Com|Pl|i|t DX

## ΠΛΗΡΟΦΟΡΙΕΣ

| | | | |
|---|---|---|---|
| **Εξεταζόμενος** | - | **Ημ. Συλλογής Δείγμ.** | - |
| **ΑΜΚΑ** | - | **Παραπέμπων** | - |
| **Ημερ. Γέννησης** | - | **Barcode** | 26xxxxxxGR |
| **Τύπος Δείγμ. #1** | ΙΣΤΟΣ ΕΓΚΛΕΙΣΜΕΝΟΣ ΣΕ ΜΠΛΟΚ ΠΑΡΑΦΙΝΗΣ | **Ημερ. Παρ. Δειγμ.** | - |
| **Τύπος Δείγμ. #2** | - | **Ημερ. Αποτελ.** | - |
| **Κωδικός Δείγμ. #1** | - | **Τύπος όγκου** | Ca παχέος εντέρου |

**Com.Pl.i.t. Dx (77 genes, 19 fusions) | Comprehensive Panel for Individualized Treatment**

## Αποτελέσματα και ερμηνεία*

| Βιοδείκτης | Αποτέλεσμα | Θεραπείες με ισχυρή κλινική σημασία | | Θεραπείες με πιθανή κλινική σημασία | Θεραπείες με πιθανή αντίσταση |
|---|---|---|---|---|---|
| | | Εγκεκριμένες/ βάσει οδηγιών θεραπείες για την ένδειξη (Level A) | Θεραπείες σε ισχυρές μελέτες (Level B) | Εκτός ένδειξης θεραπείες (Level C) | |
| ***KRAS*** | Exon 2<br>p.G12D<br>VAF: 9% | - | - | Avutometinib+Defactinib | Cetuximab<br>Tucatinib+Trastuzumab<br>Panitumumab |
| **Μικροδορυφορική Αστάθεια (MSI)** | χωρίς μικροδορυφορική αστάθεια (MSS) | - | - | - | - |
| **Ανοσοϊστοχημικοί Βιοδείκτες** | | | | | |
| **Έκφραση ERBB2 (IHC) (Πίνακας S1)** | Αρνητικό (Score 0) | - | - | - | - |

Στο πλαίσιο της Επικουρικής/Νεοεπικουρικής θεραπείας για καρκίνο του παχέος εντέρου και του ορθού, λάβετε υπόψη τους ακόλουθους βιοδείκτες για πιθανή θεραπεία: dMMR/MSI-H, POLE/POLD1 και PIK3CA. Για όλα τα άλλα ευρήματα, δεν υπάρχουν επί του παρόντος εγκεκριμένες θεραπείες ή καθιερωμένες κατευθυντήριες γραμμές για ασθενείς με νόσο πρώιμου σταδίου. Ωστόσο, αυτά τα ευρήματα μπορεί να αποκτήσουν κλινική σημασία σε περίπτωση υποτροπής ή εξέλιξης της νόσου σε μεταστατική.

*Σημείωση: Το επίπεδο σημαντικότητας των παραλλαγών (Level of Evidence, LoE) (π.χ. A, B, C κλπ) βασίζονται στις οδηγίες για την αναφορά γενετικών παραλλαγών στον καρκίνο που δόθηκαν με κοινή συναίνεση των AMP, ACMG, ASCO και CAP. Για λεπτομερή περιγραφή των οδηγιών αυτών, ανατρέξτε στην Εικόνα 1.

^ Η ανάλυση συγκεκριμένων βιοδεικτών του παρόντος πολυγονιδιακού πάνελ πραγματοποιείται στο πλαίσιο διαπίστευσης κατά ΕΛΟΤ EN ISO 15189:2022 (βλ. ενότητα «Methodology»). Η διαπίστευση κατά ΕΛΟΤ EN ISO 15189:2022 αφορά αποκλειστικά τον αναλυτικό προσδιορισμό των εν λόγω βιοδεικτών. Οι ερμηνείες που σχετίζονται με την πρόβλεψη ανταπόκρισης σε θεραπεία δεν περιλαμβάνονται στο πεδίο διαπίστευσης.



