.e., phosphorylation sites) are often altered in cancer. Creixell and colleagues reported a novel algorithm, ReKINect, to detect network-attacking mutations in phosphorylation-based signaling networks [226]. Using ReKINect, their quantitative analysis of (phospho-)proteomes of five ovarian cancer cell lines and other available tumor sequencing profiles experimentally identified several network-attacking mutations that alter specific switches, analogous to de novo appearance of kinases within the kinome [226]. In summary, the experimentally identified network perturbations altered by genomic changes offer novel diagnostic tools and therapeutic targets for more personalized treatments (i.e., PPI inhibitors).

**CRISPR-Cas9 genome editing**

Owing to their high efficacy (i.e., low off-target effects) and ease of use compared with other tools, clustered regularly interspaced short palindromic repeat-associated 9 (CRISPR-Cas9) systems have become the most popular genome-editing tool (Fig 4D). CRISPR-Cas9 offers multiple ways to functionally inspect whether a mutation is a causal tumorigenic mechanism. In principle, CRISPR-Cas9 knock-in of alternate mutations (i.e., alleles of missense mutations) into cellular or animal models, generates wild-type and variant models that are isogenic, i.e., of matched genetic background. As the only difference between the models is with respect to the missense mutations, in principle any tumorigenic differences observed between the models can be attributed to the single missense mutations. For example, homology-directed repair-mediated KRAS G12D mutations using CRISPR-Cas9 lead to macroscopic tumors with adenocarcinoma pathology, revealing that Cas9 mice empower a wide range of biological and disease modeling applications [227]. Recently, a guide RNA and catalytically impaired CRISPR-Cas9 were used to convert A-T base pairs to G-C in the genome, enabling the editing of single point mutations without inducing double-stranded DNA breaks [228]. Gebler and colleagues first
reported the inactivation of cancer mutations using CRISPR-Cas9 that combines comprehensive single-guide RNA (sgRNA) design and an efficient reporter assay [229]. However, it is challenging to design unique sgRNAs for every mutation using additional orthogonal CRISPR-Cas systems, and control the subsequent repair after Cas9-mediated DNA cleavage. Moreover, a potential risk of oncogenic phenotypes owing to possible off-target cleavage has to be evaluated in vivo prior to a clinical setting in cancer patients. The resulting large-scale functional data can be combined with computational strategies and clinical knowledge for the development of integrative approaches for accurate pathogenicity predictions.

An ensemble view of precision oncology

An introduction to the protein landscape

A protein executes its function when it populates a distinct conformational state. The landmark work of Frauenfelder, Slijard, and Wolynes [230] portrayed protein ensembles in terms of the free energy landscape. The landscape idea is compelling because it describes the mapping of all possible conformations, native and nonnative, that the protein can populate as a function of their corresponding energy levels, on a two (or three)-dimensional Cartesian coordinate system. The picture that the landscape paints of the protein states is powerful because it clarifies the physical basis of the ensemble around the native state of the protein. That picture captures the ensemble under a certain set of conditions. However, the picture is static; as such it is unable to account for biological function, which is based on cooperativity. This led us to propose that the landscape is dynamic, and that the relative distributions of the conformational states can change in response to intra- and extramolecular events, and that this is the origin of cooperativity, i.e., allostery [231, 232]. These events can be binding (noncovalent, e.g., ligands, lipids, proteins, water, and nucleic acids or covalent, e.g., mutations, post-translational modifications [PTMs], such as phosphorylation, farnesylation, palmitoylation, and ubiquitination) [233], changes in temperature, pH, and other environmental events [234]. The outcome of these events is a shift of the populations of the states, which we termed "population shift" (Fig 5). This results in redistributions of the populations of the states. Therefore, an allosteric effector exerts its functional consequence by shifting the ensemble from a highly populated inactive state to a highly populated active state or vice versa [235].

Allostery is vital for biological processes at all stages. We proposed a unified view of allostery, which integrates three perspectives: thermodynamics, free energy landscape of population shift, and structural—all with exactly the same allosteric descriptors [236]. The novelty of the unified perspective rests in its linkage of these elements. The thermodynamic perspective quantified the binding of the ligand to the active (or inactive) states by assigning experimentally measurable quantities. The free energy landscape presents the population shift (Fig 5) in terms of energy indicating the relative stabilization (or destabilization) of the two biologically relevant active (or inactive) states. The third structural component, links the first two elements, the thermodynamic and the free energy landscape perspectives, pointing to the propagation pathway between the protein active and allosteric sites. In the absence of events eliciting the population shift, the pre-existing propagation pathway does not implicate allostery; it simply points to a route between the two sites that implies that they are coupled [237]. This point is important because numerous studies focus on discovering the pathways between the two sites. Further, relevant to our point here, conformational studies of allosteric driver mutations often seek to reveal pathways linking the mutation with the active site [238–241]. Also, relevant to mutational landscapes populated by allosteric driver mutations is that the dynamic free energy landscape posits that all possible conformations that a protein can populate pre-exist, including those of conformers harboring driver mutations [234, 242–244]. An allosteric effect would
merely cause changes in the extent that these conformations are populated. An inactive protein populates its inactive state. When activated via an allosteric driver mutation, it conformation populates the now more stable active state.

Over the years, three hypotheses were proposed to describe the mechanism of protein binding. The “lock and key” theory, the protein was rigid. Therefore, its binding required that its partner has a shape that matches precisely the protein’s binding site. The “induced fit” theory hypothesized that the shape of the protein when it binds to its partner can differ from the one observed in the bound state in the complex. That is, the binding process itself induces a conformational change for optimal fit. Neither of these can explain how allosteric driver mutations can exert their oncogenic effects. Both theories overlooked the physical foundation of the free energy landscape, i.e., that in solution the protein exists in a large ensemble of dynamically interconverting states, and that populations can be redistributed, as is the case in the presence of allosteric mutations (or PTMs). Based on this notion, we theorized that the binding process can be described by a “conformational selection and population shift” [231, 245, 246]. Our concept implied that rather than evolving to support new functions, as in the case of allosteric driver mutations, distinct functions can make use of pre-existing conformations, with the requirement that their population needs to be increased [247]. This change may involve breaking and/or creating new interactions, which driver mutations in cancer encode. The “conformational selection and population shift” idea explained that because the landscape is populated by many conformations, binding will take place via those which are most compatible, with a minor conformational rearrangement for optimal fit [248]. Because the binding conformers are removed from solution, the ensemble will shift (“a population shift”) toward this state to retain the equilibrium. Population shift, which redistributes the conformational states, underlies allostery and regulation. It can clarify how allosteric driver mutations can alter signaling in cancer [101, 249] and links fundamental physicochemical principles and biological processes like signaling. By constitutively increasing the population of the active conformation, allosteric driver mutations can promote oncogenic signaling.
In line with this view, strong correlations between folding and/or binding free energy changes and probability given mutations to be disease-causing were observed [250], and the effects of mutations on protein structure, dynamics, and energy landscape were reviewed in this light [251].

**The free energy landscape and driver mutations: The kinase example**

Mutant kinase provides one example of the shift of the ensemble from the inactive to the constitutively active state, linking precision oncology to the free energy landscape via an allosteric driver mutation [249]. Kinases are vital to the life of the eukaryotic cell, accomplishing a broad range of functions [252]. They are also often involved in oncogenic signaling and are predominant molecular targets in precision oncology. Their catalytic action involves three steps, binding of ATP and the substrate, which has a Ser, Thr, or Tyr residue, transfer of the γ-phosphate of ATP to an accurately aligned hydroxyl group of the respective catalytic residue, and release of the phosphorylated substrate and ADP. ADP release is the rate determining step [253]. The conformational requirement of optimal phosphate transfer dictates the highly similar active conformations of the catalytic domain of all kinases [254, 255] (Fig 6A). By contrast, because different ligands bind and activate the diverse kinases, the inactive conformations vary [256].

In eukaryotic protein kinases (EPKs), hydrophobic contacts and electrostatic interactions accurately orient and coordinate the catalytic residues in the active conformation. The αF-helix organizes the conserved αC-helix, Gly-rich loop, catalytic loop and activation segment (Mg-binding loop, activation loop, and P+1 loop) through the regulatory-spine (R-spine), catalytic-spine (C-spine) [257], and electrostatic interactions (Fig 6A). Among the key features that differentiate between the two, active and inactive states, are the extended conformation of the activation loop, a specific salt-bridge and an organized R-spine observed in the active αC-helix-In but not in the inactive αC-helix-Out conformation (Fig 6B). Kinase activation commonly involves a rotation and shift of the αC-helix between these states, a conformational change that is governed by an allosteric switch. Driver mutations can control this switch. Allosteric inhibitors abutting the ATP binding site act by capturing the inactive αC-helix-Out state [258]. In many receptor tyrosine kinases (RTKs), juxtamembrane or the C-terminal can cis-autoinhibit catalysis. Finally, not only the structural features differentiate between the two states; the flexibility between the two lobes that are connected by a hinge linker is restrained in the active state [259–262]. Under normal physiological conditions, the catalytic core domain populates the inactive state [263]. Oncogenic driver mutations act by either stabilizing the active or disrupting interactions that stabilize the inactive conformation. Both scenarios shift the ensemble to the active state. Forty-one percent of the EGFR mutations in lung cancer present the oncogenic Leu858 driver mutation [101, 264]. This driver mutation stabilizes the αC-helix-In active conformation. By contrast, the T790M mutation in EGFR, T315I in Bcr-abl, T334I in c-Abl, T341I in Src, T670I in c-Kit (also known as CD117), and T674I in platelet-derived growth factor receptor α (PDGFRα) stabilize the hydrophobic R-spine, which destabilizes the inactive conformation (Fig 6B). The outcome is similar: the mutations allosterically switch the preferred states toward a constitutively activated kinase.

EGFR can exist as a monomer, and symmetric (inactive) and asymmetric (active) dimers (Fig 7A). The autoinhibited monomer and symmetric dimer are inactive states. Under physiological conditions, activation and signaling initiate upon ligand binding to the extracellular domain (Fig 7B). This promotes a conformational rearrangement, which is transmitted through the transmembrane domain to the juxtamembrane. The resulting conformational change stabilizes the active conformation, shifting the population from the otherwise
predominant inactive state toward the active asymmetric dimer. Driver mutations can mimic the physiological ligand, with a similar, albeit constitutive, signaling outcome. Driver mutations can achieve such activation through three scenarios. In the first, the T790M driver mutation, which introduces a hydrophobic residue, stabilizes the active monomer conformation through the R-spine, which increases the population of the asymmetric dimer. The L858R driver mutation works via a second scenario. This mutation replaces a hydrophobic residue by a charge in the hydrophobic core, thus abolishing interactions that stabilize the inactive state, also leading to a shift toward the active conformation. The L858R mutation therefore works by destabilizing the inactive symmetric dimer interface versus the asymmetric dimer. However, in a third scenario, NMR and simulations indicate that the L858R mutation can also adopt a second mechanism, stabilizing the αC-helix-In conformation similar to the T790M driver mutation resulting in asymmetric dimer formation (Fig 7B). Taken together, driver mutations can adopt one of three mechanisms: destabilize the
inactive state, stabilize the active state, or both. When viewed on the energy landscape, these can be depicted by the changes in the relative depths of minima, in this case with the outcome of favoring and/or disfavoring the asymmetric or symmetric dimer conformation [264–280].

**The structural basis of oncogenic driver mutations: The KRAS example**

Like kinases, in the cell, Ras proteins are normally in the inactive state. They are activated following an incoming signal from RTKs. Activation involves exchange of guanosine

---

**Fig 7. EGFR dimerization and activation.** (A) Model for ligand-induced homodimerization of EGFR. The inactive EGFR monomer forms a symmetric dimer in the inactive state. Ligand binding to the extracellular domain shifts the population to the active EGFR dimer. (B) EGFR or ligand binding causes an extended conformation of the extracellular domain, promoting a conformational rearrangement through the transmembrane to juxtamembrane domains [263, 270]. This results in an asymmetric assembly of the kinase domains. In the cartoons, crystal structures of extracellular domain (PDB code: 5XWD), transmembrane domain (PDB code: SLV6), and kinase domain (PDB code: 2GS7) were used to model the inactive monomer and symmetric dimer. The active asymmetric dimer model employs crystal structures of extracellular domain (PDB code: 3NJP), transmembrane/juxtamembrane domain (PDB code: 2M20), and kinase domain (PDB code: 2GS6). Spheres in the C-terminal tail represent tyrosine (Y) and phosphorylated tyrosine (pY) residues. The mutant kinase domain cartoon employs crystal structure of kinase domain with T790M/L858R mutant (PDB code: SEDP). EGFR, epidermal growth factor receptor; PDB, protein data bank. [https://doi.org/10.1371/journal.pcbi.1006658.g007](https://doi.org/10.1371/journal.pcbi.1006658.g007)
diphosphate (GDP) by guanosine triphosphate (GTP) by an exchange factor (a GEF) (Fig 8A). GDP-bound Ras proteins are inactive; GTP-bound are active [182, 281, 282]. Ras activation takes place at the membrane [283–285]. Active Ras can activate its effectors; key among these are Raf and PI3K. Their activation stimulates tumor proliferation pathways [189, 286–289]. Unlike kinases, the intrinsic catalytic activity of Ras is very low, orders of magnitude lower than GTPase-activating protein (GAP)-assisted catalysis. Hydrolysis of GTP to GDP, with the help of GAP, switches Ras back to the inactive, resting state. Catalysis requires appropriate positioning and orientation of the respective catalytic residues of Ras and GAP with respect to each other. Oncogenic mutations in Ras suppress catalysis by hindering this coordination. Mutations are common in RAS-driven human cancers [114]. The different catalytic scenarios bespeak of the differential modes of action of oncogenic mutations between the kinases and the Ras proteins [182, 290]. Even though in both cases the eventual outcomes are activated signaling, in the case of the Ras proteins, this is by suppressing, not promoting, catalysis. Driver mutations in Ras block the hydrolysis, resulting in constitutive activation. Therefore, unlike in the kinases where a distinct single active conformation with precise distances and orientations among the catalytic groups is achieved by the driver mutations through different mechanisms, in Ras, the mutations lead to multiple states, all of which can suppress catalysis.

Ras has three major isoforms: HRas, Nras, and the splice variants KRas4A and KRas4B. The amino acid sequence of their catalytic domain (residues 1–166) is highly similar, but their hypervariable regions (HVRs) vary [291], as well as their populated conformational states [292]. Hydrophobic PTMs decorate the HVRs and are essential for Ras anchoring in the plasma membrane. Despite the high sequence similarity of the catalytic domains across isoforms, and the fact that the oncogenic mutations are in this domain, their frequency and distribution vary. KRas is the most highly mutated (86%), compared to other isoforms—NRas (11%) and HRas (3%) (Fig 8B); 98% of the mutations are found at the active site Gly12, Gly13, and Gln61, obstructing GTP hydrolysis [114, 293]. KRas Gly12 mutations (89%) are the most frequent, Gly13 (9%), and Gln61 (1%). They are highly mutated in other isoforms as well, including variants, such as G13D (7%) and Q61H (0.6%). Conformational analysis indicated that mutations can change the landscape of proteins [51, 99, 290, 294–306], and this has been shown for HRas, KRas, and NRas [307–311]. Work has especially centered on how key driver mutations, such as those involving substitutions of Gly12, Gly13, and Gln61, abolish GAP-catalyzed hydrolysis [312–315]. In addition, all driver (or other) mutations, wherever they occur, including functional sites as in the case here, have allosteric effects. This has been shown in the case of Q61L in HRas, in which the mutation also allosterically influences the interaction with Raf Ras binding domain (RBD). The timescales (approximately 1 ns) of early molecular dynamics simulations focusing on GAP-mediated hydrolysis were limited, but they did point out that Gln61 of HRas and Arg789 of GAP are vital in GTP hydrolysis [316, 317]. Nonetheless, their brevity limited their ability to observe the influence of GAP binding on Ras conformational transitions. NMR experiments observed that in solution, GppNHp-bound HRas has two interconverting conformations, inactive and active. In a similar vein, simulation of GTP-bound KRas showed that in solution, GTP-bound KRas4B exists in the inactive and active states [182]. The active state of KRas4B in the simulation resembles that of HRas.

Simulations of the GTP-bound wild-type KRas4B complexed with GAP indicated that GAP not only inserts its arginine finger Arg789, aligning the catalytic Gln61 with Arg789 for GTP hydrolysis but also helps stabilize the active state [182]. This arginine finger insertion is thwarted by Ras driver mutations such as those involving Gly12, Gly13, and Gln61 (Fig 8A). In the highly flexible Switch II in the GAP-free state, only a few H-bonds between Gln61 and the GTP γ-phosphate are picked up in the simulations; however, when GAP-bound, the Switch II fluctuations are reduced, permitting formation of the H-bonds. Oncogenic driver mutations
disrupt the coordination of the arginine finger Arg789 and the catalytic Gln61, blocking GTP hydrolysis.

