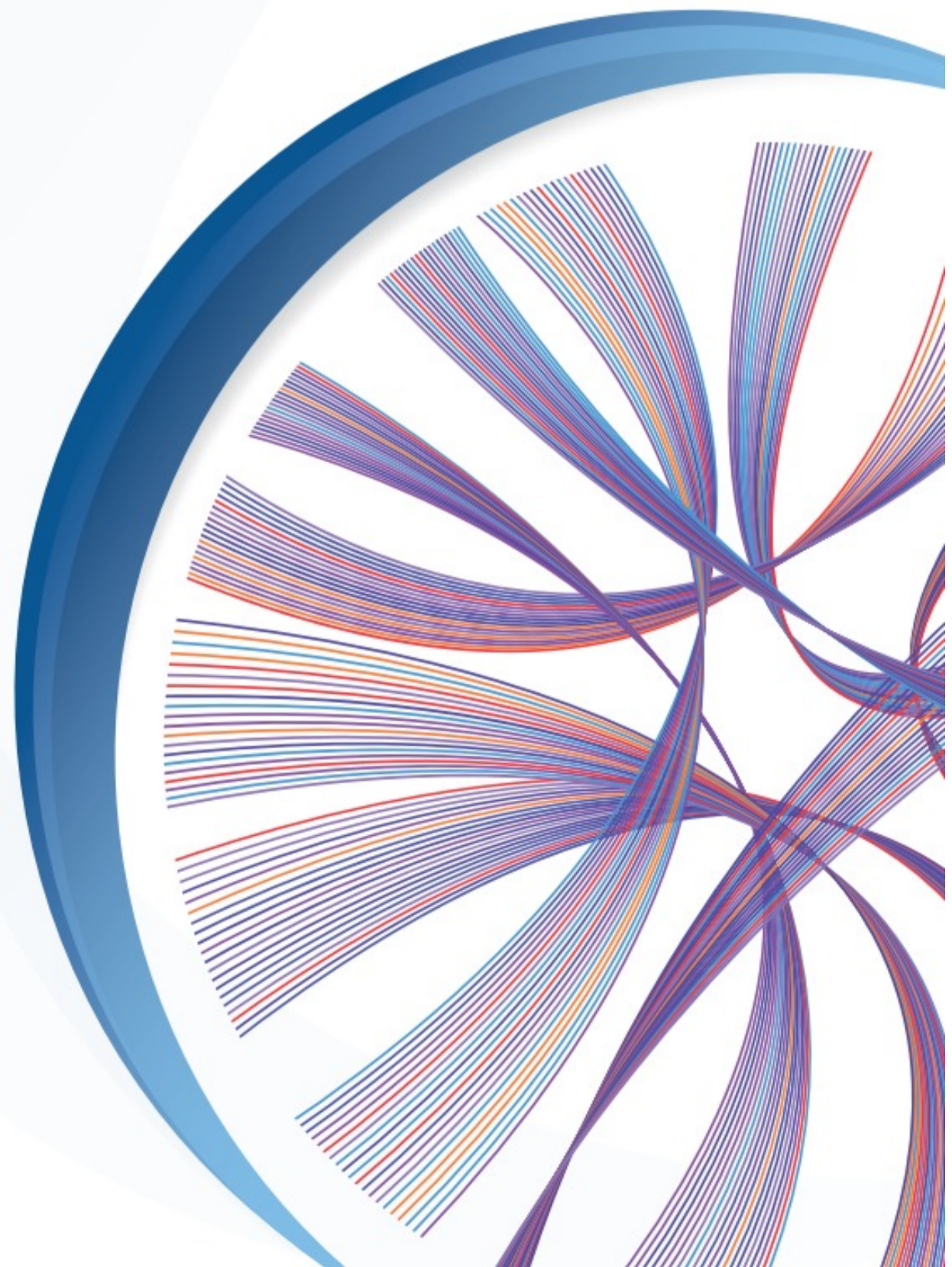


TARGET FIRST

TEST REPORT



SCOPE OF THE TEST

SNVs, InDels, CNAs, Gene Fusions status

CLINICAL INDICATION

Adenocarcinoma of rectum

REPORT DETAILS

Name : NIKITA

Gender : Female

Age/DOB : 32 Years

Reporting Date : 16/03/2023

Cancer Celltype : Adenocarcinoma, NOS

Sample Source : B-3517/23

Tumor content : 50%

Consulting Clinician : Dr. Amit Jain

Hospital : Valentis Cancer Hospital, Mussoorie Mawana Road

RESULTS
GENOMIC FINDINGS FROM TUMOR PROFILING
Genomic Alteration
Relevant Therapies (in Same Cancer Type)
Relevant Therapies (in Different Cancer)

