INTERPRETATION FRAMEWORK OF THE MOLECULAR TUMOR BOARD PORTAL

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The Molecular Tumor Board Portal (MTBP) is a CDSS developed by the Cancer Core Europe

- CCE was established to **co-develop** new precision oncology **trials** across European cancer centers under an **unified framework**

- The MTBP is the CDSS for these initiatives and also supports this 'virtual hospital' model pursued by CCE

  *(as it creates a **single technological, scientific and legal infrastructure** shared between centers)*

The MTBP automates data transfer, interpretation and reporting across CCE centers

Molecular Tumor Board Portal

> **Retrieval** from **clinical**, **pathology** & **molecular data** files from CCE and external labs

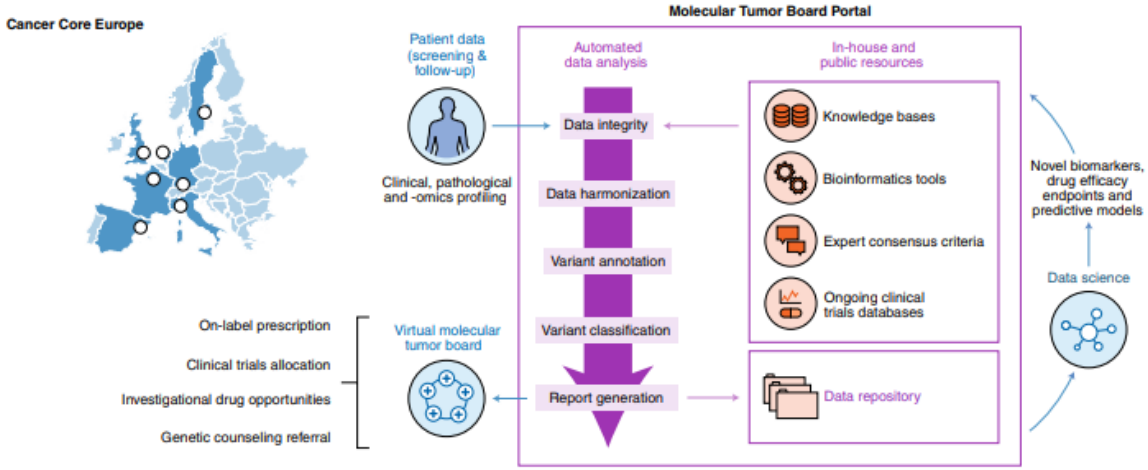
> Data **integrity** & **harmonization** processes

> Variant annotation following a workflow **pre-agreed** among CCE **experts**

> **Report generation** & **sharing** with clinical investigators

correspondence

Support systems to guide clinical decision-making in precision oncology: The Cancer Core Europe Molecular Tumor Board Portal



Tamborero et al. **Nature Medicine** 2020

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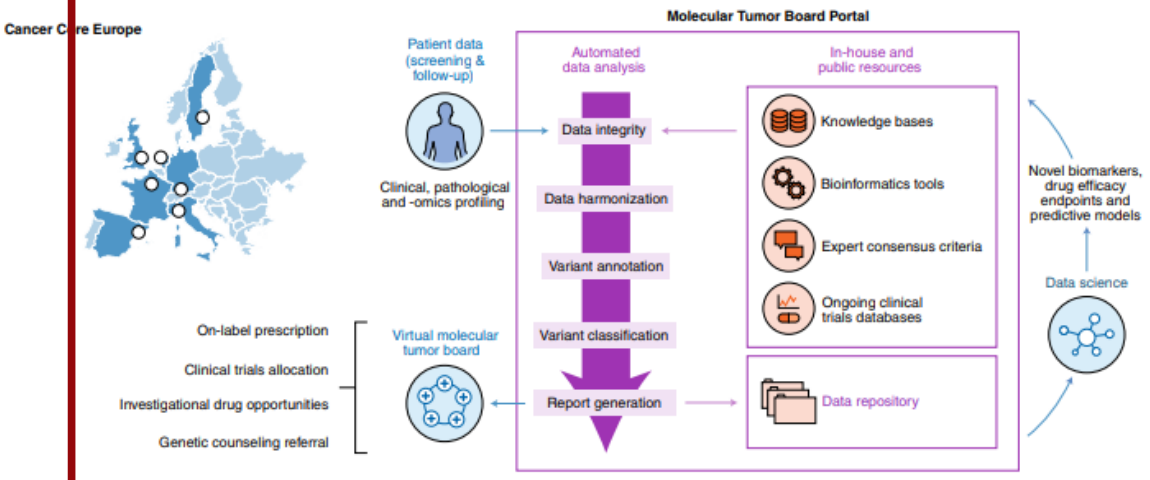


Tamborero et al. **Nature Medicine** 2020

*Fully **automated** process !*

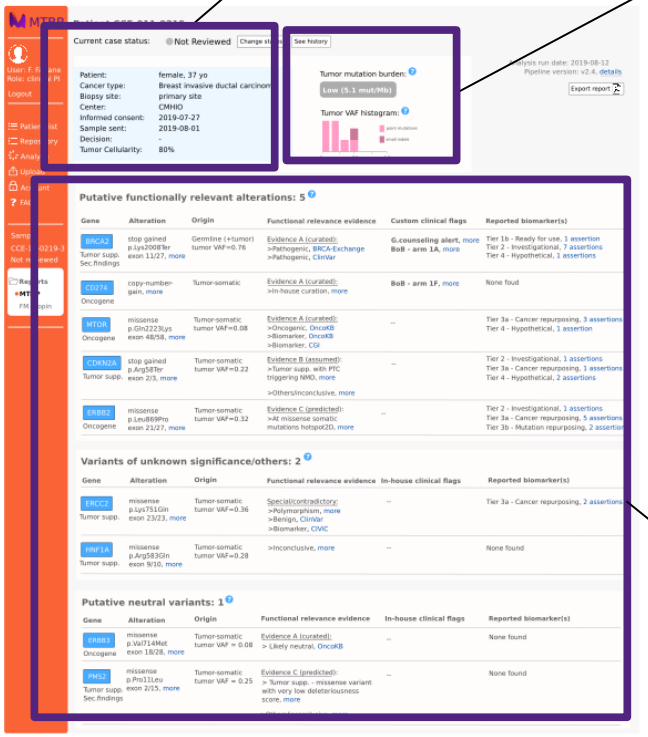
(time, accuracy and systematic advantages)

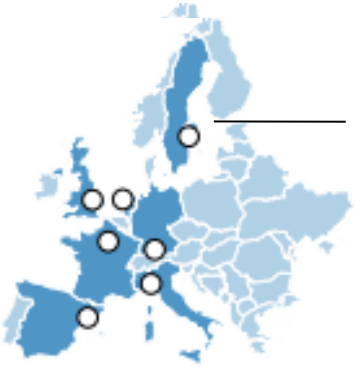
The MTBP reports are accessed via an online platform and discussed during (virtual) meetings

Interactive reports

dashboard with patient's clinical & pathology data

'broad' **genomic signatures**





Variants classified according to their predictive **relevance**

Representatives from each CCE center connect via an online platform to discuss clinical recommendations

The report classifies the individual variants for their functional and predictive relevance

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
BRCA2 Tumor supp. Sec.findings	stop gained p.Lys2008Ter exon 11/27, more	Germline (+tumor) tumor VAF=0.76	Evidence A (curated): >Pathogenic, BRCA-Exchange >Pathogenic, ClinVar	**G.counseling alert**, more **HRR- arm1** , more	Tier 1 - Ready for use, 1 assertion Tier 2 - Investigational, 7 assertions Tier 4 - Hypothetical, 1 assertions
CD274 Oncogene	copy-number-gain, more	Tumor-somatic	Evidence A (curated): >In-house curation, more	**BoB - arm 1F**, more	None foud
MTOR Oncogene	missense p.Gln2223Lys exon 48/58, more	Tumor-somatic tumor VAF=0.08	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, OncoKB >Biomarker, CGI	--	Tier 3a - Cancer repurposing, 3 assertions Tier 4 - Hypothetical, 1 assertion
CDKN2A Tumor supp.	stop gained p.Arg58Ter exon 2/3, more	Tumor-somatic tumor VAF=0.22	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more >Others/inconclusive, more	--	Tier 2 - Investigational, 1 assertions Tier 3a - Cancer repurposing, 1 assertions Tier 4 - Hypothetical, 2 assertions
ERBB2 Oncogene	missense p.Leu869Pro exon 21/27, more	Tumor-somatic tumor VAF=0.32	Evidence C (predicted): >At missense somatic mutations hotspot2D, more	--	Tier 2 - Investigational, 1 assertions Tier 3a - Cancer repurposing, 5 assertions Tier 3b - Mutation repurposing, 2 assertions

Female, 42y, ovarian cancer
Disease progression after initially successful PARP-inhibitor treatment