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**Γιώργος Νασιούλας, PhD Μοριακός Βιολόγος, Επιστημονικός Διευθυντής, ΑΜΚΑ:26025301255**

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| SAMPLE INFORMATION | | | |
|---|---|---|---|
| **Name** | - | **Date Sp. Extracted** | - |
| **ID/Medical ID** | - | **Req. Physician** | - |
| **Date of Birth** | - | **Report No** | 26xxxxxGR |
| **Material #1** | PARAFFIN EMBEDDED TISSUE-BLOCK | **Date Received** | - |
| **Material #2** | | **Date of Report** | - |
| **Sample #1 ID** | - | **Tumor type** | Colon Cancer |

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## 1. Clinically Significant Biomarkers*

| Biomarker | Result | Therapies with strong clinical significance | | Therapies with potential significance | Therapies with potential resistance |
|---|---|---|---|---|---|
| | | Approved/ Standard of care therapies (Level A) | Therapies in well powered studies (Level B) | Off label/ Investigational therapies (Level C) | |
| ***KRAS*** | Exon 2<br>p.G12D<br>VAF: 9% | - | - | Avutometinib+Defactinib | Cetuximab<br>Tucatinib+Trastuzumab<br>Panitumumab |
| **Microsatellite Instability (MSI)** | Stable (MSS) | - | - | - | - |
| **Immunohistochemistry Biomarkers** | | | | | |
| **HER2 expression (IHC) (Table S1)** | Negative (Score 0) | - | - | - | - |

In the Adjuvant/Neoadjuvant setting for colon and rectal cancers consider the following biomarkers for possible treatment: dMMR/MSI-H, POLE/ POLD1 and PIK3CA. For all other findings, there are currently no approved therapies or established guidelines for patients with early-stage disease. However, these findings may become clinically relevant in the event of disease relapse or progression to metastatic disease.

*Note: Variants' Level of Evidence (LoE) (e.g. A, B, C etc) are based on the Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. For a detailed description of the recommendation please refer to Fig. 1

^ The analysis of selected biomarkers included in the present multigene panel is performed within the scope of accreditation according to ELOT EN ISO 15189:2022 (see «Methodology» section). Accreditation according to ELOT EN ISO 15189:2022 applies exclusively to the analytical determination of the selected biomarkers. Interpretations related to the prediction of therapeutic response are not included in the scope of accreditation.



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## 2. Methodology

**NGS analysis**

DNA is extracted from the sample under investigation using the Qiamp DNA FFPE Kit (Qiagen). RNA was extracted using the RNeasy FFPE Kit (Qiagen). A total of 83 unique genes is analyzed, including the entire coding sequences of 77 genes and 19 RNA fusions, using the Pan Cancer Panel NGS Assay (Genes2Me). This is a CE-IVD hybrid capture–based assay, in which target genomic regions are enriched through probe hybridization technology. The assay enables the detection of single nucleotide variants (SNVs), insertions/deletions (indels), copy number variations (CNVs), and RNA fusions (Table 1).

Sequencing is performed on the DNBSEQ-T7 next-generation sequencing platform (MGI). Raw sequencing data are processed and analyzed using the CliSeq Interpreter, a companion software for automated, cloud-based analysis. In addition, the SeqPilot software (Version 4.4, Build 505; JSI Medical Systems) is used for independent variant calling and result confirmation.

Alteration- and tumor type-specific therapeutic implications are classified using the OncoKB™ Levels of Evidence system, which assigns clinical actionability to individual mutational events. For additional details about the OncoKB™ curation process, please refer to the version-controlled OncoKB™ Curation Standard Operating Procedure.

**Genes Analyzed**

| **77 Gene Alterations** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ABL1 | AKT1 | ALK | APC | ARAF | ATM | BRAF* | BRCA2* | CCNE1* | CDH1 |
| CDKN2A* | CSF1R | CTNNB1 | DDR2 | DICER1 | EGFR* | EIF1AX | ERBB2* | ERBB3 | ERBB4 |
| EZH2 | FBXW7 | FGFR1* | FGFR2* | FGFR3 | FLT3 | FOXL2 | GNA11 | GNAQ | GNAS |
| HGF | HNF1A | HRAS | IDH1 | IDH2 | JAK2 | JAK3 | KDR | KEAP1 | KIT |
| KRAS | LAG3 | MAP2K1 | MDM2 | MET* | MLH1* | MPL | MTAP* | MYC | NOTCH1 |
| NPM1 | NRAS | NTRK1 | NTRK2 | NTRK3 | PDGFRA | PIK3CA | PIK3R1 | POLD1 | POLE |
| PTEN* | PTPN11 | RAC1 | RAF1 | RB1 | RET | ROS1 | SMAD4 | SMARCA4* | SMARCB1 |
| SMO | SPOP | STK11 | SRC | TERT | TP53 | VHL | | | |
| **19 Fusion Genes** | | | | | | | | | |
| ALK | BRAF | EGFR | ERG | FGFR1 | FGFR2 | FGFR3 | MET | NRG1 | NTRK1 |
| NTRK2 | NTRK3 | PBX1 | PPARG | PRKACA | RAF1 | RET | ROS1 | TFE3 | |