The simulations also offer detailed scenarios of how distinct oncogenic mutations differentially suppress catalysis by disturbing the catalytically competent arrangements of Gln61 and Arg789 [182]. Analysis of the snapshots indicates that in the G12C mutant, Arg789 moves away from GTP, preventing the formation of an H-bond between the side chain NE2 atom of Gln61 and the γ-phosphate of GTP; in other mutants, Arg789 still retains its salt-bridge interactions with α- and γ-phosphates of GTP. However, in G12D and G12V, the Gln61 OE1 atom (which extracts hydrogen from the catalytic water) moves away from the γ-phosphorus of GTP; in G13D, the interaction between Gln61 and GTP is lost; in Q61H, His61 cannot coordinate the catalytic water due to distance and/or angle change. Overall, Gly12/Gly13 mutations disturb Gln61 side chain. Mutations can redistribute conformational substates [99, 305, 306], as the KRas simulations of oncogenic mutants also indicate. They can also alter the dynamics. The simulations indicate that G12D, G12V, G13D, and Q61H are more likely to shift the GTP-bound KRas4B ensemble toward the active state than G12C. G12C and G12D trigger larger conformational changes of the GDP-bound state of KRas4B than G12V, G13D, and Q61H. Furthermore, G12C and G12D not only shifted the distribution with large conformational

Fig 8. Ras activation and oncogenic mutations. (A) KRas4B is activated by the son of sevenless 1 (SOS1) nucleotide exchange factor (GEF), while GAP inactivates KRas4B via the GTP → GDP hydrolysis. KRas4B oncogenic mutations at the active site Gly12, Gly13, and Gln61 aborts the hydrolysis reaction, keeping the Ras in a constitutively active GTP-bound state. (B) Of those with mutant Ras, KRas is the most highly mutated in cancer. In KRas mutation, Gly12 mutations are the most frequent. (C) The interactions of KRas4B with the anionic membrane composed of DOPC:DOPS (4:1 molar ratio). In the active GTP-bound state, the HVR is in contact with the membrane, but the catalytic domain is away, exposing the effector binding site. In contrast, autoinhibition persists in the inactive GDP-bound state with occluded catalytic domain conformation, yielding the effector binding site is inaccessible. In cartoons, the modeled KRas4B structures were adopted from our simulations [182, 285]. DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPS, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; GAP, GTPase-activating protein; GEF, Guanine nucleotide exchange factors; HVR, hyper variable region; SOS1, son of sevenless 1.

https://doi.org/10.1371/journal.pcbi.1006658.g008
changes but also elicited larger exposure of the nucleotide-binding site, which would facilitate
the nucleotide exchange thus activation. Notably, the G12D mutation is the most frequent in
KRas4B driven cancers [114] (Fig 8B). Taken together, oncogenic mutations disturb the
proper organization of the catalytic residue Gln61 and Arg789 of GAP, impairing GTP hydro-
lysis, thus retaining Ras in the catalytically active state (Fig 8A). Furthermore, as noted above
for HRas Gln61 mutation, even mutations that are directly at functional sites, such as Ras’
Gly12, Gly13, and Gln61 are likely to have additional allosteric effects that would affect their
interactions.

Active GTP-bound KRas interacts with the plasma membrane through its farnesylated
HVR [285, 318–323]. In vitro and in silico observations suggest that membrane-attached full-
length wild-type GDP-bound KRas4B is autoinhibited by its HVR, which is sandwiched
between the effector binding site and the membrane [285] (Fig 8C). However, in the GTP-
bound state, the interaction weakens, releasing the attachment of the HVR to the catalytic
domain. This results in the catalytic domain fluctuating uncontrollably at the membrane with
the effector binding surface becoming available. NMR residue chemical shift perturbation
analysis and simulations observed that oncogenic mutations shift the landscape toward the
exposed effector binding site, with the HVR membrane attached. Therefore, driver mutations
can work in multiple ways to both block GTP hydrolysis and ease effector binding.

In resting cells, GDP-bound KRas is inactive, with the equilibrium constant of $k_{\text{off}}$ (unre-
leased) significantly larger than the $k_{\text{on}}$ (released) [324]. In the GTP-bound state, the HVR
detaches from the catalytic domain, the farnesyl anchors into the membrane and the catalytic
domain can bind its effectors. Oncogenic mutants weaken the HVR interactions with the cata-
ytic domain, increasing the fluctuations of the catalytic domain and thus the population of the
exposed site, versus the wild type.

Low frequency drivers and the concept of “latent driver” mutations

Mutations are classified as being “passengers” or “drivers.” However, biology educated us that
it does not pursue a binary “Yes” or “No” game plan. In line with physics and chemistry, con-
ditions matter and changes in the environment will alter the functional outcome. Therefore,
the question is then should allosteric mutations be distinguished based on such an unyielding,
inflexible, and resolute line?

The free energy landscape suggests that this binary distinction paradigm may not be accu-
rate [40]. As such, driver mutations may be misclassified and not accounted for in cancer treat-
ments [51]. Two issues are at play. For the first, the mutation acts allosterically, except that its
prevalence is low. That is, the frequency of the mutation may not surpass a certain threshold to
be classified as a driver; however, it acts as a conformational switch, shifting the ensemble of
the protein molecule from highly populated inactive to highly populated active state or vice
versa. In principle, such mutations can be revealed by NMR or, e.g., through long timescale
molecular dynamics simulations. The challenge is which mutation to test out of the many low
frequency ones. Detailed analysis of the protein structure may provide clues based on the loca-
tion, type of residue substitution, and if multiple structures exist, through protein structure
comparisons. For the second, the mutation is conformationally “silent”; its frequency is low,
and its effect on the ensemble minor. However, should some other cooperative “silent” muta-
tion take place during cancer development, together their effect on the ensemble can be signifi-
cant. Like driver mutations, together they can turn on (or off) a signaling pathway [247].
Unravelling such “hidden” or “latent driver” mutations among the population of the passenger
mutations, and their looming partners, is a compelling aim. Their discovery would extend the
standard statistics-based prediction defying the binary Yes/No driver/passenger classification
paradigm. As such it can inspire new effective treatments. The free energy landscape theory argues that actionable mutations are not limited to drivers. Even though statistically, and conformationally, a passenger mutation does not correlate with a clinical observation, when coupled with another allosteric effector, such as mutation, pathogen protein, or PTM, it can become actionable. Their underlying mechanisms can be AND (i.e., additive) and graded logic gate integration mechanisms (but not OR) [325].

To understand how an apparent somatic passenger mutation can turn into a driver, we mull over cellular allosteric scenarios [102]. Signaling is often not the outcome of one event; it reflects a combination of events. These can include binding of other protein factors, small molecules, ions, membrane lipids, water molecules, as well as PTMs [233, 326]. A silent mutation can couple with a newly appearing mutation, which is also silent, or with other allosteric events due to changes in the environment in the tumor cell, such as drug or radiation effects. The mutational landscape of cancer is complex, and it is reasonable to expect that among the newly evolved mutations, there will be some that can act cooperatively with existing ones.

Therefore, even though detection of actionable mutations should rest on informatics platforms and clinical trials, fundamental chemical physical considerations of the principles of the behavior of proteins in the cell argue that the annotation should also account for latent drivers that act cooperatively. It is unclear how many patients would benefit from improved identification of actionable mutations incorporating latent drivers; nonetheless, the premise of precision oncology contend that they should not be overlooked. One example is non-hotspot Akt1 mutations that confer constitutive membrane localization acting like the E17K driver mutation [327] (Fig 9), indicating that treatment decisions based only on genetics may overlook vital actionable components. As we discuss below, others include switching pathways in drug resistance. “Latent driver” mutations can also offer an explanation to how impeding an addicted growth pathway can result in rewiring the oncogenic network within short time frames in drug resistance, as well as bear on the question of the rapid evolution of cancers. In both cases, pre-existing, silent, presumably “passenger” mutations partner with newly evolved ones. Identification of a patient’s pre-existing latent drivers can powerfully equip the oncologist with foresight, helping to anticipate drug resistance and decide on drug regimes. The latent drivers’ concept calls for reassessment of genetics-based analysis; however, because their detection requires residue combinations, considerably more genomic sequence data is essential for reliable prediction. Structural data as well as powerful sampling protocols can be invaluable in annotating these mutations.

That non-hotspot, lower frequency mutations presumed passengers may indeed be functional drivers as illustrated by three examples: (i) The Akt1 pleckstrin homology (PH) domain mutants, L52R, C77F, and Q79K, increased membrane localization of Akt 1 and activated it like the E17K driver did (Fig 9). (ii) In contrast, the PH domain mutants, D32Y, K39N, and P42T, at the interface between the PH and kinase domains abolished the interactions with the membrane, which stabilized the inactive Akt conformation [51, 328]. (iii) The third involved four rare mutations in KRas [329]. Two (K117N and A146T) resemble drivers, one (L19F) presented weakened phenotype, and one (R164Q) resembled wild-type K-Ras. Notably, KRasD33E is also a rare driver mutation [330].

The conformational basis of targeting in cancer

Exploiting allosteric drugs in precision oncology rests on the premise that even though each driver mutation shifts the ensemble from an inactive to a specific active site conformational state, the conformations of the proteins will vary to a certain extent. This can have two possible consequences: either the location of the allosteric cavity will vary, or more likely, its detailed
conformation will differ, resulting in distinct drug preference at the allosteric pocket. This underscores the challenge that precision pharmacology faces in developing allosteric driver mutation-specific drug.

Not all driver mutations are allosteric. Even though no statistics were carried out, it is reasonable to expect that most of the identified, statistically significant driver mutations are not allosteric. These are in the active, or functional site, even though these too will have allosteric effects. Accordingly, drugs can be orthosteric or allosteric. Here our focus is on allosteric drugs. Although the principles of allosteric drugs actions differ from those of orthosteric drugs, to date, allosteric drug discovery has largely mimicked orthosteric drug design protocols [331].
Allosteric drugs are more specific; therefore, they are safer. Orthosteric drugs are competitive. They block active sites, turning off protein activity; allosteric drugs act by shifting the population of the active site, impeding its binding to substrates. Allosteric drugs are modulators of function. They can enhance or reduce activity. The structural/chemical difference between allosteric drugs that bind at the same allosteric site and lead to opposing effects can be very small, highlighting the conformational sensitivity in precision oncology [49, 332]. The key determinant of allosteric drugs is the extent of stabilization of the active (or inactive) conformation. This contrasts with orthosteric drugs whose key determinant is affinity [333]. To shift the conformation of the active site, the structures of allosteric drugs include “anchors” and “drivers” [49]. In the allosteric site, the binding of the drug “anchor” atom(s) stabilizes the protein conformation and does not change it as the protein transitions from the inactive state to the active. By contrast, the perturbations caused by the “driver” component “push” or “pull” actions propagate in the protein structure, shifting the conformation to the active (or inactive) state. Allosteric drugs can be noncovalent or covalent. As we discuss below, a recent KRas example epitomizes a specific covalent allosteric action against the G12C driver mutation [116]. Whether noncovalent or covalent, allosteric drugs are uniquely fit to not only target drivers but also latent drivers in precision pharmacology.

The residence times of allosteric drugs in the allosteric pocket, and thus time span of their action, are decided by the drug’s affinity and concentration; however, these do not decide the type of outcome (agonist or antagonist) nor its extent. These are determined by the specific drug—protein interactions [49]. Anchor drug atoms bind to a populated active (or inactive) allosteric site into which their shape and chemistry snugly fit; by contrast, the drug’s driver atoms either sterically collide with protein atoms, in which case they repulsively “push” them, or when attractive, they “pull” them. Anchor atoms stabilize the protein conformation but do not initiate allosteric propagation. On the other hand, the driver atoms’ “pull” or “push” actions lead to allosteric propagation that initiates at these points in the allosteric site and results in a conformational change of the active site. The shape changes it will cause are determined by the distinct push and/or pull actions, which depend on the type of atoms and/or atom groups that execute them.

Despite their advantages, allosteric drugs also present challenges [331]. Critical among these are identification of the mutation-specific allosteric cavity [334–349], which is often transient [350, 351] as well as its conformational details, the drug’s driver atoms, and their protein contacts. These will promote the desired allosteric communication. Correct identification is critical, because a misassignment can convert a drug from an agonist to an antagonist. Further, a minor population of the allosteric cavity can imply low affinity, requiring larger drug concentration, thus higher likelihood of toxicity. How to increase drug potency is also unclear. In the case of orthosteric drugs, there are no such conformational hurdles. Experimental structures can provide the active site structures in atomistic detail. These impediments mirror the nature of a drug that aims to tune a conformational ensemble to precisely and potently shift the ensemble toward a specific active site shape. Allosteric drugs exert their action by selecting one—active or inactive—state [49]. An agonist selects an active state, stabilizes it, and increases its population; inverse agonist selects an inactive state, and an antagonist either state. Whether agonist or antagonist, the interactions that anchors form are likely to be unchanged between the active and inactive states, which is not the case for drivers. A minor conformational change at the allosteric site of the inactive conformation by “pushing” a destabilizing collision or an attractive “pulling” of protein atoms can lead to a populated active state with large conformational change. Ras catalytic domain can provide an example. Ras is activated via the exchange of GDP by GTP (Fig 8). It is therefore reasonable that the γ-phosphate acts as the driver. When tested on the GNP-bound versus the GDP-bound Ras crystal structures, two oxygen atoms in
the γ-phosphate of GNP were identified as the driver. Their pulling stabilizes the active conformation, with a minor conformational change [49]. By contrast, the crystal structures of protein kinase, phosphoinositide-dependent protein kinase-1 (PDK1) exemplifies a pushing driver. PDK1 is implicated in signaling pathways, which are frequently altered in cancer, such as PI3K and Akt, Ras and MAPK, and Myc (Fig 10), with several identified driver mutations [352].

Ras also provides an example of covalent, irreversible small molecule drug specifically targeting G12C driver mutation in the GDP but not GTP bound state [333]. Crystallography discovered a small pocket that was not seen in earlier crystal structures, just under the Switch II region (Fig 11). The covalent inhibitors linked to the cysteine, influenced the Switch I and Switch II regions, resulting in the active site favoring GDP rather than GTP, thereby inhibiting Raf’s interaction, thus MAPK signaling [116]. To overcome the challenge in development of covalent inhibitors at flexible sites, covalent docking was compared with empirical electrophile screening for the flexible KRas G12C site, discovering a new, irreversible covalent compound. That compound altered the inactive, GDP-bound state of the G12C mutant, surprisingly accelerating, rather than reducing, SOS-mediated GDP by GTP exchange, thus activation [353].

Further exploration of disulfide tethering of a nonnatural cysteine (KRas<sup>M72C</sup>) detected a new compound (2C07) that binds at the Switch II pocket in the GTP- (or GppNHp) bound state and hampers SOS nucleotide exchange. The attachment of the compound to HRas (at M72C) revealed a new transient cavity. The compound's binding altered Switch II and by abolishing key polar interactions, drove Switch I further from the GTP. Subsequent design successfully modified the compound such that it could bind both nucleotide states. The ability of the compound to bind to GTP-bound Ras is significant, because in this state Ras activates its effectors, and this is the predominant oncogenic form [354]. This further illustrates the sensitivity of the ensemble, in which a covalent change increases the population times of pre-existing—albeit earlier not captured—pockets near flexible sites.

**Cellular networks**

Mutations can inactivate or constitutively activate pathways, as demonstrated for frequent mutations in cancer, such as in <i>BRAF</i> and <i>PIK3CA</i>, that play casual roles in tumorigenesis. Both orthosteric and allosteric driver mutations and drugs influence cell signaling [103]. Highly oncogenic proteins are often key cellular nodes that link several pathways. Examples include the p53 [355, 356], the Toll-like receptor [357], and Ras proteins [358–361]. Central nodes are hub proteins, which interact with multiple partners [362]. Commonly, interactions take place through shared binding sites [363]. For example, the interactions of Ras with Raf, PI3K, Ras guanine nucleotide dissociation stimulator (RalGDS), Ras association domain-containing protein 5 (RASSF5, also known as NORE1A), and additional effectors are via the same β-sheet surface motif [364]. Multiple factors determine which interaction occurs at any given time, including the effective local concentration, association with, and organization at the plasma membrane, PTMs, and more, which decide and coordinate cell signaling. Wild-type Ras binds and activates Raf, turning on the MAPK pathway; it also binds and activates PI3Kα, turning on the PI3Kα/Akt/mTOR pathway (Fig 10). A KRas molecule binds one effector at a time at a shared site [364]. The affinities of the effectors binding at the same site vary [365]; binding of wild-type Ras to Raf is in the low nanomolar range (approximately 20 nM), whereas it is in the high micromolar for PI3Kα [109, 285, 366, 367] and high nanomolar affinity (approximately 200 nM) for RASSF5 [368, 369]. If the concentrations of Raf and PI3Kα are comparable, MAPK will take place first, followed by PI3Kα/Akt/mTOR [365]. This order does not only reflect the greater affinity of Ras to Raf but also their differential functions in cell life.
The distinct affinities of the effectors at the same Ras surface reflect the different interaction details.

We consider how a driver mutation can alter cell signaling [370–373]. Because the specific residue interactions of the partners with Ras vary, a driver mutation which is at the active site may directly influence partners’ bindings, weakening (or strengthening) one versus the other. Because an effector activates a specific signaling pathway, this can alter signaling and cell fate. If, however, it is an allosteric driver mutation, it will work by shifting the ensemble. The outcome may be a slightly altered surface shape of the active site, which will disfavor binding to an otherwise preferred effector. In a competitive binding scenario as in this case,
Fig 11. Crystal structures of KRas4B<sub>G12C</sub> and covalent inhibitors. Cartoon (left panel) and surface (middle panel) representations of the crystal structure of KRas4B<sub>G12C</sub>-GDP in complex with covalently linked inhibitors (right panel) of (A) compound 4, (B) compound 9, and (C) compound 16 (PDB codes: 4LV6, 4LYJ, and 4M22, respectively) [116]. In the protein structures, light green, blue, and orange colors denote the P-loop, Switch I, and Switch II regions, respectively. In the compound structures, C, N, and O atoms are denoted as white, blue, and red spheres, respectively. Hydrogen atom is represented as an edge of stick. Other heavy atoms, S, Cl, and I atoms are marked on the sphere.

https://doi.org/10.1371/journal.pcbi.1006658.g011
balanced cell signaling can be disrupted. Therefore, the fallout of a genomic mutation in a protein coding region can quell and trample normal cell signaling through a redistribution of the ensemble.