KRAS, NRAS and BRAF Wild type

Therapy	Clinical Relevance
Cetuximab	RESPONSIVE
Panitumumab	RESPONSIVE

Therapy	Clinical Relevance	Cancer Type
NA	NA	NA

 ATM Exon 4 (p.Gln65Ter)
 Allelic burden: 22%

Therapy	Clinical Relevance
NA	NA

Therapy	Clinical Relevance	Cancer Type
Olaparib	RESPONSIVE	Prostate cancer

 ATM Exon 10 (p.Arg457Ter)
 Allelic burden: 22%

Therapy	Clinical Relevance
NA	NA

Therapy	Clinical Relevance	Cancer Type
Olaparib	RESPONSIVE	Prostate cancer

 RAD54L Exon 10
 (p.Leu301GlnfsTer6)
 Allelic burden: 30%

Therapy	Clinical Relevance
NA	NA

Therapy	Clinical Relevance	Cancer Type
Olaparib	RESPONSIVE	Prostate cancer

*NA: Not Applicable

STATUS OF VARIANTS IN CANCER RELATED BIOMARKERS

Gene	ERBB2	NRAS	KRAS	MLH1	MSH6	PMS2	MSH2	PIK3CA
Status	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Gene	BRAF	BRCA2	BRCA1	PMS1	ATM
Status	Not Detected	Not Detected	Not Detected	Not Detected	Pathogenic

VARIANT DETAILS:

Gene	Variant Location	Variant Consequence	Clinical Significance	Variant Type	Reference
ATM	chr11:g.108099912C>T, ENST00000278616, Exon 4	c.193C>T, p.Gln65Ter , 22%	Pathogenic	Stopgain	rs775248597, VCV000265585.9
ATM	chr11:g.108121561C>T, ENST00000278616, Exon 10	c.1369C>T, p.Arg457Ter , 22%	Pathogenic	Stopgain	rs749036865, VCV000219999.36
RAD54L	chr1:g.46733135_46733138dup, ENST00000371975, Exon 9	c.896_899dup, p.Leu301GlnfsTer6 , 30%	Pathogenic	Frameshift Insertion	rs1557704699, ACMG Guidelines
PDGFRA	chr4:g.55133505A>C, ENST00000257290, Exon 6	c.809A>C,p.Lys270Thr , 37%	VUS	Nonsynonymous SNV	rs545359247, VCV000571306.9

*NA: Not Applicable

VARIANTS WITH CLINICALLY RELEVANT THERAPIES

Gene (Variant) - Drug association

- *KRAS, NRAS and BRAF Wild type* :
Cetuximab - RESPONSIVE

- *KRAS, NRAS and BRAF Wild type* :
Panitumumab - RESPONSIVE

- *ATM* :
Olaparib - RESPONSIVE
Prostate cancer

- *ATM* :
Olaparib - RESPONSIVE
Prostate cancer

- *RAD54L Mutation/Deletion* :
Olaparib - RESPONSIVE
Prostate cancer

Summary

Cetuximab has been approved for the treatment of patients with *KRAS* wild-type and EGFR expressing metastatic colorectal cancer (mCRC). The absence of activating mutations in both the *KRAS* and *NRAS* genes has been linked to Cetuximab response (FDA). Further, NCCN clinical practice guidelines recommend testing for *BRAF* mutation status before administering Cetuximab and suggest that mCRC patients with *BRAF* wild type status are only eligible for Cetuximab therapy.

Panitumumab has been approved for the treatment of patients with *KRAS* wild-type and EGFR expressing metastatic colorectal cancer (mCRC). The absence of activating mutations in both the *KRAS* and *NRAS* genes has been linked to Panitumumab response (FDA). Further, NCCN clinical practice guidelines recommend testing for *BRAF* mutation status before administering Panitumumab and suggest that mCRC patients with *BRAF* wild type status are only eligible for Panitumumab therapy.

The drug olaparib has been approved for the treatment of adult patients with deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene-mutated metastatic castration-resistant prostate cancer, who show disease progression after enzalutamide or abiraterone treatment (FDA).