*CNV (amplification/deletion) analysis is included for these genes



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**Biomarkers within the scope of accreditation (ISO15189:2022)**

-*AKT1, EGFR, ERBB2, KRAS, NRAS, METex14 skipping HRAS, IDH1, IDH2, KIT, PIK3CA, PDGFRA* (SNV/indel detection by NGS)
-*ALK, ROS1, RET, FGFR1,2,3, NTRK1,2,3, METex14 skipping* (Fusion detection by NGS)
-MSI (HRM Real-time PCR)
-PDL1 SP263 & SP142 (IHC)
-*ALK* (FISH)
-*HER2* (IHC, FISH, DISH)

**Sensitivity**: Positive reference standards are tested with the assay, all corresponding mutation sites can be accurately detected, and the positive percent agreement (PPA) for all variants (SNVs, Indels, fusions and CNVs) assessed was 100%.
**Specificity**: Negative reference standards are tested with the assay, and the negative percent agreement (NPA) of SNVs, Indels, fusions and CNVs was 100%.
**Limit of Detection (LoD):** The limit of detection (LoD) of this assay is listed in the table below. The LoD is based on as low as 50 ng of gDNA input for library preparation. The assay can also be used to test microsatellite instability (MSI) with a tumor cell content as low as 10%.

The performance characteristics of the assay are summarized in the following table:

| Feature | Performance |
|---|---|
| **Precision (%)** | >96 |
| **Reproducibility (%)** | 97 |
| **On-Target Ratio (%)** | 85–95 |
| **Analytical Sensitivity (%)** | 96 |
| **Analytical Specificity (%)** | 100 |
| **Repeatability (%)** | 96 |
| **Limit of Detection (LoD, VAF)** | ≥ 1% |

**Disclaimer**

1. This test is mainly used to assist clinical decision-making, and the result does not represent clinical decision.
2. The test should be interpreted by combining the actual patient context. The medication information provided only on the basis of genetic test results, and the actual medication should follow the physician's instructions.
3. The clinical trials only present partial relevant clinical recruitment trials. For more comprehensive and updated information, please refer to the website: https://clinicaltrials.gov/.
4. As evidence on variants and drugs evolves, previous classifications may later be modified. The interpretation of a variant is based on current available evidence.



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5. Sequence variants were reported using Human Genome Variation Society (HGVS) nomenclature. Classification and interpretation of variants follow guidelines of American College of Medical Genetics and Genomics (ACMG), Association of Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).
6. Database and references used: Reference genome (GRCh37), annotation using A Locus Reference Genomic (LRG), database referencing 1000G (phaseIII-ucsc), EXAC (0.3.1), dbSNP (147), PolyPhen2/SIFT (ensdb v73), PhyloP (2013-12-06), Clinvar (2018-8) and Cosmic(V80).

**Limitations**

1. Limited tissue detection may not represent the whole DNA variations of lesions because of tumor heterogeneity.
2. Scientific data show that not all patients carry genomic variations that are associated with targeted drug, therefore not all subjects can be matched with targeted therapies or clear resistance mechanism.
3. Genetic variation beyond the detection range of this test or some non-gene mutation related factors such as drug interactions may affect the clinical effects of drugs.
4. The detection could not distinguish between somatic mutations and germline mutations effectively without control sample analysis.
5. Every molecular test has an internal 0.5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.



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## 3. Quality Control Results

| Quality Control Index | Result | Criterion |
|---|---|---|
| Average effective sequencing depth[1] | 598 | ≥ 500 |
| Tumor cell content[2] | 20% | ≥20% |
| Overall Assessment[3] | PASS | |

**Note** :

1. Average effective sequencing depth: Average sequencing depth on target without duplicated reads.
2. An overall tumor cell content percentage of ≥ 20% is recommended for the efficient detection of somatic alterations in the sample analyzed.
3. Overall Assessment: The quality control overall assessment results are divided into two levels: "PASS" and "RISK". When the overall quality assessment result is "RISK", 94-96% of coverage was achieved in the genes analysed, hence there is a small range where clinical actionable variations could be undetected.

