National Cancer Center Japan

Towards high-quality clinical trials and
implementation of genomic medicine

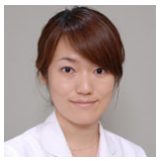
ATLAS Training Program

Cancer Genome-based Medicine Course
Lecture Title : Procedures and Interpretation of Next Generation Sequencing Results
Speaker : Kuniko Sunami

National Cancer Center Japan

Kuniko Sunami, M.D., Ph.D.

Department of Laboratory Medicine, National Cancer Center Hospital



EDUCATION

Yokohama City University School of Medicine, Japan (2001–2007)
Juntendo University Graduated School of Medicine, Japan (2013–2016)

WORK EXPERIENCE

Senior Resident, Respiratory Medicine and Medical Oncology, Tokyo Metropolitan Komagome Hospital (2009–2013)
Chief Resident, Thoracic Oncology, National Cancer Center (2013–2015)
Postdoctoral Fellow, Division of Genome Biology, National Cancer Center Research Institute (2014–2015)
Medical Staff, Division of Clinical Laboratory, National Cancer Center (2015–present)
Medical Staff, Genetic Medicine and Services, National Cancer Center (2015–present)

BOARD CERTIFICATION

Diplomate, Subspecialty Board of Medical Oncology, JSMO (2014)
Fellow of the Japanese Society of Internal Medicine (2015)
Board Certified Member of the Japanese Respiratory Society (2015)

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Goal

- Looking at a gene panel test report…
  - You can determine the recommended treatment
  - You can determine whether there are secondary findings (→Dr. Hirata)

Therefore…

- You can determine the pathogenic significance of the detected mutation
- You can extract mutations associated with therapeutic agents
- You can determine candidate drugs and the evidence level of these drugs

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Q: What is the biological significance of the variants detected in Cases 1 and 2, and what are the candidate drugs?

Simulated cases

Case 1

BIOMARKER FINDINGS	ACTIONABILITY	
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section	
Tumor Mutational Burden - TMB-Low (5 Muts/Mb)	No therapies or clinical trials. see Biomarker Findings section	
GENOMIC FINDINGS	**THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)**	**THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)**
PALB2 - L9F	Niraparib	Talazoparib
10 Trials see p. 16	Olaparib	
	Rucaparib	
PIK3CA - H1047R	none	Alpelisib
		Everolimus
10 Trials see p. 19		Temsirolimus

Case 2

RG summary report draft

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Gene mutation

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
APC	72.1 (782/1,084)	exon10:c.1312+1G>A		4166472 (1)
ATM	14.3 (51/350)	exon3:c.103C>T	R35*	922965 (2)

Number of somatic cell mutations

Region division		SNV		InDel		Total	
		Number of mutation	Mutation appearanc	Number of mutation	Mutation appearanc	Number of mutation appearances	Mutation appearanc
Exon	nonsyn	0	0.0/Mb	0	0.0/Mb	0	0.0/Mb
	syn	0	0.0/Mb				
Non-exon		2	2.1/Mb	1	1.1/Mb	3	3.2/Mb
Entire region		2	1.6/Mb	1	0.8/Mb	3	2.3/Mb

*2 Mutation appearance rate = number of mutations per 1 Mbp

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Overview

- Understand the characteristics of gene panel tests and know how to interpret the results
- Determining the pathogenic significance of the detected mutation
- Extracting the mutation associated with therapeutic agents
- Selecting candidate drugs accompanied by their evidence levels

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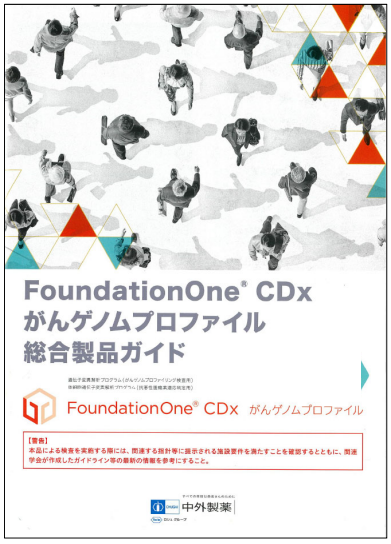
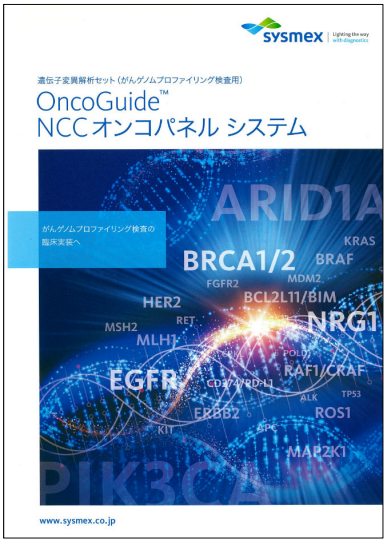
Gene panel tests covered by nation-wide insurance in Japan





All oncogene panel tests are based on the same basic concepts.

Characteristics of each Cancer Genome Profiling Test

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*As of September 2020

Test Name	FoundationOne® CDx Cancer Genome Profile	OncoGuide™ NCC Oncopanel System
Tested sample	Tumor (FFPE) Analyzes tumor only	Tumor (FFPE) + Normal (peripheral blood) Matched pair analysis
Number of genes (Number of genes targeted for fusion detection)	324 (36)	114 (12)
Role of companion diagnosis	Non-small cell lung cancer: *EGFR* (exon19del, L858R, T790M), *ALK* fusion, *ROS1* fusion, MET skipping Malignant melanoma: *BRAF* V600E/K Breast cancer: *ERBB2* copy number variation Colorectal cancer: *KRAS/NRAS* wild-type Solid cancer: *NTRK1/2/3* fusion Ovarian cancer/prostate cancer: *BRCA1/2*	-
Tumor mutational burden (/Mb)	○	○
Microsatellite instability	○	–
Germline pathogenic variants of hereditary tumor-causing genes (reportable genes)	–	○ (*APC*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *PTEN*, *RB1*, *RET*, *STK11*, *SMAD4*, *TP53*, *TSC1*, *VHL*)

Characteristics of each Cancer Genome Profiling Test

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Correctly understanding the test characteristics is essential for accurately interpreting the test results

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Items reported in Gene Panel Tests

- Abnormalities in the Gene of Interest (mutation, fusion, copy number variation, etc.)
  - Detected as “differences” compared to the reference genome sequence
  - Pathogenic significance is determined using algorithms unique to each test, but this may include mutations of unknown significance and polymorphisms
- Gene Mutation Level (Tumor Mutational Burden, TMB)
- Presence of Microsatellite Instability (MSI)
  - Some tests are not subject to analysis

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Tumor Tissue Only and Tumor/Normal Tissue Pair Analysis Somatic Mutations

Tumor Tissue Only Analysis

X Variants that may be genetic abnormalities

X Genetic abnormalities associated with cancer cells variants only observed in cancer cells)

X Polymorphisms not registered in the database, which could not be subtracted (inherited differences)

Tumor/Normal Pair Analysis

X Variants that may be genetic abnormalities

X Polymorphisms (inherited differences)

X Genetic abnormalities associated with cancer cells (variants only observed in cancer cells)

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Tumor Tissue Only and Tumor/Normal Tissue Pair Analysis Germline Changes

Tumor Tissue Only Analysis

X Variants that may be genetic abnormalities

X genetic abnormality

May contain a germline pathogenic variant (unable to differentiate between the two)

Tumor/Normal Pair Analysis

X Variants that may be genetic abnormalities

X Polymorphisms (inherited differences)

X **Germline pathogenic variant (inherited pathogenic variant)**

X Genetic abnormalities associated with cancer cells (variants only observed in cancer cells)

Can be differentiated

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Public Databases Related to Assessment of Biological Significance

Type	Name	URL
Polymorphism DB	gnomAD (formerly ExAC)	https://gnomad.broadinstitute.org/
Somatic mutation DB	COSMIC	https://cancer.sanger.ac.uk/cosmic/
Pathogenic variant DB	ClinVar	https://www.ncbi.nlm.nih.gov/clinvar/

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ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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COSMIC

Catalogue of Somatic Mutations In Cancer

- A somatic gene mutation database
- Human genome assembly version is also available

  (GRCh37 or GRCh38)
- Operated by the UK Wellcome Sanger Institute
- Regularly updated
- Possible to check whether each variant has been detected more than once in other cases

In case of oncogenes, variants with multiple cases listed may be pathogenic
Often, truncating mutations in tumor suppressor genes are not registered, even if they are pathogenic

