

Identifying Actionable Variants in Cancer - The Dual Web and Batch Processing Tool MTB -Report

Nadine S. KURZ^{a,*}, Júlia PERERA-BEL^{b,*}, Charlotte HÖLTERMANN^a, Tim TUCHOLSKI^a, Jingyu YANG^a, Tim BEISSBARTH^a and Jürgen DÖNITZ^{a,1}

^a*Dept. of Medical Bioinformatics, University Medical Center Göttingen, Germany*

^b*MARGenomics, Hospital del Mar Medical Research Institute (IMIM), Spain*

Abstract. Next-generation sequencing methods continuously provide clinicians and researchers in precision oncology with growing numbers of genomic variants found in cancer. However, manually interpreting the list of variants to identify reliable targets is an inefficient and cumbersome process that does not scale with the increasing number of cases. Support by computer systems is needed for the analysis of large scale experiments and clinical studies to identify new targets and therapies, and user-friendly applications are needed in molecular tumor boards to support clinicians in their decision-making processes. The MTB-Report tool annotates, filters and sorts genetic variants with information from public databases, providing evidence on actionable variants in both scenarios. A web interface supports medical doctors in the tumor board, and a command line mode allows batch processing of large datasets. The MTB-Report tool is available as an R implementation as well as a Docker image to provide a tool that runs out-of-the-box. Moreover, containerization ensures a stable application that delivers reproducible results over time. A public version of the web interface is available at: <http://mtb.bioinf.med.uni-goettingen.de/mtb-report>

Keywords. Variant interpretation, molecular tumor board, actionable variants, molecular profiles, next-generation sequencing, precision oncology

1. Introduction

Next-generation sequencing (NGS) platforms are capable of generating large amounts of clinically relevant data, especially in cancer genomics. The gained knowledge is being deposited in a vast variety of databases and knowledge base resources [1], complicating the task of gathering relevant information for clinicians and researchers alike. Specifically the identification of actionable variants, i.e. genomic variants with relevance for clinical decisions and cancer research, remains a challenge. Actionability in this context may range from biomarkers for approved drugs following the indications of cancer type, to cancer types not included in the drug indication (off-label use), or to variants being studied in clinical trials.

The bioinformatician, pathologist or physician who obtained genomic data from a patient tumor faces an overwhelming task. Having evaluated and filtered the data in

¹ Corresponding Author, Jürgen Dönitz, University Medical Center Göttingen (UMG), Goldschmidtstr. 1, 37077 Göttingen, Germany; E-mail: juergen.doenitz@bioinf.med.uni-goettingen.de. * equal contribution.

terms of quality, the identified genomic variants need to be interrogated for biological and clinical implications. Mutations with well-established biological impact can be identified by accessing public knowledge sources (e.g. dbSNP [2], COSMIC [3], TCGA [4]) or by applying prediction models (e.g. SIFT [5], PolyPhen-2 [6], IntOGen [7]). A detailed overview of available data resources and tools for cancer variant interpretation is provided by [8].

However, in case the variant under investigation is not associated with clear clinical evidence (e.g. an ALK mutation or amplification instead of rearrangements, unknown BRAF mutation), a range of questions arises: is this mutation a loss or gain of function (LoF and GoF, respectively)? Does it confer sensitivity to any targeted drug? Is there any existing evidence on this variant in another cancer type? Does it result in resistance, or in susceptibility to certain treatments?

Many institutions are therefore making efforts to gather and structure information from clinical trials, case reports, publications of preclinical experimental research as well as guidelines and approval organizations in online databases. Among these essential efforts we find CIViC [9], GDKD [10], OncoKB [11], My Cancer Genome [12] and PMKB [13]. Yet, these resources are not aimed at querying multiple variants at a time, as is usually the case when a tumor genome is sequenced. Tools to interpret tumor genomes are arising, such as Cancer Genome Interpreter [14] and Personal Cancer Genome Reporter (PCGR) [15], however none of these tools offer the option to be run locally on large amounts of variant data, as is often needed in cancer research and bioinformatics.