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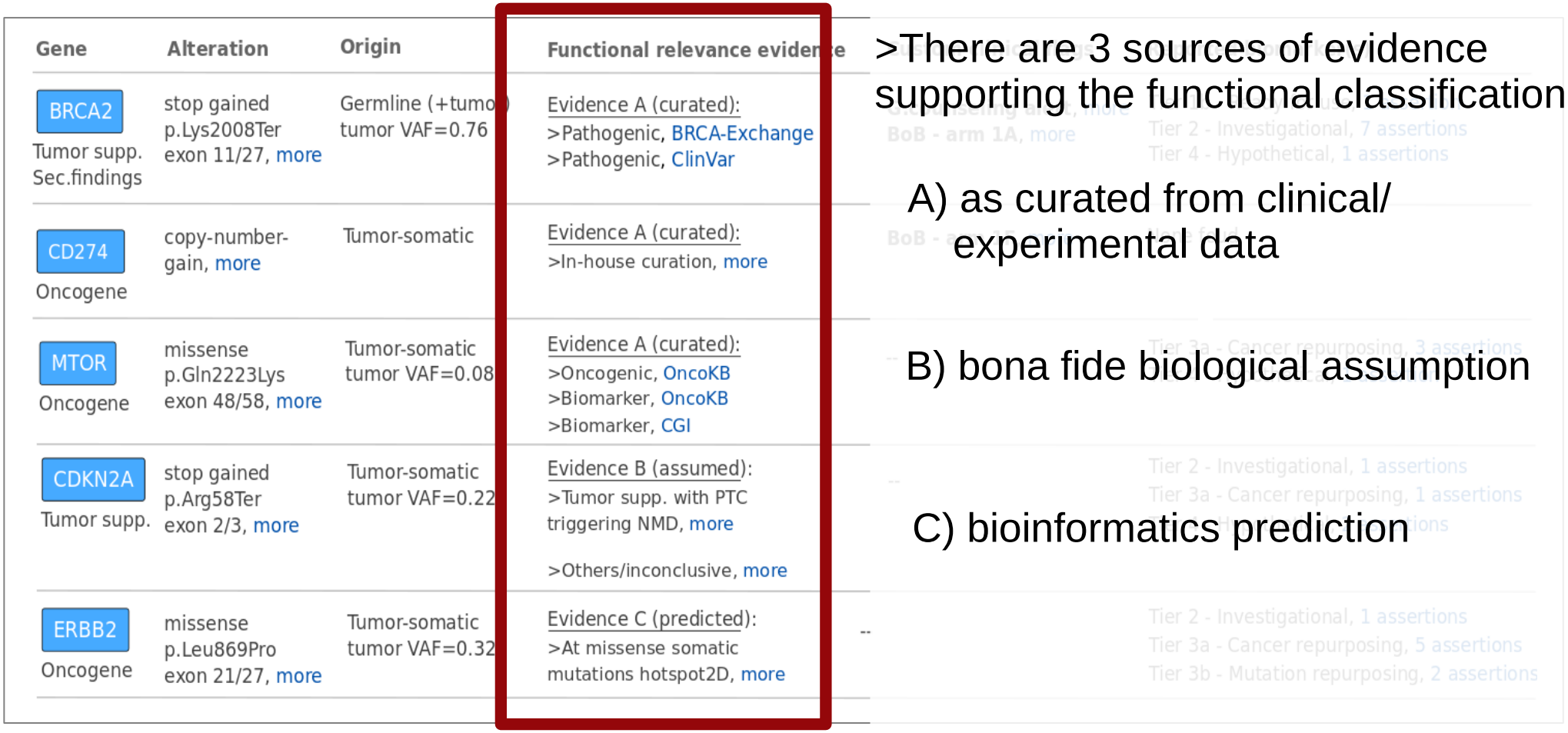
Descriptives of the gene and the variant

(MTBP variant mapping via Ensembl-VEP)

The first step is to classify the variants that are functionally relevant for the tumor phenotypes



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>There are 3 sources of evidence supporting the functional classification:

A) as curated from clinical/ experimental data

B) bona fide biological assumption

C) bioinformatics prediction

>It is a clinical decision which evidence you require to pursue an action in the variant !

Functional relevance call based on known variant effects

> Any reported effect compatible with the variant being functional regardless of the "context"*

(pathogenicity, experimental assays, clinical data, biomakers,…)

CIViC
CLINICAL INTERPRETATIONS OF VARIANTS IN CANCER

CANCER GENOME INTERPRETER

BRCA Exchange

OncoKB

ClinVar
Clinically relevant variation

** example: a BRCA1 variant can be considered loss-of-function if already:*

> described as pathogenic in germline genetic studies

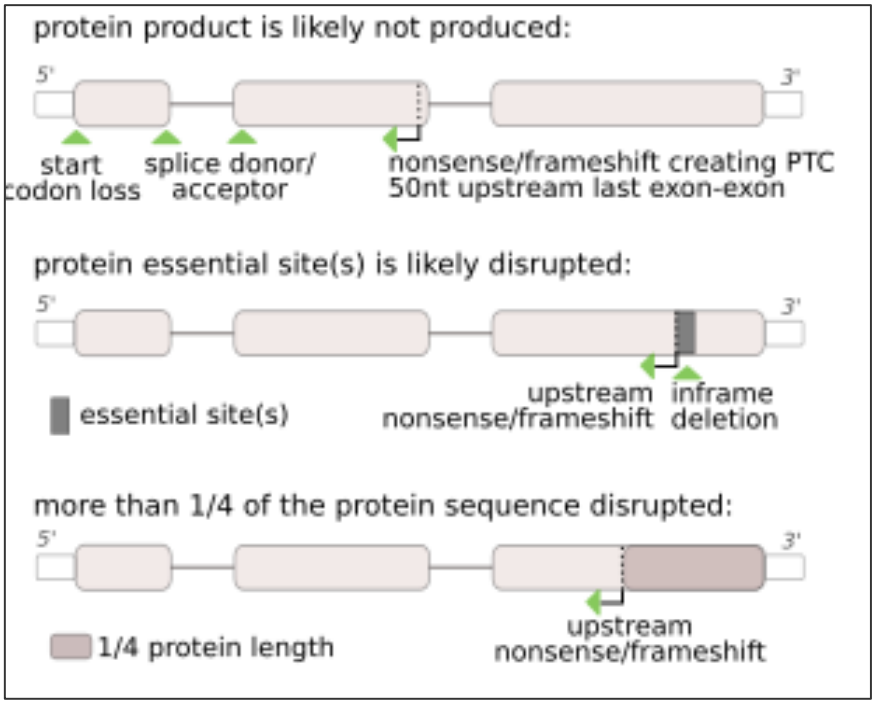
> found to disrupt WT protein in functional assays

> reported as biomarker of PARPinh in a particular cancer type(s)

The context (origin, clonality, cancer type, co-occuring alterations..)
is considered when evaluating the clinical relevance of such loss-of-function

Functional relevance call based on assumed effects

> These are largely based on **sedimented** criteria established to identify loss-of-function events in Mendelian disease genes (99% PPV)



e.g. > when a PTC leads to NMD?

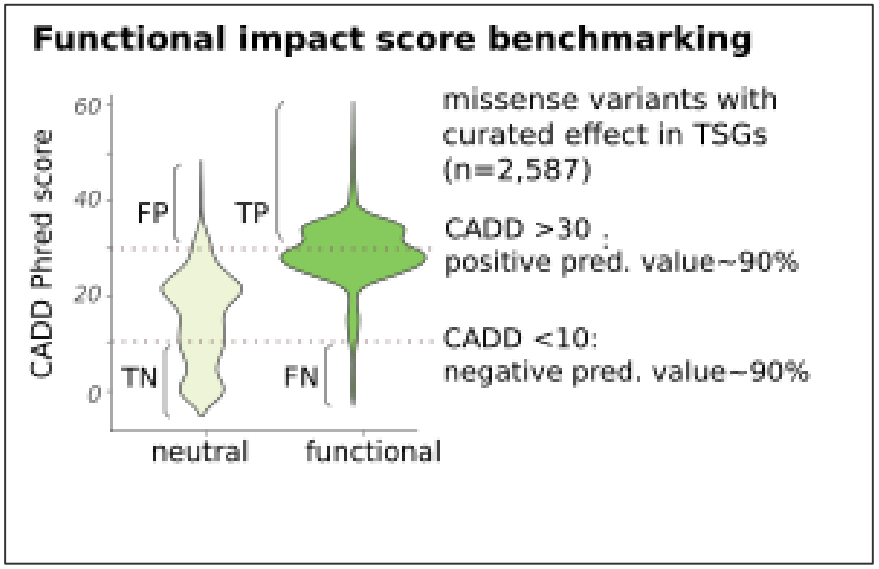
> when an essential protein region is disrupted?

Functional relevance call based on bioinformatic predictions

> Important: use those providing robust results (prioritizing PPV)

> Mutation **hotspots**: use statistical methods that consider genomic background mutational processes to call an observed accumulation as significant

> **Functional impact** scores: use a method benchmarked for you specific Analytical question and with thresholds adapted to the required PPV



Tumor variants are then matched with cancer biomarkers reported at present

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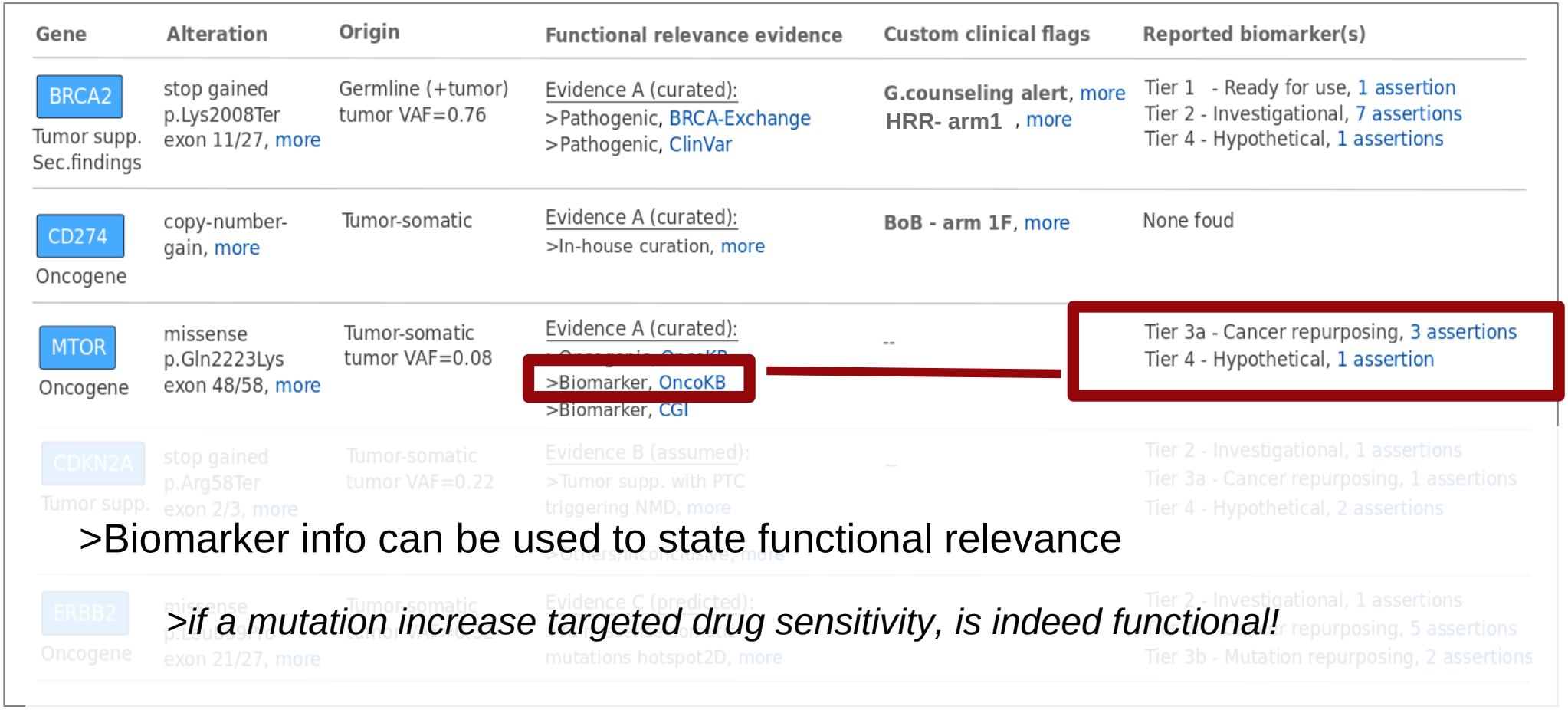
>Cancer biomarkers means that the variant is functional *AND* shapes diagnosis, prognosis or drug response

Actionability must be tiered for clinical utility



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ERBB2 Oncogene	missense p.Leu869Pro exon 21/27, more	Tumor-somatic tumor VAF=0.52	Evidence C (predicted): mutations hotspot2D, more		Tier 2 - Investigational, 1 assertions repurposing, 5 assertions Tier 3b - Mutation repurposing, 2 assertions

>Biomarker info can be used to state functional relevance

>if a mutation increase targeted drug sensitivity, is indeed functional!