RASopathies can provide an example of how a covalent linkage of the PTM at specific residue can shift Ras signaling. RASopathies are developmental nononcogenic syndromes commonly caused by germline mutations (rather than somatic mutations found in cancer). They involve dysregulation of the Ras/MAPK pathway. Normal development requires both MAPK and PI3Kα/Akt pathways (Fig 10). In RASopathies, rather than switching between these signaling pathways, the MAPK pathway dominates, stymieing physiological cell cycle regulation. Germline mutations resulting in aberrant ubiquitination are among those documented as associated with RASopathy [374]. Large-scale proteomics [375] identified Ras ubiquitination at lysines 128, 147, and 170 in wild-type HRas. One way through which ubiquitination can work, especially if occurring in the HVR, is impeding membrane anchorage, thus hindering Raf’s activation and MAPK signaling. Therefore, inhibition of ubiquitination would promote MAPK signaling at the expense of PI3Kα/Akt. Furthermore, in line with this, earlier work indicated that inhibiting Ras ubiquitination results in sequestration of Ras on the plasma membrane, promoting its activation, increased levels of extracellular signal-regulated kinase (ERK) 1/2 phosphorylation and lung cancer in cells harboring wild-type Ras [376]. Altogether, this illustrates how alteration of the chemistry of a single residue by relinquishing its charge and adding a bulky group (such as ubiquitin) affect its interaction with the membrane, which can shift signaling to an alternative pathway via effector binding (here Raf rather than PI3Kα) at the same shared site.

The strict definition of precision oncology involves differentiating among distinct driver mutations. A broadened definition may include differentiating among isoforms. HRas, Kras, and NRas have distinct preferred signaling pathways, which is reflected by their patterns of occurrence in specific cancers. Because, however, the sequences of the catalytic domains of the isoforms are highly similar, targeting a specific isoform is challenging. Concomitantly targeting all, as is the practice today, results in high toxicity. This problem is particularly at the forefront for Kras, and especially its Kras4B splice variant, the most highly oncogenic Ras isoform in human cancers. Even though the sequence of the catalytic domains across the isoforms are very similar, the HVR of Kras, but not HRas or NRas, is highly positively charged, populated with several lysines that interact favorably with acidic cell membranes, which is where KRas preferentially locates. Growth factor activation of RTKs triggers many signaling pathways [377] that act in concert in cell proliferation and survival, including the Ras/Raf/MEK/ERK and Ras/PI3K/Akt [249]. Under physiological conditions, all Ras isoforms are activated by stimulated RTK. Oncogenic Ras is activated by its driver mutations, independent of RTK. However, RTK’s role does not end with wild-type Ras activation; it also collaborates with Ras in the activation of PI3Kα. Activation of PI3K requires both binding of PI3K to the C-terminal tyrosine-phosphorylated RTK (e.g., PDGFR) and GTP-bound Ras [378–380]. The Ras binding domain of PI3Kα binds to Ras and gets allosterically activated; however, additionally, RTK’s C-terminal phosphorylated C-terminal motif interacts with the two SH2 domains of the p85 regulatory subunit of PI3Kα, relieving their autoinhibition of the p110 catalytic subunit [381]. In the scenario of oncogenic Ras activating PI3Kα, this interaction is not there; however, uniquely for KRas, phosphorylated Ca2+-bound calmodulin (CaM) can act instead. Because CaM is negatively charged, only the HVR of KRas, but not HRas or Nras, can assist in full activation [382, 383]. The resulting quaternary complex of CaM—KRas—PI3Kα can serve as a KRAS-specific drug target [384].
Molecularly targeted therapy, immunotherapy, and gene therapy

Immunotherapy-mediated precision oncology follows different principles and adheres to distinct guidelines [385]. To date, immunotherapy is selective against certain cancers in which the aberrant protein is reachable and can be manipulated by the immune response. Immuno-modulatory antibodies targeting T-cell coinhibitory receptors CTLA-4 and PD-1 (programmed death-1) have elicited optimism in several cancers in certain patients, stimulating antitumor immune responses and appearing to offer durable disease control. On the downside, the genomic features and clear specific and robust biomarkers in cancer immunotherapy are still incomplete and challenging to obtain, even though there has been progress in identifying the genomic determinants that can affect the response and resistance of the tumor cells to the treatment. Among the genomic determinants are microsatellite instability, gene copy-number alterations (CNA), and mutations that may affect the immunological response. Here we will not elaborate on this venue of precision medicine, nor on gene therapy, except to note that antibody design is an area our community can consider.

Conclusions

Translation of personal genomics to precision medicine is fraught with challenges. The underlying complex biology is still lacking, and despite progress, to date, many cell-specific factors are still unknown. Among the advances, are capable and intelligent computer-science—based algorithms to rank molecular alterations that act as drivers of cancer by integrating genomic and transcriptomic data. Such analyses benefit precision oncology, as do the integration of cancer genomics and clinical oncology. They provide comprehensive statistical assessments coupled with phenomenological descriptions. The underlying mechanistic underpinning can amplify them, expanding and substantiating the predictions of potentially actionable mutations, and molecular targets. Here we clarify the physicochemical basis of driver mutations. We relate the free energy landscape of the oncogenic protein to precision oncology, with the hope that this would inspire and dynamize contributions from the chemistry and biophysics communities. Our aim is to articulate a path forward in an area now dominantly populated by other disciplines, which overlook the fundamental underlying behavior of macromolecules in the cell. Together, the life sciences and physicochemical sciences may secure a better future.

Still, challenges abound [51]. Among these are the changes in patterns of methylation epigenetics that influence chromatin reorganization and thereby gene accessibility and silencing, post-transcriptional regulation of signaling molecules by microRNAs, histone modifications, and alternative RNA splicing [386–388]. At least to date, it is not possible to correct aberrant epigenetic marks in a precise, sequence-specific manner. Another obstacle is posed by cell-specific network dynamics, pathway redundancy, and cross-talk, which can be shifted in distinct cell states. Network science and systems-biology—based approaches, e.g., using machine learning and network science principles [2], can integrate multiple data sources and uncover complex changes in a biological system. However, the underlying complex biology is still lacking, and despite progress, to date, many cell-specific factors are still unknown. Furthermore, from our standpoint here, identifying and cataloging driver, passenger, and pre-existing latent driver mutations is enormously difficult. Beyond supervised machine learning to prioritize somatic missense mutations, scores from multiple sequence (protein or DNA) alignments, local amino acid sequence composition, and static physiochemical properties, identifying specific rare mutations that trigger conformational changes that would influence activity is an important mission. Identifying latent driver mutations is particularly challenging. Not only are they rare, at the tail of the distribution curve, but they involve cooperative mutational effects.
These may require extensive, long timescale molecular dynamics simulations. NMR experiments can also be helpful in testing predictions.

Cancer develops from genetic alterations, which affect the cell through its signaling network. Targeted therapeutics aim to block aberrant deregulated protein signaling, and via drug cocktails regimens, redundant pathways that can take over in drug resistance of surviving cancer cells might be stalled \([389–405]\). Collectively, the basis of actionable mutations and selection of molecular targets should indeed be the informatics definition of driver mutations and precision oncology platforms \([24, 406]\) and the associated clinical trials \([158, 407, 408]\); however, rare drivers and latent drivers argue that, for higher accuracy, these should be considered as well.

The Pan-Cancer analysis of over 9,000 tumors of 33 different types described the landscape of driver mutations or genes \([409]\). It indicated an overwhelming presence of nonsynonymous mutations in at least one significantly mutated gene with 2 to 6 mutations per tumor. This further substantiates the vision of precision therapies, which rests on identification and characterization of the drivers \([410]\). Almost all recurring mutations are highly frequent in different tumor types. To date, the key question is how consistent the correlations between these mutations and therapeutic responses in different cancers are. However, dysregulation mechanisms not considered in the high frequency driver mutation paradigm, such as those in the long tail, may be individually or collectively of high importance too. Classifying them—as well as their combinatorial incidences—may also converge into distinct therapeutic strategies. Our ambition is to reveal these through their underlying conformational mechanisms. The future of precision oncology has been in doubt \([411]\); we believe that its solid genomic basis, coupled with a grasp of its conformational behavior, will help in better matching the critical genomic alterations with the most beneficial available drug combinations.

References

1. Vincent JL (2017) The coming era of precision medicine for intensive care. Crit Care 21: 314. https://doi.org/10.1186/s13054-017-1910-z PMID: 29297399
2. Manem VSK, Salgado R, Affimios P, Sotiriou C, Haibe-Kains B (2017) Network science in clinical trials: A patient-centered approach. Semin Cancer Biol.
3. Wouters RHP, Bijlsma RM, Frederix GWJ, Ausems M, van Delden JJM, Voest EE, et al. (2018) Is It Our Duty To Hunt for Pathogenic Mutations? Trends Mol Med 24: 3–6. https://doi.org/10.1016/j.molmed.2017.11.008 PMID: 29246758
4. Martin SD, Coukos G, Holt RA, Nelson BH (2015) Targeting the undruggable: immunotherapy meets personalized oncology in the genomic era. Ann Oncol 26: 2367–2374. https://doi.org/10.1093/annonc/mdv382 PMID: 26371284
5. Senft D, Leiserson MDM, Ruppin E, Ronai ZA (2017) Precision Oncology: The Road Ahead. Trends Mol Med 23: 874–898. https://doi.org/10.1016/j.molmed.2017.08.003 PMID: 28887051
6. Hampel H, O’Bryant SE, Durrieu S, Younesi E, Rojkova K, Escott-Price V, et al. (2017) A Precision Medicine Initiative for Alzheimer’s disease: the road ahead to biomarker-guided integrative disease modeling. Climacteric 20: 107–118. https://doi.org/10.1080/13697137.2017.1287866 PMID: 28269989
7. Broes S, Lacombe D, Verlinden M, Huys I (2018) Toward a Tiered Model to Share Clinical Trial Data and Samples in Precision Oncology. Front Med (Lausanne) 5: 6.
8. Vargas AJ, Harris CC (2016) Biomarker development in the precision medicine era: lung cancer as a case study. Nat Rev Cancer 16: 525–537. https://doi.org/10.1038/nrc.2016.56 PMID: 27386699
9. Hyman DM, Taylor BS, Baselga J (2017) Implementing Genome-Driven Oncology. Cell 168: 584–599. https://doi.org/10.1016/j.cell.2016.12.015 PMID: 28187282
10. Prasad V (2016) Perspective: The precision-oncology illusion. Nature 537: S63. https://doi.org/10.1038/537S63a PMID: 27602743
11. Tannock IF, Hickman JA (2016) Limits to Personalized Cancer Medicine. N Engl J Med 375: 1289–1294. https://doi.org/10.1056/NEJMab1607705 PMID: 27682039
12. Voest EE, Bernards R (2016) DNA-Guided Precision Medicine for Cancer: A Case of Irrational Exuberance? Cancer Discov 6: 130–132. https://doi.org/10.1158/2159-8290.CD-15-1321 PMID: 26851184

13. Yu L, Li K, Zhang X (2017) Next-generation metabolomics in lung cancer diagnosis, treatment and precision medicine: mini review. Oncotarget 8: 115774–115786. https://doi.org/10.18632/oncotarget.22404 PMID: 29383200

14. Nakagawa H, Fujita M (2018) Whole genome sequencing analysis for cancer genomics and precision medicine. Cancer Sci 109: 513–522. https://doi.org/10.1111/cas.13505 PMID: 29345757

15. Hsieh JJ, Le V, Cao D, Cheng EH, Creighton CJ (2018) Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 244: 525–537. https://doi.org/10.1002/path.5022 PMID: 29266437

16. Del Vecchio F, Mastroiacco V, Di Marco A, Compagnoni C, Capece D, Zazzeroni F, et al. (2017) Next-generation sequencing: recent applications to the analysis of colorectal cancer. J Transl Med 15: 246. https://doi.org/10.1186/s12967-017-1353-y PMID: 29221448

17. Low SK, Zembutsu H, Nakamura Y (2018) Breast cancer: The translation of big genomic data to cancer precision medicine. Cancer Sci 109: 497–506. https://doi.org/10.1111/cas.13463 PMID: 29215763

18. Forrest SJ, Geoerger B, Janeway KA (2018) Precision medicine in pediatric oncology. Curr Opin Pediatr 30: 17–24. https://doi.org/10.1097/MOP.0000000000000570 PMID: 29189430

19. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT, Montironi R (2018) Molecular testing to inform melanoma treatment decisions: a move towards precision medicine. Mod Pathol 31: 24–38. https://doi.org/10.1038/modpathol.2017.104 PMID: 29148538

20. Collins FS, Varmus H (2015) A new initiative on precision medicine. N Engl J Med 372: 793–795. https://doi.org/10.1056/NEJMp1500523 PMID: 25635347

21. Prasad V, Gale RP. What Precisely Is Precision Oncology—and Will It Work? 2017. http://www.ascopost.com/issues/january-25-2017/what-precisely-is-precision-oncology-and-will-it-work/. [cited 2017 Jan 25].

22. Sohal DP, Rini BI, Khoraana AA, Dreicer R, Abraham J, Procop GW, et al. (2015) Prospective Clinical Study of Precision Oncology in Solid Tumors. J Natl Cancer Inst 108.

23. Garraway LA (2013) Genomics-driven oncology: framework for an emerging paradigm. J Clin Oncol 31: 1806–1814. https://doi.org/10.1200/JCO.2012.46.8934 PMID: 23589557

24. Tsang H, Addepalli K, Davis SR (2017) Resources for Interpreting Variants in Precision Genomic Oncology Applications. Front Oncol 7: 214. https://doi.org/10.3389/fonc.2017.00214 PMID: 28975082

25. Le Tourneau C, Delord JP, Goncalves A, Gavoille C, Dubot C, Isambert N, et al. (2015) Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 16: 1324–1334. https://doi.org/10.1016/S1470-2045(15)00188-6 PMID: 26342236

26. T PA, M SS, Jose A, Chandran L, Zachariah SM (2009) Pharmacogenomics: the right drug to the right person. J Clin Med Res 1: 191–194. https://doi.org/10.4021/jocm2009.08.1255 PMID: 22461867

27. Drew L (2016) Pharmacogenetics: The right drug for you. Nature 537: S60–62. https://doi.org/10.1038/s41573-016-0020-z PMID: 27082742

28. Dickmann LJ, Ware JA (2016) Pharmacogenomics in the age of personalized medicine. Drug Disc Today Technol 21–22: 11–16.

29. Schaffhausen J (2017) What Precisely Is Precision Medicine? Trends Pharmacol Sci 38: 1–2. https://doi.org/10.1016/j.tips.2016.11.004 PMID: 27955860

30. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. (2013) Mutational landscape and significance across 12 major cancer types. Nature 502: 333–339. https://doi.org/10.1038/nature12634 PMID: 24132290
34. Gale RP. Recent Progress and Concepts in Pancreatic Cancer 2016. http://www.ascopost.com/ issues/november-25-2016/recent-progress-and-concepts-in-pancreatic-cancer/. [cited 2016 Nov 15].

35. Felsenstein KM, Theodorescu D (2018) Precision medicine for urothelial bladder cancer: update on tumour genomics and immunotherapy. Nat Rev Urol 15: 92–111. https://doi.org/10.1038/nruro.2017.179 PMID: 29133939

36. Tao JJ, Schram AM, Hyman DM (2018) Basket Studies: Redefining Clinical Trials in the Era of Genome-Driven Oncology. Annu Rev Med 69: 319–331. https://doi.org/10.1146/annurev-med-062016-050343 PMID: 29120700

37. Van Waes C, Musbah O (2017) Genomics and advances towards precision medicine for head and neck squamous cell carcinoma. Laryngoscope Invest Otolaryngol 2: 310–319. https://doi.org/10.1002/lio2.86 PMID: 29094075

38. Shukla HD (2017) Comprehensive Analysis of Cancer-Proteogenome to Identify Biomarkers for the Early Diagnosis and Prognosis of Cancer. Proteomes 5: 28.