**Microsatellite instability**

Genomic DNA was extracted from the examined formalin-fixed, paraffin-embedded (FFPE) tumor tissue sample. Assessment of microsatellite instability (MSI) was performed using the MicroSight MSI - 1-step HRM Analysis method, which is based on real-time PCR followed by high-resolution melting (HRM) curve analysis.
The test targets five mononucleotide microsatellite markers (BAT25, BAT26, NR22, NR24, and MONO27), known for their high sensitivity in MSI detection. Differences are detected using specific fluorescent EasyBeacon probes with Intercalating Nucleic Acid (INA) technology, which enhances specificity and curve resolution during melting analysis.
Interpretation is based on comparing the melting profile of the tumor sample to a predefined universal reference DNA. Each microsatellite marker is classified as either stable (MSS) or unstable (MSI-H), and the overall classification of the sample depends on the number of unstable markers:
-Sample with 0-2 unstable markers: classified as microsatellite stable (MSS)
-Sample with ≥3 unstable markers (≥60%): classified as microsatellite instability-high (MSI-H).



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**ERBB2 (HER2) expression by IHC**

ERBB2 staining is performed in a VENTANA BenchMark Series automated staining instrument using the ERBB2 clone 4B5, on formalin-fixed, paraffin-embedded (FFPE) tissue. The IHC test gives a score of 0 to 3+ that measures the amount of HER2 receptor protein on the surface of cancer cells. Scoring interpretation is as follows:

HER2 IHC positive (score 3+)
HER2 IHC equivocal (score 2+)
HER2 IHC negative (score 0 or 1+)
Scoring is based on the ASCO-CAP HER2 testing guidelines (PMID: 27841667, 37303228).
For mCRC the HERACLES criteria are also used (PMID: 26449765).

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## 4. Important biomarkers findings

| Gene | Finding (VAF) | Gene | Finding (VAF) |
|---|---|---|---|
| *KRAS* | **p.G12D (9%)** | *ERBB2* amplification | Not Detected |
| *NRAS* | Not Detected | *MSI* status | **MSS** |
| *BRAF*_V600E | Not Detected | *NTRK1/2/3* rearrangement | Not Detected |

## 5. Immune Checkpoint inhibitors biomarkers

| Biomarker/Variant | Result | Clinical Interpretation |
|---|---|---|
| **Treatment effect - positive correlation** | | |
| *MSI* status | MSS | - |
| *POLE* mutation (driver) | Not Detected | - |
| *TP53* mutation | Not Detected | - |
| *KRAS* mutation | **Detected** | **May increase the benefit rate of PD-1/PD-L1 inhibitors** |
| **Treatment effect - negative correlation** | | |
| *PTEN* inactivating mutation | Not Detected | - |
| *JAK2* inactivating mutation | Not Detected | - |
| *EGFR* mutation (L858R/EX19del) | Not Detected | - |
| *ALK* rearrangement | Not Detected | - |
| *STK11* inactivating mutation | Not Detected | - |
| *KEAP1* inactivating mutation | Not Detected | - |
| *MDM2* amplification | Not Detected | - |

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## 6. Interpretations for targeted therapies

| Genetic Variation: | *KRAS*: c.35G>A (p.G12D) | VAF*: 9% | OncoKB |
|---|---|---|---|