Here, we present MTB-Report, an application that can be used on the one hand as a web interface aiming for the manual preparation of patient data for a molecular tumor board. On the other hand, it can be used as a command line tool to process large data sets to analyze data of wet lab experiments and large-scale screens. The tool is containerized as a Docker image which switches to one of these two modi based on the container runtime parameters.

2. Methods

2.1. Input data structure

The provided variants for MTB-Report may be Single Nucleotide Variants (SNVs), Copy Number Variants (CNVs) or fusion proteins. Input data can be uploaded in several tabular data formats (TXT, CSV, XLSX), yet they require a certain structure. SNVs are accepted as Variant Call Format (VCF), Mutation Annotation Format (MAF) files or as text files with a table with at least three columns (gene, variant type, variant). The genomic locations that are stored in VCF or MAF files are internally converted to protein locations, the typical variant format in public databases, in an automated fashion. CNVs must be a table with at least two columns: The first column contains gene symbols, the second column specifies the type of copy number alteration (amplification or deletion). Gene fusions must be a table with two columns, each contains one of the two affected genes of a fusion gene. For the gene names, the gene names defined by the HUGO Gene Nomenclature Committee [16] at the European Bioinformatics Institute should be used.

2.2. Databases

Genomic input data are queried against databases specialized on cancer predictive biomarkers. The user can select among the following databases:

- Gene Drug Knowledge Database - GDKD [10]: Expert-curated database that focuses exclusively on somatic variants which predict response to anti-cancer drugs (genomic predictive biomarkers). Variants with preclinical evidence are filtered based on their scientific soundness and translational power.
- Clinical Interpretation of Variants in Cancer - CIViC [9]: Expert-crowdsourced knowledgebase for the curation of genomic biomarkers in cancer: prognostic, diagnostic, predisposing and predictive. It also contains both germline and somatic variants.
- OnkoKB [11] is another database for precision medicine from the Memorial Sloan Kettering Cancer Center. For nearly 700 genes with around 5700 alterations are evidence-based information. Due to license limitations, the database is not distributed as part of the MTB-Report implementation, but can be provided by the user.
- Tumor Alterations Relevant for Genomics-driven Therapy - TARGET [17]: Consists of a list of 135 genes manually curated by experts from the Dana-Farber Cancer Institute with predictive, prognostic and diagnostic implications in cancer.
- Meric-Bernstam et al., 2015 [18]: List of therapeutically actionable genes (i.e. predictive biomarkers) with a focus on genes included as selection criteria in clinical trials.

By default, MTB-Report embeds the latest database versions. Apart from [11], the databases are included by default and can optionally be provided by the user in case a specific database version is required.

3. Results

The MTB-Report tool is a convenient tool which supports the two important use cases of precision oncology - supporting molecular tumor boards by providing actionable variants to clinicians as well as reporting actionability for large scale data analyses in cancer research. The underlying computational algorithm and methods have previously been published in [19], where an evaluation of the results showed very high sensitivity in the detection of actionable variants.

3.1. Code availability and portability

The application is available as an R implementation published as Open Source. Additionally, the implementation is available as a Docker image to provide a platform-independent environment for the application. The dependencies are conveniently shipped with the application, and thus ensure a stable tool out of the box and allow results to be reproducible. The Docker image can be used to start both the web server for interactive analysis or batch processing mode on the command line. A public instance of the web application is available at <http://mtb.bioinf.med.uni-goettingen.de/mtb-report>. The source code is available at <https://gitlab.gwdg.de/MedBioinf/mtb/mtb-report>.

A

1. Clinical 2. SNVs 3. CNVs 4. Fusions

Upload Data

Click to get example CSV file

Browse... TCGA.KICH.muse.3f2602f

Upload complete

File extensions accepted are: .xls, .xlsx, .csv, .dat, .tsv, .maf, .vcf

Separator

Comma

Semicolon

Tab

Header

B

SNVs CNVs Fusions **Browse Genomic Data**

Single nucleotide variants of the patient's tumor:

Search:

Hugo_Symbol	Variant_Classification	Protein_Change
ENO1	Missense_Mutation	L381F
CCR2	Missense_Mutation	R276K
SPATA31E1	Missense_Mutation	L281V
FOLR2	Missense_Mutation	G84S
ZNF106	Missense_Mutation	T1639I

