Resources for Interpreting Variants in Precision Genomic Oncology Applications

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August 4, 2017

Abstract

Precision genomic oncology–applying high throughput sequencing (HTS) at the point-of-care to inform clinical decisions–is a developing precision medicine paradigm that is seeing increasing adoption. Simultaneously, new developments in targeted agents and immunotherapy, when informed by rich genomic characterization, offer potential benefit to a growing subset of patients. Multiple previous studies have commented on methods for identifying both germline and somatic variants. However, interpreting individual variants remains a significant challenge, relying in large part on the integration of observed variants with biological knowledge. A number of data and software resources have been developed to assist in interpreting observed variants, determining their potential clinical actionability, and augmenting them with ancillary information that can inform clinical decisions and even generate new hypotheses for exploration in the laboratory. Here, we review available variant catalogs, variant and functional annotation software and tools, and databases of clinically actionable variants that can be used in an ad hoc approach with research samples or incorporated into a data platform for interpreting and formally reporting clinical results.

1 Introduction

Genomic technologies and approaches have transformed cancer research and have led to the production of large-scale cancer genomics compendia (*International Cancer Genome Consortium* n.d.; Cancer Genome Atlas Research Network et al. 2013). The resulting molecular characterization and categorization of individual samples from such compendia has driven development of molecular subtypes cancers as well as enhanced understanding of the molecular etiologies of carcinogenesis (Cancer Genome Atlas Network 2012; Cancer Genome Atlas Research Network 2015; "Comprehensive genomic characterization defines human glioblastoma genes and core pathways" 2008). The development of novel and effective targeted therapies has proceeded in parallel with and been accelerated by deeper, faster, and broader genomic characterization (Blumenthal, Mansfield, and Pazdur 2016), enabling early application of molecular characterization at the point of care to inform clinical decision-making (Flaherty et al. 2012; Shaw et al. 2013; Maemondo et al. 2010; Druker et al. 2006)

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and to address resistance to primary therapy (Ai and Tiu 2014). Genomic characterization also has applications in immune approaches to cancer. For example, chimeric antigen receptor T-cell (CARt) therapy have shown great success in diseases with well-characterized antigens that are relatively tumor-specific (Grupp et al. 2013) as identified by genomic profiling. Variously referred to as precision oncology (Sohal et al. 2015), genomics-driven oncology (Garraway 2013), genomic oncology, and even simply as precision medicine, the paradigm of applying high-throughput genomic approaches to patient samples is rapidly changing the landscape of oncology care and clinical oncology research.

Conventional approaches to clinical trials design may be inadequate due to molecular heterogeneity of tumors derived from a single primary tissue (Simon 2016), leading to the adoption of basket, umbrella, and hybrid trials designs. A number of studies are ongoing to determine feasibility and potential impact of precision genomic oncology at the point-of-care (Cheng et al. 2015; *NCI-MATCH Trial (Molecular Analysis for Therapy Choice)* n.d.; Lopez-Chavez et al. 2015). In addition to studies focused on identifying targetable mutations, immune-based therapeutic approaches are also being informed by HTS applied to patient samples (Bethune and Joglekar 2017; Chalmers et al. 2017; Faltas et al. 2016).

One of the most recent developments in the field of precision oncology is the approval of Pembrolizumab (Keytruda), a anti-PD-1 antibody that functions as a checkpoint inhibitor, by the US Food and Drug Administration for treatment of solid tumors that show genetic evidence of mismatch repair and therefore carry very high mutational burdens (Le et al. 2017). Pembrolizumab was previously approved for use in melanoma, but the most recent approval is the first that is targeting allows a drug to be used in a non-tissue-specific context in patients showing a specific genomic marker in any solid tumor (Garber 2017).