**Treatment Information**

The presence of KRAS mutations is associated with a high likelihood of resistance to therapies targeting EGFR (Panitumumab, Cetuximab) (PMID: 18202412, 24024839, 18316791).According to MOUNTAINEER clinical trial, KRAS and NRAS mutations may confer resistance to the anti-HER2 therapy Tucatinib & Trastuzumab. To date, most efforts to treat cancers with RAS mutations have focused on targeting downstream effectors of mutant RAS, such as RAF, MEK, or PI3K, each of which is druggable. MEK inhibitors have been the most widely investigated, typically as a combination therapy, despite the presence of multiple inhibitors that are being explored to target different KRAS-activated pathways. In the IMblaze370 trial, which evaluated Atezolizumab alone and in combination with Cobimetinib against monotherapy use of Regorafenib, the combination did not demonstrate improved overall survival (PMID: 31003911). Currently, there are no other clinical trials including cobimetinib for colorectal cancer. The MEK inhibitor binimetinib is being examined in a number of clinical trials for patients with KRAS-mutated colorectal cancer, including studies looking at the agent in combination with mFOLFIRIl (NCT02613650) and with palbociclib (NCT03981614). Clinical trials evaluating the efficacy of trametinib, a potent MEK inhibitor clinically approved for BRAF mutant cancers (mainly melanoma), in colorectal cancer have been conducted (NCT03317119, NCT03668431, NCT03714958, NCT04111458). Avutometinib is an inhibitor of Ras-Raf-MEK-ERK signaling being developed as a potential treatment for cancer. Defactinib is a small-molecule, oral focal adhesion kinase (FAK) inhibitor. It works by blocking FAK, a tyrosine kinase involved in cell adhesion and signaling pathways, including RAS/MEK/ERK and PI3K/Akt. Defactinib has been studied in various clinical trials for its potential anti-tumor and anti-angiogenic activities. In 2025 FDA approved defactinib in combination with avutometinib for the treatment of adult patients with recurrent low-grade serous ovarian cancer. A phase I clinical trial of RMC-6236 in patients with advanced refractory solid cancers harboring specific KRAS mutations (G12A, G12D, G12R, G12S, G12V) is ongoing (NCT05379985). Finally, a phase I/II trial of SX-628 (CXCR1/2 inhibitor) in combination with nivolumab is ongoing in refractory RAS mutant, MSS CRC (NCT04599140). Efforts to induce an immune response against these tumors are under investigation (PMID: 36638742).

**Gene information**

KRAS is a member of the RAS family of small GTPases, which catalyze the hydrolysis of GTP to GDP. Under physiologic conditions, these RAS proteins cycle between an active (GTP-bound) and an inactive (GDP-bound) state, to activate the MAPK and PI3K oncogenic pathway signaling downstream of Receptor Tyrosine Kinases (RTKs) (PMID: 22189424). The function of RAS enzymes is regulated by guanine nucleotide exchange factors (GEFs), such as SOS, which enable the exchange of GDP to GTP, as well as by GTPase activating proteins, such as NF1, which increase the ability of RAS to hydrolyze GTP. Once activated, RAS mediates the regulation of cellular proliferation and other cellular functions through the activation of distinct intracellular signaling pathways, including the RAF/MEK/ERK and PI3K/AKT/mTOR pathways. Transforming mutations in KRAS are frequently found in pancreatic, colon, endometrial, ovarian and biliary cancers; almost all of these mutations result in constitutive activation of KRAS, thereby promoting cell proliferation (PMID: 21993244, 24651010). KRAS mutations occur primarily in three major hotspots, G12, G13 and Q61, the first two of which occur in the GTP-ase domain and the latter interferes with the ability of NF1 to bind and regulate KRAS (PMID: 22589270, 21993244, 24651010). Other less well-characterized mutations such as A146T/V/P and L19F have also been characterized and found to be activating albeit at lower frequencies (PMID: 20147967, 20432530, 20570890). Germline KRAS mutations are responsible for both Noonan syndrome (NS)



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and cardio-facio-cutaneous (CFC) syndrome (PMID: 16474405, 16474404, 19467855, 17056636) that are associated with hyperactivated MAPK pathway, distinctive clinical characteristics and a predisposition to cancer (PMID: 19467855, 17704260).

**Variant Information**

The KRAS G12D mutation is known to be oncogenic. The KRAS G12D mutation is located in the P-loop of the catalytic G-domain of the protein. This mutation has been found in lung, colorectal, pancreatic and ovarian cancer (PMID: 28572459). Expression of this mutation in cell lines and mouse models demonstrated that it is activating, as measured by increased downstream pathway activation, colony formation and in vivo tumor development across multiple lineages compared to wildtype (PMID: 20570890, 20147967, 11751631, 15093544, 19296721, 17349581, 32792368). In vitro studies have demonstrated that this mutation confers resistance to BRAF inhibition in lymphoid cells as measured by decreased pathway activation in the presence of the drug, but is sensitive to the MEK inhibitor cobimetinib (PMID: 30341394). A patient with hairy cell leukemia acquired a KRAS G12D mutation following relapse on the BRAF inhibitor vemurafenib but was successfully treated with the cobimetinib for at least twelve months (PMID: 30341394). Preclinical studies with xenograft mouse models expressing KRAS G12D demonstrated sensitivity to treatment with setidegrasib as measured by reduced tumor volume following treatment (Abstract: Nagashima et al. Abstract# 5735, AACR 2023.).