Showing 1 to 5 of 1,313 entries

C

Summary of Actionable Variants: Filter & Selection

Select Actionable Variants

Single Nucleotide Variants

Gene	Patient's Variant	Level of Evidence
NRAS	Q61K	B1,B2,B3
CTNNB1	R212H	B2,B3
CDK6	R220C	B2,B3
SETD2	R2399*	B3
CHEK2	R406H	B2

Showing 11 to 15 of 25 entries

Copy Number Variants

Gene Pat Var level.col

No data available in table

Showing 0 to 0 of 0 entries

Select Levels of Evidence

Evidence on SAME cancer type

A1) FDA & Guidelines

A2) Clinical Trials

A3) Pre-clinical

Evidence on OTHER cancer types

B1) FDA & Guidelines

B2) Clinical Trials

B3) Pre-clinical

Display other genes without evidence level

FINDINGS BY LEVEL

1 Approved 2 Clinical 3 Pre-clinical

Sort results by:

Genes

Drugs

Levels of Evidence

Download results

Download report (.pdf)

Download report (.csv)

Download metadata (.yaml)

D

Details of Actionable Variants

Search:

Gene	Pat Var	Cancer	Known Var	Predicts	Drugs	Evidence	Ref	Origin Database	level
CHEK2 (PMKB,DGIdb)	R406H	prostate	any variant (LoP)	response	PARP inhibitors	early trials	26510020	GDKD	B2
CTNNB1 (PMKB,DGIdb)	R212H	endometrial	H36Y, S37C, S37Y, D32V (GoF)	response	everolimus + letrozole	early trials	25624430	GDKD	B2
CTNNB1 (PMKB,DGIdb)	R212H	melanoma	activating any mut.	resistance	Anti-CTLA-4 Monoclonal Antibody, Anti-PD-L1 Monoclonal Antibody	preclinical	25970248	CVIC	B3
CTNNB1 (PMKB,DGIdb)	R212H	colorectal	any variant (GoF)	resistance	Tankyrase inhibitors	preclinical	23539443	GDKD	B3

Showing 1 to 4 of 4 entries

Figure 1. User interface of MTB-Report, showing A) the input form for patient data, B) a genome browser, and C) the results section of a query displaying the identified actionable variants. D) Upon selection of one or multiple variants, the details of all evidence linked to these variants are displayed in the table of results.

3.2. The web application

The interactive web application is written in R using the Shiny framework [20]. It is launched automatically by the Docker container when no input files are provided, and can then be accessed locally using a browser at <http://localhost:3838/mtb-report>. The graphical user interface (GUI) guides the user through several steps of data input, enabling a convenient environment for uploading genomic variants and adding metadata about the patient. Of note, this patient data is not used in querying the databases and thus

does not influence the results, but is instead appended to the output tables to be used in subsequent analyses. The application does not apply quality filters to the variants provided: it assumes that the input only contains high quality variants. Additionally, the user can explore sample data from the TCGA [4] project.

After providing the variant and patient data in the input form, MTB-Report provides multiple possibilities to browse the parsed genomic data. In a first summary box (“Summary of Actionable Variants: Filter & Selection”, Figure 1) the identified actionable variants are listed. This summary by variant displays the evidence level associated to that variant, and any genomic quality provided as input by the user. An additional summary by level of evidence is depicted in a figure (with the number of findings at each level). By selecting a variant or a level of evidence, the detailed information of the actionability of a variant is displayed in the box “Details of Actionable Variants” (Figure 1). The table can be filtered by selecting one or several variants and levels of evidence. If more than one selection is applied, the union of the selection is displayed. The results can be sorted by genes, by drugs or by levels of evidence (though the interactive table allows sorting by each column, the download option will retain this selection for the generated report file). The detailed table can further be sorted according to columns or searched for specific patterns. Links to important databases of clinical implications of genomic variants such as PMKB [13] and DGIdb [21] are provided. Furthermore, the user can verify the variant-drug association with the original source. A report in Portable Document Format (PDF) or Comma-separated values (CSV) format of these results can be downloaded, where actionable variants are sorted according to their relevance in the final report.

```
data:
  path: input.maf
  cancer_type: pancreas # only for documentation
  variation_type: snv
  ref_genome: hg38 # only for documentation
tool:
  git_checksum: aac6608c
  date: '2022-02-27'
databases:
  gdkd: v20.0
  civic: 15-jan-01
  target_meric: v3
  oncokb: v1
```

Figure 2. Part of a sample metadata file generated by MTB-Report. The first section describes the input data, the following sections are generated by MTB-Report and define the software and databases’ versions.