As with any clinical testing modality, whether in a research setting or at the point-of-care, a clear understanding of the goals of applying the test is necessary when first designing the test and its validation. However, the flexibility and number of potential data items that arise from even a limited application of HTS has lead the US Food and Drug Administration (FDA) has begun to define its regulatory role (FDA 2015) and, critically, how existing knowledge bases can be applied in real time to address findings from clinical HTS testing (FDA 2016).

This review aims to provide an organized set of biological knowledge bases with relevance to the interpretation of small variants, defined as single nucleotide variants or short (on the order of 20 base pairs or fewer) insertions and deletions. The catalogs of observed variants section lists large-scale catalogs of variants, useful for filtering known common polymorphisms and identifying previously-identified cancer variants. When a variant observed in a clinical sample has not been seen but appears to affect the protein coding sequence, the functional annotation resources section presents a sampling of some of the most common software and databases for predicting the impact on protein function. Finally, we catalog several data products and knowledgebases have been developed to provide decision support (with strong disclaimers and caveats) directly linking observed variants to clinical intervention in point-of-care HTS applications. Integrating the various data sources described in this review with variants observed in individual patients can be accomplished with combinations of software tools for the manipulation of variant datasets.

1.1 Catalogs of observed germline and somatic variants

Databases of observed variation in normal populations, diseased individuals, and cancer compendia form the map onto which observed variants in patients are projected. Because of the vast quantities of genomic data and, specifically, DNA variants, there is a tension between providing rich, highly-curated information about individual variants and producing the largest possible catalog of variants with manageable levels of curation. This section reviews some of the available catalogs (Table 1) of genomic variation observed in the germline as well as those that appear in tumors as somatic mutations. Note that many of the databases mentioned below overlap in data sources (some nearly completely), but they may differ in the amount and depth of curation, additional metadata added

to each variant, speed of updates, and methods or formats for access.

1.2 Germline

Comprehensive catalogs of germline variants inform decisions about the frequency of variants as seen in the general population as well as to identify variants that are annotated as cancer-associated. In the context of tumor sequencing, common variants are unlikely to be genomic drivers of carcinogenesis and are often filtered from a report of potential somatic variants. This filtering process is particularly important when tumor sequencing is not accompanied by matched normal sequencing. Additional germline databases that catalog disease-associated variants can be useful to begin to address familial risk and potentially pharmacogenomic loci (Wheeler et al. 2013; Relling and Evans 2015).

Perhaps the oldest of the variant catalogs, dbSNP contains 325,658,303 individual variant records (build 150, accessed May 30, 2017) and is available in multiple formats, searchable, and linked to records in literature and other data resources and databases. While the vast majority of variants in dbSNP have been observed in individuals without cancer, somatic variants are included and annotated in the database. Because dbSNP is driven by community submission of variants, levels of evidence vary among individual variants. The genome Aggregation Database, or gnomAD, (Lek et al. 2016; *gnomAD browser* n.d.) contains information from 123,136 exomes and 15,496 whole-genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies (accessed May 30, 2017). These data were collected by numerous collaborations, underwent standard processing, and unified quality control and results area accessible as a searchable online database and as a downloadable VCF-format text file. ClinVar (Landrum et al. 2016), maintained by the NIH National Center for Biotechnology Information (NCBI), is a freely available archive for interpretations of clinical significance of variants for reported conditions. Entries in ClinVar are taken directly from submitters and represent the relationship between variants and clinical significance. When multiple submissions concerning a single variant are available, ClinVar supplies high-level summaries of agreement or disagreement across submitters. Importantly, though, clinical significance in ClinVar is reported as supplied by the submitter. The Personalized Genome Project (Church 2005) provides a limited number of fully open-access genome sequencing results provided by volunteers with trait surveys and even some microbiome surveys of participants. A catalog of germline variants derived from 69 genomes sequenced using the Complete Genomics sequencing platform (Drmanac et al. 2010) may be useful for groups who have data generated from the same platform, particularly for identifying sequencing-platform-specific false positive results.