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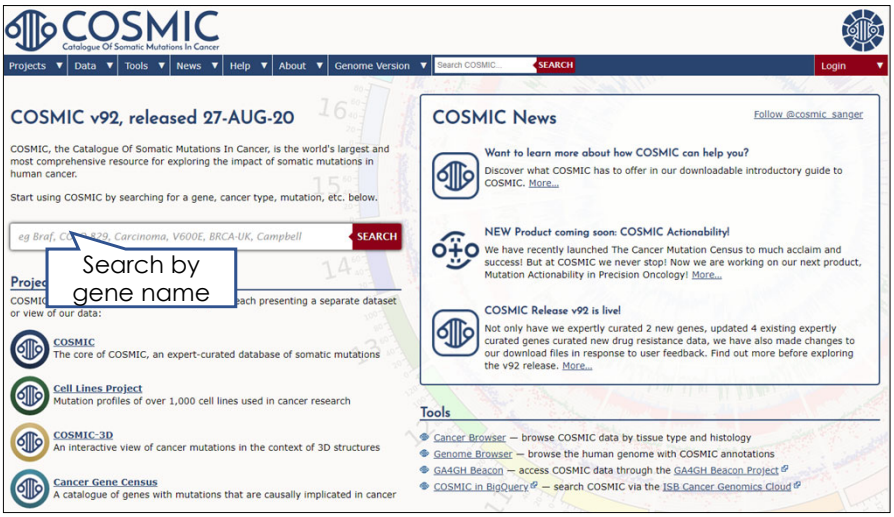
How to use COSMIC

http://cancer.sanger.ac.uk/cosmic/



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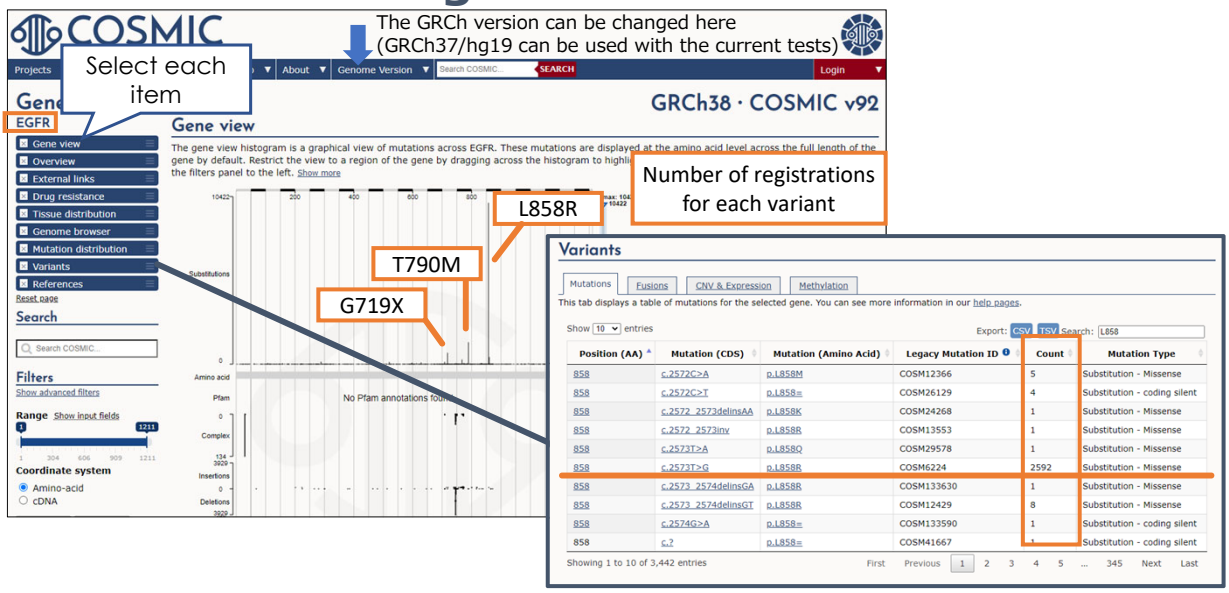
Searching “EGFR” in COSMIC

Pathogenic variant



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Searching “EGFR L858R” in COSMIC

Pathogenic variant

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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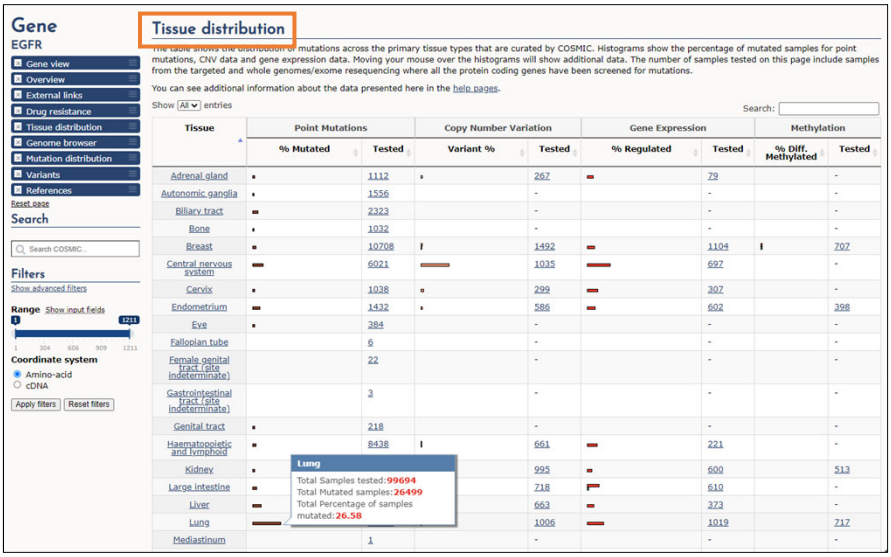
Searching “EGFR L858R” in COSMIC

Pathogenic variant



ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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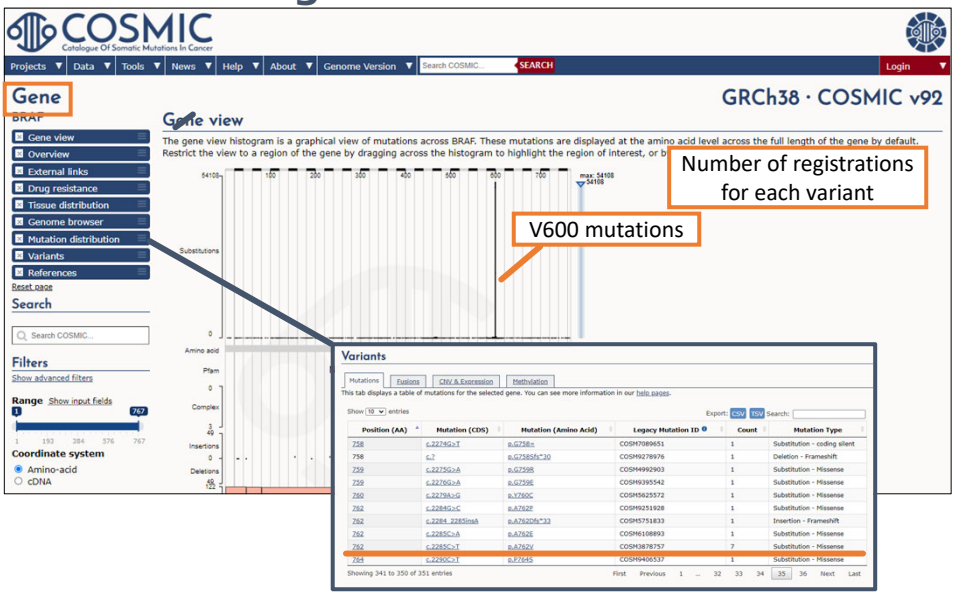
Searching “BRAF A762V” in COSMIC