39. Nussinov R, Wolynes PG (2014) A second molecular biology revolution? The energy landscapes of biomolecular function. Phys Chem Chem Phys 16: 6321–6322. https://doi.org/10.1039/c4cp90027h PMID: 24608340

40. Nussinov R, Tsai CJ (2015) ‘Latent drivers’ expand the cancer mutational landscape. Curr Opin Struct Biol 32: 25–32. https://doi.org/10.1016/j.sbi.2015.01.004 PMID: 25661093

41. Greenman C, Stephens P, Smith R, Daigleish GL, Hunter C, Bignell G, et al. (2007) Patterns of somatic mutation in human cancer genomes. Nature 446: 153–158. https://doi.org/10.1038/nature05610 PMID: 17344846

42. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. (2008) Somatic mutations affect key pathways in lung adenocarcinoma. Nature 455: 1069–1075. https://doi.org/10.1038/nature07423 PMID: 18948947

43. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321: 1801–1806. https://doi.org/10.1126/science.1164368 PMID: 18772397

44. Huang S, Ernberg I, Kauffman S (2009) Cancer attractors: a systems view of tumors from a gene network dynamics and developmental perspective. Semin Cell Dev Biol 20: 869–876. https://doi.org/10.1016/j.semcdb.2009.07.003 PMID: 19595782

45. Carter H, Chen S, Isik L, Tyekucheva S, Velculescu VE, Kinzler KW, et al. (2009) Cancer-specific high-throughput annotation of somatic mutations: computational prediction of driver missense mutations. Cancer Res 69: 6660–6667. https://doi.org/10.1158/0008-5472.CAN-09-1133 PMID: 19654296

46. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW (2013) Cancer genome landscapes. Science 339: 1546–1558. https://doi.org/10.1126/science.1235122 PMID: 23539594

47. Jones JA, Byrd JC (2014) How will B-cell-receptor-targeted therapies change future CLL therapy? Blood 123: 1455–1460. https://doi.org/10.1182/blood-2013-09-543092 PMID: 24394667
55. Fratta E, Sigalotti L, Covre A, Parisi G, Coral S, Maio M (2013) Epigenetics of melanoma: implications for immune-based therapies. Immunotherapy 5: 1103–1116. https://doi.org/10.2217/imt.13.108 PMID: 24088079

56. Sette C (2013) Alternative splicing programs in prostate cancer. Int J Cell Biol 2013: 458727. https://doi.org/10.1155/2013/458727 PMID: 23983695

57. Sutton LA, AgathangeliD A, BelessI C, Darzentas N, Davi F, Ghia P, et al. (2013) Antigen selection in B-cell lymphomas—tracing the evidence. Semin Cancer Biol 23: 399–409. https://doi.org/10.1016/j.semcancer.2013.07.006 PMID: 23932942

58. Misso G, Zappavigna S, Castellano M, De Rosa G, Di Martino MT, Tagliaferri P, et al. (2013) Emerging pathways as individualized therapeutic target of multiple myeloma. Expert Opin Biol Ther 13 Suppl 1: S95–109.

59. Garagnani P, Pirazzini C, Franceschi C (2013) Colorectal cancer microenvironment: among nutrition, gut microbiota, inflammation and epigenetics. Curr Pharm Des 19: 765–778. PMID: 23016865

60. Fendler B, Atwal G (2012) Systematic deciphering of cancer genome networks. Yale J Biol Med 85: 339–440. PMID: 23012582

61. Swanton C (2012) Intratumor heterogeneity: evolution through space and time. Cancer Res 72: 4875–4882. https://doi.org/10.1158/0008-5472.CAN-12-2217 PMID: 23002210

62. Shibata D (2011) Molecular tumor clocks to study the evolution of drug resistance. Mol Pharm 8: 2050–2054. https://doi.org/10.1021/mp200296n PMID: 21732670

63. Manfredi JJ (2010) The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev 24: 1580–1589. https://doi.org/10.1101/gad.1941710 PMID: 20679392

64. Hoivik EA, Kusonmano K, Halle MK, Berg A, Wik E, Werner HM, et al. (2014) Hypomethylation of the CTCFL/B ORIS promoter and aberrant expression during endometrial cancer progression suggests a role as an Epi-driver gene. Oncotarget 5: 1052–1061. https://doi.org/10.18632/oncotarget.1697 PMID: 24658009

65. D’Costa ZC, Higgins C, Ong CW, Irwin GW, Boyle D, McArt DG, et al. (2014) TBX2 repression of CST6 resulting in uncontrolled legumain activity to sustain breast cancer proliferation: a novel cancer-selective target pathway with therapeutic opportunities. Oncotarget 5: 1609–1620. https://doi.org/10.18632/oncotarget.1707 PMID: 24742492

66. Verbruggen P, Heinemann T, Manders E, von Bornstaedt G, van Driel R, Hofer T (2014) Robustness of DNA repair through collective rate control. PLoS Comput Biol 10: e1003438. https://doi.org/10.1371/journal.pcbi.1003438 PMID: 24499930

67. Zinovyev A, Kuperstein I, Barillot E, Heyer WD (2013) Synthetic lethality between gene defects affecting a single non-essential molecular pathway with reversible steps. PLoS Comput Biol 9: e1003016. https://doi.org/10.1371/journal.pcbi.1003016 PMID: 23592964

68. Cheng WY, Yu Yang TH, Anastassiou D (2013) Biomolecular events in cancer revealed by attractor metagenes. PLoS Comput Biol 9: e1002920. https://doi.org/10.1371/journal.pcbi.1002920 PMID: 23468608

69. Thomas LF, Saetrom P (2012) Single nucleotide polymorphisms can create alternative polyadenylation signals and affect gene expression through loss of microRNA-regulation. PLoS Comput Biol 8: e1002621. https://doi.org/10.1371/journal.pcbi.1002621 PMID: 22915998

70. Hsu HY, Lin TY, Wu YC, Tsao SM, Hwang PA, Shih YW, et al. (2014) Fucoidan inhibition of lung cancer in vivo and in vitro: role of the Smurf2-dependent ubiquitin proteasome pathway in TGFbeta receptor degradation. Oncotarget 5: 7870–7885. https://doi.org/10.18632/oncotarget.2317 PMID: 25149540

71. Miller MS, Schmidt-Kittler O, Bolduc DM, Brower ET, Chaves-Moreira D, Allaire M, et al. (2014) Structural basis of nSH2 regulation and lipid binding in PI3Kalpha. Oncotarget 5: 5198–5208. https://doi.org/10.18632/oncotarget.2263 PMID: 25105564

72. Soutto M, Chen Z, Saleh MA, Katsha A, Zhu S, Zaika A, et al. (2014) TFF1 activates p53 through down-regulation of miR-504 in gastric cancer. Oncotarget 5: 5663–5673. https://doi.org/10.18632/oncotarget.2156 PMID: 25015107

73. Shahbazi J, Scarlett CJ, Norris MD, Liu B, Haber M, Tee AE, et al. (2014) Histone deacetylase 2 and N-Myc reduce p53 protein phosphorylation at serine 46 by repressing gene transcription of tumor
protein 53-induced nuclear protein 1. Oncotarget 5: 4257–4268. https://doi.org/10.18632/oncotarget.1991 PMID: 24952595

76. Treude F, Kappes F, Fahrenkamp D, Muller-Newen G, Dajas-Bailador F, Kramer OH, et al. (2014) Caspase-8-mediated PAR-4 cleavage is required for TNFalpha-induced apoptosis. Oncotarget 5: 2988–2999. https://doi.org/10.18632/oncotarget.1634 PMID: 24931006

77. Le Grand M, Rovini A, Bourgarel-Rey V, Honore S, Bastonero S, Brauguer D, et al. (2014) ROS-mediated EB1 phosphorylation through Akt/GSK3beta pathway: implication in cancer cell response to microtubule-targeting agents. Oncotarget 5: 3408–3423. https://doi.org/10.18632/oncotarget.1982 PMID: 24930764

78. Leontieva OV, Demidenko ZN, Blagosklonny MV (2014) Contact inhibition and high cell density deactivate the mammalian target of rapamycin pathway, thus suppressing the senescence program. Proc Natl Acad Sci U S A 111: 8832–8837. https://doi.org/10.1073/pnas.1405723111 PMID: 24889617

79. Tran EJ, King MC, Corbett AH (2014) Macromolecular transport between the nucleus and the cytoplasm: Advances in mechanism and emerging links to disease. Biochim Biophys Acta 1843: 2784–2795. https://doi.org/10.1016/j.bbamcr.2014.08.003 PMID: 25116306

80. Noguchi M, Hirata N, Suzui F (2014) The links between AKT and two intracellular proteolytic cascades: ubiquitination and autophagy. Biochim Biophys Acta 1846: 342–352. https://doi.org/10.1016/j.bbcan.2014.07.013 PMID: 25109892

81. Serviss JT, Johnsson P, Grander D (2014) An emerging role for long non-coding RNAs in cancer metastasis. Front Genet 5: 234. https://doi.org/10.3389/fgene.2014.00234 PMID: 25101115

82. Janich P, Meng QJ, Benitah SA (2014) Circadian control of tissue homeostasis and adult stem cells. Curr Opin Cell Biol 31: 8–15. https://doi.org/10.1016/j.cceb.2014.06.010 PMID: 25016176

83. Merid SK, Goranskaia D, Alexeyenko A (2014) Distinguishing between driver and passenger mutations in individual cancer genomes by network enrichment analysis. BMC Bioinformatics 15: 308. https://doi.org/10.1186/1471-2105-15-308 PMID: 25236784

84. Tokheim CJ, Papadopoulos N, Kinzler KW, Vogelstein B, Karchin R (2016) Evaluating the evaluation of cancer driver genes. Proc Natl Acad Sci U S A 113: 14330–14335. https://doi.org/10.1073/pnas.1616440113 PMID: 27911828

85. Raphael BJ, Dobson JR, Oesper L, Vandin F (2014) Identifying driver mutations in sequenced cancer genomes: computational approaches to enable precision medicine. Genome Med 6: 5. https://doi.org/10.1186/gm524 PMID: 24479672

86. Dimitrakopoulos CM, Beerenwinkel N (2017) Computational approaches for the identification of cancer genes and pathways. Wiley Interdiscip Rev Syst Biol Med 9.

87. Bozic I, Gerold JM, Nowak MA (2016) Quantifying Clonal and Subclonal Passenger Mutations in Cancer Evolution. PLoS Comput Biol 12: e1004731. https://doi.org/10.1371/journal.pcbi.1004731 PMID: 26828429

88. Krishnan VG, Ng PC (2012) Predicting cancer drivers: are we there yet? Genom Med 4: 88. https://doi.org/10.1186/gm389 PMID: 23181697

89. Abate F, Zairis S, Ficarra E, Acquaviva A, Wiggins CH, Frattini V, et al. (2014) Pegasus: a comprehensive annotation and prediction tool for detection of driver gene fusions in cancer. BMC Syst Biol 8: 97. https://doi.org/10.1186/s12918-014-0097-z PMID: 25183062

90. Reva B (2013) Revealing selection in cancer using the predicted functional impact of cancer mutations. Application to nomination of cancer drivers. BMC Genomics 14 Supp 3: S8.

91. Sakoparnig T, Fried P, Beerenwinkel N (2015) Identification of constrained cancer driver genes based on mutation timing. PLoS Comput Biol 11: e1004027. https://doi.org/10.1371/journal.pcbi.1004027 PMID: 25569148

92. Mao Y, Chen H, Liang H, Merc-Bernstam F, Mills GB, Chen K (2013) CanDrA: cancer-specific driver missense mutation annotation with optimized features. PLoS ONE 8: e77945. https://doi.org/10.1371/journal.pone.0077945 PMID: 24205039

93. Reva B, Antipin Y, Sander C (2011) Predicting the functional impact of protein mutations: application to cancer genomes. Nucleic Acids Res 39: e118. https://doi.org/10.1093/nar/gkr407 PMID: 21727090

94. Hudson AM, Wirth C, Stephenson NL, Fawdar S, Brognard J, Miller CJ (2015) Using large-scale genomics data to identify driver mutations in lung cancer: methods and challenges. Pharmacogenomics 16: 1149–1160. https://doi.org/10.2217/pgs.15.60 PMID: 26230733

95. Anoosha P, Huang LT, Sakhthivel R, Karunagaran D, Gromiha MM (2015) Discrimination of driver and passenger mutations in epidermal growth factor receptor in cancer. Mutat Res 780: 24–34. https://doi.org/10.1016/j.mrfmm.2015.07.005 PMID: 26264175
96. Poulos RC, Wong JWH (2018) Finding cancer driver mutations in the era of big data research. Biophys Rev.
97. Caskey T (2018) Precision Medicine: Functional Advancements. Annu Rev Med 69: 1–18. https://doi.org/10.1146/annurev-med-041316-090905 PMID: 29261360
98. Wei G, Xi W, Nussinov R, Ma B (2016) Protein Ensembles: How Does Nature Harness Thermodynamic Fluctuations for Life? The Diverse Functional Roles of Conformational Ensembles in the Cell. Chem Rev 116: 6516–6551. https://doi.org/10.1021/acs.chemrev.5b00562 PMID: 26807783
99. Tsai CJ, Nussinov R (2014) The free energy landscape in translational science: how can somatic mutations result in constitutive oncogenic activation? Phys Chem Chem Phys 16: 6332–6341. https://doi.org/10.1039/c3cp54253 PMID: 24445437
100. Liu J, Nussinov R (2016) Allostery: An Overview of Its History, Concepts, Methods, and Applications. PLoS Comput Biol 12: e1004966. https://doi.org/10.1371/journal.pcbi.1004966 PMID: 27253437
101. Nussinov R, Tsai CJ (2013) Allostery in disease and in drug discovery. Cell 153: 293–305. https://doi.org/10.1016/j.cell.2013.03.034 PMID: 23582321
102. Nussinov R, Tsai CJ, Ma B (2013) The underappreciated role of allostery in the cellular network. Annu Rev Biophys 42: 169–189. https://doi.org/10.1146/annurev-biophysics-060812-143407 PMID: 23451894
103. Nussinov R, Tsai CJ (2013) Allostery in disease and in drug discovery. Cell 153: 293–305. https://doi.org/10.1016/j.cell.2013.03.034 PMID: 23451894
104. Nussinov R, Tsai CJ, Csermely P (2011) Allo-network drugs: harnessing allostery in cellular networks. Trends Pharmacol Sci 32: 686–693. https://doi.org/10.1016/j.tips.2011.08.004 PMID: 21925743
105. Tsai CJ, Del Sol A, Nussinov R (2009) Protein allostery, signal transduction and dynamics: a classification scheme of allosteric mechanisms. Mol Biosyst 5: 207–216. https://doi.org/10.1039/b819720b PMID: 26807783
106. Spencer-Smith R, O’Bryan JP (2017) Direct inhibition of RAS: Quest for the Holy Grail? Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2017.1012.1005
107. Waters AM, Ozkan-Dagliyan I, Vaseva AV, Fer N, Strathern LA, Hobbs GA, et al. (2017) Evaluation of the selectivity and sensitivity of isoform- and mutation-specific RAS antibodies. Sci Signal 10.
108. McCormick F (2016) K-Ras protein as a drug target. J Mol Med (Berl) 94: 253–258.
109. Kauke MJ, Traximayr MW, Parker JA, Kiefer JD, Knihitila R, McGee J, et al. (2017) An engineered protein antagonist of K-Ras/B-Raf interaction. Sci Rep 7: 5831. https://doi.org/10.1038/s41598-017-05889-7 PMID: 28724936
110. Lu S, Jang H, Gu S, Zhang J, Nussinov R (2016) Drugging Ras GTPase: a comprehensive mechanistic and signaling structural view. Chem Soc Rev 45: 4929–4952. https://doi.org/10.1039/c5cs00911a PMID: 27396271
111. Lu S, Jang H, Zhang J, Nussinov R (2016) Inhibitors of Ras-SOS Interactions. ChemMedChem 11: 814–821. https://doi.org/10.1002/cmdc.201500481 PMID: 26630662
112. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ (2014) Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov 13: 828–851. https://doi.org/10.1038/nrd4389 PMID: 25323927
113. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3: 11–22. https://doi.org/10.1038/nrc8969 PMID: 12509763
114. Prior IA, Lewis PD, Mattos C (2012) A comprehensive survey of Ras mutations in cancer. Cancer Res 72: 2457–2467. https://doi.org/10.1158/0008-5472.CAN-11-2612 PMID: 22589270
115. Thompson H (2013) US National Cancer Institute’s new Ras project targets an old foe. Nat Med 19: 949–950. https://doi.org/10.1038/nn0813-949 PMID: 23921727
116. Ostrem J, Peters U, Sos ML, Wells JA, Shokat KM (2013) K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 503: 548–551. https://doi.org/10.1038/nature12796 PMID: 24256730
117. Patgiri A, Yadav KK, Arora PS, Bar-Sagi D (2011) An orthosteric inhibitor of the Ras-Sos interaction. Nat Chem Biol 7: 585–587. https://doi.org/10.1038/nchembio.612 PMID: 21765406
118. Leshchiner ES, Parkhitchko A, Bird GH, Luccarelli J, Bellairs JA, Escudero S, et al. (2015) Direct inhibition of oncogenic KRAS by hydrocarbon-stapled SOS1 helices. Proc Natl Acad Sci U S A 112: 1761–1766. https://doi.org/10.1073/pnas.1413185112 PMID: 25624486
119. Sun Q, Burke JP, Pian J, Burns MC, Olejniczak ET, Waterson AG, et al. (2012) Discovery of small molecules that bind to K-Ras and inhibit Ras-mediated activation. Angew Chem Int Ed Engl 51: 6140–6143. https://doi.org/10.1002/anie.201201358 PMID: 22566140
120. Upadhyaya P, Qian Z, Seiner NG, Clippinger SR, Wu Z, Briesewitz R, et al. (2015) Inhibition of Ras signaling by blocking Ras-effector interactions with cyclic peptides. Angew Chem Int Ed Engl 54: 7602–7606. https://doi.org/10.1002/anie.201502763 PMID: 25950772

121. Shima F, Yoshikawa Y, Ye M, Araki M, Matsumoto S, Liao J, et al. (2013) In silico discovery of small-molecule Ras inhibitors that display antitumor activity by blocking the Ras-effector interaction. Proc Natl Acad Sci U S A 110: 8182–8187. https://doi.org/10.1073/pnas.1217730110 PMID: 23630290

122. Upadhyaya P, Bedewy W, Pei D (2016) Direct Inhibitors of Ras-Effector Protein Interactions. Mini Rev Med Chem 16: 376–382. PMID: 26423701

123. Welsch ME, Kaplan A, Chambers JM, Stokes ME, Bos PH, Zask A, et al. (2017) Multivalent Small-Molecule Pan-RAS Inhibitors. Cell 168: 878–889.e829. https://doi.org/10.1016/j.cell.2017.02.006 PMID: 28235199

124. Zhang J, Yang PL, Gray NS (2009) Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer 9: 28–39. https://doi.org/10.1038/nrc2559 PMID: 19104514

125. Peri F, Airoldi C, Colombo S, Martegani E, van Neuren AS, Stein M, et al. (2005) Design, synthesis and biological evaluation of sugar-derived Ras inhibitors. Chembiochem 6: 1839–1848. https://doi.org/10.1002/cbic.200400420 PMID: 16196015

126. Ostrem JM, Shokat KM (2016) Direct small-molecule inhibitors of KRAS: from structural insights to mechanism-based design. Nat Rev Drug Discov 15: 771–785. https://doi.org/10.1038/nrd.2016.139 PMID: 27469033

127. Hunter JC, Gurbani D, Ficarro SB, Carrasco MA, Lim SM, Choi HG, et al. (2014) In situ selectivity profiling and crystal structure of SML-8-73-1, an active site inhibitor of oncogenic K-Ras G12C. Proc Natl Acad Sci U S A 111: 8895–8900. https://doi.org/10.1073/pnas.1404639111 PMID: 24889603

128. Lim SM, Westover KD, Ficarro SB, Harrison RA, Choi HG, Pacold ME, et al. (2014) Therapeutic targeting of oncogenic K-Ras by a covalent catalytic site inhibitor. Angew Chem Int Ed Engl 53: 199–204. https://doi.org/10.1002/anie.201307387 PMID: 24259466

129. Xiong Y, Lu J, Hunter J, Li L, Scott D, Choi HG, et al. (2017) Covalent Guanosine Mimetic Inhibitors of G12C KRAS. ACS Med Chem Lett 8: 61–66.