1.3 Somatic

Whereas databases of germline variants are useful to filter out variants unlikely to be directly involved in carcinogenesis, databases of somatic variants are useful to identify variants and their frequencies as observed in tumors. In some cases, identified variants may be associated with specific tumor types, offering mechanistic clues, particularly in the rare cancer setting where biological understanding may be limited.

Several catalogs of somatic variants have, at their core, variants derived from The Cancer Genome Atlas (TCGA). These databases vary in the pipelines used to define the variants, the level of annotation associated with individual variants, the proportion of TCGA included, and methods for accessing or querying. Recently, National Cancer Institute (NCI) has established the Genomic Data Commons (GDC) to harmonize clinical information and genomic results across enterprise cancer datasets (Grossman et al. 2016), particularly those funded by NCI, such as TCGA. In addition to the adult tumors profiled as part of the TCGA, the NCI GDC also contains data from several pediatric tumors profiled as part of the Therapeutically Applicable Research To Generate Effective Treatments (TARGET) project (*Therapeutically Applicable Research to Generate Effective Treat-*

ments (TARGET) n.d.). Cancer cell line data from the Cancer Cell Line Encyclopedia (CCLE) are also included (Barretina et al. 2012) in the GDC data collection. The GDC is a modern data platform that provides multiple access methods including a programmatic application programming interface (API), data file download, and web browser based text and graphical queries and visualization. The International Cancer Genome Consortium (ICGC) is a large, international collaboration with a collection of 76 studies (including TCGA studies) encompassing 21 tissue primary sites. Like the NCI GDC, the ICGC data portal provides modern data platform approaches to data access, visualization, and query (Zhang et al. 2011). The Catalog of Somatic Mutations in Cancer (COSMIC) database is perhaps the largest and best-known cancer variant database. It presents a unified dataset consisting of curated cancer variants for specific genes as well as genomic screens from projects such as TCGA. Several other cancer variant data resources are listed in Table 1.

2 Functional Annotation Resources

When faced with variants with little or no literature or database support, differentiating those that variants that are likely to be deleterious, perhaps contributing to carcinogenesis, versus those that likely are tolerated by the cell is a critical task, particularly in the setting of clinical precision genomic oncology. Note that determing that a variant is deleterious is not likely to result in a change in diagnosis, prognosis, or therapy. However, prioritizing variants for further study, research interest, and for discussion in forums such as a molecular tumor board is a valuable and necessary aspect of applying genomic technologies in the clinical arena.

A number of algorithms and methods have been developed to predict the effect of observed variants on protein structure and function as well as the potential for clinical impact. These prediction methods utilize features of the variant and its context such as sequence identity, sequence conservation, evolutionary relationship, protein primary and secondary structure, entropy based protein stability and approaches such as clustering based on sequence alignments and machine learning. Some of them are specific to the type of variant or mutation, some to a disease type, and some more general. Therefore, applying these functional annotational tools and interpreting the results in a clinical or research setting may require significant human curation before being recognized as clinically actionable. Here we present a review of a representative set of approaches for predicting pathogenicity of different variants. For a comprehensive list of prediction tools and their details see Table 2. For more detailed scientific and technical explanations of these methods, we refer the reader to a comprehensive review (Addepalli 2014).

2.1 SIFT

Sorting Intolerant From Tolerant, or SIFT, predicts functional impacts of amino acid substitutions (Ng and Henikoff 2003) is one of the earliest variant effect prediction tools and represents the class of prediction algorithms that utilizes protein conservation. It has since been updated and an online version of the tool is available (Kumar, Henikoff, and Ng 2009). SIFT uses sequence homology, as measured by protein-level conservation, to classify variants based as tolerated or deleterious based on the associated protein coding changes. SIFT has served as a benchmark against which other methods are compared because of its relative simplicity. SIFT considers the type of amino acid change induced by a genomic variant and the position at which the change/mutation occurs. SIFT relies on the presence of sequences from which conservation can be determined; variants for which such databases are limited will potentially lack robust predictions.

