TÜM GENOM ANALİZİ ve KLİNİK KULLANIMI

'whole genom sequencing (WGS)'

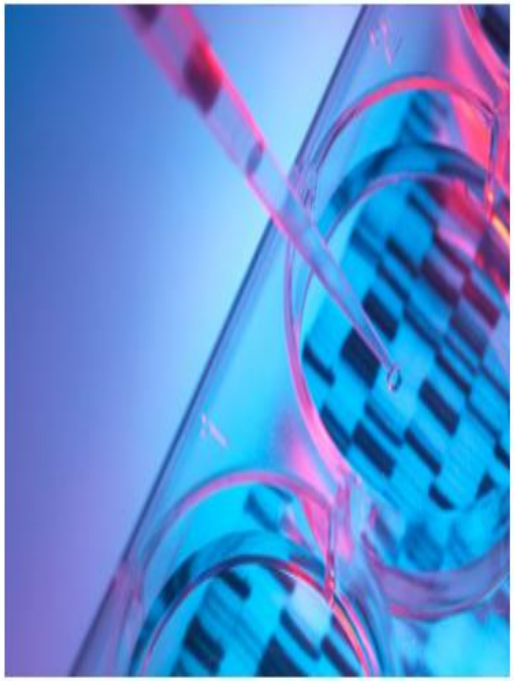
Dr. Mutlu DOĞAN

SBÜ Dr AY Ankara Onkoloji EAH

Tıbbi Onkoloji Kliniği

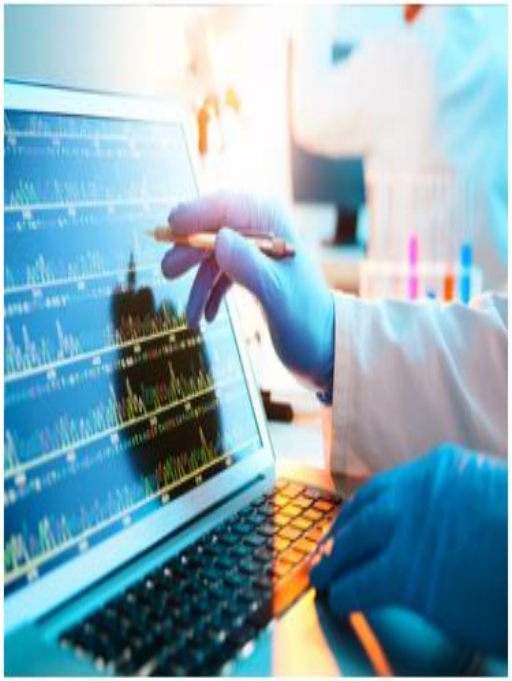
14 Kasım 2020

‘HUMAN GENOM PROJECT’



What is the Human Genome Project?›

The Human Genome Project was the international research effort to determine the DNA sequence of the entire human genome.



Human Genome Project Results›

In 2003, an accurate and complete human genome sequence was finished two years ahead of schedule and at a cost less than the original estimated budget.

Human Genome Project Timeline of Events›

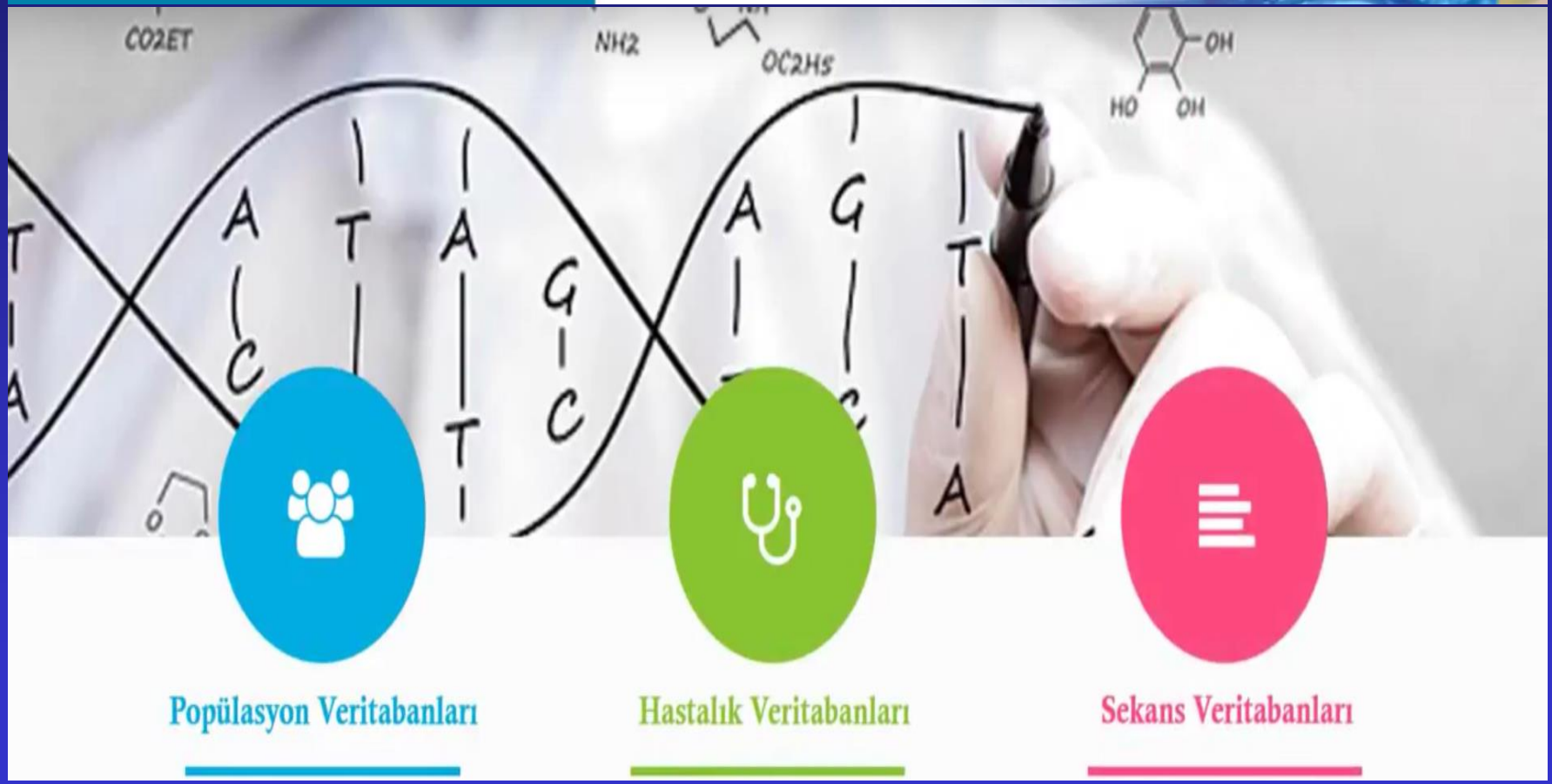
Key moments and press releases from the history of the Human Genome Project.