130. Muller MP, Jeganathan S, Heidrich A, Campos J, Goody RS (2017) Nucleotide based covalent inhibitors of KRas can only be efficient in vivo if they bind reversibly with GTP-like affinity. Sci Rep 7: 3687. https://doi.org/10.1038/s41598-017-03973-6 PMID: 28623374

131. Lito P, Solomon M, Li LS, Hansen R, Rosen N (2016) Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. Science 351: 604–608. https://doi.org/10.1126/science.aad6204 PMID: 26841430

132. Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10: 789–799. https://doi.org/10.1038/nm1087 PMID: 15286780

133. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314: 268–274. https://doi.org/10.1126/science.1133427 PMID: 16959974

134. Mauro MJ, O'Dwyer ME, Druker BJ (2001) ST1571, a tyrosine kinase inhibitor for the treatment of chronic myelogenous leukemia: validating the promise of molecularly targeted therapy. Cancer Chemother Pharmacol 48 Suppl 1: S77–78.

135. Dagher R, Cohen M, Williams G, Rothmann M, Gobburu J, Robbie G, et al. (2002) Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. Clin Cancer Res 8: 3034–3038. PMID: 12374669

136. Moscow JA, Fojo T, Schilsky RL (2018) The evidence framework for precision cancer medicine. Nat Rev Clin Oncol 15: 183–192. https://doi.org/10.1038/nrclinonc.2017.186 PMID: 29255239

137. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, et al. (2011) Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 29: 3366–3373. https://doi.org/10.1200/JCO.2011.35.0986 PMID: 21768458

138. Perez EA, Romond EH, Suman VJ, Jeong JH, Sledge G, Geyer CE Jr, et al. (2014) Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned
joint analysis of overall survival from NSABP B-31 and NCCTG N9831. J Clin Oncol 32: 3744–3752. https://doi.org/10.1200/JCO.2014.55.5730 PMID: 25332249

142. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. (2012) Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13: 239–246. https://doi.org/10.1016/S1470-2045(11)70393-X PMID: 22885168

143. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, et al. (2011) Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 29: 2866–2874. https://doi.org/10.1200/JCO.2010.33.4235 PMID: 21670455

144. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, et al. (2002) A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. Blood 100: 1965–1971. https://doi.org/10.1182/blood-2001-12-0181 PMID: 12200353

145. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, et al. (2002) Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukaemia in myeloid blast crisis: results of a phase II study. Blood 99: 3530–3539. PMID: 11986204

146. O’Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukaemia. N Engl J Med 348: 994–1004. https://doi.org/10.1056/NEJMoa022457 PMID: 12657609

147. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. (2012) Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380: 358–365. https://doi.org/10.1016/S0140-6736(12)60869-X PMID: 22739584

148. Nikanjam M, Liu S, Yang J, Kurzrock R (2017) Dosing de novo combinations of two targeted drugs: Towards a customized precision medicine approach to advanced cancers. Oncotarget 7: 11310–11320. https://doi.org/10.18632/oncotarget.20723 PMID: 28424323

149. Liu S, Nikanjam M, Kurzrock R (2016) Dosing de novo combinations of two targeted drugs: Towards a customized precision medicine approach to advanced cancers. Oncotarget 7: 11130–11132. https://doi.org/10.18632/oncotarget.7023 PMID: 26824502

150. Toda K, Kawada K, Iwamoto M, Inamori S, Sasazuki T, Shirasawa S, et al. (2016) Metabolic Alterations Caused by KRAS Mutations in Colorectal Cancer Contribute to Cell Adaptation to Glutamine Depletion by Upregulation of Asparagine Synthetase. Neoplasia 18: 655–665. https://doi.org/10.1016/j.neo.2016.09.004 PMID: 27764698

151. Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, et al. (2016) Environment Impacts the Metabolic Dependencies of Ras-Driven Non-Small Cell Lung Cancer. Cell Metab 23: 517–528. https://doi.org/10.1016/j.cmet.2016.01.007 PMID: 26853747

152. Wolpaw AJ, Dang CV (2018) Exploiting Metabolic Vulnerabilities of Cancer with Precision and Accuracy. Trends Cell Biol 28: 201–212. https://doi.org/10.1016/j.tcb.2017.11.006 PMID: 29229182

153. Wright GM, Do H, Weiss J, Alam NZ, Rathi V, Walkiewicz M, et al. (2014) Mapping of actionable mutations to histological subtype domains in lung adenocarcinoma: implications for precision medicine. Oncotarget 5: 2107–2113. https://doi.org/10.18632/oncotarget.1840 PMID: 24749233

154. Sharma SV, Settleman J (2007) Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes Dev 21: 3214–3231. https://doi.org/10.1101/gad.1609907 PMID: 18079171

155. Torti D, Trusolino L (2011) Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. EMBO Mol Med 3: 623–636. https://doi.org/10.1002/emmm.201001176 PMID: 21953712

156. Vos S, van Delden JJM, van Diest PJ, Bredenoord AL (2017) Moral Duties of Genomics Researchers: Why Personalized Medicine Requires a Collective Approach. Trends Genet 33: 118–128. https://doi.org/10.1016/j.tig.2016.11.006 PMID: 28017938

157. Singer J, Irmsch A, Ruscheweyh HJ, Singer F, Toussaint NC, Levesque MP, et al. (2017) Bioinformatics for precision oncology: Brief Bioinform.

158. Chae YK, Pan AP, Davis AA, Patel SP, Carneiro BA, Kurzrock R, et al. (2017) Path toward Precision Oncology: Review of Targeted Therapy Studies and Tools to Aid in Defining “Actionability” of a Molecular Lesion and Patient Management Support. Mol Cancer Ther 16: 2645–2655. https://doi.org/10.1158/1535-7163.MCT-17-0597 PMID: 29205694

159. Ding L, Raphael BJ, Chen F, Wendl MC (2013) Advances for studying clonal evolution in cancer. Cancer Lett 340: 212–219. https://doi.org/10.1016/j.canlet.2012.12.028 PMID: 23353056

160. Nowell PC (1976) The clonal evolution of tumor cell populations. Science 194: 23–28. PMID: 959840
161. Ding L, Wendl MC, McMichael JF, Raphael BJ (2014) Expanding the computational toolbox for mining cancer genomes. Nat Rev Genet 15: 556–570. https://doi.org/10.1038/nrg3767 PMID: 25001846

162. Greenman C, Wooster R, Futreal PA, Stratton MR, Easton DF (2006) Statistical analysis of pathogenicity of somatic mutations in cancer. Genetics 173: 2187–2198. https://doi.org/10.1534/genetics.105.044677 PMID: 16783027

163. Getz G, Hofling H, Mesirov JP, Golub TR, Meyerson M, Tibshirani R, et al. (2007) Comment on “The consensus coding sequences of human breast and colorectal cancers”. Science 317: 1500.

164. Davoli T, Xu AW, Mewasser KE, Sack LM, Yoon JC, Park PJ, et al. (2013) Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. Cell 155: 948–962. https://doi.org/10.1016/j.cell.2013.10.011 PMID: 24183448

165. Ye J, Pavlicek A, Lunney EA, Rejto PA, Teng CH (2010) Statistical method on nonrandom clustering with application to somatic mutations in cancer. BMC Bioinformatics 11: 11. https://doi.org/10.1186/1471-2105-11-11 PMID: 20053295

166. Ryslik GA, Cheng Y, Cheung KH, Modis Y, Zhao H (2013) Leveraging protein quaternary structure to identify onco-genic driver mutations. BMC Bioinformatics 14: 190. https://doi.org/10.1186/1471-2105-14-190 PMID: 23758891

167. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, et al. (2014) Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 505: 495–501. https://doi.org/10.1038/nature12912 PMID: 24390350

168. Carter H, Karchin R (2014) Predicting the functional consequences of somatic missense mutations found in tumors. Methods Mol Biol 1101: 135–159. https://doi.org/10.1007/978-1-62703-721-1_8 PMID: 24233781

169. Keskin O, Ma B, Nussinov R (2005) Hot regions in protein—protein interactions: the organization and contribution of structurally conserved hot spot residues. J Mol Biol 345: 1281–1294. https://doi.org/10.1016/j.jmb.2004.10.077 PMID: 15644221

170. Keskin O, Gursoy A, Ma B, Nussinov R (2008) Principles of protein-protein interactions: what are the preferred ways for proteins to interact? Chem Rev 108: 1225–1244. https://doi.org/10.1021/cr040409x PMID: 18355092

171. Kim E, Ilic N, Shrestha Y, Zou L, Kamburov A, Zhu C, et al. (2016) Systematic Functional Interrogation of Rare Cancer Variants Identifies Oncogenic Alleles. Cancer Discov 6: 1281–1294. https://doi.org/10.1016/j.canc res.2016.07.001 PMID: 27281633
181. Liu J, Nussinov R (2008) Allosteric effects in the marginally stable von Hippel-Lindau tumor suppressor protein and allostery-based rescue mutant design. Proc Natl Acad Sci U S A 105: 901–906. https://doi.org/10.1073/pnas.0707401105 PMID: 18195360

182. Lu S, Jang H, Nussinov R, Zhang J (2016) The Structural Basis of Oncogenic Mutations G12, G13 and Q61 in Small GTPase K-Ras4B. Sci Rep 6: 21949. https://doi.org/10.1038/srep21949 PMID: 26902995

183. Lu S, Jang H, Muratciglu S, Gursoy A, Keskin O, Nussinov R, et al. (2016) Ras Conformational Ensembles, Allostery, and Signaling. Chem Rev 116: 6607–6665. https://doi.org/10.1021/acs.chemrev.5b00542 PMID: 26815308

184. Park MJ, Shen H, Spaeth JM, Tolvanen JH, Fairolc C, Knudtson JF, et al. (2018) Oncogenic exon 2 mutations in Mediator subunit MED12 disrupt allosteric activation of Cyclin C-CDK8/19. J Biol Chem. 185. Xu Q, Tang Q, Katsonis P, Lichtarge O, Jones D, Bovo S, et al. (2017) Benchmarking predictions of allostery in liver pyruvate kinase in CAGI4. Hum Mutat 38: 1123–1135. https://doi.org/10.1002/humu.23222 PMID: 28370845

186. Shen Q, Cheng F, Song H, Lu W, Zhao J, An X, et al. (2017) Proteome-Scale Investigation of Protein Allosteric Regulation Perturbed by Somatic Mutations in 7,000 Cancer Genomes. Am J Hum Genet 100: 5–20. https://doi.org/10.1016/j.ajhg.2016.09.020 PMID: 27939638

187. Zhan C, Qi R, Wei G, Guven-Maivor E, Nussinov R, Ma B (2016) Conformational dynamics of cancer-associated MyD88-TIR domain mutant L252P (L265P) allosterically tilts the landscape toward homo-dimerization. Protein Eng Des Sel 29: 347–354. https://doi.org/10.1093/protein/gzw033 PMID: 27503954

188. Tsai CJ, Nussinov R (2017) Allostery modulates the beat rate of a cardiac pacemaker. J Biol Chem 292: 6429–6430. https://doi.org/10.1074/jbc.H116.773697 PMID: 28411213

189. Fetics SK, Guterres H, Kearney BM, Buhrmann G, Ma B, Nussinov R, et al. (2015) Allosteric effects of the oncogenic RasQ61L mutant on Raf-RBD. Structure 23: 505–516. https://doi.org/10.1016/j.str.2014.12.017 PMID: 25684575

190. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38: e164. https://doi.org/10.1093/nar/gkq603 PMID: 20601685

191. Blucher AS, Choonoo G, Kulesz-Martin M, Wu G, McWeeny SK (2017) Evidence-Based Precision Oncology with the Cancer Targetome. Trends Pharmacol Sci 38: 1085–1099. https://doi.org/10.1016/j.tips.2017.08.006 PMID: 28646459

192. Shrestha R, Hodzic E, Sauerwald T, Dao P, Wang K, Yeung J, et al. (2017) HIT’nDRIVE: patient-specific multidriver gene prioritization for precision oncology. Genome Res 27: 1573–1588. https://doi.org/10.1101/gr.221218.117 PMID: 28768687

193. Zhao J, Cheng F, Zhao Z (2017) Tissue-Specific Signaling Networks Rewired by Major Somatic Mutations in Human Cancer Revealed by Proteome-Wide Discovery. Cancer Res 77: 2810–2821. https://doi.org/10.1158/0008-5472.CAN-16-2460 PMID: 28364002

194. Roychowdhury S, Chinnaiyan AM (2014) Translating genomics for precision cancer medicine. Annu Rev Genomics Hum Genet 15: 395–415. https://doi.org/10.1146/annurev-genom-090413-025552 PMID: 25184532

195. Porta-Pardo E, Garcia-Alonso L, Hrabe T, Dopazo J, Godzik A (2015) A Pan-Cancer Catalogue of Cancer Driver Protein Interaction Interfaces. PLoS Comput Biol 11: e1004518. https://doi.org/10.1371/journal.pcbi.1004518 PMID: 26485003

196. Korthauer KD, Kendziorski C (2015) MADGiC: a model-based approach for identifying driver genes in cancer. Bioinformatics 31: 1526–1535. https://doi.org/10.1093/bioinformatics/btu858 PMID: 25573922

197. Li M, Kales SC, Ma K, Shoemaker BA, Crespo-Barreto J, Cangelosi AL, et al. (2016) Balancing Protein Stability and Activity in Cancer: A New Approach for Identifying Driver Mutations Affecting CBL Ubiquitin Ligase Activation. Cancer Res 76: 561–571. https://doi.org/10.1158/0008-5472.CAN-14-3812 PMID: 26676746

198. Li M, Simonetti FL, Goncarenco A, Panchenko AR (2016) MutaBind estimates and interprets the effects of sequence variants on protein-protein interactions. Nucleic Acids Res 44: W494–501. https://doi.org/10.1093/nar/gkw374 PMID: 27150810

199. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. (2017) Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9: 34. https://doi.org/10.1186/s13073-017-0424-2 PMID: 28420421
200. Mochizuki AY, Frost IM, Mastrodimos MB, Plant AS, Wang AC, Moore TB, et al. (2018) Precision Medicine in Pediatric Neurooncology: A Review. ACS Chem Neurosci 9: 11–28. https://doi.org/10.1021/acschemneuro.7b00388 PMID: 29199818

201. Cohen KJ, Pollack IF, Zhou T, Buxton A, Holmes EJ, Burger PC, et al. (2011) Temozolomide in the treatment of high-grade gliomas in children: a report from the Children’s Oncology Group. Neuro Oncol 13: 317–323. https://doi.org/10.1093/neuonc/noq191 PMID: 21339192

202. Lashford LS, Thiesse P, Jouvet A, Jaspan T, Couanet D, Griffiths PD, et al. (2002) Temozolomide in malignant gliomas of childhood: a United Kingdom Children’s Cancer Study Group and French Society for Pediatric Oncology Intergroup Study. J Clin Oncol 20: 4684–4691. https://doi.org/10.1200/JCO.2002.08.141 PMID: 12488414

203. Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J, et al. (2010) Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. J Clin Oncol 28: 3061–3068. https://doi.org/10.1200/JCO.2009.26.7252 PMID: 20479398

204. Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, et al. (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482: 226–231. https://doi.org/10.1038/nature10833 PMID: 22286061

205. Coveney PV, Dougherty ER, Highfield RR (2016) Big data need big theory too. Philos Trans A Math Phys Eng Sci

206. Barone L, Williams J, Micklos D (2017) Unmet needs for analyzing biological big data: A survey of 704 NSF principal investigators. PLoS Comput Biol 13: e1005755. https://doi.org/10.1371/journal.pcbi.1005755 PMID: 29049281

207. Yakhini Z, Jurisica I (2011) Cancer computational biology. BMC Bioinformatics 12: 120. https://doi.org/10.1186/1471-2105-12-120 PMID: 21521513

208. Hogeweg P (2011) The roots of bioinformatics in theoretical biology. PLoS Comput Biol 7: e1002021. https://doi.org/10.1371/journal.pcbi.1002021 PMID: 21483479

209. Winter C, Kristiansen G, Kersting S, Roy J, Aust D, Knosel T, et al. (2012) Google goes cancer: improving outcome prediction for cancer patients by network-based ranking of marker genes. PLoS Comput Biol 8: e1002511. https://doi.org/10.1371/journal.pcbi.1002511 PMID: 22615549

210. Mathew JP, Taylor BS, Bader GD, Pyarajan S, Antonotti M, Chinnaiyan AM, et al. (2007) From bytes to bedside: data integration and computational biology for translational cancer research. PLoS Comput Biol 3: e12. https://doi.org/10.1371/journal.pcbi.0030012 PMID: 17319736

211. Stefl S, Nishi H, Petukh M, Panchenko AR, Alexov E (2013) Molecular mechanisms of disease-causing missense mutations. J Mol Biol 425: 3919–3936. https://doi.org/10.1016/j.jmb.2013.07.014 PMID: 23871686

212. Zhao F, Zheng L, Goncearenco A, Panchenko AR, Li M (2018) Computational Approaches to Prioritize Cancer Driver Missense Mutations. Int J Mol Sci 19.