2.2 PolyPhen-2

Polymorphism Phenotyping v2, or PolyPhen2, predicts the effecting of coding nonsynonymous SNPs on protein structure and function and annotates them (Adzhubei, Jordan, and Sunyaev 2013). This

algorithm uses a naive Bayes approach to combine information across a panel of 3D structural, sequence-based, and conservation-based features. Trained on two datasets, HumDiv and HumVar, and associated non-deleterious controls, the PolyPhen2 algorithm represents a class of multivariate prediction algorithms that employ machine learning and multiple features of variant impact.

2.3 Mutation Assessor

Mutation Assessor is an algorithm and tool that, like SIFT, uses a conservation-based approach. However, Mutation Assessor also incorporates evolutionary information in an attempt to account for shifts in function between subfamilies of proteins (Reva, Antipin, and Sander 2011), potentially extending the functional annotation of variants to "switch of function" as well as loss or gain of function. By quantifying the impact to conserved residues both globally and within subfamilies (residues that distinguish subfamilies from each other are thought to be less tolerant to change), Mutation Assessor defines a functional impact score to predict which variants are likely to be deleterious.

2.4 CONDEL

The CONsensus DELeteriousness, or CONDEL score, is an integrated prediction method for missense mutations that is relatively easy to extend with additional prediction resources (Gonzlez-Prez and Lpez-Bigas 2011). Originally implemented as a weighted average of the normalized scores from the output of two computational tools, Mutation Assessor and FATHMM, CONDEL can be extended or adapted to data at hand and represents an "aggregator" approach to variant effect prediction. Condel scores can be derived for a limited set of specified mutations via an online web application. The Ensembl database provides a variation of position-specific CONDEL predictions that combine SIFT and Polyphen-2 for every possible amino acid substitution in all human proteins.

2.5 CHASM

Cancer-specific High-throughput Annotation of Somatic Mutations, or CHASM, is a computational method that identifies and prioritizes the missense mutations likely to enhance tumor cell proliferation (Carter et al. 2009). CHASM uses machine learning to classify putative "driver" cancer mutations as distinct from "passenger" mutations. Training the CHASM model employed in-silico simulation to generate realistic "passenger" mutations, specifically modeled to represent variant context and genes that are observed in cancer settings. Multiple features of the variants including their DNA and protein contexts were then used to build a machine learning approach that attempted to maximize the specificity of separating driver mutations from passenger mutations. CHASM represents a relatively specific algorithm focused not on "deleteriousness" but, rather, on the likelihood that an observed variant is a cancer "driver".

2.6 dbNSFP

Recognizing that applying all of the effect prediction tools available is potentially challenging, Liu et al. 2016 developed a database that aggregates predictions for *all* possible SNVs associated with coding changes (in Gencode gene models). With more than ten different prediction algorithms and extensive additional annotation, this database can be a useful one-stop-shop for adding annotations to variant datasets. The snpEff suite (described below) can be used in conjunction with dbNSFP to efficiently annotate SNPs with the potential to effect coding genes.

3 Clinical Actionability

The ultimate goal for many of the above-mentioned resources is to develop an individualized approach to the diagnosis, prevention and treatment of cancer, or precision oncology. However, despite recent advances in HTS, determining the clinical relevance of experimentally observed cancer variants remains a challenge in the application of HTS in clinical practice. Difficulties in differentiating driver and passenger mutations, lack of standards and guidelines in reporting and interpretation of genomic variants, lack of clinical evidence in associating genomic variants to clinical outcome, lack of resources to disseminate clinical knowledge to the cancer community and the precise definition of actionability have been reported to contribute to the bottleneck (M. M. Li et al. 2017; Prawira et al. 2017; Uzilov et al. 2016; Hedley Carr et al. 2016). Comprehensive resources linking experimentally determined cancer variants and clinical actionability have been developed to address some of these challenges and address various aspects of translating research results into clinical valuable information to support clinical decisions in precision oncology (See Table 3). In recognition of the fact that central curation of information regarding actionability is extremely challenging, several of the resources below use crowdsourcing as a means of gathering updates and enhancing curation efforts. In addition to a web interface, some tools provide additional access via API, mobile app, and/or social media tagging to facilitate dissemination of information and enhance accessibility. While some of these tools share similar functions, in the section below, we highlight distinct features and capabilities for a representative set of resources.