3.3. Batch processing

Providing the files to be analyzed as command line arguments, the MTB-Report results are processed without launching the GUI, returning the results in CSV-formatted tables. The reported actionability of queried variants is identical in the web and batch processing modes, however, the CSV output generated by the batch mode is more suitable for downstream analysis, and can be further processed. Additional information, corresponding to the patient and cancer type information that can be provided in the web version, may be provided by passing an optional metadata file in YAML format as a command line parameter. The metadata file can contain several sets of input files for

different patients or different cell lines. This way, the simultaneous analysis of large-scale studies with multiple patients or biological model systems is possible.

The metadata file is not only used to structure the input, the MTB-Report tool also writes relevant information back into the YAML file. The data generated by MTB-Report includes all relevant information to make the results fully reproducible, including the MTB-Report version, the consulted databases and their versions as well as the input and output files. An example metadata file is partially listed in Figure 2.

4. Discussion

With MTB-Report we have implemented a tool for precision oncology, with an implementation of an R-based web application for the evidence-based reporting strategy for molecular tumor boards and a command line tool for large scale data projects. MTB-Report focuses on finding evidence-based actionable variants and provides an expanded catalog by reporting cancer type repurposing and low evidence levels. MTB-Report is available as a public domain tool and a stand-alone tool, a public instance is available on the institute's web page. In this way, it fits the needs of both clinicians who may use an up-to-date version of the web application, and bioinformaticians, who can install the application locally and integrate it in in-house-pipelines and custom analyses.

As the protection of sensitive patient data is one of the most important requirements in clinical deployments, all database files may be included based on the local host file system. MTB-Report can thus be installed in protected local area networks and is fully functional with restricted or no internet connections. Furthermore, the source code is freely available, which allows insights into the code and a custom local build of the Docker image.

More tools aim to provide web applications and tools for precision oncology, examples are cBioPortal [22], MTB Portal [23], the Cancer Genome Interpreter [14], PanDrugs [24], IMPACT [25,26] and MIRACUM-Pipe [27]. These tools are comparable to MTB-Report, as they focus on cancer variants, allowing a multi-query of genomic variants against selected databases and applying algorithms (heuristic or predictive) to prioritize drugs. With regard to approved drugs, all tools rely on the same resources. However, expanding the therapeutic landscape, each tool uses its own approach. For instance, the Cancer Genome Interpreter identifies driver mutations and uses the Catalog of Validated Oncogenic Mutations and the Cancer Biomarkers database [14], maintained by the same group, to identify actionable driver variants. The PCGR [15] performs multiple oncology-relevant annotations (CIViC [9], Cancer Biomarkers database [14], ClinVar [28], COSMIC [3], DGIdb [21], etc.) from VCF files. VCF files allow PCGR to include additional information retrieved from the genomic data (e.g. MSI status, mutational signatures, and mutational burden). The output is a tiered interactive report in Hypertext Markup Language (HTML) format that is intended for clinical translation. PanDrugs [24] integrates multiple resources and provides a curated model for drug annotations. This tool puts emphasis on indirect targeting and pathway repurposing, and ranks drugs according to a weighted model that considers the number of alterations supporting evidence for a drug. The IMPACT pipeline [25,26] and web portal have a strong focus on pharmacogenomic (drug-target) interactions and rank drugs as well, by computing a hypergeometric test that considers the number of alterations supporting evidence for a drug. The advantage of MTB-Report is its dual use feature to use the same methods for single patients and large-scale data projects.