>Biomarker actionability must factor in **additional considerations** beyond the variant matching (aka, the "context")

> co-occuring alterations, cancer type match, biomarker clinical evidence, etc

Guidelines to tier biomarker actionability

Annals of Oncology 29: 1895–1902, 2018
doi:10.1093/annonc/mdy263
Published online 21 August 2018

SPECIAL ARTICLE

A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT)

For non-standard therapies, we prioritize allocation to clinical trials (recruiting across CCE centers)

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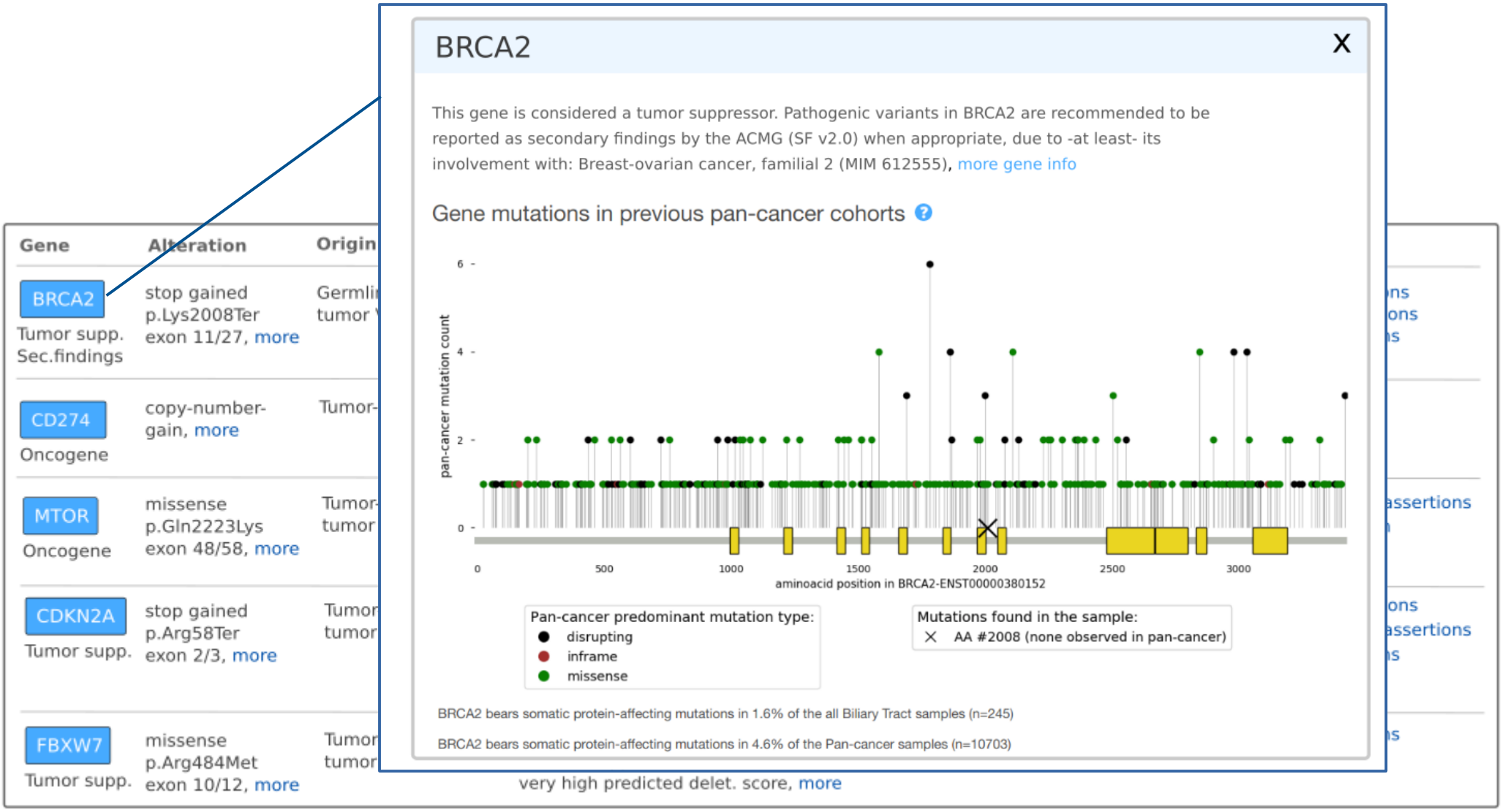
> Variants matching **trial eligibility** are flagged & prioritized in the MTBP reports

***(clinical, pathological and molecular** criteria passed via 'custom actionability' files)*

MTBP reports are HTML documents: use of interactive elements



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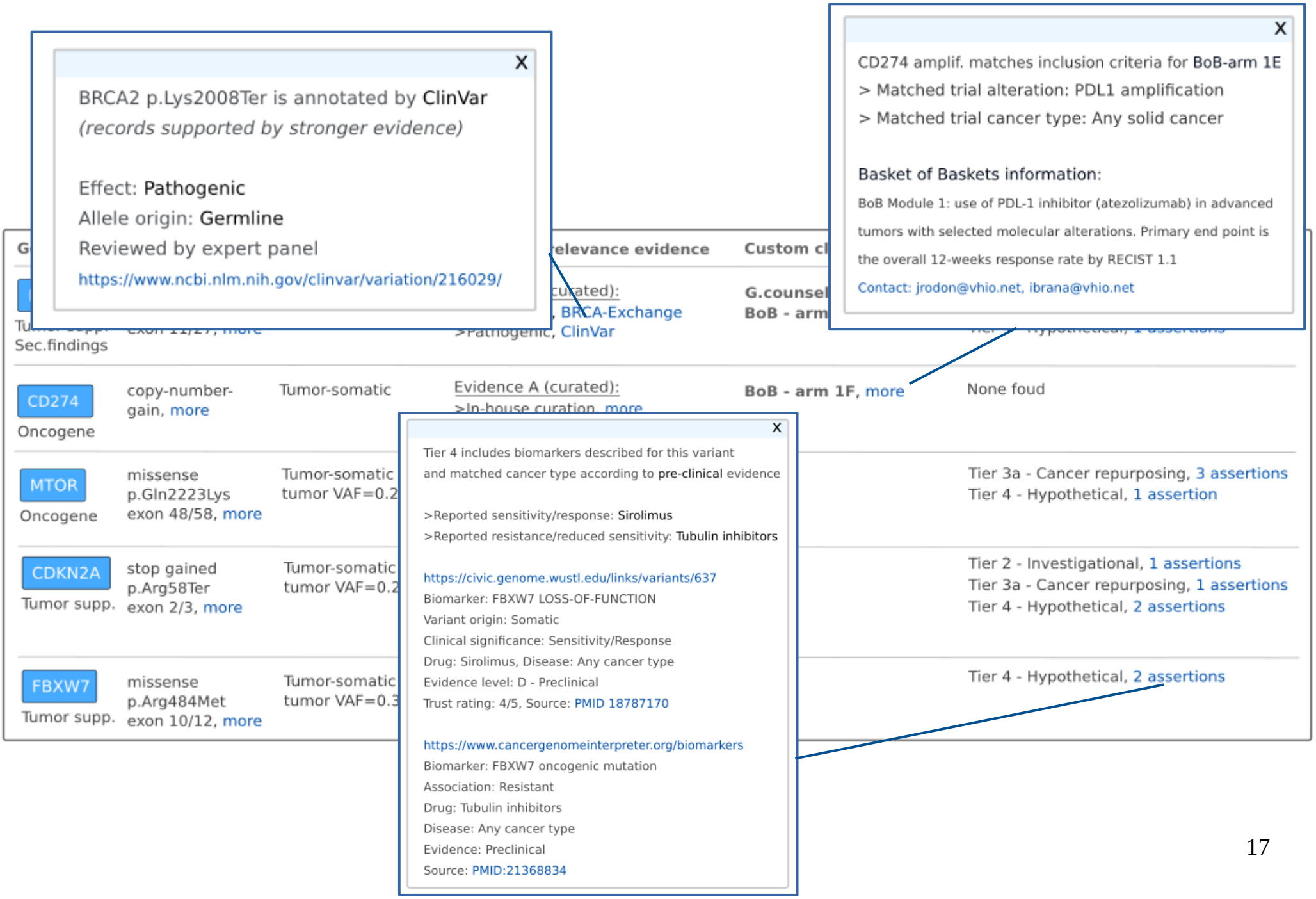