Clinical Interpretation of Variants in Cancer (CIViC) is an open access and open source platform for community-driven curation and interpretation of cancer variants. It is based on a crowdsourcing model where individuals in the community can contribute to produce a centralized knowledge base with the goal of disseminating knowledge and encouraging active discussion. Users, including patients, patient advocates, clinicians and researchers, can participate, along with community editors, in various stages of interpreting the clinical significance of cancer variants using standards and guidelines developed by community experts (M. M. Li et al. 2017; M. Griffith, Spies, et al. 2016).

The Drug Gene Interaction Database (DGIdb) is an open source and open access platform for gene and drug annotation for known interaction and potential druggability. Users can can cross-reference genes of interest and drugs against up to 15 sources and in functionally classified gene categories (Wagner et al. 2016; M. Griffith, O. L. Griffith, et al. 2013). Cancer Genome Interpreter (CGI) identifies mutational events that are biomarkers of drug response or interact with known chemical compounds (Tamborero et al. 2017). PharmGKB is a pharmacogenomic resource for building clinical implementation and interpretation based on annotating, integrating and aggregating knowledge extracted from research-level publications. It provides scored clinical annotation, prescription annotation (drug dosing, prescribing information), as well as pharmacokinetics/pharmacodynamics (PK/PD) annotation, with primary literature reference.

OncoKb contains information on the clinical implication of specific genetic alterations in cancer. Each variant is annotation from multiple sources and scored using Levels of Evidence ranging from Level 1, which includes FDA approved biomarker predictive of response to an FDA-approved drug, to Level 2, which includes variants for which an FDA-approved or standard of care treatment is available, Level 3 and Level 4 contain variants with investigational and hypothetical therapeutic implications, respectively. A similarly structured scoring system is available for indicating therapeutic implications for variants associated with resistance (Chakravarty et al. 2017). Cancer Driver Log (CanDL), an expert-curated database for potential driver mutations in cancer, employs a similar four-level scoring system based on FDA approval, clinical, pre-clinical and experimental functional evidence (Damodaran et al. 2015).

MyCancerGenome (MCG) is a knowledge resource highlighting the implication of tumor mutation on cancer care. It allows users to access its content via a mobile app and provide patient-focused information. Patients can access a database entitled DNA-mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT) for Epidermal Growth Factor Receptor (EGFR) mutation for non-

small cell lung cancer (NSCLC). Personalized Cancer Therapy (PCT) at the MD Anderson Cancer Center is a resource for clinical response associated with cancer variants and aims to facilitate patient involvement in biomarker-related clinical trials. Drug effectiveness is associated with a specific biomarker and scored based on prospective clinical study as well as Food and Drug Administration (FDA) approval.

4 Tools for manipulating variant datasets

Processing sequence data with the goal of determining variants (somatic or germline) often ends with a file in Variant Call Format (VCF format), a loose, self-describing data standard describing variants along a genome, associated statistical and numeric metrics for each variant, and information integrated from data resources such as those described in the preceding sections (Danecek et al. 2011). An ecosystem of tools, listed in Table 4, has been developed for basic transformations, manipulations, merge operations, and for adding transcript, protein, and higher-level functional annotations to variants in a VCF file. The vt and bcftools software suites perform operations such as slicing by genomic coordinate, data compression, and, importantly variant normalization, rendering variants more readily comparable across resources. Annovar (Yang and Wang 2015; Wang, M. Li, and Hakonarson 2010) and the SnpEff suite (Cingolani 2012) add annotations relative to gene annotations, including information about transcript and protein-coding changes. Recently, several software developers of variant annotation tools have developed a standard for reporting gene-centric annotations that has simplified post-processing of variants after annotation. Finally, tools such as Vcfanno (Pedersen, Layer, and Quinlan 2016) have been developed that can flexibly add fields to variants in a VCF file based on relatively sophisticated logic and data transformations, reducing the number of tools required to bring a new data resource into the annotation pipeline.