VUS



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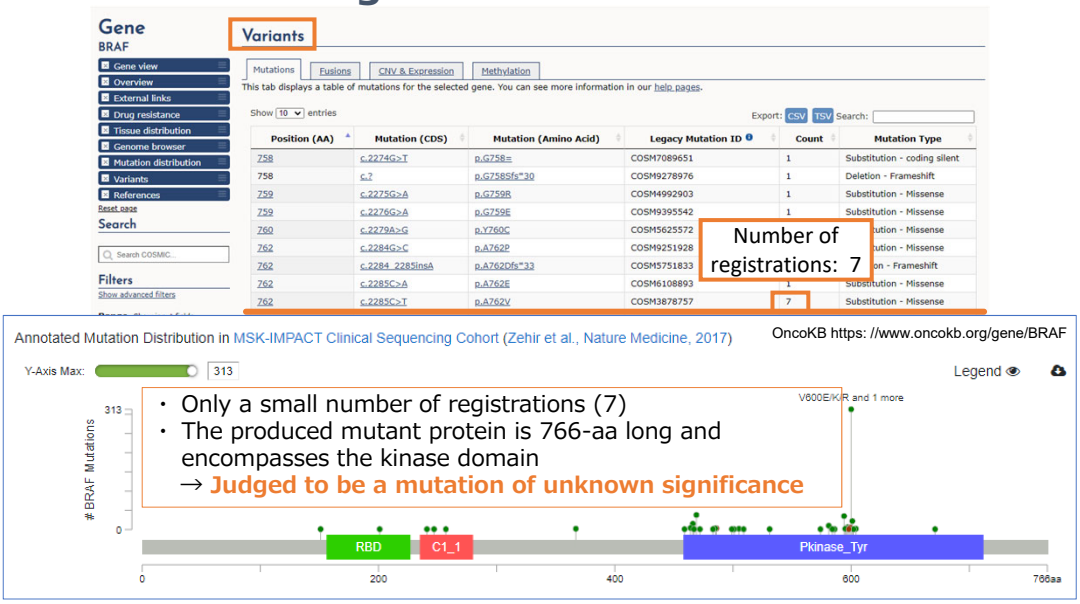
Searching “BRAF A762V” in COSMIC

VUS



- Only a small number of registrations (7)
- The produced mutant protein is 766-aa long and encompasses the kinase domain
  → **Judged to be a mutation of unknown significance**

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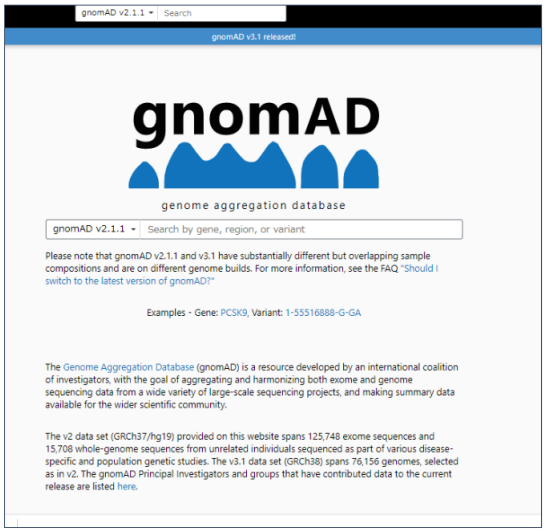
gnomAD

The Genome Aggregation Database

- Database containing whole-exon / whole-genome sequence data
- v.2 data set with GRCh37/hg19 as the reference genome and v.3 data set with GRCh38 as the reference genome, have been released
- Variant registration frequency can be checked by ethnicity



This database is useful for determining whether the variant of interest is a polymorphism, based on the registration frequency in the concerned ethnicity

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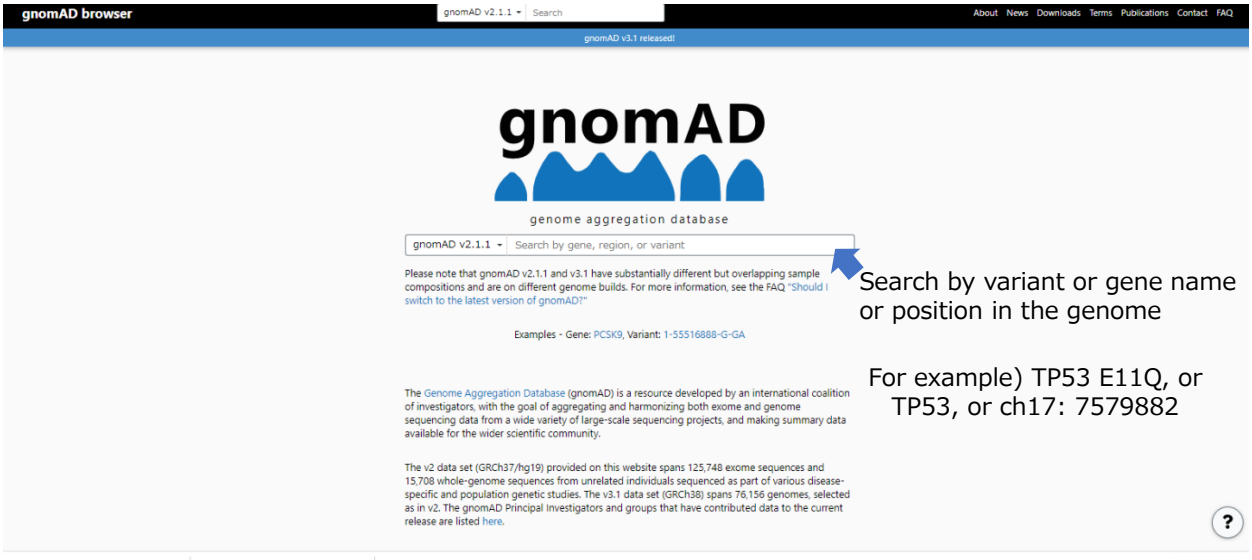
How to use gnomAD



Search by variant or gene name or position in the genome

For example) TP53 E11Q, or TP53, or ch17: 7579882

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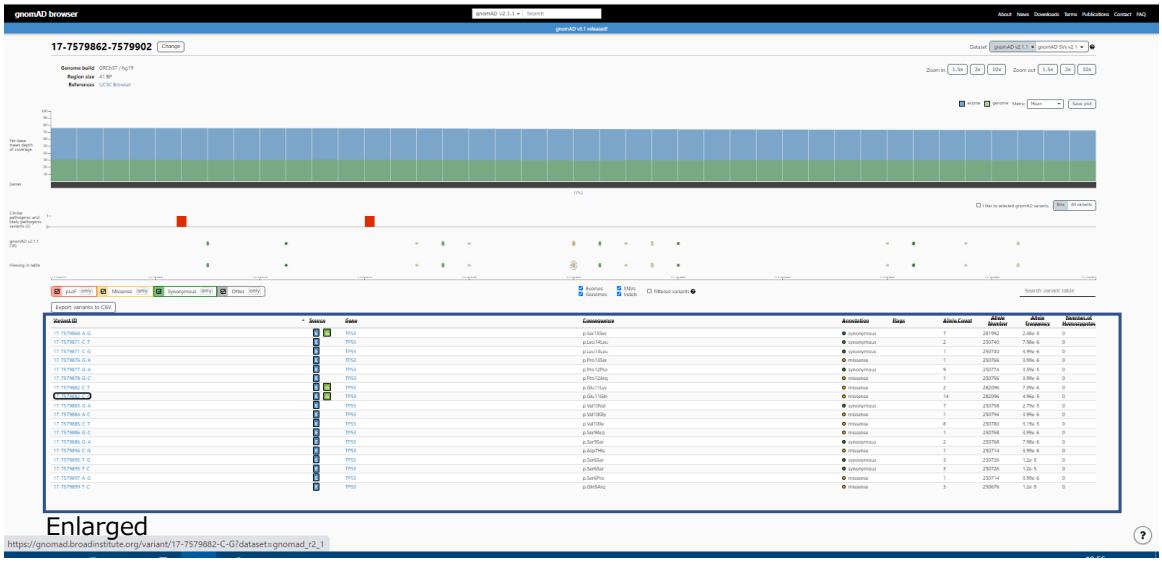
Searching for “TP53 E11Q” in gnomAD