The approval was based on the clinical study PROfound (NCT02987543). The study included patients harbouring *BRCA1*, *BRCA2* and *ATM* mutations (cohort A) and patients harbouring mutations in 12 genes related to HRR pathway (cohort B). A median of 7.4 months progression free survival was recorded based on imaging (HR 0.34; 95% CI, 0.25, 0.47; p<0.001) with median overall survival of 18.5 months in cohort A. Olaparib treatment had shown a good response in both cohorts A and B.

The drug olaparib has been approved for the treatment of adult patients with deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene-mutated metastatic castration-resistant prostate cancer, who show disease progression after enzalutamide or abiraterone treatment (FDA).

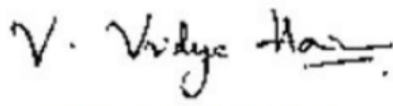
The approval was based on the clinical study PROfound (NCT02987543). The study included patients harbouring *BRCA1*, *BRCA2* and *ATM* mutations (cohort A) and patients harbouring mutations in 12 genes related to HRR pathway (cohort B). A median of 7.4 months progression free survival was recorded based on imaging (HR 0.34; 95% CI, 0.25, 0.47; p<0.001) with median overall survival of 18.5 months in cohort A. Olaparib treatment had shown a good response in both cohorts A and B.

The drug olaparib has been approved for the treatment of adult patients with deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene-mutated metastatic castration-resistant prostate cancer, who show disease progression after enzalutamide or abiraterone treatment (FDA).

The approval was based on the clinical study PROfound (NCT02987543). The study included patients harbouring *BRCA1*, *BRCA2* and *ATM* mutations (cohort A) and patients harbouring mutations in 12 genes related to HRR pathway (cohort B). A median of 5.8 months progression free survival was recorded based on imaging (HR 0.49; 95% CI: 0.38, 0.63; p<0.0001) in cohort A+B. Olaparib treatment had shown a good response in both cohorts A and B.

REFERENCES

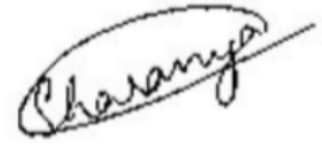
- Hampel, Heather, et al. "A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment." *Genetics in Medicine* 17.1 (2015): 70.
- Hoffman-Andrews, Lily. "The known unknown: the challenges of genetic variants of uncertain significance in clinical practice." *Journal of Law and the Biosciences* 4.3 (2017): 648.
- Landrum M. J. et al., ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.*, 44(D1):D862-8, 2015.
- Li, Marilyn M., et al. "Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists." *The Journal of molecular diagnostics* 19.1 (2017): 4-23.
- Nykamp, K., Anderson, M., Powers, M., Garcia, J., Herrera, B., Ho, Y. Y., Topper, S. (2017). Sherlock: a comprehensive refinement of the ACMG-AMP variant classification criteria. *Genetics in medicine: official journal of the American College of Medical Genetics*, 19(10), 1105-1117. doi:10.1038/gim.2017.37
- Richards S. et al. Standards and Guidelines for Interpretation of Sequence Variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and Association for Molecular Pathology. *Genetics in Medicine* (2015); 17: 405- 423
- Spratt, Daniel E., et al. "Racial/ethnic disparities in genomic sequencing." *JAMA oncology* 2.8 (2016): 1070-1074
- The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discovery*. 2017;7(8):818-831.
- The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Research*. 2019;47: D506-D515



Vidya H Veldore, PhD
Clinical Director



Vyomesh Javle
TL - Clinical Bioinformatician



Sharanya J
Team Lead - Clinical Reporting

TEST DESCRIPTION

TARGET First is a Next Generation Sequencing based test which identifies genetic alterations in a comprehensive panel of well curated 53 tumor genes which can impact response to approved therapy for a particular cancer type. Some of the alterations detected may have bearing on prognosis and/or therapeutic options and may provide relevant information that allows oncologists/clinicians to consider various lines of targeted treatment for the patient.

GENES EVALUATED

TARGET First detects mutations (SNVs and Short Indels), Copy Number Variations (CNVs), gene fusions and splice variants in the 53 genes :