5 Discussion

5.1 Pragmatic details

Despite advanced toolsets for manipulating variant files and increasing adoption available standard formats, practical pitfalls and challenges remain to the basic manipulation of variant datasets. Some data resources are available in multiple formats and not all formats contain identical information. Matching variants between resources and observed variants can be challenging, as some variants can be represented validly in multiple forms. Ideally, variants are cataloged with clarity with respect to a reference genome and, whenever possible, using HGVS nomenclature (Dunnen et al. 2016). In spite of increasing awareness and uptake of HGVS standard nomenclature, the critical step of matching variants across tools and databases in assessing clinical significance is still hampered by inconsistencies across tools and databases (Yen et al. 2017). Particularly when handling clinical samples, an information system that provides results from multiple resources when assessing novel variants, incorporates in silico controls when adding or updating data resources (to avoid introducing errors), and adheres to HGVS nomenclature wherever possible in data processing pipelines can increase the likelihood of discovering potentially relevant variants.

This review is meant to be comprehensive, so the reader might wonder "Where do we start?". It is difficult to make hard-and-fast recommendations about what resources, tools, and databases are "the best" given the lack of gold-standard datasets on which to base such evalutations. Furthermore, the context for sequencing (clinical or not, targeted mutations, trial setting, or novel variant and biomarker discovery) will drive annotation pipeline development. Not all data resources need to be added simultaneously if developing a pipeline for annotating cancer variants for precision oncology applications. In a clinical setting, targeting the reporting workflow and working with clinicians to understand the most relevant annotations is the most efficient approach to determin-

ing relevant resources for annotation. In the more general sequencing applications, starting with some key resource (dbSNP, COSMIC, ClinVar, dbNSFP, for example) and extending with additional resources as they become available or as time and interest permits is a reasonable approach. Developing a modular informatics pipeline, perhaps using a computational workflow framework (`https://github.com/pditommaso/awesome-pipeline`) that can be easily extended and re-run on previously annotated data is helpful to keep pace with the rapidly changing and growing collection of annotation resources.

5.2 Conclusion

Robust sequencing technologies and increasingly reliable bioinformatics pipelines, combined with parallel development of therapeutics and diagnostics has bolstered the field of precision genomic oncology. However, the sheer number of resources available that can inform the interpretation of small variants is staggering, except for the very few variants with well-established clinical relevance or an associated targeted therapy. This review has highlighted a number of important data resources individually. For other variants, data integration remains a significant hurdle to the rapid turnaround required to apply HTS in a clinical context. Expert panel review (the molecular tumor board) has been effective for some groups (Knepper et al. 2017; Beltran et al. 2015; Sohal et al. 2015) while other groups have adopted a protocol-based approach (Ghazani et al. 2017). Even when molecularly targetable lesions are identified, barriers to delivering therapy have been observed, limiting the impact of precision genomic oncology in some settings (Bryce et al. 2017).