All these examples show the variability between reporting tools with regard to the databases used, the prioritization rules, and the visualization approaches. It is important to note that MTB-Report is a reporting tool, and, as such, does not provide any treatment suggestions and leaves the decision to the liable person. It serves as a tool to compile and visualize available information for the treating physicians or researchers. The quality of the displayed information is therefore heavily dependent on the quality of the provided databases. In contrast, so-called treatment algorithms incorporate expert rules into the NGS bioinformatic pipeline with the final aim of assigning a treatment to a patient. Treatment algorithms ensure standardization and reproducibility by regulating technical aspects of data processing such as minimum coverage, allele frequency, fold change, size of amplicons, prediction scores, as well as the rules to match variants to drug prescription. Hence, treatment algorithms are suitable for clinical trials. The methods presented here and in [19] are still under development and for research purpose only.

5. Declarations

Ethical vote: Not applicable

Conflict of interest: The authors declare that they have no competing interests.

Authors contributions: JPB, TB: design and concept of the application and user interface; CH, JD: concept of the batch processing; NSK, JPB, CH, TT, JY: R software implementation; NSK, JPB, CH, JD, TB writing the manuscript; All authors approved the manuscript in the submitted version and take responsibility for the scientific integrity of the work.

Funding: This work was supported by the Volkswagen Foundation within research project MTB-Report (ZN3424) and by the German Ministry of Education and Research (BMBF) projects Genoperspektiv [01GP1402], MyPathSem [031L0024], HER2LOW [031A429] and MMLL-Demonstrators [031A428].

References

- [1] Good BM, Ainscough BJ, McMichael JF, Su AI, Griffith OL. Organizing knowledge to enable personalization of medicine in cancer. *Genome Biol.* 2014 Aug 27;15(8):438, doi: 10.1186/s13059-014-0438-7.
- [2] Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 2001 Jan 1;29(1):308–11, doi:10.1093/nar/29.1.308.
- [3] Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res.* 2017 Jan 4;45(D1):D777–83, doi: 10.1093/nar/gkw1121.
- [4] Weinstein JN, Collisson EA, Mills GB, Shaw KM, Ozenberger BA, Ellrott K, et al. The Cancer Genome Atlas Pan-Cancer Analysis Project. *Nat Genet.* 2013 Oct;45(10):1113–20, doi: 10.1038/ng.2764.
- [5] Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003 Jul 1;31(13):3812–4, doi: 10.1093/nar/gkg509.
- [6] Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010 Apr;7(4):248–9, doi: 10.1002/0471142905.hg0720s76.
- [7] Gonzalez-Perez A, Perez-Llamas C, Deu-Pons J, Tamborero D, Schroeder MP, Jene-Sanz A, et al. IntOGen-mutations identifies cancer drivers across tumor types. *Nat Methods.* 2013 Nov;10(11):1081–2, doi: 10.1038/nmeth.2642.

