# **Supplementary Table S1: Command line options of each tools used for the sequencing data calling**

| **Tool** | **Option** |
| --- | --- |
| GroupReadByUMI | adjacency strategy |
| CallMolecularConsensus | --cc 3 |
| FilterConsensusRead | -M 1  -N 40 |
| BWA | -M |
| Freebayes | -F 2  -C 2  --genotype-qualities  --pooled-continuous  --pooled-discrete |
| Mutect2 | --max-reads-per-alignment-start 0  --max-mnp-distance 3  --pcr-snv-qual 70  --pcr-indel-qual 70 |
| STAR | --twopassMode Basic  --alignSplicedMateMapLminOverLmate 0.5  --peOverlapNbasesMin 10  --outFilterMultimapNmax 100  --outFilterMismatchNmax 33  --seedSearchStartLmax 12  --alignSJoverhangMin 15  --outFilterMatchNminOverLread 0  --outFilterScoreMinOverLread 0.3  --alignSJDBoverhangMin 1  --outFilterType BySJout  --chimSegmentMin 10  --chimOutType WithinBAM SoftClip  --chimJunctionOverhangMin 10  --chimScoreDropMax 30  --chimScoreJunctionNonGTAG 0  --chimScoreSeparation 1  --alignSJstitchMismatchNmax 5 -1 5 5  --chimSegmentReadGapMax 3  --chimMultimapNmax 50 |
| Arriba | -u with blacklist from original github repository |
| Minimap2 | -ax sr |

# **Supplementary Table S2: List of features required for drug prioritization**

| Database or tool used | Description | Feature |
| --- | --- | --- |
| VEP | Effect prediction and description of variants | Variant impact |
| Variant coordinates (coordinates, exons, codons…) |
| Pfam | Protein domain database | Protein domain annotation |
| COSMIC Fusion | Database of gene fusions involved in cancer | Fusion involvement in cancer |
| Cancer Gene Census (COSMIC) | Annotation of genes involved in cancer | CNV cancer involvement |
| Laboratory gene panel annotation (COSMIC Gene Census, Vogelstein, Cancermine, ONGene, TCGA, TSGDatabase, JaxCKB and OncoKB, see Supplementary Figure 4) | Genes of the panel annotated depending on their cancer role | Consensus of Gene Role (oncogene / TSG) |
| RefSeq | Gene and transcript database | Transcript annotation |
| Last exon annotation |
| Ensembl | Gene and transcript database | Transcript annotation |
| Custom disease ontology | Cancer ontology | Type of cancer (disease name) |
| Name of the organ |
| Liquid tumor or solid tumor |
| ComPerMed CPV list | List of pathogenic variants according to ComPerMed publication | Pathogenic variant annotation |
| GnomAD | Population database | Average of all population frequency |
| ClinVar | Clinical database | ClinVar description: (Likely) pathogenic, unknown, (likely) benign |
| CIViC | Cancer variant database | CIViC description: (Likely) pathogenic, unknown, (likely) benign |
| Sift | Variant predictor | Impact of the mutation over protein |
| MutationTaster |
| fathmm-MKL |
| LRT |
| MetaLR |
| MetaSVM |
| PROVEAN |
| FATHMM |
| DANN |
| MutationAssessor |
| MolecularMatch | Therapeutic database | Drug name |
| Clinical evidence tier |
| Disease name |
| Variant coordinates (gene, coordinates, exon and codon) |
| CIViC actionability | Therapeutic database | Drug name |
| Clinical evidence tier |
| Disease name |
| Variant coordinates (gene, coordinates, exon and codon) |

**Supplementary Table S3: Filters used for CFB cohort variant interpretation**

| **Filter** | **Value** |
| --- | --- |
| Coverage | ≥ 30x |
| Read alternate observation | ≥ 2 |
| Quality phred score | ≥ Medium |
| Allele frequency | ≥ 4% |
| Variant frequency in the cohort | ≤ 50% |
| Variant effect (VEP) | missense, intronic, splice polypyrimidine, splice region, 5 prime UTR, stop gained, frameshift, inframe deletion, splice donor region, splice acceptor, start lost, splice donor, stop lost, inframe insertion, stop retained, coding sequence |
| Relative population frequency | ≤ 10-3 |