Enlarged

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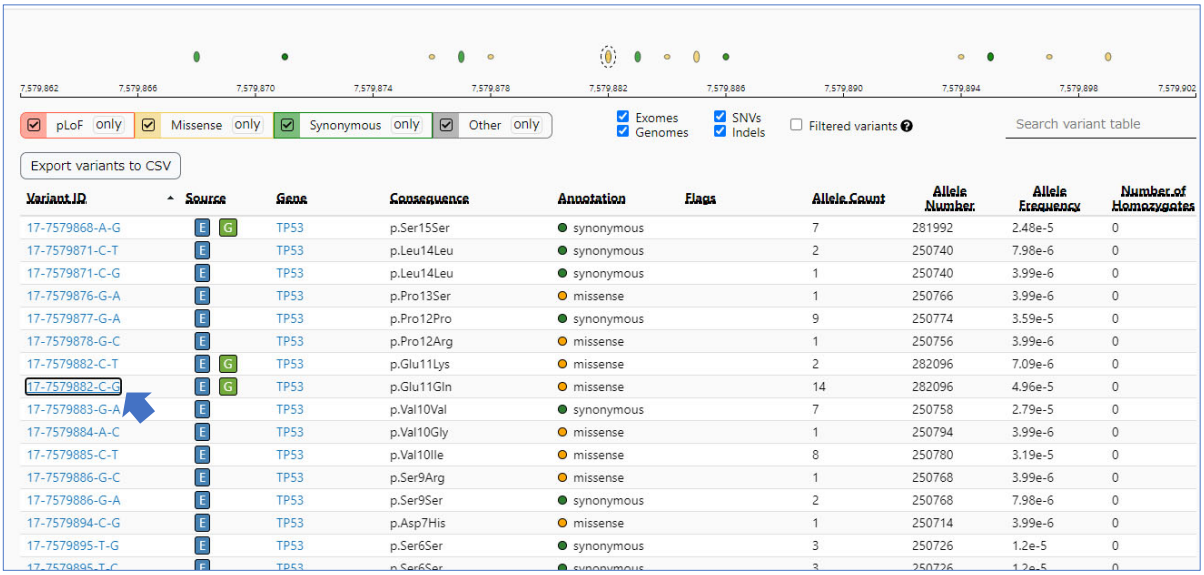
Searching for “TP53 E11Q” in gnomAD



7,579,862 7,579,866 7,579,870 7,579,874 7,579,878 7,579,882 7,579,886 7,579,890 7,579,894 7,579,898 7,579,902

pLoF only | Missense only | Synonymous only | Other only | Exomes | Genomes | SNVs | Indels | Filtered variants | Search variant table

Export variants to CSV

Variant ID	Source	Gene	Consequence	Annotation	Flags	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
17-7579868-A-G	E G	TP53	p.Ser15Ser	synonymous		7	281992	2.48e-5	0
17-7579871-C-T	E	TP53	p.Leu14Leu	synonymous		2	250740	7.98e-6	0
17-7579871-C-G	E	TP53	p.Leu14Leu	synonymous		1	250740	3.99e-6	0
17-7579876-G-A	E	TP53	p.Pro13Ser	missense		1	250766	3.99e-6	0
17-7579877-G-A	E	TP53	p.Pro12Pro	synonymous		9	250774	3.59e-5	0
17-7579878-G-C	E	TP53	p.Pro12Arg	missense		1	250756	3.99e-6	0
17-7579882-C-T	E G	TP53	p.Glu11Lys	missense		2	282096	7.09e-6	0
17-7579882-C-G	E G	TP53	p.Glu11Gln	missense		14	282096	4.96e-5	0
17-7579883-G-A	E	TP53	p.Val10Val	synonymous		7	250758	2.79e-5	0
17-7579884-A-C	E	TP53	p.Val10Gly	missense		1	250794	3.99e-6	0
17-7579885-C-T	E	TP53	p.Val10Ile	missense		8	250780	3.19e-5	0
17-7579886-G-C	E	TP53	p.Ser9Arg	missense		1	250768	3.99e-6	0
17-7579886-G-A	E	TP53	p.Ser9Ser	synonymous		2	250768	7.98e-6	0
17-7579894-C-G	E	TP53	p.Asp7His	missense		1	250714	3.99e-6	0
17-7579895-T-G	E	TP53	p.Ser6Ser	synonymous		3	250726	1.2e-5	0

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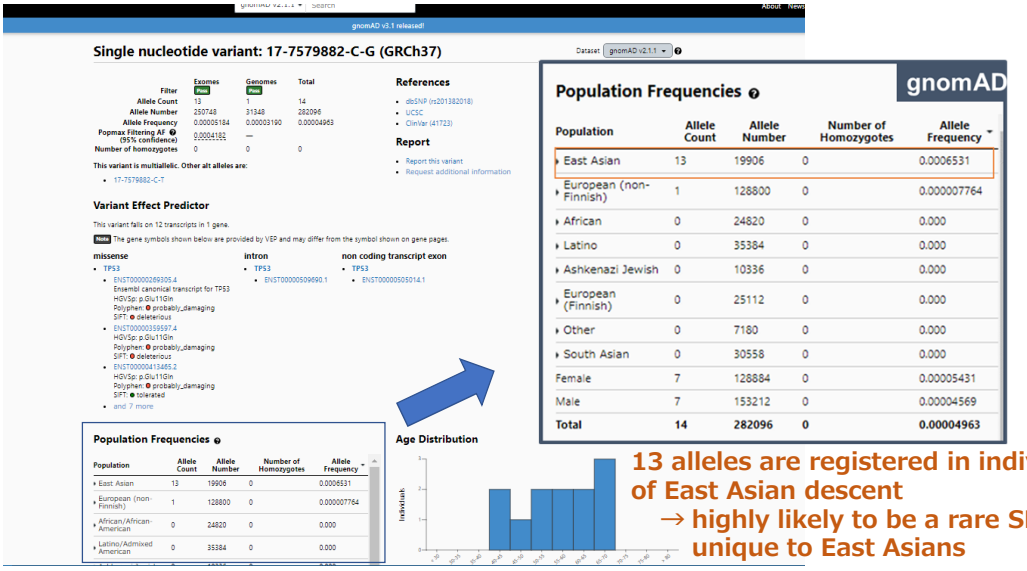
Searching for “TP53 E11Q” in gnomAD



Single nucleotide variant: 17-7579882-C-G (GRCh37)

Dataset: gnomAD v2.1.1

gnomAD

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
East Asian	13	19906	0	0.0006531
European (non-Finnish)	1	128800	0	0.000007764
African	0	24820	0	0.000
Latino	0	35384	0	0.000
Ashkenazi Jewish	0	10336	0	0.000
European (Finnish)	0	25112	0	0.000
Other	0	7180	0	0.000
South Asian	0	30558	0	0.000
Female	7	128884	0	0.00005431
Male	7	153212	0	0.00004569
Total	14	282096	0	0.00004963

References

Report

Variant Effect Predictor

Age Distribution

13 alleles are registered in individuals of East Asian descent
→ highly likely to be a rare SNP unique to East Asians

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Overview

- Understand the characteristics of gene panel tests and know how to interpret the results
- Determining the pathogenic significance of the detected mutation
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- Selecting candidate drugs accompanied by their evidence levels

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Databases Providing Therapeutic Options Accompanied by Evidence Level

Type	Name	URL
Knowledge database	**CIViC**	**https://civicdb.org/home**
Knowledge database	**OncoKB**	**https://www.oncokb.org/**

These databases can be used to search for the currently available therapeutic options that can be expected to be effective based on the detected genetic abnormality and the rationale for the efficacy (evidence level)

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CIViC

Overview

- Knowledge database based on expert crowd sourcing
- Operating organization: Washington University School of Medicine
- Five levels of evidence (A to E) are presented, depending on clinical usefulness.

Features

- Cleaned up by experts
- Provides evidence level and type

Griffith et al., *Nat Genet 2017*

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How to use CIViC

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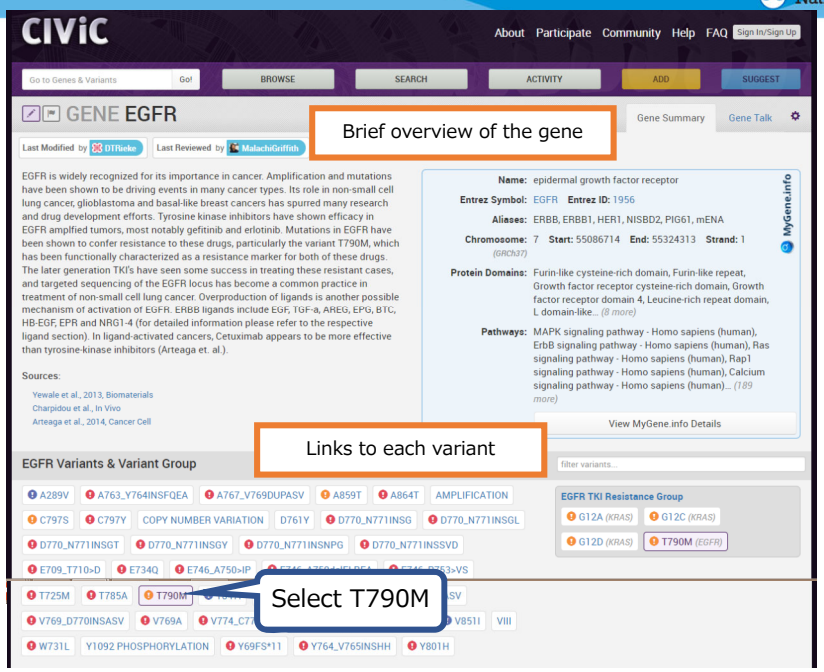