http://www.hgvs.org/mutnomen

https://mutalyzer.nl

HGVS

HUMAN GENOME VARIATION SOCIETY



VARYANT NİTELİKLERİ

LP
muhtemel patojenik varyant

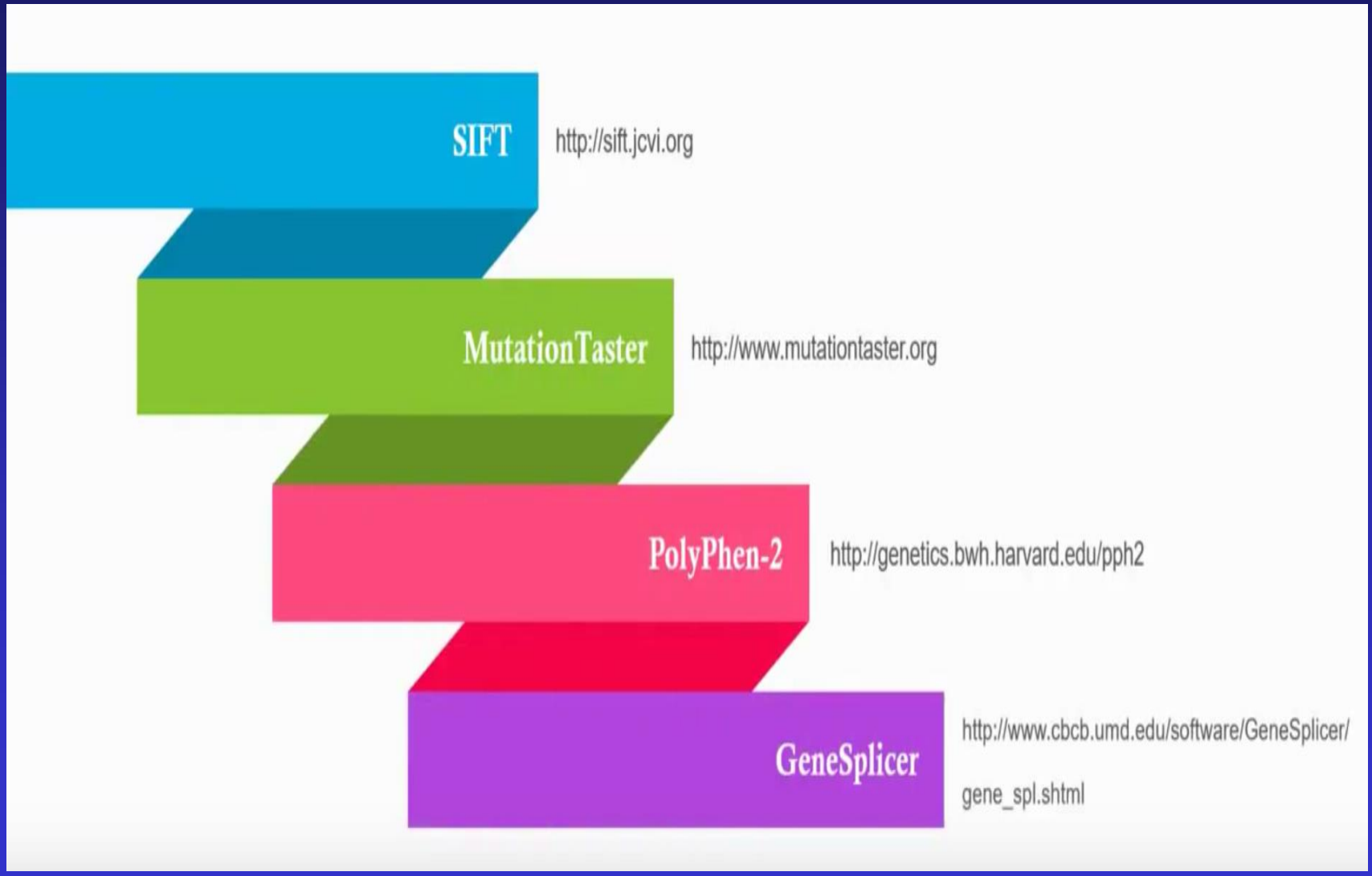
P
patojenik varyant

VUS
'Variant of Unknown Significance'