213. Ng PK, Li J, Jeong KJ, Shao S, Chen H, Tsang YH, et al. (2018) Systematic Functional Annotation of Somatic Mutations in Cancer. Cancer Cell 33: 450–462.e410. https://doi.org/10.1016/j.ccell.2018.01.021 PMID: 29533785

214. Alvarez MJ, Shen Y, Giorgi FM, Lachmann A, Ding BB, Ye BH, et al. (2016) Functional characterization of somatic mutations in cancer using network-based inference of protein activity. Nat Genet 48: 838–847. https://doi.org/10.1038/ng.3593 PMID: 27322546

215. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, et al. (2014) Proteogenomic characterization of human colon and rectal cancer. Nature 513: 382–387. https://doi.org/10.1038/nature13438 PMID: 25043054

216. Nesvizhskii AI (2014) Proteogenomics: concepts, applications and computational strategies. Nat Methods 11: 1114–1125. https://doi.org/10.1038/nmeth.3144 PMID: 25357241

217. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clausner KR, Wang P, et al. (2016) Proteogenomics connects somatic mutations to signalling in breast cancer. Nature 534: 55–62. https://doi.org/10.1038/nature18003 PMID: 27251275
221. Zhang H, Liu T, Zhang Z, Payne SH, Zhang B, McDermott JE, et al. (2016) Integrated Proteogenomic Characterization of Human High-Grade Serous Ovarian Cancer. Cell 166: 755–765. https://doi.org/10.1016/j.cell.2016.05.069 PMID: 27372738

222. Cheng F, Jia P, Wang Q, Lin CC, Li WH, Zhao Z (2014) Studying tumorigenesis through network evolution and somatic mutational perturbations in the cancer interactome. Mol Biol Evol 31: 2156–2169. https://doi.org/10.1093/molbev/msu167 PMID: 24881052

223. Cheng F, Liu C, Lin CC, Zhao J, Jia P, Li WH, et al. (2015) A Gene Gravity Model for the Evolution of Cancer Genomes: A Study of 3,000 Cancer Genomes across 9 Cancer Types. PLoS Comput Biol 11: e1004497. https://doi.org/10.1371/journal.pcbi.1004497 PMID: 26352260

224. Piazza I, Kochanowski K, Cappelletti V, Fuhrer T, Noor E, Sauer U, et al. (2018) A Map of Protein-Metabolite Interactions Reveals Principles of Chemical Communication. Cell 172: 358–372. https://doi.org/10.1016/j.cell.2017.12.006 PMID: 29307493

225. Sahni N, Yi S, Taipale M, Fuxman Bass JI, Coulombe-Huntington J, Yang F, et al. (2015) Widespread macromolecular interaction perturbations in human genetic disorders. Cell 161: 647–660. https://doi.org/10.1016/j.cell.2015.04.013 PMID: 25910212

226. Creixell P, Schoof EM, Simpson CD, Longden J, Miller CJ, Lou HJ, et al. (2015) Kinome-wide decoding of network-attacking mutations rewiring cancer signaling. Cell 163: 202–217. https://doi.org/10.1016/j.cell.2015.08.056 PMID: 26388441

227. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. (2014) CRISP R-Cas9 knockin mice for genome editing and cancer modeling. Cell 160: 440–455. https://doi.org/10.1016/j.cell.2014.09.014 PMID: 25263330

228. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. (2017) Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature 551: 464–471. PMID: 29160308

229. Frauenfelder H, Sligar SG, Wolynes PG (1991) The energy landscapes and motions of proteins. Science 254: 1598–1603. PMID: 1749933

230. Kumar S, Ma B, Tsai CJ, Sinha N, Nussinov R (2000) Folding and binding cascades: dynamic landscapes and population shifts. Protein Sci 9: 10–19. https://doi.org/10.1110/ps.9.1.10 PMID: 10739242

231. Gunasekaran K, Ma B, Nussinov R (2004) Is allostery an intrinsic property of all dynamic proteins? Proteins 57: 433–443. https://doi.org/10.1002/prot.20232 PMID: 15382234

232. Nussinov R, Tsai CJ, Xin F, Radivojac P (2012) Allosteric post-translational modification codes. Trends Biochem Sci 37: 447–455. https://doi.org/10.1016/j.tibs.2012.07.001 PMID: 22884395

233. Tsai CJ, Ma B, Nussinov R (1999) Folding and binding cascades: shifts in energy landscapes. Proc Natl Acad Sci U S A 96: 9970–9972. PMID: 10468538

234. Nussinov R, Tsai CJ (2015) Allostery without a conformational change? Revisiting the paradigm. Curr Opin Struct Biol 30: 17–24. https://doi.org/10.1016/j.sbi.2014.11.005 PMID: 25500675

235. Tsai CJ, Nussinov R (2014) A unified view of “how allostery works”. PLoS Comput Biol 10: e1003394. https://doi.org/10.1371/journal.pcbi.1003394 PMID: 24516370

236. Weinkam P, Pons J, Sali A (2012) Structure-based model of allostery predicts coupling between distant sites. Proc Natl Acad Sci U S A 109: 4875–4880. https://doi.org/10.1073/pnas.1116274109 PMID: 22403063

237. Collier G, Ortiz V (2013) Emerging computational approaches for the study of protein alloster. Arch Biochem Biophys 538: 6–15. https://doi.org/10.1016/j.abb.2013.07.025 PMID: 23933229

238. Tehver R, Chen J, Thirumalai D (2009) Allostery wiring diagrams in the transitions that drive the GroEL reaction cycle. J Mol Biol 387: 390–406. https://doi.org/10.1016/j.jmb.2008.12.032 PMID: 19121324

239. Feher VA, Durrant JD, Van Wart AT, Amaro RE (2014) Computational approaches to mapping allosteric pathways. Curr Opin Struct Biol 25: 98–103. https://doi.org/10.1016/j.abb.2014.02.004 PMID: 24667124

240. Morra G, Verkhiiver G, Colombo G (2009) Modeling signal propagation mechanisms and ligand-based conformational dynamics of the Hsp90 molecular chaperone full-length dimer. PLoS Comput Biol 5: e1000323. https://doi.org/10.1371/journal.pcbi.1000323 PMID: 19360478

241. Tsai CJ, Kumar S, Ma B, Nussinov R (1999) Folding funnels, binding funnels, and protein function. Protein Sci 8: 1181–1190. https://doi.org/10.1110/ps.8.6.1181 PMID: 10386868
243. Boehr DD, Nussinov R, Wright PE (2009) The role of dynamic conformational ensembles in biomolecular recognition. Nat Chem Biol 5: 789–796. https://doi.org/10.1038/nchembio.232 PMID: 19841628

244. del Sol A, Tsai CJ, Ma B, Nussinov R (2009) The origin of allosteric functional modulation: multiple pre-existing pathways. Structure 17: 1042–1050. https://doi.org/10.1016/j.str.2009.06.008 PMID: 19679084

245. Ma B, Kumar S, Tsai CJ, Nussinov R (1999) Folding funnels and binding mechanisms. Protein Eng 12: 713–720. PMID: 10506280

246. Ma B, Shatsky M, Wolfson HJ, Nussinov R (2002) Multiple diverse ligands binding at a single protein site: a matter of pre-existing populations. Protein Sci 11: 184–197. https://doi.org/10.1110/ps.21302 PMID: 11790828

247. Nussinov R, Tsai CJ (2014) Free energy diagrams for protein function. Chem Biol 21: 311–318. https://doi.org/10.1016/j.chembio.2013.12.015 PMID: 24508196

248. Csermely P, Palotai R, Nussinov R (2010) Induced fit, conformational selection and independent dynamic segments: an extended view of binding events. Trends Biochem Sci 35: 539–546. https://doi.org/10.1016/j.tibs.2010.04.009 PMID: 20541943

249. Tsai CJ, Nussinov R (2013) The molecular basis of targeting protein kinases in cancer therapeutics. Semin Cancer Biol 23: 235–242. https://doi.org/10.1016/j.semcancer.2013.04.001 PMID: 23651790

250. Petukh M, Kucukkal TG, Alexov E (2015) On human disease-causing amino acid variants: statistical study of sequence and structural patterns. Hum Mutat 36: 524–534. https://doi.org/10.1002/humu.22770 PMID: 25689729

251. Kucukkal TG, Petukh M, Li L, Alexov E (2015) Structural and physico-chemical effects of disease and non-disease nsSNPs on proteins. Curr Opin Struct Biol 32: 18–24. https://doi.org/10.1016/j.sbi.2015.01.003 PMID: 25658850

252. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. Science 298: 1912–1918. https://doi.org/10.1126/science.1075762 PMID: 12471243

253. Grant BD, Adams JA (1996) Pre-steady-state kinetic analysis of cAMP-dependent protein kinase using rapid quench flow techniques. Biochemistry 35: 2022–2029. https://doi.org/10.1021/bi952144+ PMID: 8639687

254. Huse M, Kuriyan J (2002) The conformational plasticity of protein kinases. Cell 109: 275–282. PMID: 12015977

255. Nolen B, Taylor S, Ghosh G (2004) Regulation of protein kinases: controlling activity through activation segment conformation. Mol Cell 15: 661–675. https://doi.org/10.1016/j.molcel.2004.08.024 PMID: 15350212

256. Kuriyan J, Eisenberg D (2007) The origin of protein interactions and allostery in colocalization. Nature 450: 983–990. https://doi.org/10.1038/nature06524 PMID: 18075577

257. Taylor SS, Kornev AP (2011) Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci 36: 65–77. https://doi.org/10.1016/j.tibs.2010.09.006 PMID: 20971646

258. Liu Y, Gray NS (2006) Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol 2: 358–364. https://doi.org/10.1038/nchembio799 PMID: 16783341

259. Deindl S, Kadlecek TA, Brdicka T, Cao X, Weiss A, Kuriyan J (2007) Structural basis for the inhibition of tyrosine kinase activity of ZAP-70. Cell 129: 735–746. https://doi.org/10.1016/j.cell.2007.03.039 PMID: 17512407

260. Brasher BB, Van Etten RA (2000) c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the Src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. J Biol Chem 275: 35631–35637. https://doi.org/10.1074/jbc.M005401200 PMID: 10964922

261. Lietha D, Cai X, Ceccarelli DF, Li Y, Schaller MD, Eck MJ (2007) Structural basis for the autoinhibition of focal adhesion kinase. Cell 129: 1177–1187. https://doi.org/10.1016/j.cell.2007.05.041 PMID: 17574028

262. Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, et al. (2003) Structural basis for the autoinhibition of c-Abi tyrosine kinase. Cell 112: 859–871. PMID: 12654251

263. Jura N, Zhang X, Endres NF, Seeliger MA, Schindler T, Kuriyan J (2011) Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. Mol Cell 42: 9–22. https://doi.org/10.1016/j.molcel.2011.03.004 PMID: 21474065

264. Shan Y, Eastwood MP, Zhang X, Kim ET, Arkhipov A, Dror RO, et al. (2012) Oncogenic mutations counteract intrinsic disorder in the EGFR kinase and promote receptor dimerization. Cell 149: 860–870. https://doi.org/10.1016/j.cell.2012.02.063 PMID: 22579287
265. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141: 1117–1134. https://doi.org/10.1016/j.cell.2010.06.011 PMID: 20602996

266. Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2: 127–137. https://doi.org/10.1038/35052073 PMID: 1125954

267. Hynes NE, MacDonald G (2009) ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol 21: 177–184. https://doi.org/10.1016/j.ceb.2008.12.010 PMID: 19208461

268. Yarden Y, Schlessinger J (1987) Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation. Biochemistry 26: 1434–1442. PMID: 3494472

269. Yarden Y, Schlessinger J (1987) Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. Biochemistry 26: 1443–1451. PMID: 3494473

270. Jura N, Endres NF, Engel K, Deindl S, Das R, Lamers MH, et al. (2009) Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment. Cell 137: 1293–1307. https://doi.org/10.1016/j.cell.2009.04.025 PMID: 19563760

271. Endres NF, Engel K, Das R, Kovacs E, Kuriyan J (2011) Regulation of the catalytic activity of the EGF receptor. Curr Opin Struct Biol 21: 777–784. https://doi.org/10.1016/j.sbi.2011.07.007 PMID: 21868214

272. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. Cell 125: 641–651. PMID: 16777603

273. Chung I, Akita R, Vanden D, Toomre D, Schlessinger J, Mellman I (2010) Spatial control of EGF receptor activation by reversible dimerization on living cells. Nature 464: 783–787. https://doi.org/10.1038/nature08827 PMID: 2020817

274. Mi LZ, Lu C, Li Z, Nishida N, Walz T, Springer TA (2011) Simultaneous visualization of the extracellular and cytoplasmic domains of the epidermal growth factor receptor. Nat Struct Mol Biol 18: 984–989. https://doi.org/10.1038/nsmb.2092 PMID: 21822280

275. Wang Z, Longo PA, Tarrant MK, Kim K, Head S, Leahy DJ, et al. (2011) Mechanistic insights into the activation of oncogenic forms of EGF receptor. Nat Struct Mol Biol 18: 1388–1393. https://doi.org/10.1038/nsmb.2168 PMID: 22101934

276. Red Brewer M, Choi SH, Alvarado D, Moravcevic K, Pozzi A, Lemmon MA, et al. (2009) The juxtamembrane region of the EGF receptor functions as an activation domain. Mol Cell 34: 641–651. https://doi.org/10.1016/j.molcel.2009.04.034 PMID: 19560417

277. Dixit A, Yi L, Gowthaman R, Torkamani A, Schork NJ, Verkhivker GM (2009) Sequence and structure signatures of cancer mutation hotspots in protein kinases. PLoS One 4: e7485. https://doi.org/10.1371/journal.pone.0007485 PMID: 19834613

278. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, et al. (2007) Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. Cancer Cell 11: 217–227. https://doi.org/10.1016/j.ccr.2006.12.017 PMID: 17349580

279. Shan Y, Seelig MA, Eastwood MP, Frank F, Xu H, Jensen MO, et al. (2009) A conserved protonation-dependent switch controls drug binding in the Abl kinase. Proc Natl Acad Sci U S A 106: 139–144. https://doi.org/10.1073/pnas.0811223106 PMID: 19109437

280. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. (2008) The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 105: 2070–2075. https://doi.org/10.1073/pnas.0709662105 PMID: 18227510

281. Milburn MV, Tong L, deVos AM, Brunger A, Yamaizumi Z, Nishimura S, et al. (1990) Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins. Science 247: 939–945. PMID: 2406906

282. Cherfils J, Zeghouf M (2013) Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiol Rev 93: 269–309. https://doi.org/10.1152/physrev.00003.2012 PMID: 23303910

283. Bos JL, Rehmman H, Wittinghofer A (2007) GEFs and GAPs: critical elements in the control of small G proteins. Cell 129: 865–877. https://doi.org/10.1016/j.cell.2007.05.018 PMID: 17540168

284. Sondermann H, Soisson SM, Boykevich S, Yang SS, Bar-Sagi D, Kuriyan J (2004) Structural analysis of autoinhibition in the Ras activator Son of sevenless. Cell 119: 393–405. https://doi.org/10.1016/j.cell.2004.10.005 PMID: 15507210

285. Jang H, Banerjee A, Chavan TS, Lu S, Zhang J, Gaponenko V, et al. (2016) The higher level of complexity of K-Ras4B activation at the membrane. FASEB J 30: 1643–1655. https://doi.org/10.1096/fj.15-279091 PMID: 2671888
286. Pacold ME, Suire S, Perisic O, Lara-González S, Davis CT, Walker EH, et al. (2000) Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell 103: 931–943. PMID: 11136978

287. Nassar N, Horn G, Herrmann C, Block C, Janknecht R, Wittinghofer A (1996) Ras/Rap effector specificity determined by charge reversal. Nat Struct Biol 3: 723–729. PMID: 8756332