Civic Evidence Level Classifications

Level	Definition	Examples and further comments
A Validated association	Proven/consensus association in human medicine.	*"AML with mutated NPM1" is a provisional entity in WHO classification of acute myeloid leukemia (AML). This mutation should be tested for in clinical trials and is recommended for testing in patients with cytogenetically normal AML.* Validated associations are often in routine clinical practice already or are the subject of major clinical trial efforts.
B Clinical evidence	Clinical trial or other primary patient data supports association.	*BRAF V600E is correlated with poor prognosis in papillary thyroid cancer in a study of 187 patients with PTC and other thyroid diseases.* The evidence should be supported by observations in multiple patients. Additional support from functional data is desirable but not required.
C Case study	Individual case reports from clinical journals.	*A single patient with FLT3 over-expression responded to the FLT3 inhibitor sunitinib.* The study may have involved a large number of patients, but the statement was supported by only a single patient. In some cases, observations from just a handful of patients (e.g. 2-3) or a single family may also be considered a case study/report.
D Preclinical evidence	*In vivo* or *in vitro* models support association.	*Experiments showed that AG1296 is effective in triggering apoptosis in cells with the FLT3 internal tandem duplication.* The study may have involved some patient data, but support for this statement was limited to in vivo or in vitro models (e.g. mouse studies, cell lines, molecular assays, etc.).
E Inferential association	Indirect evidence.	*CD33 and CD123 expression were significantly increased in patients with NPM1 mutation with FLT3-ITD, indicating these patients may respond to combined anti-CD33 and anti-CD123 therapy.* The assertion is at least one step removed from a direct association between a variant and clinical relevance.

Nature Genetics 49, 170–174 (2017)

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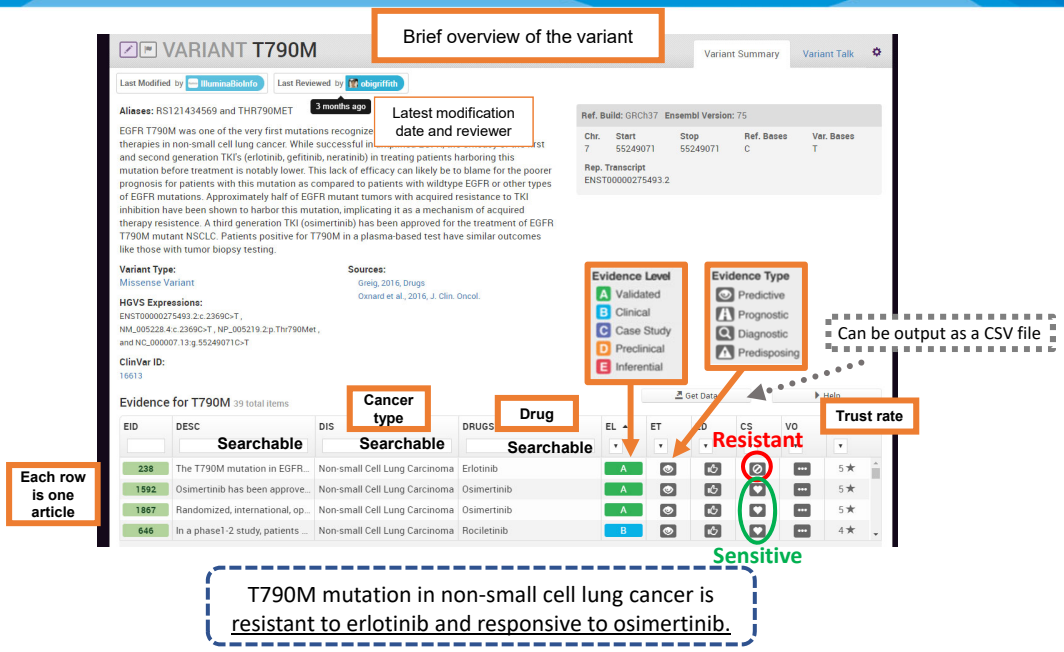

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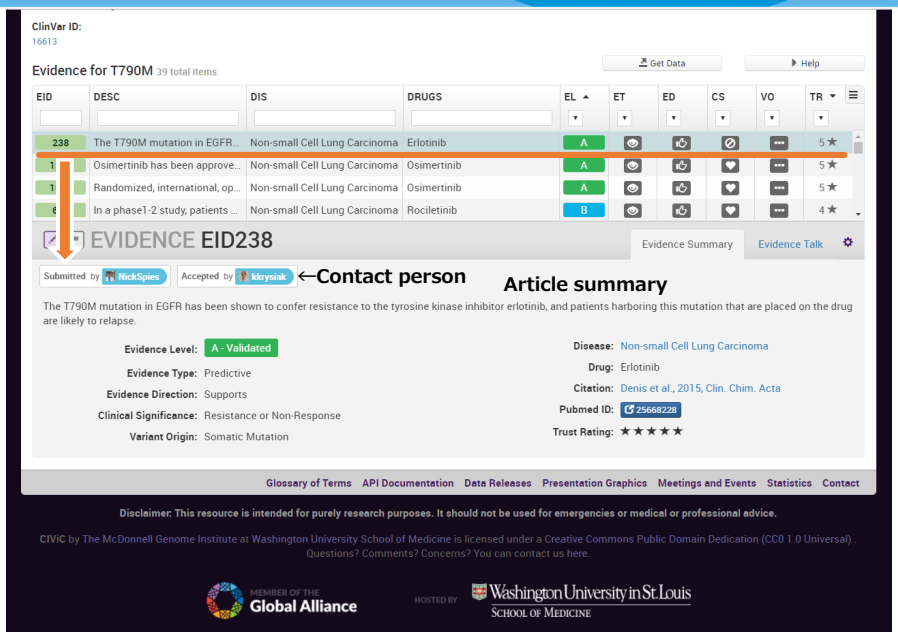

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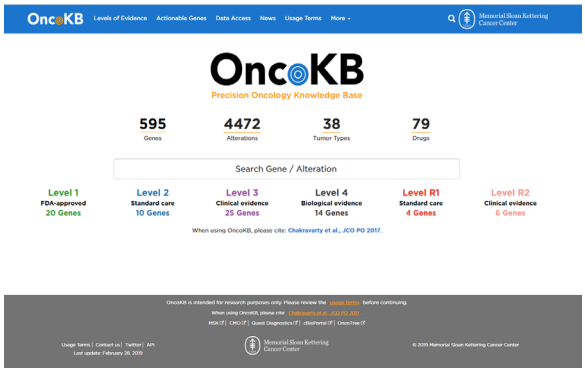
OncoKB

- Knowledge database curated by MSKCC clinical fellows and research fellows
- Operating organization: Memorial Sloan Kettering Cancer Center (MSKCC)
- Four evidence levels regarding clinical usefulness (1 to 4) and two evidence levels regarding resistance (R1 and R2) are presented



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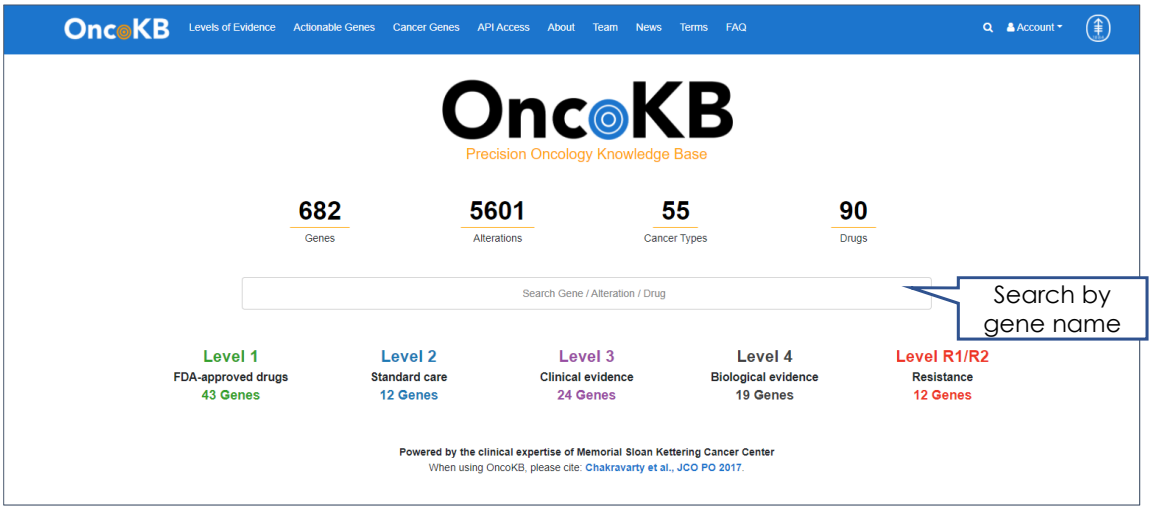
How to use OncoKB