Cancer arises in an individual after a typically complex and incompletely understood set of oncogenic events that are increasingly observable at the molecular level. Progress in cancer prevention, early detection, diagnosis, prognosis, and treatment is increasingly driven by insight gained through the analysis and interpretation of large genomic, proteomic, and pharmacological knowledge bases. Reductionist approaches to cancer biology can achieve only limited success in understanding cancer biology and improving therapy. Cancer is a disease associated with disruption of normal cellular circuitry and processes that leads to abnormal or uncontrolled proliferative growth, characterized by a complex spectrum of biochemical alterations that affects biological processes at multiple scales from the molecular activity and cellular homeostasis to intercellular and inter-tissue signaling. The cancer research community has made great strides in measuring the oncogenic events that lead to the development of cancer and therapy resistance. However, the challenges brought by complexity inherent in protein networks, intercellular signaling, cellular heterogeneity, and the dynamic nature of cancer demand multiscale systems and modeling approaches that address the interrelatedness of the biological processes underlying cancer.

6 Conflict of Interest

This work was performed while KA and HT were employed by Attain, LLC, in support of bioinformatics projects at the National Cancer Institute. The authors declare that the work was conducted in the absence of any commercial or financial relationships that constitute a potential conflict of interest.

7 Acknowledgments

This work was supported by the National Cancer Institute Center for Biomedical Informatics and Information Technology and the National Cancer Institute Center for Cancer Research in the Intramural Research Program at the National Institutes of Health.

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Resource	Variant Type	URL	Citation
dbSNP	Germline and somatic	https://www.ncbi.nlm.nih.gov/projects/SNP/	(Sherry et al. 2001)
COSMIC	Somatic	http://cancer.sanger.ac.uk/cosmic	(Reva, Antipin, and Sander 2011)
gnomAD	Germline	http://gnomad.broadinstitute.org/	(Lek et al. 2016)
69 genomes from CGI	Germline	http://www.completegenomics.com/public-data/69-genomes/	(Drmanac et al. 2010)
Personalized Genome Project	Germline	http://www.personalgenomes.org/	(Church 2005)
ClinVar	Germline predisposition and somatic	https://www.ncbi.nlm.nih.gov/clinvar/intro/	(Landrum et al. 2016)
NCI Genomic Data Commons	Germline and somatic	https://portal.gdc.cancer.gov/	(Grossman et al. 2016)
cBioPortal	Somatic	http://www.cbioportal.org	(Cerami et al. 2012; Gao et al. 2013)
Intogen (Partial TCGA dataset)	Somatic	https://www.intogen.org/search	(Rubio-Perez et al. 2015; Gonzalez-Perez et al. 2013)
Pediatric Cancer Genome Project	Somatic	http://explorepcgp.org	(Downing et al. 2012)

Table 1: Catalogs of germline and somatic variants. The most commonly used catalogs include dbSNP, COSMIC, ClinVar, and gnomAD.

Resource	URL	Citation	Notes
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2	(Adzhubei, Jordan, and Sunyaev 2013)	Bayesian classification
SIFT	http://sift.jcvi.org	(Ng and Henikoff 2003)	Alignment scores
MutationAssessor	http://mutationassessor.org	(Reva, Antipin, and Sander 2011)	conservation, naive Bayes classifier
MutationTaster	http://www.mutationtaster.org	(Schwarz et al. 2014)	
PROVEAN	http://provean.jcvi.org/index.php	(Choi et al. 2012)	
CADD	http://cadd.gs.washington.edu	(Kircher et al. 2014)	
GERP++	http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html	(Davydov et al. 2010)	
PhyloP and PhastCons	http://compgen.cshl.edu/phast/index.php	(Siepel et al. 2005; Pollard et al. 2010)	
nsSNPAnalyzer	http://snpanalyzer.uthsc.edu/	(Bao, Zhou, and Cui 2005)	Random Forest
SNPs&GO	http://snps-and-go.biocomp.unibo.it/snps-and-go/	(Calabrese et al. 2009)	SVM
SNAP2	https://rostlab.org/services/snap2web/	(Hecht, Bromberg, and Rost 2015)	Neural Networks
SNPs3D	http://www.snps3d.org/	(Yue, Melamud, and Moult 2006)	Structure and sequence analysis
MutPred2	http://mutpred.mutdb.org/	(Pejaver et al. 2017)	Random Forest
AUTO-MUTE	http://binf2.gmu.edu/automute/	(Masso and Vaisman 2010)	Topology and statistical contact potential
Panther	http://www.pantherdb.org/tools/csnpScoreForm.jsp	(Thomas et al. 2003)	Hidden Markov Model
stSNP	http://ilyinlab.org/StSNP/	(Uzun et al. 2007)	comparative modelling of protein structure
Integrated predictive methods and aggregated databases			
Condel	http://bg.upf.edu/fannsdb/	(Gonzlez-Prez and Lpez-Bigas 2011)	a weighted average of multiple methods
CoVEC	https://sourceforge.net/projects/covec/files		
CAROL	http://www.sanger.ac.uk/science/tools/carol	(Lopes et al. 2012)	combines PolyPhen-2 and SIFT
dbNSFP	https://sites.google.com/site/jpopgen/dbNSFP	(Liu et al. 2016)	Aggregated database of variant information
Cancer-specific prediction tools			
CHASM	http://wiki.chasmsoftware.org/index.php/Main_Page	(Carter et al. 2009)	Random Forest
CanDrA	http://bioinformatics.mdanderson.org/main/CanDrA#CanDrA	(Mao et al. 2013)	96 structural, evolutionary and gene features