# **Supplementary Table S4: Adapted ComPerMed score table for non-clear LoF mutations**

| **Parameter** | **Score +2** | **Score +1** | **Score +0.5** |
| --- | --- | --- | --- |
| Mutation tier from COSMIC Mutation Census | 1, 2, 3 | Other tier | / |
| *In-silico* variant damaging prediction tools\* | / | / | ⅔ of the prediction as damaging |
| ClinVar pathogenic annotation | / | / | Pathogenic / Likely Pathogenic |
| Present in CIViC | / | / | Yes |

# \*In silico variant damaging prediction tools used : MutationTaster, fathmm-MKL, LRT, MetaLR, MetaSVM, PROVEAN, FATHMM, DANN, MutationAssessor

# **Supplementary Table S5: CIViC clinical evidence (B) not identified by DrugOrder**

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| **Sample** | **Variant\_Name** | **HGVS** | **Drug(s)** | **CIViC evidence level** | **Disease** | **OncoKB level** |
| --- | --- | --- | --- | --- | --- | --- |
| tumor56 | 1\_H3-3A\_226064434\_A\_T | K28M | Akt/ERK Inhibitor ONC201 | B | glioma | None |
| tumor57 | 11\_GSTP1\_67352689\_A\_G | I105V | FOLFOX Regimen | B | carcinoma of colon | None |
| tumor99 | 20\_GNAS\_57428713\_T\_C | 393T>C | Cisplatin Fluorouracil | B | neoplasm of esophagus | None |
| tumor196 | 19\_ERCC2\_45854919\_T\_G | K751Q | Paclitaxel Carboplatin | B | non-small cell lung cancer | 3A\* |
| tumor239 | 1\_NRAS\_115258747\_C\_T | G12D | Cetuximab | B | carcinoma of colon | R1\*\* |
| tumor282 | 19\_ERCC2\_45854919\_T\_G | K751Q | Cisplatin | B | neoplasm of bone | 3A |
| tumor302 | 20\_GNAS\_57428713\_T\_C | 393T>C | Erlotinib Gefitinib | B | non-small cell lung cancer | None |
| tumor318 | 7\_ABCB1\_87160618\_A\_T | S893T | Paclitaxel | B | neoplasm of ovary | None |
| tumor356 | 7\_ABCB1\_87138645\_A\_G | I1145I | Carboplatin Cisplatin | B | non-small cell lung cancer | None |

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**\*** Compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication but neither biomarker and drug are standard of care

**\*\*** Standard of care biomarker predictive of resistance to an FDA-approved drug in this indication

# **Supplementary Table S6: Filters used for FMI tumor interpretation**

| **Filter** | **Value** |
| --- | --- |
| Coverage | ≥ 10x |
| VAF | ≥ 5% |
| Allele strand ration | ≥ 10/90 |
| Population frequency | ≤ 0.01% |

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# **Supplementary Figure S1: Cancer gene role distribution among genes of laboratory panel**

We utilized resources such as COSMIC Cancer Gene Census, Vogelstein, Cancermine, ONGene, TCGA, TSGDatabase, JaxCKB, and OncoKB for the annotations of TSG and Oncogenes. It's important to note that a single gene can fall into multiple categories. For the identification of genes implicated in oncogenic fusions, we specifically referred to the annotations provided by the COSMIC Cancer Gene Census. Unknown status is given for genes without any annotation in these databases.

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# **Supplementary Figure S2: Therapeutic database heterogeneity**

This figure illustrates the diversity within our therapeutic database, as depicted by an upset plot. The bar chart on the lower left shows the total count of drugs per database. The lower section of the plot represents a specific intersection of databases. The upper section displays the count of drugs that intersect with the database selected in the lower section. Blue bars signify each intersection that involves DrugOrder, while the sea-blue bars represent intersections that exclude DrugOrder.

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# **Supplementary Figure S3: Heatmap of the 20 best DOscored variants on patient without conclusions in clinical reports**

The first column on the left illustrates the variant type, with SNV in blue, CNV in sea-blue, and fusion in red. The columns within the heatmap represent the DOscore and its corresponding tiers. DOscore is normalized based on the highest sample DOscore, while framework part tier scores are normalized based on the best framework part tier score. The heatmap is divided into three distinct clusters, each determined by the sample DOscore.