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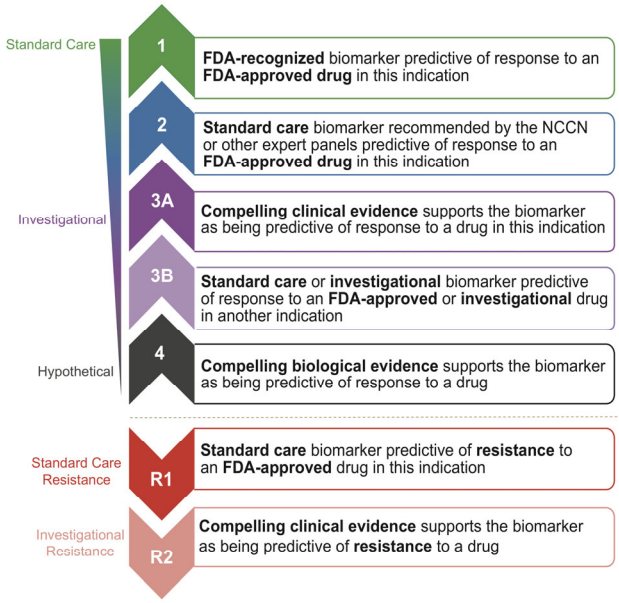
Oncokb Evidence Level Classifications



https: //www.oncokb.org/levels

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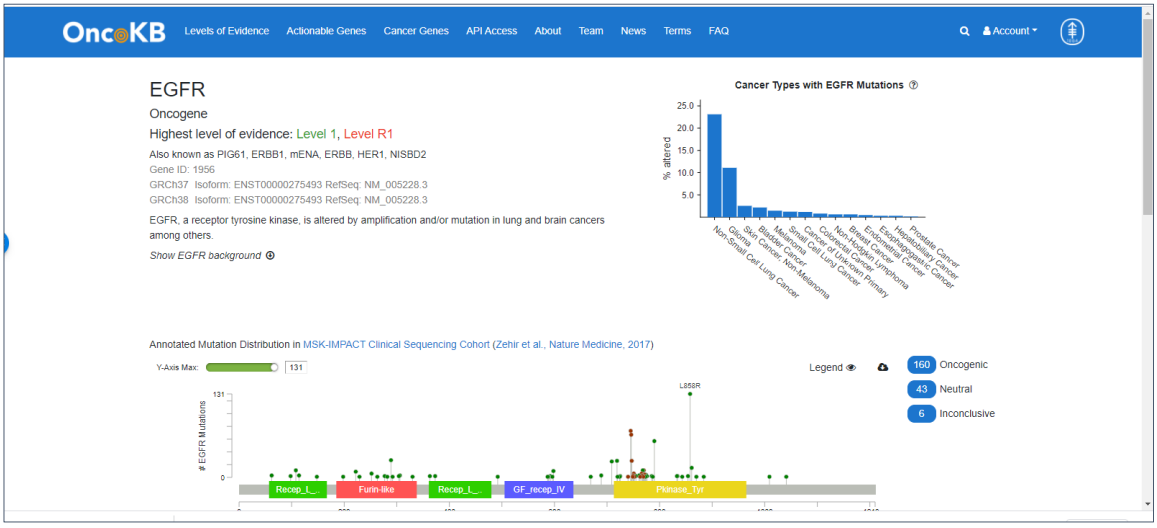
How to use OncoKB



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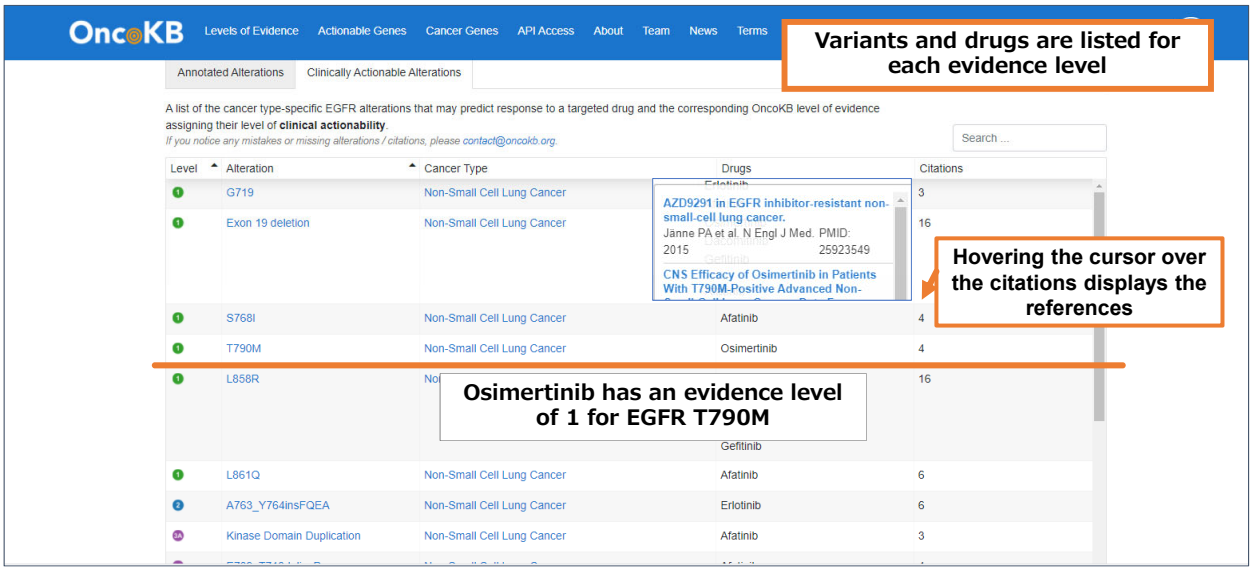
How to use OncoKB



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Simulated cases

Case 1

BIOMARKER FINDINGS	ACTIONABILITY
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section
Tumor Mutational Burden - TMB-Low (5 Muts/Mb)	No therapies or clinical trials. see Biomarker Findings section

GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
PALB2 - L9F	Niraparib	Talazoparib
10 Trials see p. 16	Olaparib	
	Rucaparib	
PIK3CA - H1047R	none	Alpelisib
10 Trials see p. 19		Everolimus
		Temsirolimus

Case 2

RG summary report draft

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Gene mutation

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
APC	72.1 (782/1,084)	exon10:c.1312+1G>A		4166472 (1)
ATM	14.3 (51/350)	exon3:c.103C>T	R35*	922965 (2)

Number of somatic cell mutations

Region division		SNV		InDel		Total	
		Number of mutation	Mutation appearanc	Number of mutation	Mutation appearanc	Number of mutation appearances	Mutation appearanc
Exon	nonsyn	0	0.0/Mb	0	0.0/Mb	0	0.0/Mb
	syn	0	0.0/Mb				
Non-exon		2	2.1/Mb	1	1.1/Mb	3	3.2/Mb
Entire region		2	1.6/Mb	1	0.8/Mb	3	2.3/Mb

*2 Mutation appearance rate = number of mutations per 1 Mbp

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Information written in the report for Cases 1 and 2

Foundation One CDx — **Tumor Analysis Only**

BIOMARKER FINDINGS

① Microsatellite status - MS-Stable

② Tumor Mutational Burden - TMB-Low (5 Muts/Mb)

GENOMIC FINDINGS

③ ④ *PALB2* - L9F

③ ④ *PIK3CA* - H1047R

10 Trials see p. 19

ACTIONABILITY	
No therapies or clinical trials. see Biomarker Findings section	
No therapies or clinical trials. see Biomarker Findings section	
THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Niraparib	Talazoparib ⑤
Olaparib ⑤	
Rucaparib	
none ⑤	Alpelisib
	Everolimus ⑤
	Temsirolimus

Items mentioned:

① Whether there is microsatellite instability
② Tumor mutational burden
③ Gene name
④ Amino acid variant
⑤ Name of candidate drug