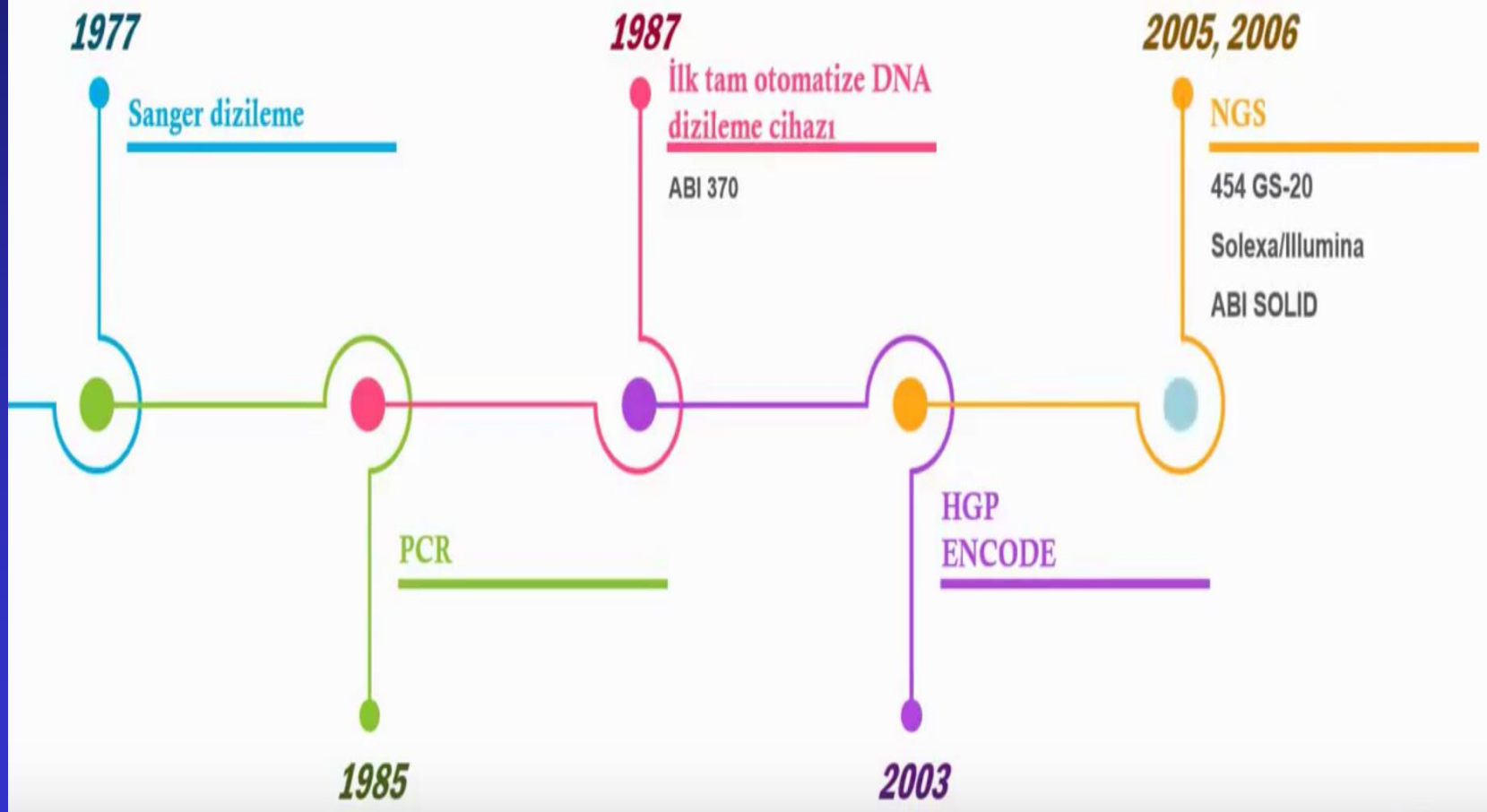
LB
muhtemel benign varyant

B
benign varyant

BİLGİSAYAR TAHMİN PROGRAMLARI



Dizileme teknolojilerindeki gelişmeler



SBÜ Dr AY Ankara Onkoloji EAH, Tıbbi Genetik

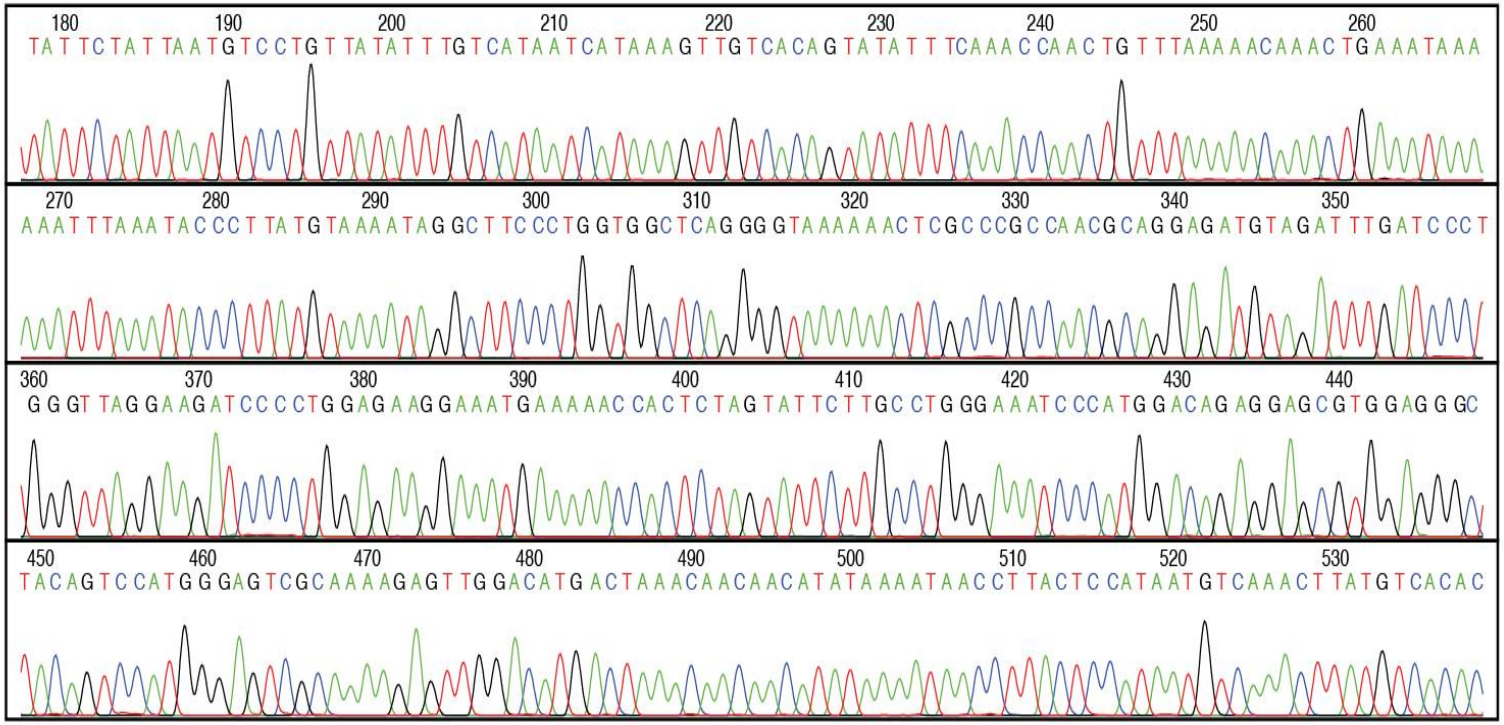
GENOMİK TESTLER

GENETİK BİLGİ

KLİNİK PRATİK

Yeni Nesil Dizileme

'Next Generation Sequencing' (NGS)



DNA sequence data from an automated sequencing machine

American Journal of Epidemiology

Vol. 186, No. 8
DOI: 10.1093/aje/kww224
Advance Access publication:
May 10, 2017

Practice of Epidemiology

Human Genome Sequencing at the Population Scale: A Primer on High-Throughput DNA Sequencing and Analysis