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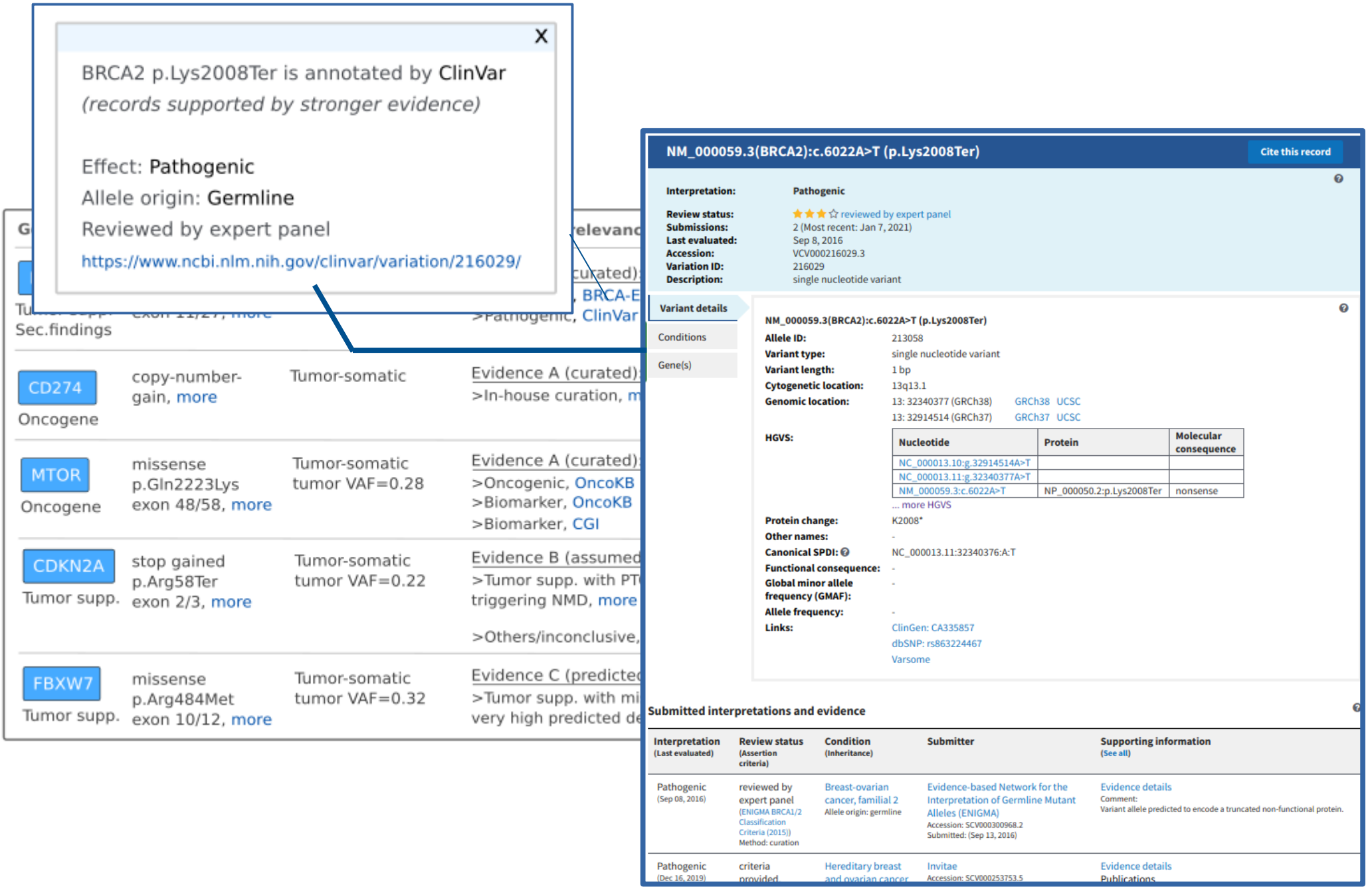


CLINICAL INTERPRETATION OF NGS RESULTS

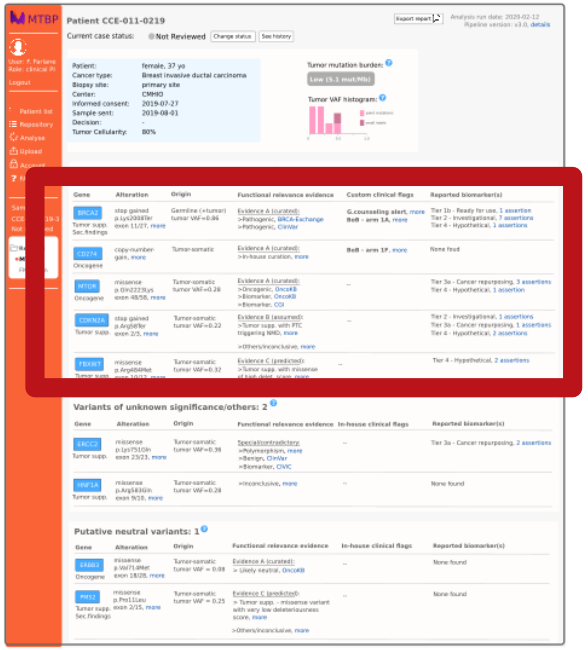
EXAMPLES

Molecular Tumor Board Portal

CASE 1

> 49 years woman, advanced **ovarian cancer** with a **known** cancer family history

> **Panel sequencing results** – breast tumor biopsy (sample purity ~90%)
+ paired germline control



5 genomic alterations classified as functionally relevant

Zoom-in in the MTBP report: **Putative Functional Variants table**

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
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MTOR Oncogene	missense p.Gln2223Lys exon 48/58, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, CGI	--	Tier 3a - Cancer repurposing, 3 assertions Tier 4 - Hypothetical, 1 assertion
CDKN2A Tumor supp.	stop gained p.Arg58Ter exon 2/3, more	Tumor-somatic tumor VAF=0.22	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more >Others/inconclusive, more	--	Tier 2 - Investigational, 1 assertions Tier 3a - Cancer repurposing, 1 assertions Tier 4 - Hypothetical, 2 assertions
FBXW7 Tumor supp.	missense p.Arg484Met exon 10/12, more	Tumor-somatic tumor VAF=0.32	Evidence C (predicted): >Tumor supp. with missense of very high predicted delet. score, more	--	Tier 4 - Hypothetical, 2 assertions

> **BRCA2** nonsense, germline origin;

> tumor VAF compatible with LoH

> already reported as pathogenic

BRCA2 p.Lys2008Ter

https://www.ncbi.nlm.nih.gov/clinvar/variation/216029
Effect: Pathogenic
Allele origin: germline
Review status: reviewed by expert panel

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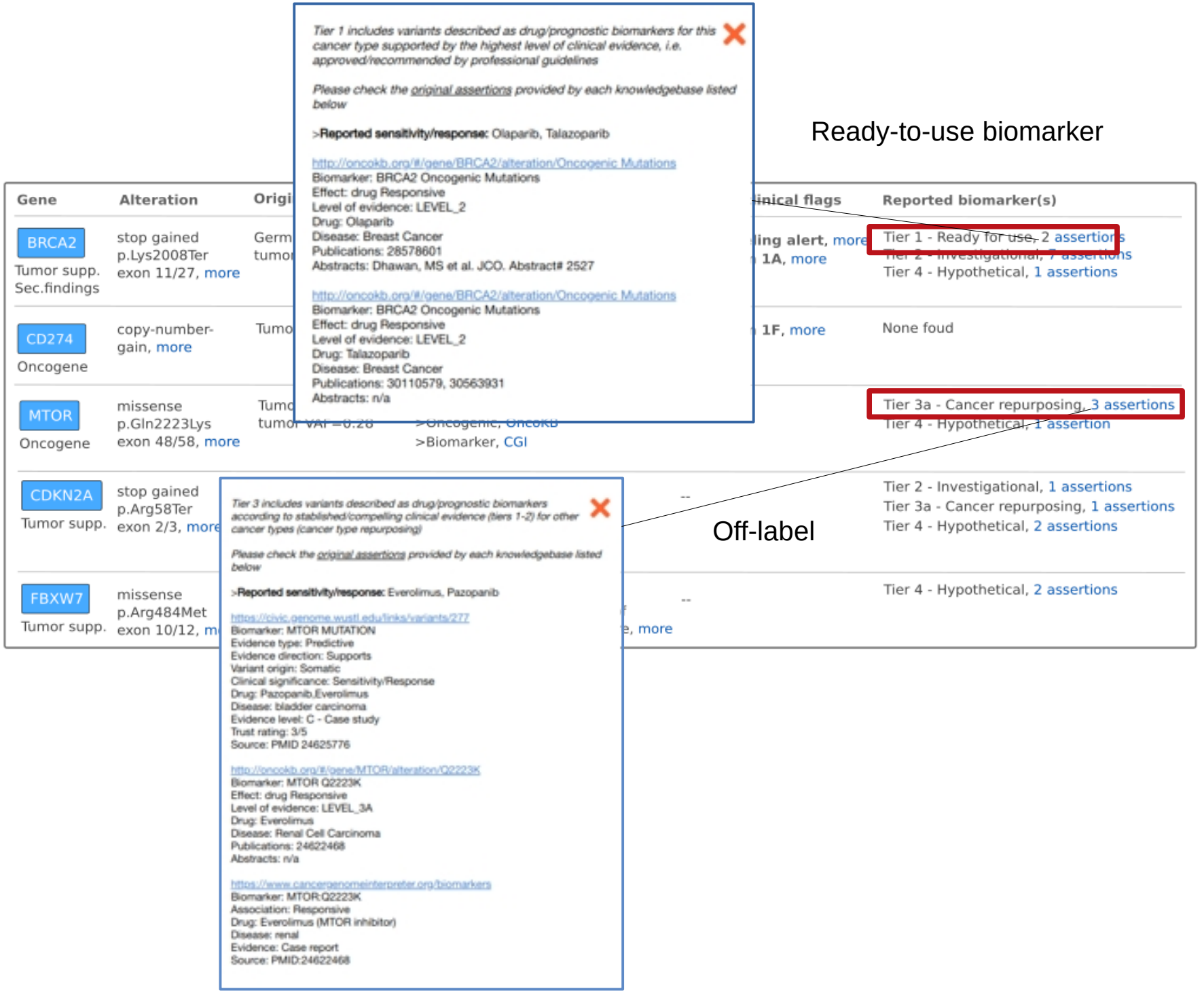
> **CD274** amplification (estimated DNA material gain >6 copies)

> **MTOR** somatic missense variant known to be oncogenic (evidence A - curated)

> **CDKN2A** somatic nonsense “fair” to assume that is LoF (evidence B - assumed)

> **FBXW7** somatic missense **predicted** to be LoF (evidence C - predicted)