- [8] Borchert F, Mock A, Tomczak A., Hügel J, Alkarkoukly S, Knurr A, et al. Knowledge bases and software support for variant interpretation in precision oncology. *Brief Bioinform.* 2021 Nov 5;22(6), doi:10.1093/bib/bbab134.
- [9] Griffith M, Spies NC, Krysiak K, McMichael JF, Coffman AC, Danos AM, et al. CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat Genet.* 2017 Feb;49(2):170–4, doi: 10.1038/ng.3774.
- [10] Dienstmann R, Jang IS, Bot B, Friend S, Guinney J. Database of genomic biomarkers for cancer drugs and clinical targetability in solid tumors. *Cancer Discov.* 2015 Feb 1;5(2):118–23, doi: 10.1158/2159-8290.CD-14-1118.
- [11] Chakravarty D, Gao J, Phillips S, Kundra R, Zhang H, Wang J, et al. OncoKB: A Precision Oncology Knowledge Base. *JCO Precision Oncology.* 2017 May 16;1(1):1–16, doi: 10.1200/PO.17.00011.
- [12] Holt ME, Mittendorf KF, LeNoue-Newton M, Jain NM, Anderson I, Lovly CM, et al. My Cancer Genome: Coevolution of precision oncology and a molecular oncology knowledgebase. *JCO Clinical Cancer Informatics.* 2021 Dec;(5):995-1004, doi: 10.1200/CCL.21.00084.
- [13] Huang L, Fernandes H, Zia H, Tavassoli P, Rennert H, Pisapia D, et al. The cancer precision medicine knowledge base for structured clinical-grade mutations and interpretations. *J Am Med Inform Assoc.* 2017 May 1;24(3):513–9, doi: 10.1093/jamia/ocw148.
- [14] Tamborero D, Rubio-Perez C, Deu-Pons J, Schroeder MP, Vivancos A, Rovira A, et al. Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations. *Genome Medicine.* 2018 Mar 28;10(1):25, doi: 10.1186/s13073-018-0531-8.
- [15] Nakken S, Fournous G, Vodák D, Aasheim LB, Myklebost O, Hovig E. Personal Cancer Genome Reporter: variant interpretation report for precision oncology. *Bioinformatics.* 2018; 34(10), 1778-1780, doi: 10.1093/bioinformatics/btx817.
- [16] Povey S, Lovering R, Bruford E, Wright M, Lush M, Wain H. The HUGO gene nomenclature committee (HGNC). *Hum Genet.* 2001 Dec;109(6):678-80, doi: 10.1007/s00439-001-0615-0.
- [17] Van Allen EM, Wagle N, Stojanov P, Perrin DL, Cibulskis K, Marlow S, et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. *Nat Med.* 2014 Jun;20(6):682-8, doi: 10.1038/nm.3559.
- [18] Meric-Bernstam F, Johnson A, Holla V, Bailey AM, Brusco L, Chen K, et al. A decision support framework for genomically informed investigational cancer therapy. *J Natl Cancer Inst.* 2015 Jul;107(7), doi: 10.1093/jnci/djv098.
- [19] Perera-Bel J, Hutter B, Heining C, Bleckmann A, Fröhlich M, Fröhling S, et al. From somatic variants towards precision oncology: Evidence-driven reporting of treatment options in molecular tumor boards. *Genome Med.* 2018; 10(1), 1-15, doi: 10.1186/s13073-018-0529-2.
- [20] Chang W, Cheng J, Allaire JJ, Sievert C, Schloerke B, Xie Y, Allen J, McPherson J, Dipert A, Borges B. shiny: Web Application Framework for R [Internet]. 201821. Available from: <https://CRAN.R-project.org/package=shiny>
- [21] Griffith M, Griffith OL, Coffman AC, Weible JV, McMichael JF, Spies NC, et al. DGIdb: mining the druggable genome. *Nat Methods.* 2013 Dec;10(12):1209–10, doi: 10.1038/nmeth.2689.
- [22] Gao, J, Aksoy, BA, Dogrusoz, U, Dresdner, G, Gross, B, Sumer, SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science signaling.* 2013 Apr 2;6(269):p11-p11, doi: 10.1126/scisignal.2004088.
- [23] Tamborero D, Dienstmann R, Rachid MH, Boekel J, Lopez-Fernandez A, Jonsson M et al. The Molecular Tumor Board Portal supports clinical decisions and automated reporting for precision oncology. *Nat Cancer.* 2022 Feb;3(2):251-61, doi: 10.1038/s43018-022-00332-x.
- [24] Piñeiro-Yáñez E, Reboiro-Jato M, Gómez-López G, Perales-Patón J, Troulé K, Rodríguez JM, et al. PanDrugs: a novel method to prioritize anticancer drug treatments according to individual genomic data. *Genome Med.* 2018 May 31;10(1):41, doi:10.1186/s13073-018-0546-1.
- [25] Hintzsche J, Kim J, Yadav V, Amato C, Robinson SE, Seelenfreund E, et al. IMPACT: a whole-exome sequencing analysis pipeline for integrating molecular profiles with actionable therapeutics in clinical samples. *J Am Med Inform Assoc.* 2016 Jul;23(4):721-30, doi:10.1093/jamia/ocw022.
- [26] Hintzsche JD, Yoo M, Kim J, Amato CM, Robinson WA, Tan AC. IMPACT web portal: oncology database integrating molecular profiles with actionable therapeutics. *BMC Medical Genomics.* 2018 Apr 20;11(2):26, doi: 10.1186/s12920-018-0350-1.
- [27] Metzger P, Scheible R, Hess M, et al. Miracum-Pipe. <https://github.com/AG-Boerries/MIRACUM-Pipe> (10 May 2022, date last accessed).
- [28] Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res.* 2014 Jan;42(D1):D980-985, doi: 10.1093/nar/gkt1113.