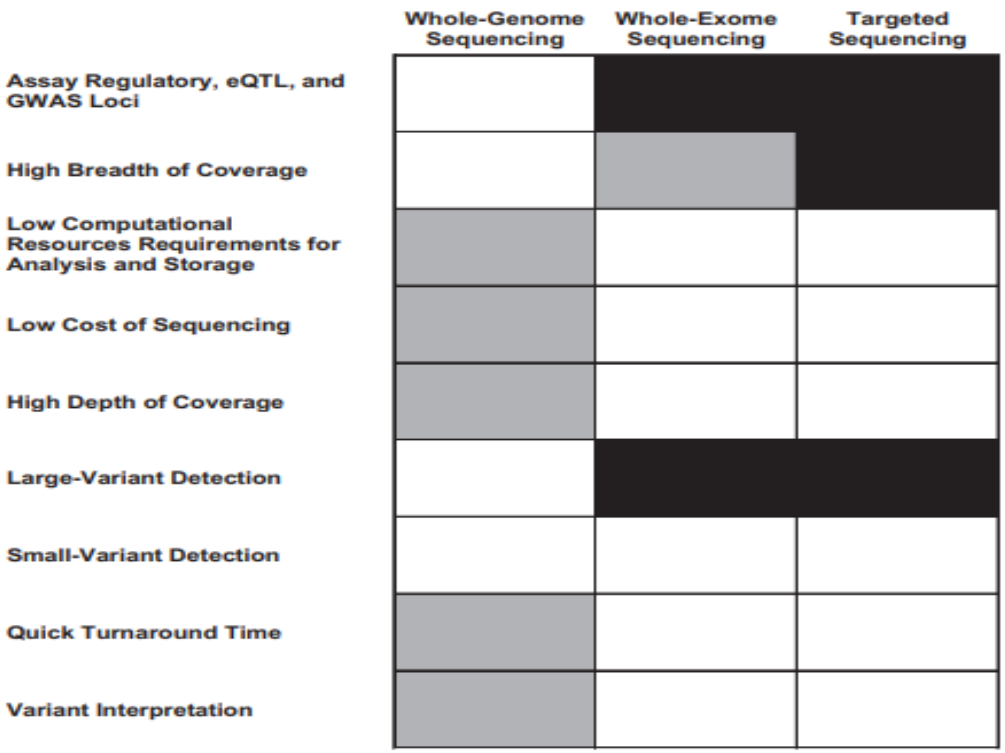


Figure 2. Suitability of whole-genome sequencing, whole-exome sequencing, and targeted sequencing for various applications. Lighter shading indicates that the sequencing approach is more suitable for the task, and darker shading indicates that the approach is less suitable. eQTL, expression quantitative trait loci; GWAS, genome-wide association studies.

NGS KLİNİK KULLANIMI

- Patojenik gen taraması
- Kompleks hastalıklar
- Enfeksiyon
- Kanser

NGS Teknikleri

Table 1. Characteristics (Preparation, Sequencing Method, Results, and Typical Uses) of Several Available DNA Sequencing Platforms