Tier 1 includes variants described as drug/prognostic biomarkers for this cancer type supported by the highest level of clinical evidence, i.e. approved/recommended by professional guidelines
Please check the original assertions provided by each knowledgebase listed below
>Reported sensitivity/response: Olaparib, Talazoparib
http://oncokb.org/#/gene/BRCA2/alteration/Oncogenic Mutations
Biomarker: BRCA2 Oncogenic Mutations
Effect: drug Responsive
Level of evidence: LEVEL_2
Drug: Olaparib
Disease: Breast Cancer
Publications: 28578601
Abstracts: Dhawan, MS et al. JCO. Abstract# 2527
http://oncokb.org/#/gene/BRCA2/alteration/Oncogenic Mutations
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Publications: 30110579, 30563931
Abstracts: n/a
Ready-to-use biomarker
Gene
Alteration
Reported biomarker(s)
BRCA2
Tumor supp.
Sec.findings
stop gained
p.Lys2008Ter
exon 11/27, more
Tier 1 - Ready for use, 2 assertions
Tier 4 - Hypothetical, 1 assertions
CD274
Oncogene
copy-number-gain, more
None foud
MTOR
Oncogene
missense
p.Gln2223Lys
exon 48/58, more
>Biomarker, CGI
Tier 3a - Cancer repurposing, 3 assertions
Tier 4 - Hypothetical, 1 assertion
CDKN2A
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Tier 2 - Investigational, 1 assertions
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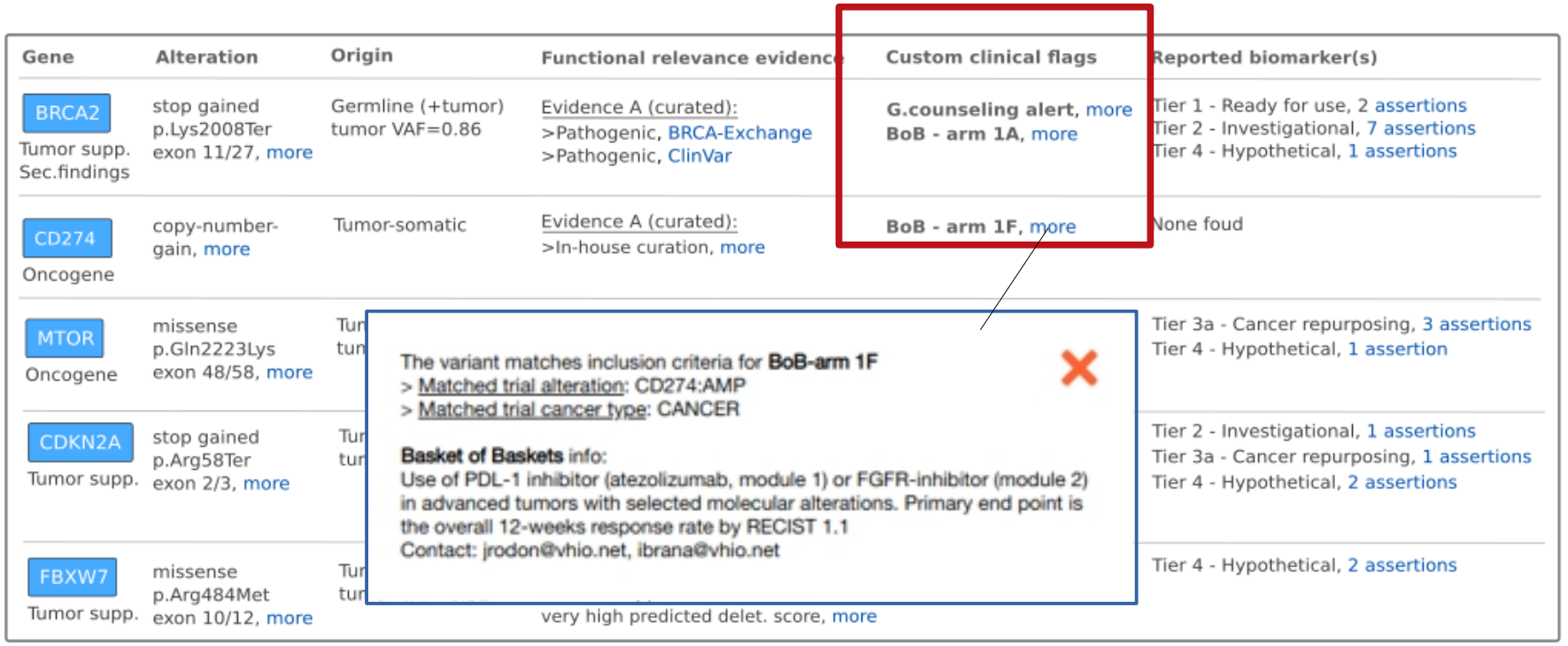
Tier 4 - Hypothetical, 2 assertions
Off-label
FBXW7
Tumor supp.
missense
p.Arg484Met
Tier 4 - Hypothetical, 2 assertions
Tier 3 includes variants described as drug/prognostic biomarkers according to stablished/compelling clinical evidence (tiers 1-2) for other cancer types (cancer type repurposing)
Please check the original assertions provided by each knowledgebase listed below
>Reported sensitivity/response: Everolimus, Pazopanib
https://civic.genome.wustl.edu/links/variants/277
Biomarker: MTOR MUTATION
Evidence type: Predictive
Evidence direction: Supports
Variant origin: Somatic
Clinical significance: Sensitivity/Response
Drug: Pazopanib,Everolimus
Disease: bladder carcinoma
Evidence level: C - Case study
Trust rating: 3/5
Source: PMID 24625776
http://oncokb.org/#/gene/MTOR/alteration/Q2223K
Biomarker: MTOR Q2223K
Effect: drug Responsive
Level of evidence: LEVEL_3A
Drug: Everolimus
Disease: Renal Cell Carcinoma
Publications: 24622468
Abstracts: n/a
https://www.cancergenomeinterpreter.org/biomarkers
Biomarker: MTOR:Q2223K
Association: Responsive
Drug: Everolimus (MTOR inhibitor)
Disease: renal
Evidence: Case report
Source: PMID:24622468

> **Genetic counseling alert** (not necessary for this case)

> **BRCA2** pathogenic qualifies for **BoB** (arm 1A)

> **PDL1** amplification qualifies for **BoB** (arm 1F)



Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
BRCA2 Tumor supp. Sec.findings	stop gained p.Lys2008Ter exon 11/27, more	Germline (+tumor) tumor VAF=0.86	Evidence A (curated): >Pathogenic, BRCA-Exchange >Pathogenic, ClinVar	**G.counseling alert**, more **BoB - arm 1A**, more	Tier 1 - Ready for use, 2 assertions Tier 2 - Investigational, 7 assertions Tier 4 - Hypothetical, 1 assertions
CD274 Oncogene	copy-number-gain, more	Tumor-somatic	Evidence A (curated): >In-house curation, more	**BoB - arm 1F**, more	None foud
MTOR Oncogene	missense p.Gln2223Lys exon 48/58, more	Tu tu			Tier 3a - Cancer repurposing, 3 assertions Tier 4 - Hypothetical, 1 assertion
CDKN2A Tumor supp.	stop gained p.Arg58Ter exon 2/3, more	Tu tu			Tier 2 - Investigational, 1 assertions Tier 3a - Cancer repurposing, 1 assertions Tier 4 - Hypothetical, 2 assertions
FBXW7 Tumor supp.	missense p.Arg484Met exon 10/12, more	Tu tu	very high predicted delet. score, more		Tier 4 - Hypothetical, 2 assertions

The variant matches inclusion criteria for **BoB-arm 1F**
> Matched trial alteration: CD274:AMP
> Matched trial cancer type: CANCER

Basket of Baskets info:
Use of PDL-1 inhibitor (atezolizumab, module 1) or FGFR-inhibitor (module 2) in advanced tumors with selected molecular alterations. Primary end point is the overall 12-weeks response rate by RECIST 1.1
Contact: jrodon@vhio.net, ibrana@vhio.net

CASE 2

> 43 years man, advanced **colorectal adenocarcinoma,** known BRAF V600mut

> Panel sequencing results – primary tumor biopsy (sample purity ~80%) + paired germline control

6 genomic alterations classified as functionally relevant

Multiple mutations classified as
VUS/likely neutral

Zoom-in in the MTBP report: Putative Functional Variants table

Tumor Mutation Burden: **High (37.83 mut/Mb)**

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
MLH1 Tumor supp. Sec.findings	frameshift p.Arg497Glyfs*11 exon 13/19, more	Germline (+tumor) tumor VAF=0.48	Evidence A (curated): >Pathogenic, ClinVar	**G.counseling alert**, more **BoB - arm 1A**, more	None found
BRAF Oncogene	missense, p.Val600Glu exon 15/18, more	Tumor-somatic tumor VAF=0.36	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, CGI >Pathogenic, ClinVar >Biomarker, CiVIC	--	Tier 1 - Ready for use, 2 assertions Tier 2 - Investigational, 27 assertions Tier 3a - Cancer repurposing, 90 assertions Tier 4 - Hypothetical, 1 assertions
MEN1 Tumor supp. Sec.findings	frameshift p.Arg521Profs*15 exon 10/10, more	Tumor-somatic tumor VAF=0.35	Evidence A (curated): >Pathogenic, ClinVar	--	None found
MAP2K4 Ambiguous	missense p.Pro326Leu exon 9/11, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Likely Oncogenic, OncoKB	--	None found
TP53 Tumor supp. Sec.findings	frameshift p.Ser90Profs*33 exon 4/11, more	Tumor-somatic tumor VAF=0.35	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	Tier 3 - Cancer repurposing, 6 assertions
RNF43 Tumor supp.	frameshift p.Gly659Valfs+41 exon 9/10, more	Tumor-somatic tumor VAF=0.32	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	None found

> Germline **MLH1** frameshift variant, reported as pathogenic; not previously known for this patient

Tumor Mutation Burden: **High (37.83 mut/Mb)**

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
MLH1 Tumor supp. Sec.findings	frameshift p.Arg497Glyfs*11 exon 13/19, more	Germline (+tumor) tumor VAF=0.48	Evidence A (curated): >Pathogenic, ClinVar	**G.counseling alert**, more **BoB - arm 1A**, more	None found
BRAF Oncogene	missense, p.Val600Glu exon 15/18, more	Tumor-somatic tumor VAF=0.36	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, CGI >Pathogenic, ClinVar >Biomarker, CiVIC	--	Tier 1 - Ready for use, 2 assertions Tier 2 - Investigational, 27 assertions Tier 3a - Cancer repurposing, 90 assertions Tier 4 - Hypothetical, 1 assertions
MEN1 Tumor supp. Sec.findings	frameshift p.Arg521Profs*15 exon 10/10, more	Tumor-somatic tumor VAF=0.35	Evidence A (curated): >Pathogenic, ClinVar	--	None found
MAP2K4 Ambiguous	missense p.Pro326Leu exon 9/11, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Likely Oncogenic, OncoKB	--	None found
TP53 Tumor supp. Sec.findings	frameshift p.Ser90Profs*33 exon 4/11, more	Tumor-somatic tumor VAF=0.35	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	Tier 3 - Cancer repurposing, 6 assertions
RNF43 Tumor supp.	frameshift p.Gly659Valfs+41 exon 9/10, more	Tumor-somatic tumor VAF=0.32	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	None found