NCC Oncopanel — **Tumor/Normal tissue Pair Analysis**

RG summary report draft

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Gene mutation ① ② ③ ④ ⑤

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
APC	72.1 (782/1,084)	exon10:c.1312+1G>A		4166472 (1)
ATM	14.3 (51/350)	exon3:c.103C>T	R35*	922965 (2)

Number of somatic cell mutations ⑥

Region division		SNV		InDel		Total	
		Number of mutation	Mutation appearanc	Number of mutation	Mutation appearanc	Number of mutation appearances	Mutation appearanc
Exon	nonsyn	0	0.0/Mb	0	0.0/Mb	0	0.0/Mb
	syn	0	0.0/Mb				
Non-exon		2	2.1/Mb	1	1.1/Mb	3	3.2/Mb
Entire region		2	1.6/Mb	1	0.8/Mb	3	2.3/Mb

*2 Mutation appearance rate = number of mutations per 1 Mbp

Items mentioned:

① Gene name
② Mutation/allele frequency
③ CDS variant
④ Amino acid variant
⑤ COSMIC ID (number of registrations)
⑥ Tumor mutational burden

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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A. Case 1

Tumor analysis only

Foundation One CDx

Woman in her 50s, stomach cancer

BIOMARKER FINDINGS

Microsatellite status - MS-Stable

Tumor Mutational Burden - TMB-Low (5 Muts/Mb)

GENOMIC FINDINGS

PALB2 - L9F

10 Trials see p. 16

PIK3CA - H1047R

10 Trials see p. 19

ACTIONABILITY	
No therapies or clinical trials. see Biomarker Findings section	
No therapies or clinical trials. see Biomarker Findings section	
THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Niraparib	Talazoparib
Olaparib	
Rucaparib	
none	Alpelisib
	Everolimus
	Temsirolimus

Candidate drug with PIK3CA^mutation and evidence level (CIViC)

DIS	DRUGS	DESC	EL	ET	ED	CS	VO	ER
Breast Cancer	Alpelisib, Fulvestrant (Combination)		A					5★
Her2-receptor Positive Breast Cancer	Everolimus		B					4★
Colorectal Cancer	Anti-EGFR Monoclonal Antibody		B					4★
Cancer	Capivasertib		B					4★

Access date: 2020/12/25

Interpretation

- No microsatellite instability
- Tumor mutational burden 5/Mb: Not high

○ Assignment of biological significance

- *PALB2* L9F
  Missense mutation in a tumor suppressor gene
  No. of COSMIC registrations: 1
  gnomAD registrations: None
  → Mutation of unknown significance
- *PIK3CA* H1047R
  Oncogene missense mutation
  No. of COSMIC registrations: 3656
  → Activating mutation

○ Candidate drug and evidence (see CIViC)

- Only evidence for combination therapy with other drugs for PIK3CA activating mutation
  → No recommended treatment

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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A. Case 2

NCC Oncopanel

RG summary report draft

Test information

Test name	NCC Oncopanel Test
System name	OncoGuide TM NCC Oncopanel System

Gene mutation

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
APC	72.1 (782/1,084)	exon10:c.1312+1G>A		4166472 (1)
ATM	14.3 (51/350)	exon3:c.103C>T	R35*	922965 (2)

Number of somatic cell mutations

Region division		SNV		InDel		Total	
		Number of mutation	Mutation appearanc	Number of mutation	Mutation appearanc	Number of mutation appearances	Mutation appearanc
Exon	nonsyn	0	0.0/Mb	0	0.0/Mb	0	0.0/Mb
	syn	0	0.0/Mb				
Non-exon		2	2.1/Mb	1	1.1/Mb	3	3.2/Mb
Entire region		2	1.6/Mb	1	0.8/Mb	3	2.3/Mb

*2 Mutation appearance rate = number of mutations per 1 Mbp

Interpretation

○ Assignment of biological significance

- *APC* c.1312+1G>A
  Splicing site base substitution mutation in a tumor suppressor gene
  → Truncating loss-of-function mutation
- *ATM* R35*
  Tumor suppressor gene truncating mutation
  → Loss-of-function mutation
- Tumor mutational burden 2.3/Mb: Not high

○ Candidate drug and evidence level

- There are no candidate drugs for *APC* loss-of-function mutations
- Candidate drug for *ATM* loss-of-function mutation

Olaparib: Evidence level A/1 in prostate cancer (CIViC/OncoKB)

→ **Evidence level 3B** in other cancers **(OncoKB)**

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Reference Material

- NCC Oncopanel Report Explanation

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

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Results Report: Sequencing Report①

Sequencing report			
	Reference No.	[redacted]	Report draft creation date	[redacted]
Sample information			
Sample	Test registration No. (B):	[redacted]	Facility patient	[redacted]
Tumor tissue sequencing information			
Panel	NCC oncopanel v4		
Reagent	SureSelect XT HS Reagent		
Sequencer run date	10-Dec-18		
Name of read data	[redacted]		
Total number of reads	3,19,22,686		
Read mapping rate (%)	78.26		
Duplication rate (%)	72.55		
Discordance rate (%)	0.3		
Mismatch rate (%)	0.29		
Deletion rate (%)	0.00		
Insertion rate (%)	0.00		
Mean read depth	706.6		
Median read depth	616.0		
Mean insert size	1297.7		
Median insert size	206.0		
Normal tissue sequencing information			
Panel	NCC oncopanel v4		
Reagent	SureSelect XT HS Reagent		
Sequencer run date	10-Dec-18		
Name of read data	[redacted]		
Total number of reads	95,84,714		
Read mapping rate (%)	86.99		
Duplication rate (%)	12.53		
Discordance rate (%)	0.29		
Mismatch rate (%)	0.28		
Deletion rate (%)	0.01		
Insertion rate (%)	0.00		
Mean read depth	706.6		
Median read depth	616.0		
Mean insert size	1297.7		
Median insert size	206.0		
Data analysis			
Module	cisCall-7.1.6, cisGermline-1.0.1, cisAnnotate-1.1.3,		
Dataset	Dataset-1.00-180411		
Genetic abnormality selection conditions (SNV, InDel)	Exon/Splicing, -Syn, -SNP(+COSMIC), VAF≥0.05		
Genetic abnormality selection conditions (CNV)	[redacted] CNR≥4.0		
Genetic abnormality selection conditions (Fusion)	target		

Sequencing information
Tumor tissue
Mean read depth, etc.

Sequencing information
Normal tissue

Data analysis
Analysis pipeline version information, detection conditions of each mutation

- Describes information regarding NGS analysis and candidate genetic abnormalities.
- Describes which detected genetic abnormalities might be pathogenic.
- Does not describe when the likelihood of polymorphism is extremely high (frequencies of 1% or higher registered in either SNP database).
- Includes mutations of unknown significance, rare SNPs (frequency of less than 1%).

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Results Report: Sequencing Report②

Gene Mutation entry example

Genetic mutation information		
1	Gene name (Ensemble Expression ID)	KRAS (COSMIC-R, ENST00000311936)
	Mutation type	nonsynonymous SNV
	Physical location (Chromosome: base	12:25,398,284
	Gene copy number ratio (Corrected	1.12
	Mutation allele frequency (%)	11.3 (42/371)
	CDS variant	exon2:c.35G>T
	Amino acid variant	G12V
	COSMIC|ClinVar Registration ID	520|RCV000013413.4
	COSMIC|ClinVar Registration No.	7,326|9
	COSMIC Status|ClinVar Significance	Confirmed_somatic_variant|Pathogenic
	SNP database	
	Detection method	matched, known (somatic)
2	Gene name (Ensemble Expression ID)	TP53 (COSMIC-R, ENST00000269395)
	Mutation type	frameshift insertion
	Physical location (Chromosome: base	17:7,579,315
	Gene copy number ratio (Corrected	1.02
	Mutation allele frequency (%)	15.5 (159/1,023)
	CDS variant	exon4:c.371_372insG
	Amino acid variant	C124fs*25
	COSMIC|ClinVar Registration ID	1268350|-
	COSMIC|ClinVar Registration No.	3|-
	COSMIC Status|ClinVar Significance	Variant_of_unknown_origin|-
	SNP database	
	Detection method	matched

Germline genetic mutation information
TSC1 R692Q
BRCA1 E797fs*3

Registration status in COSMIC and ClinVar databases.
Describes the ID, number of registrations, and additional information in each database.

COSMIC Status : Whether the somatic mutation has been confirmed
Confirmed_somatic_variant – Confirmed
Variant_of_unknown_origin – Non confirmed

ClinVar Significance : Clinically significant variant
Pathogenic, Likely pathogenic – pathogenic variant (likely)
Uncertain_significance – May be pathogenic, but evidence is lacking
Likely_benign, benign – Not a pathogenic variant (unlikely)

This shows the components of the analysis program that was used to detect the variant.