Platform	Preparation for Sequencing	Sequencing	Read Length, base pairs	Raw Error Rate, %	Error Mode	Throughput, GB	Time, hours	Typical Uses
Illumina (Illumina Inc., San Diego, California)	DNA is isolated from a sample and fragmented into smaller pieces, adapter sequences are ligated, and then DNA is inserted into a flowcell and clonally amplified to create clusters.	Reversible terminator technology	36, 75, 100, 150, 250, 300	0.1–1	Higher error rate at the ends of reads; most errors are substitutions.	MiSeq Reagent Kit v2 (2 × 150): 4.5–5.1 HiSeq X: 1,600–1,800	MiSeq Reagent Kit v2 (2 × 150): 24 HiSeq X: <72	Cost-effective for whole human genomes or whole human exomes. Strengths in SNV and small INDEL detection. Difficulties in aligning short reads to reference genome.
Ion Torrent (Thermo Fisher Scientific Inc., Waltham, Massachusetts)	DNA is isolated from a sample and fragmented into smaller pieces, adapter sequences are ligated, and then DNA is amplified on bead surfaces via emulsion PCR. The beads are added to wells on a semiconductor chip (1 bead per well) for sequencing.	Semiconductor sequencing	200, 400	1–2	Most errors are insertions or deletions, particularly in homopolymers.	Ion PGM 318 Chip v2: 0.6–2 Ion Proton, Ion PI Chip: approximately 10	Ion PGM 318 Chip v2: 4–7.5 Ion Proton, Ion PI Chip: 2–4	Strengths include low start-up costs for machine and reagents and short run time. Used for sequencing whole human genomes or targeted regions.
Pacific Biosciences (Pacific Biosciences of California, Inc., Menlo Park, California)	The samples do not need to be amplified before sequencing. DNA is fragmented and transformed to SMRTbell library format, which is a circularized fragment (double-stranded DNA flanked by 2 hairpin loops).	Single-molecule real-time sequencing	Read length selectable up to approximately 10,000–15,000	14–15	Errors are stochastic; most errors are insertions or deletions.	RS II (P6–C4): 0.5–1 Sequel: 5–10	RS II (P6–C4): 0.5–4 Sequel: 0.5–4	Long reads are advantageous for phasing small variants or identifying large structural variants. Sequencing (machine and reagents) is expensive, so typically used for specifically targeted regions of human genomes.
Oxford Nanopore (Oxford Nanopore Technologies, Oxford, United Kingdom)	The samples do not need to be amplified before sequencing. DNA is fragmented and adapters are ligated.	Nanopore sequencing	Read length selectable up to approximately 250,000	5–40	Errors are stochastic.	MinION Mk1B: 5–10 PromethION: 1,400–2,800	MinION Mk1B: up to 48 PromethION: up to 48	Portable device is useful for sequencing in the field, but not used for personal genomes yet due to high (and inconsistent) error rate. Default run time is 48 hours, but the user may reduce run time when less output is desired.

Abbreviations: INDEL, insertion or deletion variant; PCR, polymerase chain reaction; PGM, Personal Genome Machine; SMRT, single-molecule real-time; SNV, single-nucleotide variant.

Goldfeder RL et al, Am J Epidemiol 2017

FoundationOne®CDx

Technical Specifications

FOUNDATIONONE®CDx

Intended Use

FoundationOne®CDx (F1CDx) is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. The F1CDx assay is performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

Table 1: Companion diagnostic indications

INDICATIONS	BIOMARKER	FDA-APPROVED THERAPY
Non-Small Cell Lung Cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib) or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa®(alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta™ (capmatinib)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E or V600K	Mekinist® (trametinib) or Cotellic®(cobimetinib), in combination with Zelboraf®(vemurafenib)
Breast Cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA alterations	Piqray® (alpelisib)
Colorectal Cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3 and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3 and 4)	Vectibix® (panitumumab)
Ovarian Cancer	*BRCA1/2* alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre™ (pemigatinib)
Prostate Cancer	Homologous Recombination Repair *(HRR) gene (BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D and RAD54L)* alterations	Lynparza® (olaparib)
Solid tumors	TMB ≥ 10 mutations per megabase	Keytruda® (pembrolizumab)

The test is also used for detection of genomic loss of heterozygosity (LOH) from formalin-fixed, paraffin-embedded (FFPE) ovarian tumor tissue. Positive homologous recombination deficiency (HRD) status (defined as tBRCA-positive and/or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy in accordance with the Rubraca product label.

FOUNDATIONONE CDx™

New **FDA-Approved** Broad Companion Diagnostic (CDx) for Solid Tumors

FDA-Approved Content

Report Section 1


FOUNDATIONONE CDx™

PATIENT Jane Sample | TUMOR TYPE Lung adenocarcinoma | TRF# TRFXXXXXX

PATIENT
DISEASE Lung adenocarcinoma
NAME Not Given
DATE OF BIRTH Not Given
SEX Female
MEDICAL RECORD # Not Given

PHYSICIAN
ORDERING PHYSICIAN Not Given
MEDICAL FACILITY Not Given
ADDITIONAL RECIPIENT Not Given
MEDICAL FACILITY ID Not Given
PATHOLOGIST Not Given

SPECIMEN
SPECIMEN SITE Not Given
SPECIMEN ID Not Given
SPECIMEN TYPE Not Given
DATE OF COLLECTION Not Given
SPECIMEN RECEIVED Not Given

1

CDx Associated Findings

GENOMIC FINDINGS DETECTED	FDA-APPROVED THERAPEUTIC OPTIONS
EGFR L858R	Gilotrif® (Afatinib) Iressa® (Gefitinib) Tarceva® (Erlotinib)

2

OTHER ALTERATIONS & BIOMARKERS IDENTIFIED
Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See professional services section for additional information.

Microsatellite Status MS-Stable§
Tumor Mutation Burden 11 Muts/Mb§
CDKN2A/B loss§
EGFR amplification§
PTCH1 T416S
RBM10 Q494*
TP53 R267P