> Also, a MEN1 mutation described as pathogenic

> Reported in several individuals with multiple endocrine neoplasia type 1

> However, not found in the germline sample in this patient, and tVAF=35% → somatic event, no genetic counseling referral flag in the report

NM_001370259.2(MEN1):c.1546dup (p.Arg516fs) — Cite this record

Interpretation:	**Pathogenic**
Review status:	★★☆☆ criteria provided, multiple submitters, no conflicts
Submissions:	8 (Most recent: Jul 4, 2021)
Last evaluated:	Oct 12, 2020
Accession:	VCV000279852.15
Variation ID:	279852
Description:	1bp duplication

Comment:
This duplication of one nucleotide in MEN1 is denoted c.1546dupC at the cDNA level and p.Arg516ProfsX15 (R516PfsX15) at the protein level. The normal sequence, with the base that is duplicated in brackets, is ACCCCCC[dupC]GGAA. Using alternate nomenclature, this variant would be defined as MEN1 1561dupC, 1650insC, 1656dupC, 1657insC, 7766insC, or 7773insC. The duplication causes a frameshift which changes an Arginine to a Proline at codon 516, and creates a premature stop codon at position 15 of the new reading frame. Even though this frameshift occurs in the last exon of the gene, and nonsense-mediated decay is not expected to occur, it is significant since the last 95 amino acids are no longer translated correctly, and are replaced by 14 incorrect amino acids. This variant is predicted to cause loss of normal protein function through protein truncation. MEN1 c.1546dupC has been reported in at least one individual with familial isolated hyperparathyroidism and in many individuals with multiple endocrine neoplasia type 1 (Agarwal 1997, Bergman 2000, Warner 2004, Cardinal 2005, Pieterman 2012). This variant was reported to segregate with disease through five generations of a large Finnish MEN1 family (Kytola 2001). MEN1 c.1546dupC has been reported at a high frequency in patients with MEN1 in multiple populations and likely occurs as a result of a mutational hotspot (Giraud 1998, Kytola 2001, Ebeling 2004). In addition, in vitro functional studies show that this variant impairs nuclear localization of protein compared to wild-type (Ikeo 1999). Based on the currently available evidence, we consider this variant to be pathogenic. (less)

0 assertions

MEN1 Tumor supp. Sec.findings	frameshift p.Arg521Profs*15 exon 10/10, more	Tumor-somatic tumor VAF=0.35	Evidence A (curated): >Pathogenic, ClinVar	--	None found
MAP2K4 Ambiguous	missense p.Pro326Leu exon 9/11, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Likely Oncogenic, OncoKB	--	None found
TP53 Tumor supp. Sec.findings	frameshift p.Ser90Profs*33 exon 4/11, more	Tumor-somatic tumor VAF=0.35	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	Tier 3 - Cancer repurposing, 6 assertions
RNF43 Tumor supp.	frameshift p.Gly659Valfs+41 exon 9/10, more	Tumor-somatic tumor VAF=0.32	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	None found

Tumor Mutation Burden: **High (37.83 mut/Mb)**

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
MLH1 Tumor supp. Sec.findings	frameshift p.Arg497Glyfs*11 exon 13/19, more	Germline (+tumor) tumor VAF=0.48	Evidence A (curated): >Pathogenic, ClinVar	**G.counseling alert**, more **BoB - arm 1A**, more	None found
BRAF Oncogene	missense, p.Val600Glu exon 15/18, more	Tumor-somatic tumor VAF=0.36	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, CGI >Pathogenic, ClinVar >Biomarker, CiVIC	--	Tier 1 - Ready for use, 2 assertions Tier 2 - Investigational, 27 assertions Tier 3a - Cancer repurposing, 90 assertions Tier 4 - Hypothetical, 1 assertions
MEN1 Tumor supp. Sec.findings	frameshift p.Arg521Profs*15 exon 10/10, more	Tumor-somatic tumor VAF=0.35	Evidence A (curated): >Pathogenic, ClinVar		
MAP2K4 Ambiguous	missense p.Pro326Leu exon 9/11, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Likely Oncogenic, OncoKB		
TP53 Tumor supp. Sec.findings	frameshift p.Ser90Profs*33 exon 4/11, more	Tumor-somatic tumor VAF=0.35	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more		
RNF43 Tumor supp.	frameshift p.Gly659Valfs+41 exon 9/10, more	Tumor-somatic tumor VAF=0.32	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more		

Tier 1 includes variants described as drug/prognostic biomarkers for this cancer type supported by the highest level of clinical evidence, i.e. approved/recommended by professional guidelines

Please check the original assertions provided by each knowledgebase listed below

>**Reported sensitivity/response:** Cetuximab, Encorafenib, Panitumumab

http://oncokb.org/#/gene/BRAF/alteration/V600E
Biomarker: BRAF V600E
Effect: drug Responsive
Level of evidence: LEVEL_1
Drug: Encorafenib, Cetuximab
Disease: Colorectal Cancer
Publications: 31566309
Abstracts: n/a

http://oncokb.org/#/gene/BRAF/alteration/V600E
Biomarker: BRAF V600E
Effect: drug Responsive
Level of evidence: LEVEL_2
Drug: Encorafenib, Panitumumab
Disease: Colorectal Cancer
Publications: 29431699, 31566309
Abstracts: n/a

EGFRi + RAFi

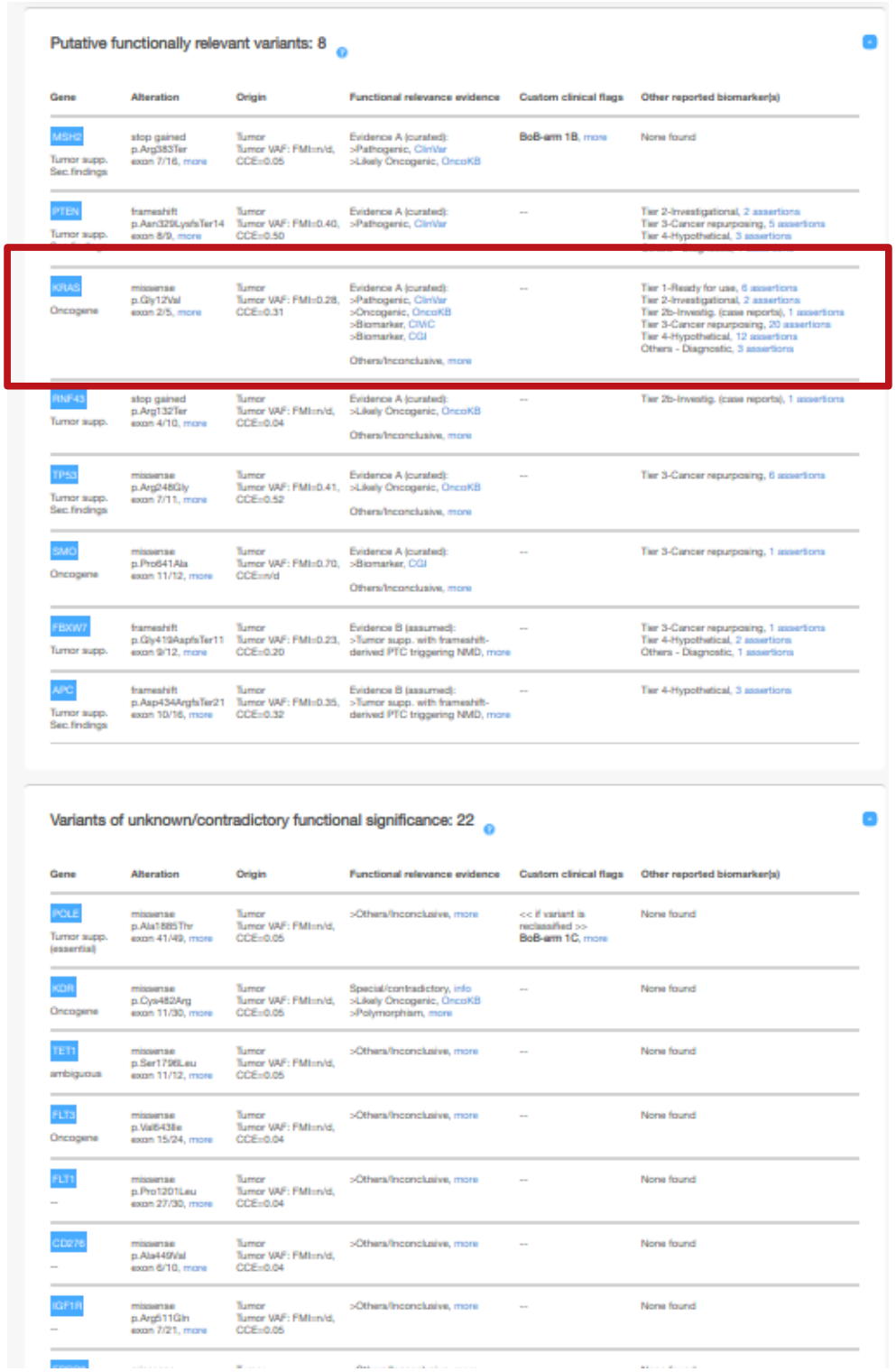
(KRAS wild-type)

Tumor Mutation Burden: **High (37.83 mut/Mb)**

genetic counseling alert;
BoB arm 1A

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Reported biomarker(s)
MLH1 Tumor supp. Sec.findings	frameshift p.Arg497Glyfs*11 exon 13/19, more	Germline (+tumor) tumor VAF=0.48	Evidence A (curated): >Pathogenic, ClinVar	**G.counseling alert**, more **BoB - arm 1A**, more	None found
BRAF Oncogene	missense, p.Val600Glu exon 15/18, more	Tumor-somatic tumor VAF=0.36	Evidence A (curated): >Oncogenic, OncoKB >Biomarker, CGI >Pathogenic, ClinVar >Biomarker, CiVIC	--	Tier 1 - Ready for use, 2 assertions Tier 2 - Investigational, 27 assertions Tier 3a - Cancer repurposing, 90 assertions Tier 4 - Hypothetical, 1 assertions
MEN1 Tumor supp. Sec.findings	frameshift p.Arg521Profs*15 exon 10/10, more	Tumor-somatic tumor VAF=0.35	Evidence A (curated): >Pathogenic, ClinVar	--	None found
MAP2K4 Ambiguous	missense p.Pro326Leu exon 9/11, more	Tumor-somatic tumor VAF=0.28	Evidence A (curated): >Likely Oncogenic, OncoKB	--	None found
TP53 Tumor supp. Sec.findings	frameshift p.Ser90Profs*33 exon 4/11, more	Tumor-somatic tumor VAF=0.35	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	Tier 3 - Cancer repurposing, 6 assertions
RNF43 Tumor supp.	frameshift p.Gly659Valfs+41 exon 9/10, more	Tumor-somatic tumor VAF=0.32	Evidence B (assumed): >Tumor supp. with PTC triggering NMD, more	--	None found