SNVs/InDels Covered in TARGET First

<i>ABL1</i>	<i>ALK</i>	<i>AR</i>	<i>ATM</i>	<i>BARD1</i>	<i>BRAF</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRIP1</i>	<i>CDK12</i>
<i>CDK4</i>	<i>CDK6</i>	<i>CDKN2A</i>	<i>CHEK1</i>	<i>CHEK2</i>	<i>EGFR</i>	<i>EPCAM</i>	<i>ERBB2</i>	<i>ERBB3</i>	<i>EZH2</i>
<i>FANCL</i>	<i>FGFR3</i>	<i>GAPDH</i>	<i>IDH1</i>	<i>IDH2</i>	<i>JAK2</i>	<i>KIT</i>	<i>KRAS</i>	<i>MAP2K1</i>	<i>MAP2K2</i>
<i>MDM2</i>	<i>MET</i>	<i>MLH1</i>	<i>MLH3</i>	<i>MSH2</i>	<i>MSH6</i>	<i>NRAS</i>	<i>PALB2</i>	<i>PDGFRA</i>	<i>PDGFRB</i>
<i>PIK3CA</i>	<i>PMS1</i>	<i>PMS2</i>	<i>PPP2R2A</i>	<i>RAD51B</i>	<i>RAD51C</i>	<i>RAD51D</i>	<i>RAD54L</i>	<i>RET</i>	<i>ROS1</i>
<i>STK11</i>	<i>TSC1</i>	<i>TSC2</i>							

CNAs Covered in TARGET First

<i>ABL1</i>	<i>AR</i>	<i>ATM</i>	<i>BARD1</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRIP1</i>	<i>CDK12</i>	<i>CDK4</i>	<i>CDK6</i>
<i>CDKN2A</i>	<i>CHEK1</i>	<i>CHEK2</i>	<i>EGFR</i>	<i>EPCAM</i>	<i>ERBB2</i>	<i>ERBB3</i>	<i>EZH2</i>	<i>FANCL</i>	<i>FGFR3</i>
<i>GAPDH</i>	<i>IDH2</i>	<i>JAK2</i>	<i>KIT</i>	<i>MDM2</i>	<i>NRAS</i>	<i>PALB2</i>	<i>PDGFRA</i>	<i>PDGFRB</i>	<i>PPP2R2A</i>
<i>RAD51B</i>	<i>RAD51C</i>	<i>RAD51D</i>	<i>RAD54L</i>	<i>STK11</i>	<i>TSC1</i>	<i>TSC2</i>			

Gene Fusions Covered in TARGET First

<i>ALK</i>	<i>MET</i>	<i>RET</i>	<i>ROS1</i>
------------	------------	------------	-------------

TEST METHODOLOGY

Sample preparation and Library preparation :

DNA isolated from FFPE, or any other fresh tumor tissue source was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >250X coverage on Illumina sequencing platform.

Bioinformatics Analysis and Reporting :

The sequences obtained are aligned to human reference genome (GRCh37/hg19) and variant analysis was performed using set of Bioinformatics Pipeline. Only non-synonymous and splice site variants found in the panel consisting of specific set of genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported. Clinically relevant mutations were annotated using published variants in literature and a set of databases – ClinVar, COSMIC and dbSNP. Common variants are filtered based on allele frequency in 1000 Genome Phase 3, ExAC, dbSNP, gnomAD, etc. In the absence of a clinically significant reported known variation(s), pathogenicity will be predicted based on in-silico gene prioritization tools: CADD, SIFT, PolyPhen-2, Condel and Mutation taster and prioritized for clinical correlation. The identified pathogenic variant will be correlated with observed phenotypic features of the patient and interpreted according to American College of Medical Genetics (ACMG) guidelines.

Somatic variants are classified into two tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics as per international guidelines:

ACMG, ASCO, AMP, CAP, NCCN and ESMO

LIMITATIONS AND DISCLAIMER

- DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of the many aspects used by the healthcare provider to help with a diagnosis and treatment plan.
- We are using the canonical transcript for clinical reporting which is usually the longest coding transcript with strong/multiple supporting evidence. However, in rare cases, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
- The contents of this test should be carefully assessed by the treating physician and further interpreted along with clinical, histopathological findings, contraindications and guidelines before deciding the course of therapy.
- The chromosomal aberrations like copy number variations and rearrangements may not be reliably detected with this assay and have to be confirmed by alternate method.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is 70-75%. The CNVs detected have to be confirmed by alternate method.
- Most recent block is recommended for testing as the mutation profile may change in response to treatment and hence differ at different sampling points.
- TARGT FIRST test has been developed, validated and performed by 4baseCare Genomics Pvt. Ltd and has not been cleared or approved by the FDA.
- The identified pathogenic variant will be correlated with observed phenotypic features of the patient and interpreted according to (ASCO) guidelines.
- Certain genes may not be covered completely, and few mutations could be missed. A negative result cannot rule out the possibility that the tested tumor sample carries mutations not previously associated with cancer and hence not included in the panel.