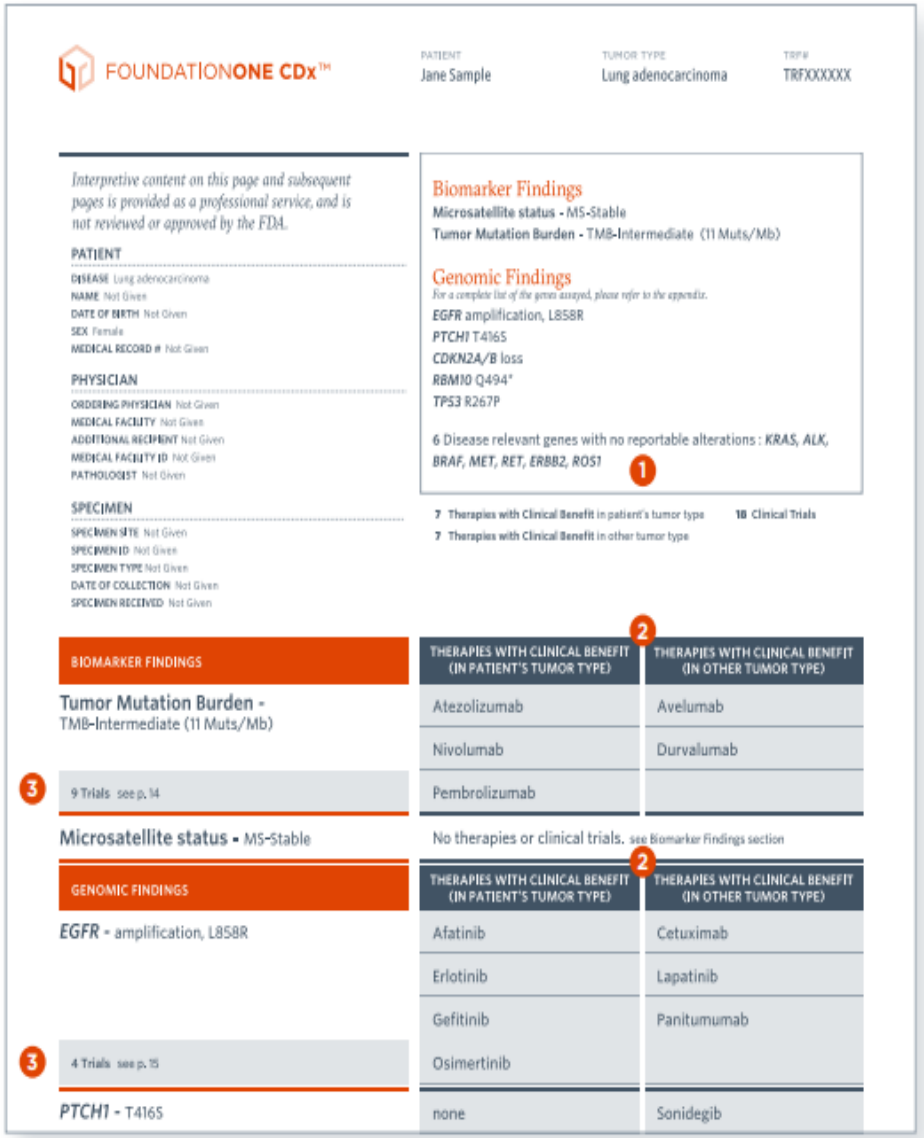
§ Refer to appendix for limitation statements related to detection of any copy number alterations, gene rearrangements, MSI or TMB result in this section.
Please refer to appendix for Explanation of Clinical Significance Classification and for variants of unknown significance (VUS).

1 **FDA-Approved Therapies**
List of FDA-approved companion diagnostics to identify patients who may benefit from associated therapies

2 **All Other Biomarkers**
All other biomarkers, including tumor mutational burden (TMB) and microsatellite instability (MSI), without companion diagnostic claims

Professional Services

Report Section 2


FOUNDATIONONE CDx™

PATIENT Jane Sample | TUMOR TYPE Lung adenocarcinoma | TRF# TRFXXXXXX

Interpretive content on this page and subsequent pages is provided as a professional service, and is not reviewed or approved by the FDA.

PATIENT
DISEASE Lung adenocarcinoma
NAME Not Given
DATE OF BIRTH Not Given
SEX Female
MEDICAL RECORD # Not Given

PHYSICIAN
ORDERING PHYSICIAN Not Given
MEDICAL FACILITY Not Given
ADDITIONAL RECIPIENT Not Given
MEDICAL FACILITY ID Not Given
PATHOLOGIST Not Given

SPECIMEN
SPECIMEN SITE Not Given
SPECIMEN ID Not Given
SPECIMEN TYPE Not Given
DATE OF COLLECTION Not Given
SPECIMEN RECEIVED Not Given

Biomarker Findings
Microsatellite status - MS-Stable
Tumor Mutation Burden - TMB-Intermediate (11 Muts/Mb)

Genomic Findings
For a complete list of the genes assayed, please refer to the appendix.
EGFR amplification, L858R
PTCH1 T416S
CDKN2A/B loss
RBM10 Q494*
TP53 R267P

6 Disease relevant genes with no reportable alterations : *KRAS, ALK, BRAF, MET, RET, ERBB2, ROS1*

1

7 **Therapies with Clinical Benefit** in patient's tumor type
7 **Therapies with Clinical Benefit** in other tumor type
18 **Clinical Trials**

2

BIOMARKER FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Tumor Mutation Burden - TMB-Intermediate (11 Muts/Mb)	Atezolizumab	Avelumab
	Nivolumab	Durvalumab
3 9 Trials see p. 14	Pembrolizumab	
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section	

2

GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
EGFR - amplification, L858R	Afatinib	Cetuximab
	Erlotinib	Lapatinib
	Gefitinib	Panitumumab
3 4 Trials see p. 15	Osimertinib	
PTCH1 - T416S	none	Sonidegib

GUARDANT360 CDx

Precision medicine is becoming increasingly important for advanced cancer patients. Certain therapies provide better patient outcomes and fewer side effects than broad-based chemotherapy. But an individual's response to a given treatment often depends on the genomic profile of their tumor.

For oncologists, the FDA-approved Guardant360® CDx provides comprehensive genomic results from a simple blood draw in seven days, helping them move beyond the limitations of tissue biopsies to match patients with the best treatments. Obtaining clinically relevant genomic information through a blood draw helps patients avoid an additional tissue or surgical biopsy and moves beyond the limitations of tissue specimens. Guardant360 CDx covers all genes recommended by the National Comprehensive Cancer Network, including the 55 genes most relevant to clinical care.