Table 2: Tools, software, and databases for functional prediction and annotation of variant impact. The most common approaches include SIFT, PolyPhen-2, CADD, and CHASM, though there is little consensus about which are "best". One of the most efficient approaches is to use an aggregated database like dbNSFP to add many annotations simultaneously.

Resource	URL	Citation	Crowd-sourcing used	Access
Cancer Driver Log	https://candl.osu.edu/	(Damodaran et al. 2015)	Yes	
Cancer Genome Interpreter	https://www.cancergenomeinterpreter.org/home	(Tamborero et al. 2017)	Yes	API
CIViC	https://civic.genome.wustl.edu/home	(M. Griffith, Spies, et al. 2016)	Yes	API
DGIdb	http://dgidb.genome.wustl.edu/	(Wagner et al. 2016; M. Griffith, O. L. Griffith, et al. 2013)	Yes	
Clinical Knowledge Base	https://www.jax.org/clinical-genomics/clinical-offerings/ckb			
My Cancer Genome	http://www.mycancergenome.org	(Micheel, Lovly, and Levy 2014)	Yes	API, app
OncoKb	http://oncokb.org/	(Chakravarty et al. 2017)		API
Personalized Cancer Therapy	https://pct.mdanderson.org			
PharmGKB	https://www.pharmgkb.org/	(Hewett et al. 2002)	Yes	
Precision Medicine Knowledge Base (Beta)	https://pmkb.weill.cornell.edu/	(Huang et al. 2016)	Yes	
TumorPortal	http://www.tumorportal.org/	(Lawrence et al. 2014)		

Table 3: Tools, software, and databases for functional prediction and annotation of variant impact. In a clinical setting, these databases are the most relevant, as they are maintained to provide clinically actionable and curated content. Evalutation of each database by both clinical and informatics team members is probably warranted when choosing which and how to include these data in the annotation pipeline and, potentially, in clinical reports.

Software	URL	Citation
vt	http://genome.sph.umich.edu/wiki/Vt	(Tan, Abecasis, and Kang 2015)
bcftools	http://www.htslib.org/download/	(H. Li et al. 2009)
ANNOVAR	http://annovar.openbioinformatics.org/en/latest/	(Wang, M. Li, and Hakonarson 2010)
SnpEff	http://snpeff.sourceforge.net/	(Cingolani 2012)
Oncotator	https://portals.broadinstitute.org/oncotator/	(Ramos et al. 2015)
vcfanno	https://github.com/brentp/vcfanno	(Pedersen, Layer, and Quinlan 2016)

Table 4: Software tools for manipulating and adding annotations to variant datasets.