- Matched : Detected in comparison with patient's normal sample = somatic mutation
- Known (somatic) : Detected as a somatic mutation (previously reported important variant)
- Known (germline) : Detected as a germline variant (as above)
- gatk : Detected as a germline variant

Candidate germline variants are summarized separately in the bottom row. (including rare SNPs with a frequency of less than 1%).

Supplement: Mentioned items

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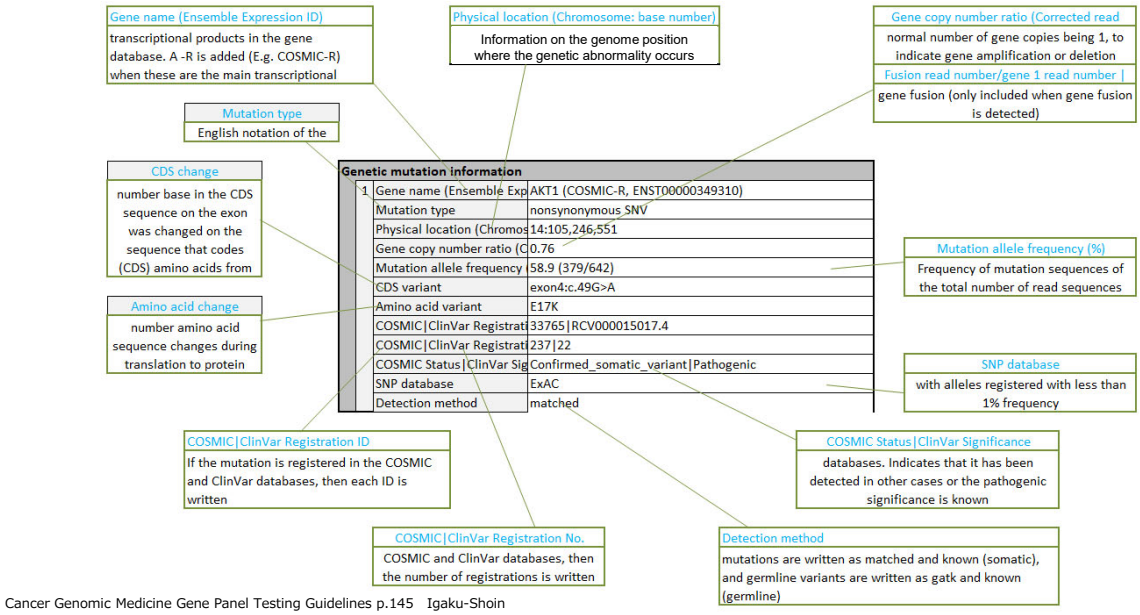

Cancer Genomic Medicine Gene Panel Testing Guidelines p.145 Igaku-Shoin

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Results Report: Sequencing Report③

Copy Number Variant

2	Gene name (Ensemble Expression ID)	EGFR	CDKN2A
	Mutation type	amplification	homozygous deletion
	Physical location (Chromosome: base number)	7:55,086,714-55,275,773	9:21,967,751-21,994,490
	Gene copy number ratio (Corrected read number ratio)	20.70	0.25
	Mutation allele frequency (%)	*-	*-
	CDS variant	*-	*-
	Amino acid variant	*-	*-
	COSMIC|ClinVar Registration ID	*-|-	*-|-
	COSMIC|ClinVar Registration No.	*-|-	*-|-
	COSMIC Status|ClinVar Significance	*-|-	*-|-
	SNP database	*-	*-
	Detection method	matched	matched

Gene copy number ratio is measured and detected.
The whole region of each exon gene is assessed, and no partial deletion is detected.

Points to note:
- Does not reflect the tumor cell ratio.
- Deletions are always described as homozygous deletions.

Copy number variant detection condition
- Amplification ≥4
- Deletion <0.5

Gene Fusion

3	Gene name (Ensemble Expression ID)	SLC34A2|ROS1
	Mutation type	fusion
	Physical location (Chromosome: base number)	4:25,679,257-6,:117,648,089
	Fusion read number/gene 1 read number | gene 2 read number	148/1,433|834
	Mutation allele frequency (%)	10.33
	CDS variant	*-
	Amino acid variant	*-
	COSMIC|ClinVar Registration ID	*-|-
	COSMIC|ClinVar Registration No.	*-|-
	COSMIC Status|ClinVar Significance	*-|-
	SNP database	*-
	Detection method	target

This is confirming genome sequence fusion, so the gene order is sometimes displayed in reverse.

It is necessary to confirm what is known.

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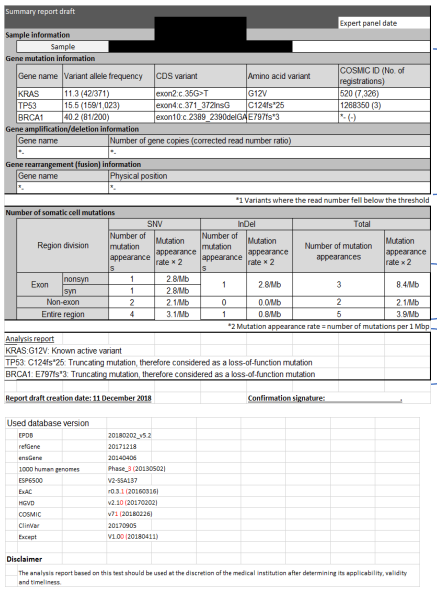
Results Report: Summary Report



Summary report draft

Expert panel date

Sample information

Sample [redacted]

Gene mutation information

Gene name	Variant allele frequency	CDS variant	Amino acid variant	COSMIC ID (No. of registrations)
KRAS	11.3 (42/371)	exon2.c.35G>T	G12V	520 (7,326)
TP53	15.5 (159/1,023)	exon4.c.371_372insG	C124fs*25	1268350 (3)
BRCA1	40.2 (81/200)	exon10 c. 2389_2390del/GA	E797fs*3	*, (-)

Gene amplification/deletion information

Gene name	Number of gene copies (corrected read number ratio)
*-	*-

Gene rearrangement (fusion) information

Gene name	Physical position
*-	*-

*1 Variants where the read number fell below the threshold

Number of somatic cell mutations

Region division		SNV		InDel		Total	
		Number of mutation appearances	Mutation appearance rate × 2	Number of mutation appearances	Mutation appearance rate × 2	Number of mutation appearances	Mutation appearance rate × 2
Exon	nonsyn	1	2.8/Mb	1	2.8/Mb	3	8.4/Mb
	syn	1	2.8/Mb				
Non-exon		2	2.1/Mb	0	0.0/Mb	2	2.1/Mb
Entire region		4	3.1/Mb	1	0.8/Mb	5	3.9/Mb

*2 Mutation appearance rate = number of mutations per 1 Mbp

Analysis report

KRAS:G12V: Known active variant

TP53: C124fs*25: Truncating mutation, therefore considered as a loss-of-function mutation

BRCA1: E797fs*3: Truncating mutation, therefore considered as a loss-of-function mutation

Report draft creation date: 11 December 2018 Confirmation signature: _____

Used database version

EPDB	20180202_v5.2
refGene	20171218
ensGene	20140406
1000 human genomes	Phase_3 (20130502)
ESP6500	V2-SSA137
ExAC	r0.3.1 (20160316)
HGVD	v2.10 (20170202)
COSMIC	v71 (20180226)
ClinVar	20170905
Except	V1.00 (20180411)

Disclaimer

The analysis report based on this test should be used at the discretion of the medical institution after determining its applicability, validity and timeliness.

The following information is extracted based on the description of the genetic abnormality in the sequencing report

Somatic Genetic Abnormality:

- Known druggable mutation
- COSMIC DB registered mutation
- Truncating mutation, splicing site mutation (tumor suppressor genes)
- Gene amplification (copy number ratio ≥4)
- Gene fusion (gene deletions are not described)

Germline Pathogenic Variant:

<Genes of interest>
APC, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *PTEN*, *RB1*, *RET*, *STK11*, *SMAD4*, *TP53*, *TSC1*, *VHL*

- "Pathogenic" in ClinVar
- Truncating mutation (tumor suppressor genes)

Not differentiated in the summary report

Number of Somatic Mutations

Analysis Report

Describes the frequency for each detected genetic abnormality

- Categorically described items are known to exhibit functional changes due to the mutation
- Mutations detectable with known companion diagnostics have "known" added at the beginning of the sentence

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