Guardant360 CDx is FDA approved for tumor mutation profiling, also known as comprehensive genomic profiling (CGP), across all solid cancers and also as a companion diagnostic to identify non-small cell lung cancer patients who may benefit from treatment with Tagrisso® (osimertinib).

Since being introduced, the Guardant360 laboratory developed test (LDT) has become widely accepted for blood-based CGP with more than 150 peer-reviewed publications. It has been trusted by more than 7,000 oncologists, with more than 150,000 tests performed to date, and is broadly covered by Medicare and many private payers, representing over 170 million lives. (Note: Coverage information for patients.)

If you are a healthcare provider in the United States or Europe looking for information about Guardant360 CDx, please visit our product website. If you are a healthcare provider in Asia, Middle East, or Africa, please visit this website.

Jones, Linda (A0123456)
Patient MRN: - | DOB: FEB-01-1956 | Gender: Female
Diagnosis: Non-small cell lung cancer (NSCLC) | Test Number 1

GUARDANT360 CDx
Therapy Finder Page

REPORTING
Report Date: JAN-28-2018
Receipt Date: JAN-21-2018
Collection Date: JAN-20-2018
Specimen: Blood
Status: FINAL

PHYSICIAN
John Miller

Account: Pleasantville Oncology
Address: 1234 Main Street
Anaheim, CA 94063, United States
Ph: (123) 456-7890 | Fax: (123) 456-7899
Additional Recipient: N/A

Complete Tumor Response Map on page 2

This content is provided as a professional service and has not been reviewed or approved by the FDA.

Summary of Detected Somatic Alterations, Immunotherapy Biomarkers & Associated Treatment Options

KEY: Approved in indication | Approved in other indication | Lack of response

Detected alteration(s)/ biomarker(s)	Associated FDA-approved therapies	Clinical trial availability (see page 3)	% cfDNA or Amplification
MSI-High	Pembrolizumab Nivolumab	Yes	—
EGFR L858R	Afatinib, Osimertinib, Erlotinib, Gefitinib Neratinib	Yes	4.9%
NF1 S365*	Cobimetinib, Everolimus, Temsirolimus, Trametinib	Yes	4.3%
TP53 Q331*	None	Yes	6.8%

Variants of Uncertain Significance
STK11 A295T (2.1%), *BRCA1* L1230V (0.2%)
The functional consequences and clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

Alterations or biomarkers that were "NOT DETECTED" have been excluded from the summary table above.

We evaluated this sample for 74 genes, including the following guideline-recommended genes for NSCLC:

EGFR (T790M and others)	*ALK*	*ROS1*	*BRAF*	*MET*	*ERBB2* (HER2)	*RET*	*NTRK*

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GUARDANT

A more detailed Guardant360 CDx Patient Report is available through our online portal: portal.guardanthealth.com or to set up an account, contact Client Services: 855.698.8887

TST-PRT-001 V22.0 | Pg 1 of 5
Professional Services

Disclaimer: Represented here is the Professional Services part of the report. The results for the biomarkers shown on this report are not prescriptive or conclusive for labeled use of any specific therapeutic product. For the Guardant360 CDx intended use statement, please refer to the Guardant360 CDx Report. For further information, see Technical Information.