CASE 3



Putative functionally relevant variants: 8

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Other reported biomarker(s)
MSH2 Tumor supp. Sec.findings	stop gained p.Arg383Ter exon 7/16, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Evidence A (curated): >Pathogenic, ClinVar >Likely Oncogenic, OncoKB	BoB-arm 1B, more	None found
PTEN Tumor supp.	frameshift p.Asn329LysfsTer14 exon 8/9, more	Tumor Tumor VAF: FMI=0.40, CCE=0.50	Evidence A (curated): >Pathogenic, ClinVar	--	Tier 2-Investigational, 2 assertions Tier 3-Cancer repurposing, 5 assertions Tier 4-Hypothetical, 3 assertions
KRAS Oncogene	missense p.Gly12Val exon 2/5, more	Tumor Tumor VAF: FMI=0.28, CCE=0.31	Evidence A (curated): >Pathogenic, ClinVar >Oncogenic, OncoKB >Biomarker, CIVIC >Biomarker, CGI Others/Inconclusive, more	--	Tier 1-Ready for use, 6 assertions Tier 2-Investigational, 2 assertions Tier 2b-Investig. (case reports), 1 assertions Tier 3-Cancer repurposing, 20 assertions Tier 4-Hypothetical, 12 assertions Others - Diagnostic, 3 assertions
RNF43 Tumor supp.	stop gained p.Arg132Ter exon 4/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 2b-Investig. (case reports), 1 assertions
TP53 Tumor supp. Sec.findings	missense p.Arg248Gly exon 7/11, more	Tumor Tumor VAF: FMI=0.41, CCE=0.52	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 6 assertions
SMO Oncogene	missense p.Pro641Ala exon 11/12, more	Tumor Tumor VAF: FMI=0.70, CCE=n/d	Evidence A (curated): >Biomarker, CGI Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 1 assertions
FBXW7 Tumor supp.	frameshift p.Gly413AspfsTer11 exon 9/12, more	Tumor Tumor VAF: FMI=0.23, CCE=0.20	Evidence B (assumed): >Tumor supp. with frameshift-derived PTC triggering NMD, more	--	Tier 3-Cancer repurposing, 1 assertions Tier 4-Hypothetical, 2 assertions Others - Diagnostic, 1 assertions
APC Tumor supp. Sec.findings	frameshift p.Asp434ArgfsTer21 exon 10/16, more	Tumor Tumor VAF: FMI=0.35, CCE=0.32	Evidence B (assumed): >Tumor supp. with frameshift-derived PTC triggering NMD, more	--	Tier 4-Hypothetical, 3 assertions

Variants of unknown/contradictory functional significance: 22

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Other reported biomarker(s)
POLE Tumor supp. (essential)	missense p.Ala1885Thr exon 41/49, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	<< if variant is reclassified >> BoB-arm 1C, more	None found
KDR Oncogene	missense p.Cys482Arg exon 11/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Special/contradictory, info >Likely Oncogenic, OncoKB >Polymorphism, more	--	None found
TET1 ambiguous	missense p.Ser1796Leu exon 11/12, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found
FLT3 Oncogene	missense p.Val643Ile exon 15/24, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
FLT1 --	missense p.Pro1201Leu exon 27/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
CD276 --	missense p.Ala449Val exon 6/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
IGF1R --	missense p.Arg511Gln exon 7/21, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found

> 52 years man, advanced
colorectal adenocarcinoma

>Known KRAS G12C mut
(also observed here, VAF compatible with clonal event)

Putative functionally relevant variants: 8

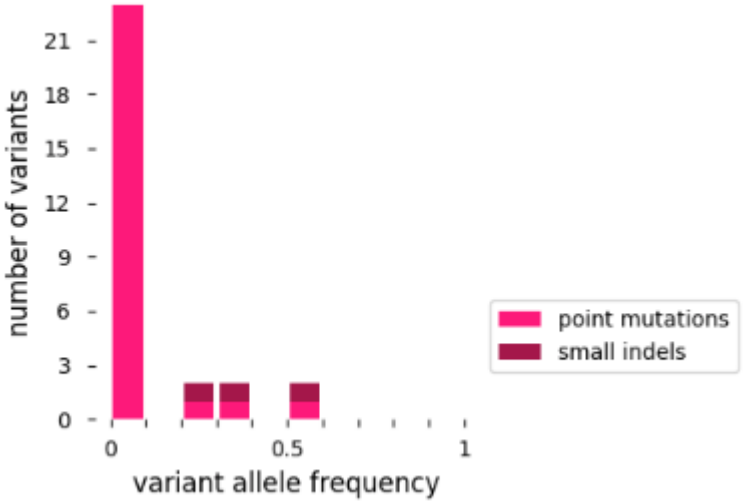
Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Other reported biomarker(s)
MSH2 Tumor supp. Sec.findings	stop gained p.Arg383Ter exon 7/16, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Evidence A (curated): >Pathogenic, ClinVar >Likely Oncogenic, OncoKB	BoB-arm 1B, more	None found
PTEN Tumor supp. Sec.findings	frameshift p.Asn329LysfsTer14 exon 8/9, more	Tumor Tumor VAF: FMI=0.40, CCE=0.50	Evidence A (curated): >Pathogenic, ClinVar	--	Tier 2-Investigational, 2 assertions Tier 3-Cancer repurposing, 5 assertions Tier 4-Hypothetical, 3 assertions Others - Diagnostic, 1 assertions
KRAS Oncogene	missense p.Gly12Val exon 2/5, more	Tumor Tumor VAF: FMI=0.28, CCE=0.31	Evidence A (curated): >Pathogenic, ClinVar >Oncogenic, OncoKB >Biomarker, CIVIC >Biomarker, CGI Others/Inconclusive, more	--	Tier 1-Ready for use, 6 assertions Tier 2-Investigational, 2 assertions Tier 2b-Investig. (case reports), 1 assertions Tier 3-Cancer repurposing, 20 assertions Tier 4-Hypothetical, 12 assertions Others - Diagnostic, 3 assertions
RNF43 Tumor supp.	stop gained p.Arg132Ter exon 4/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 2b-Investig. (case reports), 1 assertions
TP53 Tumor supp. Sec.findings	missense p.Arg248Gly exon 7/11, more	Tumor Tumor VAF: FMI=0.41, CCE=0.52	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 6 assertions
SMO Oncogene	missense p.Pro641Ala exon 11/12, more	Tumor Tumor VAF: FMI=0.70, CCE=n/d	Evidence A (curated): >Biomarker, CGI Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 1 assertions
FBXW7 Tumor supp.	frameshift p.Gly413AspfsTer11 exon 9/12, more	Tumor Tumor VAF: FMI=0.23, CCE=0.20	Evidence B (assumed): >Tumor supp. with frameshift-derived PTC triggering NMD, more	--	Tier 3-Cancer repurposing, 1 assertions Tier 4-Hypothetical, 2 assertions Others - Diagnostic, 1 assertions
APC Tumor supp. Sec.findings	frameshift p.Asp434ArgfsTer21 exon 10/16, more	Tumor Tumor VAF: FMI=0.35, CCE=0.32	Evidence B (assumed): >Tumor supp. with frameshift-derived PTC triggering NMD, more	--	Tier 4-Hypothetical, 3 assertions

Variants of unknown/contradictory functional significance: 22

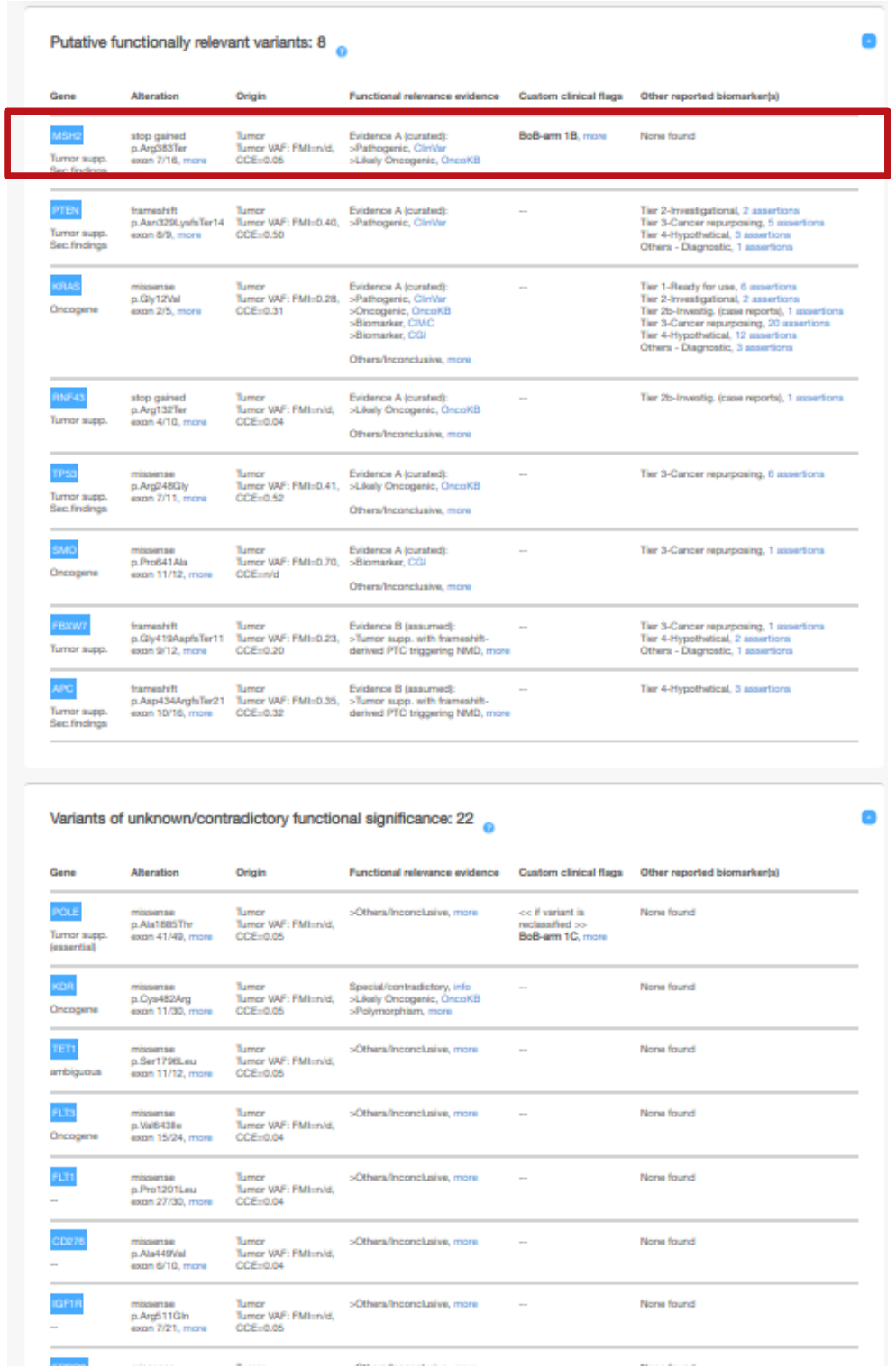
Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Other reported biomarker(s)
POLE Tumor supp. (essential)	missense p.Ala1885Thr exon 41/49, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	<< if variant is reclassified >> BoB-arm 1C, more	None found
KDR Oncogene	missense p.Cys482Arg exon 11/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Special/contradictory, info >Likely Oncogenic, OncoKB >Polymorphism, more	--	None found
TET1 ambiguous	missense p.Ser1796Leu exon 11/12, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found
FLT3 Oncogene	missense p.Val643Ile exon 15/24, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
FLT1 --	missense p.Pro1201Leu exon 27/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
CD276 --	missense p.Ala449Val exon 6/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
IGF1R --	missense p.Arg511Gln exon 7/21, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found