288. Huang L, Hofer F, Martin GS, Kim SH (1998) Structural basis for the interaction of Ras with RapGDS. Nat Struct Biol 5: 422–426. PMID: 9628477

289. Muraticciolu S, Chavan TS, Freed BC, Jang H, Khavrutskii L, Freed RN, et al. (2015) GTP-Dependent K-Ras Dimerization. Structure 23: 1325–1335. https://doi.org/10.1016/j.str.2015.04.019 PMID: 26051715

290. Parker JA, Mattos C (2017) The K-Ras, N-Ras, and H-Ras Isoforms: Unique Conformational Preferences and Implications for Targeting Oncogenic Mutants. Cold Spring Harb Perspect Med: https://doi.org/10.1101/cshperspect.a031427 PMID: 29038336

291. Nussinov R, Tsai CJ, Chakrabarti M, Jang H (2016) A New View of Ras Isoforms in Cancers. Cancer Res 76: 18–23. https://doi.org/10.1158/0008-5472.CAN-15-1536 PMID: 26659836

292. Johnson CW, Reid D, Parker JA, Salter S, Knüthila R, Kuzmic P, et al. (2017) The small GTPases K-Ras, N-Ras, and H-Ras have distinct biochemical properties determined by allosteric effects. J Biol Chem 292: 12981–12993. https://doi.org/10.1074/jbc.M117.778886 PMID: 28630043

293. Bryant KL, Mancias JD, Kimmelman AC, Der CJ (2014) KRAS: feeding pancreatic cancer proliferation. Trends Biochem Sci 39: 91–100. https://doi.org/10.1016/j.tibs.2013.12.004 PMID: 24388967

294. Kumar A, Glembo TJ, Ozkan SB (2015) The Role of Conformational Dynamics and Allostery in the Disease Development of Human Ferritin. Biophys J 109: 1273–1281. https://doi.org/10.1016/j.bpj.2015.06.060 PMID: 26255589

295. Scarabelli G, Grant BJ (2014) Kinesin-5 allosteric inhibitors uncouple the dynamics of nucleotide, microtubule, and neck-linker binding sites. Biophys J 107: 2204–2213. https://doi.org/10.1016/j.bpj.2014.09.019 PMID: 25418105

296. Alred EJ, Scheele EG, Berhanu WM, Hansmann UH (2014) Stability of Iowa mutant and wild type Abeta-peptide aggregates. J Chem Phys 141: 175101. https://doi.org/10.1063/1.4900892 PMID: 25381547

297. Gkeka P, Evangelidis T, Pavlaki M, Lazani V, Christoforidis S, Agianian B, et al. (2014) Investigating the structure and dynamics of the PIK3CA wild-type and H1047R oncogenic mutant. PLoS Comput Biol 10: e1003895. https://doi.org/10.1371/journal.pcbi.1003895 PMID: 25340423

298. Lu S, Deng R, Jiang H, Song H, Li S, Shen Q, et al. (2015) The Mechanism of ATP-Dependent Allosteric Protection of Akt Kinase Phosphorylation. Structure 23: 1725–1734. https://doi.org/10.1016/j.str.2015.06.027 PMID: 26256536

299. Blacklock K, Verkhivker GM (2014) Computational modeling of allosteric regulation in the hsp90 chaperones: a statistical ensemble analysis of protein structure networks and allosteric communications. PLoS Comput Biol 10: e1003679. https://doi.org/10.1371/journal.pcbi.1003679 PMID: 24922508

300. Invernizzi G, Tiberti M, Lambrighi M, Lindoff-Larsen K, Papaleo E (2014) Communication routes in ARID domains between distal residues in helix 5 and the DNA-binding loops. PLoS Comput Biol 10: e1003744. https://doi.org/10.1371/journal.pcbi.1003744 PMID: 25187961

301. Sun H, Tian S, Zhou S, Li Y, Li D, Xu L, et al. (2015) Revealing the favorable dissociation pathway of type II kinase inhibitors via enhanced sampling simulations and two-end-state calculations. Sci Rep 5: 8457. https://doi.org/10.1038/srep08457 PMID: 25678308

302. Liu JY, Chen XE, Zhang YL (2014) Insights into the key interactions between human protein phosphatase 5 and cantharidin using molecular dynamics and site-directed mutagenesis bioassays. Sci Rep 5: 12359. https://doi.org/10.1038/srep12359 PMID: 26190207

303. Clausen R, Ma B, Nussinov R, Shehu A (2015) Mapping the Conformation Space of Wildtype and Mutant H-Ras with a Memetic, Cellular, and Multiscale Evolutionary Algorithm. PLoS Comput Biol 11: e1004470. https://doi.org/10.1371/journal.pcbi.1004470 PMID: 26325505

304. Sun H, Li Y, Tian S, Wang J, Hou T (2014) P-loop conformation governed crizotinib resistance in G2032R-mutated ROS1 tyrosine kinase: clues from free energy landscape. PLoS Comput Biol 10: e1003729. https://doi.org/10.1371/journal.pcbi.1003729 PMID: 25033171

305. Lu S, Li S, Zhang J (2014) Harnessing allostery: a novel approach to drug discovery. Med Res Rev 34: 1242–1285. https://doi.org/10.1002/med.21317 PMID: 24827416

306. Huang W, Wang G, Shen Q, Liu X, Lu S, Geng L, et al. (2015) ASBench: benchmarking sets for allosteric discovery. Bioinformatics 31: 2598–2600. https://doi.org/10.1093/bioinformatics/btv169 PMID: 25810427
307. Kapoor A, Travesset A (2015) Differential dynamics of RAS isoforms in GDP- and GTP-bound states. Proteins 83: 1091–1106. https://doi.org/10.1002/prot.24805 PMID: 25846136

308. Gorfe AA, Grant BJ, McCammon JA (2008) Mapping the nucleotide and isoform-dependent structural and dynamical features of Ras proteins. Structure 16: 885–896. https://doi.org/10.1016/j.str.2008.03.009 PMID: 18547521

309. Raimondi F, Portella G, Orozco M, Fanelli F (2011) Nucleotide binding switches the information flow in ras GTPases. PLoS Comput Biol 7: e1001098. https://doi.org/10.1371/journal.pcbi.1001098 PMID: 21390270

310. Ma J, Karplus M (1997) Molecular switch in signal transduction: reaction paths of the conformational changes in ras p21. Proc Natl Acad Sci U S A 94: 11905–11910. PMID: 9342335

311. Hall BE, Bar-Sagi D, Nasser N (2002) The structural basis for the transition from Ras-GTP to Ras-GDP. Proc Natl Acad Sci U S A 99: 12138–12142. https://doi.org/10.1073/pnas.192453199 PMID: 12123964

312. Lukman S, Grant BJ, Gorfe AA, Grant GH, McCammon JA (2010) The distinct conformational dynamics of K-Ras and H-Ras A59G. PLoS Comput Biol 6.

313. Khrenova MG, Mironov VA, Grigorenko BL, Nemukhin AV (2014) Modeling the role of G12V and G13V Ras mutations in the Ras-GAP-catalyzed hydrolysis reaction of guanosine triphosphate. Biochemistry 53: 7093–7099. https://doi.org/10.1021/bi5011333 PMID: 25339142

314. Ford B, Hornak V, Kleinman H, Nassar N (2006) Structure of a transient intermediate for GTP hydrolysis by ras. Structure 14: 427–436. https://doi.org/10.1016/j.str.2005.12.010 PMID: 16531227

315. Buhrman G, Wink G, Mattos C (2007) Transformation efficiency of RasQ61 mutants linked to structural features of the switch regions in the presence of Raf. Structure 15: 1618–1629. https://doi.org/10.1016/j.str.2007.10.011 PMID: 18073111

316. Resat H, Straatsma TP, Dixon DA, Miller JH (2001) The arginine finger of RasGAP helps Gln-61 align the nucleophilic water in GAP-stimulated hydrolysis of GTP. Proc Natl Acad Sci U S A 98: 6033–6038. https://doi.org/10.1073/pnas.091506998 PMID: 11371635

317. Michael JV, Goldfinger LE (2017) Concepts and advances in cancer therapeutic vulnerabilities in RAS membrane targeting. Semin Cancer Biol.

318. Zhou Y, Prakash P, Liang H, Cho KJ, Gorfe AA, Hancock JF (2017) Lipid-Sorting Specificity Encoded in K-Ras Membrane Anchor Regulates Signal Output. Cell 168: 239–251.e216. https://doi.org/10.1016/j.cell.2016.11.059 PMID: 28041860

319. Wright LP, Philips MR (2006) Thematic review series: lipid posttranslational modifications. CAAX modification and membrane targeting of Ras. J Lipid Res 47: 883–891. https://doi.org/10.1194/jlr.R600004-JLR200 PMID: 16543601

320. Gulyas G, Radvanszki G, Matuska R, Balla A, Hunyadi L, Balla T, et al. (2017) Plasma membrane phosphatidylinositol 4-phosphate and 4,5-bisphosphate determine the distribution and function of K-Ras4B but not H-Ras proteins. J Biol Chem 292: 18862–18877. https://doi.org/10.1074/jbc.M117.806679 PMID: 28939768

321. Jang H, Muratcioglu S, Gursoy A, Keskin O, Nussinov R (2016) Membrane-associated Ras dimers are isoform-specific: K-Ras dimers differ from H-Ras dimers. Biochem J 473: 1719–1732. https://doi.org/10.1042/BCJ20160031 PMID: 27057007

322. Lu S, Banerjee A, Jang H, Zhang J, Gaponenko V, Nussinov R (2015) GTP Binding and Oncogenic Mutations May Attenuate Hypervariable Region (HVR)-Catalytic Domain Interactions in Small GTPase K-Ras4B, Exposing the Effector Binding Site. J Biol Chem 290: 28887–28890. https://doi.org/10.1074/jbc.M115.664755 PMID: 26453300

323. Bradshaw JM (2010) The Src, Syk, and Tec family kinases: distinct types of molecular switches. Cell Signal 22: 1175–1184. https://doi.org/10.1016/j.cellsig.2010.03.001 PMID: 20266868

324. Nussinov R, Ma B, Tsai CJ, Csermely P (2013) Allosteric conformational barcodes direct signaling in the cell. Structure 21: 1509–1521. https://doi.org/10.1016/j.str.2013.06.002 PMID: 24010710

325. Yi KH, Axtmaier J, Gustin JP, Rajpuorhit A, Lauring J (2013) Functional analysis of non-hotspot AKT1 mutants found in human breast cancers identifies novel driver mutations: implications for personalized medicine. Oncotarget 4: 29–34. https://doi.org/10.18632/oncotarget.755 PMID: 23237847
328. Parikh C, Janakiraman V, Wu WI, Foo CK, Klijavin NM, Chaudhuri S, et al. (2012) Disruption of PH-kinase domain interactions leads to oncogenic activation of AKT in human cancers. Proc Natl Acad Sci U S A 109: 19368–19373. https://doi.org/10.1073/pnas.1204384109 PMID: 23134728

329. Smith G, Bounds R, Wolf H, Steele RJ, Carey FA, Wolf CR (2010) Activating K-Ras mutations outwith ‘hotspot’ codons in sporadic colorectal tumours—implications for personalised cancer medicine. Br J Cancer 102: 693–703. PMID: 20147967

330. Lu J, Bera AK, Gondi S, Westover KD (2018) KRAS Switch Mutants D33E and A59G Crystallize in the State 1 Conformation. Biochemistry 57: 324–333. https://doi.org/10.1021/acs.biochem.7b00974 PMID: 29235861

331. Nussinov R, Tsai CJ (2012) The different ways through which specificity works in orthosteric and allosteric drugs. Curr Pharm Des 18: 1311–1316. PMID: 22316155

332. Stalnecker CA, Erickson JW, Cerione RA (2017) Conformational changes in the activation loop of mitochondrial glutaminase C: A direct fluorescence readout that distinguishes the binding of allosteric inhibitors from activators. J Biol Chem 292: 6095–6107. https://doi.org/10.1074/jbc.M116.758219 PMID: 28196863

333. Nussinov R, Tsai CJ (2015) The design of covalent allosteric drugs. Annu Rev Pharmacol Toxicol 55: 249–267. https://doi.org/10.1146/annurev-pharmtox-010814-124401 PMID: 25149918

334. Guarnera E, Berezovsky IN (2016) Allosteric sites: remote control in regulation of protein activity. Curr Opin Struct Biol 37: 1–8. https://doi.org/10.1016/j.sbi.2015.10.004 PMID: 26562539

335. Lu S, Huang W, Zhang J (2014) KRAS Switch Mutants D33E and A59G Crystallize in the State 1 Conformation. Biochemistry 57: 324–333. https://doi.org/10.1021/acs.biochem.7b00974 PMID: 29235861

336. Nussinov R, Tsai CJ (2015) The design of covalent allosteric drugs. Annu Rev Pharmacol Toxicol 55: 249–267. https://doi.org/10.1146/annurev-pharmtox-010814-124401 PMID: 25149918
349. Huang W, Nussinov R, Zhang J (2017) Computational Tools for Allosteric Drug Discovery: Site Identification and Focus Library Design. Methods Mol Biol 1529: 439–446. https://doi.org/10.1007/978-1-4939-6637-0_23 PMID: 27914066

350. Oleinikovas V, Saladino G, Cosssins BP, Gervasio FL (2016) Understanding Cryptic Pocket Formation in Protein Targets by Enhanced Sampling Simulations. J Am Chem Soc 138: 14257–14263. https://doi.org/10.1021/jacs.6b05425 PMID: 27726386

351. Tzeng SR, Kalodimos CG (2013) Allosteric inhibition through suppression of transient conformational states. Nat Chem Biol 9: 462–465. https://doi.org/10.1038/nchembio.1250 PMID: 23644478

352. Gagliardi PA, Puliafito A, Primo L (2018) PDK1: At the crossroad of cancer signaling pathways. Semin Cancer Biol 48: 27–35. https://doi.org/10.1016/j.semcancer.2017.04.014 PMID: 28473254

353. Nnadi CI, Jenkins ML, Gentile DR, Bateman LA, Zaidman D, Balius TE, et al. (2018) Novel K-Ras G12C Switch-II Covalent Binders Destabilize Ras and Accelerate Nucleotide Exchange. J Chem Inf Model 58: 464–471. https://doi.org/10.1021/acs.jcim.7b00399 PMID: 29320178

354. Gentile DR, Rathinaswamy MK, Jenkins ML, Moss SM, Siempelkamp BD, Renslo AR, et al. (2017) Ras Binder Induces a Modified Switch-II Pocket in GTP and GDP States. Cell Chem Biol 24: 1455–1466.e1414. https://doi.org/10.1016/j.chembiol.2017.08.025 PMID: 29033317

355. Tuncbag N, Kar G, Gursoy A, Keskin O, Nussinov R (2009) Towards inferring time dimensionality in protein-protein interaction networks by integrating structures: the p53 example. Mol Biosyst 5: 1770–1778. https://doi.org/10.1039/B905661K PMID: 19585003

356. Acuner Ozbabacan SE, Gursoy A, Keskin O, Nussinov R (2010) Conformational ensembles, signal transduction and residue hot spots: application to drug discovery. Curr Opin Drug Discov Devel 13: 527–537. PMID: 20812144

357. Guven-Maiorov E, Keskin O, Gursoy A, Nussinov R (2015) A Structural View of Negative Regulation of the Toll-like Receptor-Mediated Inflammatory Pathway. Biophys J 109: 1214–1226. https://doi.org/10.1016/bpj.2015.06.048 PMID: 26276688

358. Nakhaei-Rad S, Haghighi F, Nouri P, Rezaei Adariani S, Lissy J, Kazemein Jasemi NS, et al. (2018) Structural fingerprints, interactions, and signaling networks of RAS family proteins beyond RAS isoforms. Crit Rev Biochem Mol Biol 53: 130–156. https://doi.org/10.1080/10409238.2018.1431605 PMID: 29457927

359. Marin-Ramos NI, Ortega-Gutierrez S, Lopez-Rodriguez ML (2018) Blocking Ras inhibition as an anti-tumor strategy. Semin Cancer Biol: 10.

360. Chaker M, Minden A, Chen S, Weiss RH, Chini EN, Mahipal A, et al. (2018) Rho GTPase effectors and NAD metabolism in cancer immune suppression. Expert Opin Ther Targets 22: 9–17. https://doi.org/10.1080/14728222.2018.1413091 PMID: 29207896

361. Engin HB, Carlin D, Pratt D, Carter H (2017) Modeling of RAS complexes supports roles in cancer for less studied partners. BMC Biophys 10: 5. https://doi.org/10.1186/s13628-017-0037-6 PMID: 28815022

362. Keskin O, Nussinov R (2007) Similar binding sites and different partners: implications to shared proteins in cellular pathways. Structure 15: 341–354. https://doi.org/10.1016/j.str.2007.01.007 PMID: 17355869

363. Tsai CJ, Ma B, Nussinov R (2009) Protein-protein interaction networks: how can a hub protein bind so many different partners? Trends Biochem Sci 34: 594–600. https://doi.org/10.1016/j.tibs.2009.07.007 PMID: 19837592

364. Nussinov R, Tsai CJ, Muratioglu S, Jang H, Gursoy A, Keskin O (2015) Principles of K-Ras effector organization and the role of oncogenic K-Ras in cancer initiation through G1 cell cycle deregulation. Expert Rev Proteomics 12: 669–682. https://doi.org/10.1586/14798450.2015.1100079 PMID: 26496174

365. Nussinov R, Tsai CJ, Jang H (2018) Oncogenic KRas mobility in the membrane and signaling response. Semin Cancer Biol.