Liquid Biopsy to Identify Actionable Genomic Alterations

Sai-Hong Ignatius Ou, MD, PhD, Misako Nagasaka, MD, and Viola W. Zhu, MD, PhD

***sürücü mut saptanması**

***ted yanıtı izlenmesi**

***rekürrens saptanması**

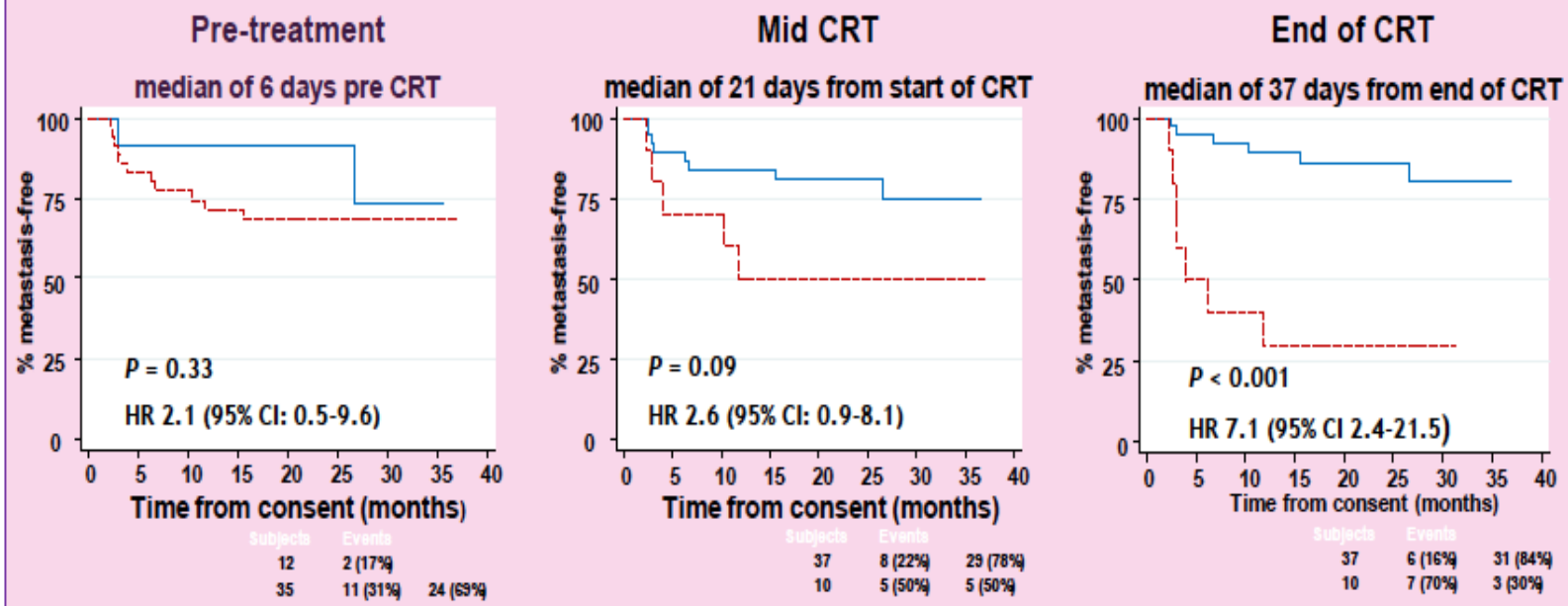
***direnç mek tanımlanması**

EGFR, RAS, BRAF(V600E), PIK3CA

Ou SHI et al, ASCO Ed book 2018, 978-987

CtDNA during and post-CRT in LARC

Metastasis free survival by ctDNA detectability at each time-point



ctDNA undetectable

ctDNA detectable

Royal Marsden Hospital Study: N=47

Khakoo et al. presented at the EACR-ESMO Joint conference on Liquid Biopsies 2019

- **MSK-IMPACT, Foundation One (F1CDx)**

  FDA onayı

- **The Catalogue of Somatic Mutations in Cancer (COSMIC)**

- **Maliyet-etkin**

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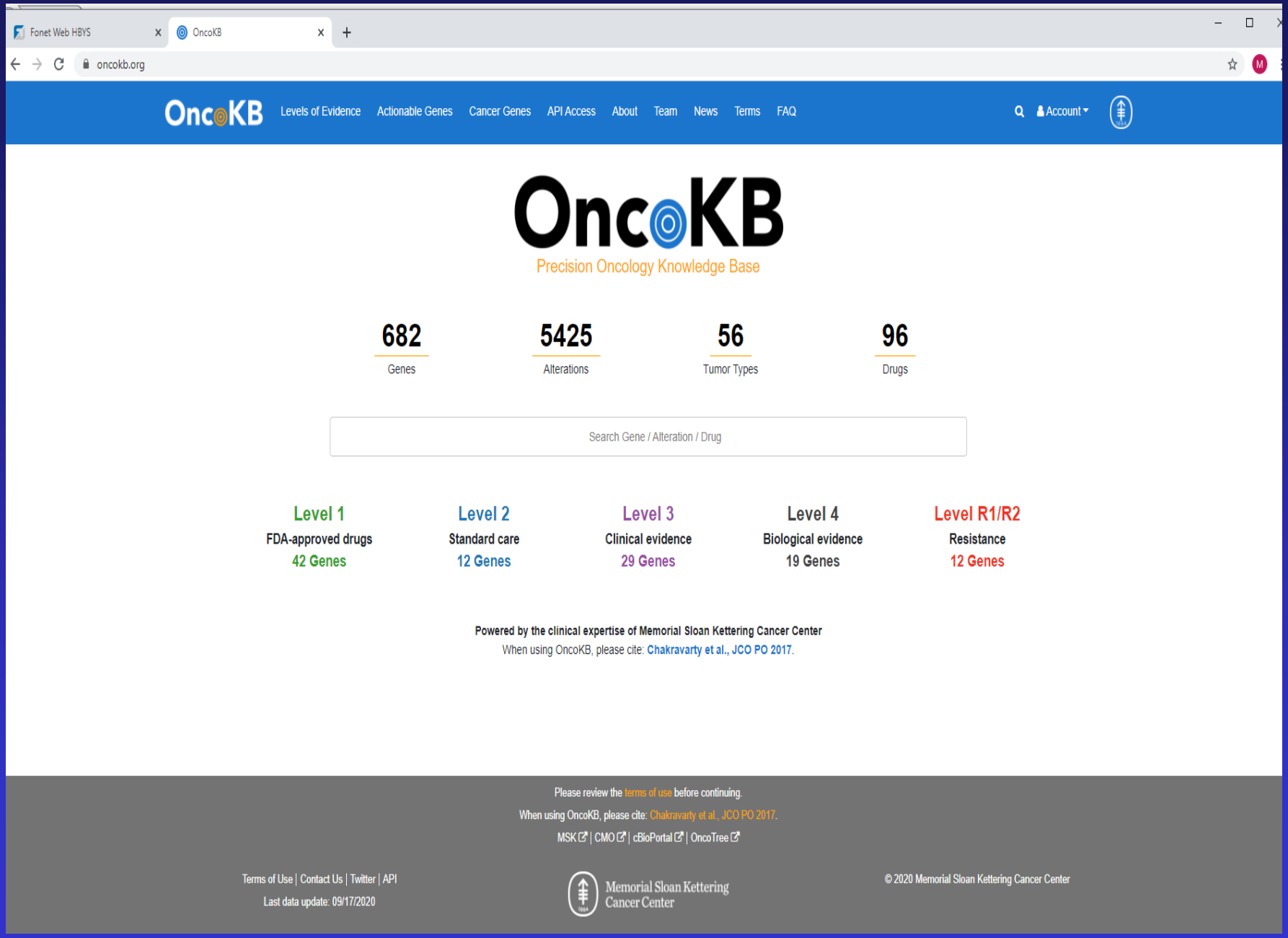
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OncoKB
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FDA-approved drugs
42 Genes
Level 2
Standard care
12 Genes
Level 3
Clinical evidence
29 Genes
Level 4
Biological evidence
19 Genes
Level R1/R2
Resistance
12 Genes
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Last data update: 09/17/2020
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ÖZET

- Genomik değişikliler
- Tüm genom analizi
- (olası) benign, (olası) patolojik, VUS
- Doku vs likit biyopsi… konkordans ↑, spesivite ↑

'kime-ne zaman- hangi genomik test'