>High number of mutations in the report
(a small proportion being functional, the remaining being VUS or neutral)

>However, most of these mutations are subclonal



Many of these are C>T or T>C,
which is the signature
of mismatch DNA repair deficiency



Putative functionally relevant variants: 8

Gene	Alteration	Origin	Functional relevance evidence	Custom clinical flags	Other reported biomarker(s)
MSH2 Tumor supp. Sec.findings	stop gained p.Arg383Ter exon 7/16, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Evidence A (curated): >Pathogenic, ClinVar >Likely Oncogenic, OncoKB	BoB-arm 1B, more	None found
PTEN Tumor supp. Sec.findings	frameshift p.Asn329LysfsTer14 exon 8/9, more	Tumor Tumor VAF: FMI=0.40, CCE=0.50	Evidence A (curated): >Pathogenic, ClinVar	--	Tier 2-Investigational, 2 assertions Tier 3-Cancer repurposing, 5 assertions Tier 4-Hypothetical, 3 assertions Others - Diagnostic, 1 assertions
KRAS Oncogene	missense p.Gly12Val exon 2/5, more	Tumor Tumor VAF: FMI=0.28, CCE=0.31	Evidence A (curated): >Pathogenic, ClinVar >Oncogenic, OncoKB >Biomarker, CIVIC >Biomarker, CGI Others/Inconclusive, more	--	Tier 1-Ready for use, 6 assertions Tier 2-Investigational, 2 assertions Tier 2b-Investig. (case reports), 1 assertions Tier 3-Cancer repurposing, 20 assertions Tier 4-Hypothetical, 12 assertions Others - Diagnostic, 3 assertions
RNF43 Tumor supp.	stop gained p.Arg132Ter exon 4/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 2b-Investig. (case reports), 1 assertions
TP53 Tumor supp. Sec.findings	missense p.Arg248Gly exon 7/11, more	Tumor Tumor VAF: FMI=0.41, CCE=0.52	Evidence A (curated): >Likely Oncogenic, OncoKB Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 6 assertions
SMO Oncogene	missense p.Pro641Ala exon 11/12, more	Tumor Tumor VAF: FMI=0.70, CCE=n/d	Evidence A (curated): >Biomarker, CGI Others/Inconclusive, more	--	Tier 3-Cancer repurposing, 1 assertions
FBXW7 Tumor supp.	frameshift p.Gly413AspfsTer11 exon 9/12, more	Tumor Tumor VAF: FMI=0.23, CCE=0.20	Evidence B (assumed): >Tumor supp. with frameshift-derived PTC triggering NMD, more	--	Tier 3-Cancer repurposing, 1 assertions Tier 4-Hypothetical, 2 assertions Others - Diagnostic, 1 assertions
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Variants of unknown/contradictory functional significance: 22

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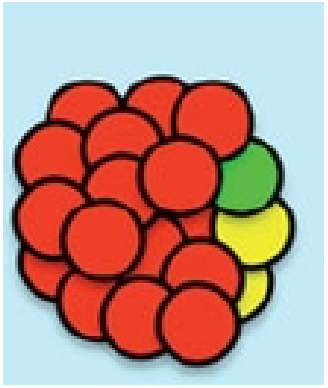
KDR Oncogene	missense p.Cys482Arg exon 11/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	Special/contradictory, info >Likely Oncogenic, OncoKB >Polymorphism, more	--	None found
TET1 ambiguous	missense p.Ser1796Leu exon 11/12, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found
FLT3 Oncogene	missense p.Val643Ile exon 15/24, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
FLT1 --	missense p.Pro1201Leu exon 27/30, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
CD276 --	missense p.Ala449Val exon 6/10, more	Tumor Tumor VAF: FMI=n/d, CCE=0.04	>Others/Inconclusive, more	--	None found
IGF1R --	missense p.Arg511Gln exon 7/21, more	Tumor Tumor VAF: FMI=n/d, CCE=0.05	>Others/Inconclusive, more	--	None found

> This can be explained by a **MSH2 nonsense mutation**, already reported as **pathogenic** and related to DNA mismatch repair deficiency

> This is not a germline event! (by age and tumor, this patient could have Lynch syndrome!)

> The mutation is a *tumor somatic* event, identified as subclonal (VAF~5%)



> A minority of the cells in the tumor have developed a *somatic* mismatch repair deficiency

> Of note, this phenotype is actionable by immune-checkpoint inhibitors

(high load of somatic mutations → high neoantigen burden → tumor sensitive to immune-recognition)

Should we use a targeted therapy for a mechanism that is not present in all the tumor cells?

CONCLUSIONS

> Clinical interpretation of NGS data (n=1) is aimed to use **current knowledge** to support the decision-making

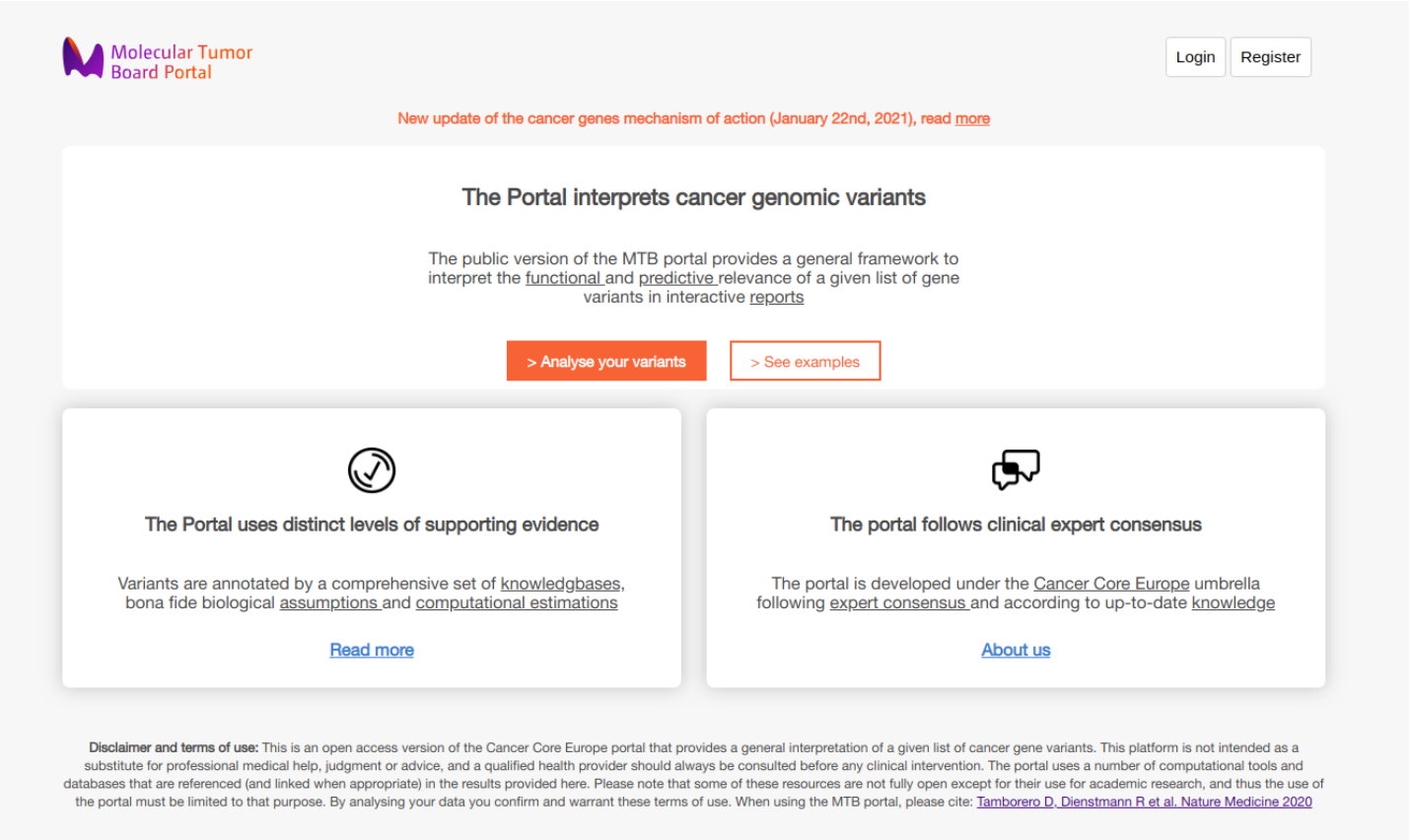
> Variant effects are supported by **distinct levels of evidence**, which may be used differently depending on the context

> The use of **CDSS** implements **efficient, accurate and comprehensive** analyses based on predefined (expert) criteria

–----Exercise with MTBP---

Public version of the MTBP

https://www.mtbp.org/



> Is a **lightweight** version as compared to the 'production' tool

(generic analysis from a generic input, so clonality, origin, trials matching etc is not performed)

> But you can upload your variants and obtain a report with their functional and predictive relevance