366. Banerjee A, Jang H, Nussinov R, Gaponenko V (2016) The disordered hypervariable region and the folded catalytic domain of oncogenic K-Ras4B partner in phospholipid binding. Curr Opin Struct Biol 36: 10–17. https://doi.org/10.1016/j.sbi.2015.11.010 PMID: 26709496

367. Herrmann C, Horn G, Spaargaren M, Wittighofer A (1996) Differential interaction of the ras family GTP-binding proteins H-Ras, Rap1A, and R-Ras with the putative effector molecules Raf kinase and Raf–guanine nucleotide exchange factor. J Biol Chem 271: 6794–6800. PMID: 8636102

368. Harjes E, Harjes S, Wohlgemuth S, Muller KH, Krieger E, Herrmann C, et al. (2006) GTP-Ras disrupts the intramolecular complex of C1 and RA domains of Nore1. Structure 14: 881–888. https://doi.org/10.1016/j.str.2006.03.008 PMID: 16698549
369. Liao TJ, Tsai CJ, Jang H, Fushman D, Nussinov R (2016) RASSF5: An MST activator and tumor suppresor in vivo but opposite in vitro. Curr Opin Struct Biol 41: 217–224. https://doi.org/10.1016/j.sbi.2016.09.001 PMID: 27643882

370. Leiserson MD, Blokh D, Sharan R, Raphael BJ (2013) Simultaneous identification of multiple driver pathways in cancer. PLoS Comput Biol 9: e1003054. https://doi.org/10.1371/journal.pcbi.1003054 PMID: 23717195

371. Hart JR, Zhang Y, Liao L, Ueno L, Du L, Jonkers M, et al. (2015) The butterfly effect in cancer: a single base mutation can remodel the cell. Proc Natl Acad Sci U S A 112: 1131–1136. https://doi.org/10.1073/pnas.1424012112 PMID: 25583473

372. Reimand J, Bader GD (2013) Systematic analysis of somatic mutations in phosphorylation signaling predicts novel cancer drivers. Mol Syst Biol 9: 637. https://doi.org/10.1038/msb.2012.68 PMID: 23340843

373. Babur O, Gonen M, Aksoy BA, Schultz N, Ciriello G, Sander C, et al. (2015) Systematic identification of cancer driving signaling pathways based on mutual exclusivity of genomic alterations. Genome Biol 16: 45. https://doi.org/10.1186/s13059-015-0612-6 PMID: 25887147

374. Brand K, Kentsch H, Glasshoff C, Rosenberg G (2014) RASopathy-associated CBL germline mutations cause aberrant ubiquitylation and trafficking of EGFR. Hum Mutat 35: 1372–1381. https://doi.org/10.1002/humu.22682 PMID: 25178484

375. Wagner SA, Beli P, Weinert BT, Scholz C, Kelstrup CD, Young C, et al. (2012) Proteomic analyses reveal divergent ubiquitylation site patterns in murine tissues. Mol Cell Proteomics 11: 1578–1585. https://doi.org/10.1074/mcp.M112.017905 PMID: 22790023

376. Baietti MF, Simicek M, Abbasi Asbagh L, Radaelli E, Lievens S, Crowther J, et al. (2016) OTUB1 triggers lung cancer development by inhibiting RAS monoubiquitination. EMBO Mol Med 8: 288–303. https://doi.org/10.15252/emmm.201505972 PMID: 26881969

377. Schlessinger J (2000) Cell signaling by receptor tyrosine kinases. Cell 103: 211–225. PMID: 11057895

378. Klinghoffer RA, Duckworth B, Vallius M, Cantley L, Kazlauskas A (1996) Platelet-derived growth factor-dependent activation of phosphatidylinositol 3-kinase is regulated by receptor binding of SH2-domain-containing proteins which influence Ras activity. Mol Cell Biol 16: 5905–5914. PMID: 8816504

379. Wagner MJ, Stacey MM, Liu BA, Pawson T (2013) Molecular mechanisms of SH2- and PTB-domain-containing proteins in receptor tyrosine kinase signaling. Cold Spring Harb Perspect Biol 5: a008987. https://doi.org/10.1101/cshperspect.a008987 PMID: 24296166

380. Lee JY, Chu TY, Asara J, Cantley LC (2011) Inhibition of PI3K binding to activators by serine phosphorylation of PI3K regulatory subunit p85alpha Src homology-2 domains. Proc Natl Acad Sci U S A 108: 14157–14162. https://doi.org/10.1073/pnas.1107747108 PMID: 21825134

381. Nussinov R, Wang G, Tsai CJ, Jang H, Lu S, Banerjee A, et al. (2017) Calmodulin and PI3K Signaling in KRAS Cancers. Trends Cancer 3: 214–224. https://doi.org/10.1016/j.treca.2017.01.007 PMID: 28462395

382. Nussinov R, Muratioglu S, Tsai CJ, Jang H, Gursoy A, Keskin O (2015) The Key Role of Calmodulin in KRAS-Driven Adenocarcinomas. Mol Cancer Res 13: 1265–1273. https://doi.org/10.1158/1541-7786.MCR-15-0165 PMID: 26085527

383. Nussinov R, Muratioglu S, Tsai CJ, Jang H, Gursoy A, Keskin O (2016) K-Ras4B/calmodulin/PI3Kalpha: A promising new adenosccinoma-specific drug target? Expert Opin Ther Targets 20: 831–842. https://doi.org/10.1517/14728222.2016.1135131 PMID: 26873344

384. Jenkins RW, Thummalapalli R, Carter J, Canadas I, Barbie DA (2018) Molecular and Genomic Determinants of Response to Immune Checkpoint Inhibition in Cancer. Annu Rev Med 69: 333–347. https://doi.org/10.1146/annurev-med-060116-022926 PMID: 29099676

385. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3: 415–428. https://doi.org/10.1038/nrg816 PMID: 12042769

386. Forde PM, Brahmer JR, Kelly RJ (2014) New strategies in lung cancer: epigenetic therapy for nonsmall cell lung cancer. Clin Cancer Res 20: 2244–2248. https://doi.org/10.1158/1078-0432.CCR-13-2088 PMID: 24644000

387. Rothbart SB, Strahl BD (2014) Interpreting the language of histone and DNA modifications. Biochim Biophys Acta 1839: 627–643. https://doi.org/10.1016/j.bbagrm.2014.03.001 PMID: 24631868
389. Nussinov R, Tsai CJ, Mattos C (2013) 'Pathway drug cocktail': targeting Ras signaling based on structural pathways. Trends Mol Med 19: 695–704. https://doi.org/10.1016/j.molmed.2013.07.009 PMID: 23953481

390. Lavi O (2015) Redundancy: a critical obstacle to improving cancer therapy. Cancer Res 75: 808–812. https://doi.org/10.1158/0008-5472.CAN-14-3256 PMID: 25576083

391. Yap TA, Omlin A, de Bono JS (2013) Development of therapeutic combinations targeting major cancer signaling pathways. J Clin Oncol 31: 1592–1605. https://doi.org/10.1200/JCO.2011.37.6418 PMID: 23509311

392. Shao DD, Xue W, Krall EB, Bhutkar A, Piccioni F, Wang X, et al. (2014) KRAS and YAP1 converge to regulate EMT and tumor survival. Cell 158: 171–184. https://doi.org/10.1016/j.cell.2014.06.004 PMID: 24954536

393. Kapoor A, Yao W, Ying H, Hua S, Liewen A, Wang Q, et al. (2014) Yap1 activation enables bypass of oncogenic Kras addiction in pancreatic cancer. Cell 158: 185–197. https://doi.org/10.1016/j.cell.2014.06.003 PMID: 24954535

394. Kaveri D, Kastner P, Dembele D, Nerlov C, Chan S, Kirstetter P (2013) beta-Catenin activation synergizes with Pten loss and Myc overexpression in Notch-independent T-ALL. Blood 122: 694–704. https://doi.org/10.1182/blood-2012-12-471904 PMID: 23801632

395. Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, Margolin A, et al. (2006) NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci U S A 103: 18261–18266. https://doi.org/10.1073/pnas.0606018103 PMID: 17142493

396. Ramaswamy B, Lu Y, Teng KY, Nuovo G, Li X, Shapiro CL, et al. (2012) Hedgehog signaling is a novel therapeutic target in tamoxifen-resistant breast cancer aberrantly activated by PI3K/AKT pathway. Cancer Res 72: 5048–5059. https://doi.org/10.1158/0008-5472.CAN-12-1248 PMID: 22875023

397. Sharma N, Nanta R, Sharma J, Gunewardena S, Singh KP, Shankar S, et al. (2015) PI3K/AKT/mTOR and sonic hedgehog pathways cooperate together to inhibit human pancreatic cancer stem cell characteristics and tumor growth. Oncotarget 6: 32039–32060. https://doi.org/10.18632/oncotarget.5055 PMID: 26451606

398. Ilic N, Utermark T, Widlund HR, Roberts TM (2011) PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. Proc Natl Acad Sci U S A 108: E699–708. https://doi.org/10.1073/pnas.1108237108 PMID: 21876152

399. Lin L, Sabnis AJ, Chan E, Olivas V, Cade L, Pazarentzos E, et al. (2015) The Hippo effector YAP promotes resistance to RAF- and MEK-targeted cancer therapies. Nat Genet 47: 250–256. https://doi.org/10.1038/ng.3218 PMID: 25665005

400. Keren-Paz A, Emmanuel R, Samuels Y (2015) YAP and the drug resistance highway. Nat Genet 47: 193–194. https://doi.org/10.1038/ng.3228 PMID: 25711863

401. Flaherty KT, Wargo JA, Bivona TG (2015) YAP in MAPK pathway targeted therapy resistance. Cell Cycle 14: 1765–1766. https://doi.org/10.1080/15384101.2015.1032644 PMID: 26036142

402. Lavi O, Greene JM, Levy D, Gottesman MM (2014) Simplifying the complexity of resistance heterogeneity in metastasis. Trends Mol Med 20: 129–136. https://doi.org/10.1016/j.molmed.2013.12.005 PMID: 24491979

403. Nussinov R, Tsai CJ, Jang H, Korcsmaros T, Csermely P (2016) Oncogenic KRAS signaling and YAP1/beta-catenin: Similar cell cycle control in tumor initiation. Semin Cell Dev Biol 58: 79–85. https://doi.org/10.1016/j.semcdb.2016.04.001 PMID: 27058752

404. Nussinov R, Tsai CJ, Jang H (2016) Independent and core pathways in oncogenic KRAS signaling. Expert Rev Proteomics 13: 711–716. https://doi.org/10.1080/14789450.2016.1209417 PMID: 27389825

405. Nussinov R, Tsai CJ, Jang H (2016) A New View of Pathway-Driven Drug Resistance in Tumor Proliferation. Trends Pharmacol Sci 38: 427–437. https://doi.org/10.1016/j.tips.2017.02.001 PMID: 28245913

406. Nakken S, Fournous G, Vodak D, Aasheim LB, Myklebost O, Hovig E (2017) Personal Cancer Genome Reporter: variant interpretation report for precision oncology. Bioinformatics.

407. Kurnit KC, Ileana Dumbrava EE, Litzenburger BC, Khotskaya YB, Johnson A, Yap TA, et al. (2018) Precision Oncology Decision Support: Current Approaches and Strategies for the Future. Clin Cancer Res.

408. Eifert C, Pantazi A, Sun R, Xu J, Cingolani P, Heyer J, et al. (2017) Clinical application of a cancer genomic profiling assay to guide precision medicine decisions. Per Med 14: 309–325. https://doi.org/10.2217/pme-2017-0011 PMID: 28890729
409. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, et al. (2018) Comprehensive Characterization of Cancer Driver Genes and Mutations. Cell 173: 371–385.e318. https://doi.org/10.1016/j.cell.2018.02.060 PMID: 29625053

410. Margolin AA (2013) Oncogenic Driver Mutations: Neither Tissue-Specific nor Independent. Sci Transl Med 5: 214ec200.

411. Warner JL (2017) Giving Up on Precision Oncology? Not So Fast! Clin Transl Sci 10: 128–129. https://doi.org/10.1111/cts.12457 PMID: 28146309

412. Hanwell MD, Curtis DE, Lonie DC, Vandermersch T, Zurek E, Hutchinson GR (2012) Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J Cheminform 4: 17. https://doi.org/10.1186/1758-2946-4-17 PMID: 22889332

413. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. (2013) Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31: 213–219. https://doi.org/10.1038/nbt.2514 PMID: 23396013

414. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297–1303. https://doi.org/10.1101/gr.107524.110 PMID: 20644199

415. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22: 568–576. https://doi.org/10.1101/gr.129684.111 PMID: 22300766

416. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK (2012) Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. Bioinformatics 28: 1811–1817. https://doi.org/10.1093/bioinformatics/bts271 PMID: 22581179

417. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. (2010) A method and server for predicting damaging missense mutations. Nat Methods 7: 248–249. https://doi.org/10.1038/nmeth0410-248 PMID: 20354512

418. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4: 1073–1081. https://doi.org/10.1038/nprot.2009.86 PMID: 19561590

419. Wong WC, Kim D, Carter H, Diekhans M, Ryan MC, Karchin R (2011) CHASM and SNVBox: toolkit for detecting biologically important single nucleotide mutations in cancer. Bioinformatics 27: 2147–2148. https://doi.org/10.1093/bioinformatics/btr357 PMID: 21685053

420. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Methods 7: 248–249. https://doi.org/10.1038/nmeth0410-248 PMID: 20354512

421. Kamburov A, Lawrence MS, Polak P, Leshchiner I, Lage K, Golub TR, et al. (2015) Comprehensive assessment of cancer missense mutation clustering in protein structures. Proc Natl Acad Sci U S A 112: E5486–E5495. https://doi.org/10.1073/pnas.1516373112 PMID: 26392535

422. Zhao J, Cheng F, Wang Y, Arteaga CL, Zhao Z (2016) Systematic Prioritization of Druggable Mutations in approximately 5000 Genomes Across 16 Cancer Types Using a Structural Genomics-based Approach. Mol Cell Proteomics 15: 642–656. https://doi.org/10.1074/mcp.M115.053199 PMID: 26657081

423. Porta-Pardo E, Hrabe T, Godzik A (2015) Cancer3D: understanding cancer mutations through protein structures. Nucleic Acids Res 43: D968–973. https://doi.org/10.1093/nar/gku1140 PMID: 25392415

424. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499: 214–218. https://doi.org/10.1038/nature12213 PMID: 23770567

425. Jia P, Wang Q, Chen Q, Hutchinson KE, Pao W, Zhao Z (2014) MSEA: detection and quantification of mutation hotspots through mutation set enrichment analysis. Genome Biol 15: 489. https://doi.org/10.1186/s13059-014-0489-9 PMID: 25348067

426. Tamborero D, Gonzalez-Perez A, Perez-Llamas C, Deu-Pons J, Kandoth C, Reimand J, et al. (2013) Comprehensive identification of mutational cancer driver genes across 12 tumor types. Sci Rep 3: 2650. https://doi.org/10.1038/srep02650 PMID: 24084849

427. Gonzalez-Perez A, Lopez-Bigas N (2012) Functional impact bias reveals cancer drivers. Nucleic Acids Res 40: e169. https://doi.org/10.1093/nar/gks743 PMID: 22904074
429. Tamborero D, Gonzalez-Perez A, Lopez-Bigas N (2013) OncodriveCLUST: exploiting the positional clustering of somatic mutations to identify cancer genes. Bioinformatics 29: 2238–2244. https://doi.org/10.1093/bioinformatics/btt395 PMID: 23884480

430. Dees ND, Zhang Q, Kandoth C, Wendl MC, Schierding W, Koboldt DC, et al. (2012) MuSiC: identifying mutational significance in cancer genomes. Genome Res 22: 1589–1598. https://doi.org/10.1101/gr.134635.111 PMID: 22759861

431. Leiserson MD, Vandin F, Wu HT, Dobson JR, Eldridge JV, Thomas JL, et al. (2015) Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. Nat Genet 47: 106–114. https://doi.org/10.1038/ng.3168 PMID: 25501392

432. Hofree M, Shen JP, Carter H, Gross A, Ideker T (2013) Network-based stratification of tumor mutations. Nat Methods 10: 1108–1115. https://doi.org/10.1038/nmeth.2651 PMID: 24037242

433. Hou JP, Ma J (2014) DawnRank: discovering personalized driver genes in cancer. Genome Med 6: 56. https://doi.org/10.1186/s13073-014-0056-8 PMID: 25177370

434. Jia P, Zhao Z (2014) VarWalker: personalized mutation network analysis of putative cancer genes from next-generation sequencing data. PLoS Comput Biol 10: e1003460. https://doi.org/10.1371/journal.pcbi.1003460 PMID: 24516372

435. Bashashati A, Haffari G, Ding J, Ha G, Lui K, Rosner J, et al. (2012) DriverNet: uncovering the impact of somatic driver mutations on transcriptional networks in cancer. Genome Biol 13: R124. https://doi.org/10.1186/gb-2012-13-12-r124 PMID: 23383675

436. Paull EO, Carlin DE, Niepel M, Sorger PK, Haussler D, Stuart JM (2013) Discovering causal pathways linking genomic events to transcriptional states using Tied Diffusion Through Interacting Events (Tie-DIE). Bioinformatics 29: 2757–2764. https://doi.org/10.1093/bioinformatics/btt471 PMID: 23986566