### Cetuximab

DrugBank

Cetuximab and panitumumab are anti-EGFR monoclonal antibodies that are FDA-approved for patients with EGFR expressing, RAS-wildtype colorectal cancer. Patients with metastatic colorectal cancer (mCRC) harboring either exon 2 (e.g. G12, G13) or non-exon 2 (e.g Q61, K117, A146) KRAS mutations do not respond favorably to the anti-EGFR therapies cetuximab (PMID: 21228335, 20619739) or panitumumab (PMID: 18316791, 20921465, 24024839).

### Tucatinib+Trastuzumab

DrugBank

Tucatinib, a small molecular inhibitor of HER2, and trastuzumab, a HER2-targeted antibody, are FDA-approved in combination for the treatment of patients with HER2 amplified metastatic colorectal cancer whose tumors are also RAS and BRAF wildtype. Patients with metastatic colorectal cancer harboring KRAS mutations do not respond favorably to trastuzumab as seen in the MyPath trial. In the multiple basket MyPath Phase IIa trial of pertuzumab and trastuzumab in which 56 metastatic colorectal cancer patients with HER2 amplification received combination therapy, the objective response rate for patients with KRAS wildtype colorectal cancer (43/56 patients) compared to KRAS mutated colorectal cancer (13/56 patients) was 40% (17/43, 95% CI, 25%–56%) versus 8% (1/13, 95% CI 0.2%–36%), respectively (PMID: 30857956).

### Panitumumab

DrugBank

Cetuximab and panitumumab are anti-EGFR monoclonal antibodies that are FDA-approved for patients with EGFR expressing, RAS-wildtype colorectal cancer. Patients with metastatic colorectal cancer (mCRC) harboring either exon 2 (e.g. G12, G13) or non-exon 2 (e.g Q61, K117, A146) KRAS mutations do not respond favorably to the anti-EGFR therapies cetuximab (PMID: 21228335, 20619739) or panitumumab (PMID: 18316791, 20921465, 24024839).

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**Avutometinib+Defactinib** DrugBank

Avutometinib, a RAF/MEK clamp inhibitor, and defactinib, a FAK inhibitor, are FDA-approved in combination for the treatment of adult patients with KRAS-mutated recurrent low-grade serous ovarian cancer (LGSOC) who have received prior systemic therapy. FDA approval was based on the results of the Phase II RAMP-201 (NCT04625270) trial of avutometinib plus defactinib in 109 patients with recurrent LGSOC.

In the Phase II RAMP-201 (NCT04625270) trial, patients with KRAS-mutated LGSOC (n=57) demonstrated an overall response rate (ORR) of 44% (n=25), with a 2% (n=2) complete response (CR) rate, 40% (n=23) partial response (PR) rate and 49% (n=28) stable disease (SD) rate, a median duration of response (DOR) of 31.0 months (95% CI=14.8-31.1) and a median progression-free survival (PFS) of 22.0 months (95% CI=11.1-36.6) (PMID: 40644648). Responders included patients with the following KRAS mutations: KRAS A146V, G12D, G12R, G12V and Q61H (PMID: 40644648). Of patients with KRAS wildtype LGSOC (n=52), the cohort demonstrated an ORR of 17% (n=9), with a 17% (n=9) PR rate and 65% (n=34) SD rate, and a median PFS of 9.2 months (95% CI=5.5-NE) (PMID: 40644648).

*VAF: Variant Allele Frequency

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## 7. Clinical Trials to consider

### KRAS associated clinical trials

| NCT05797467 | PHASE3 |
|---|---|
| **Title** | Adjuvant Chemotherapy Combined With Targeted Therapy or Not in the T3-4N2 Colorectal Cancer Patients |
| **Treatment** | FOLFOX chemotherapy regimens \| Bevacizumab |
| **Location** | China |

| NCT06555003 | PHASE3 |
|---|---|
| **Title** | DEBIRI Plus Chemotherapy vs. Chemotherapy Alone in Colorectal Cancer Liver Metastases |
| **Treatment** | Drug-Eluting Embolic Bead \| Chemotherapy drug |
| **Location** | Iran |

| NCT06973564 | PHASE1 \| PHASE2 |
|---|---|
| **Title** | JAB-23E73 in Adult Participants With Advanced Solid Tumors With KRAS Alteration |
| **Treatment** | JAB-23E73 \| JAB-23E73 \| JAB-23E73 |
| **Location** | United States |

| NCT06575127 | PHASE2 |
|---|---|
| **Title** | Modified FOLFOXIRI Plus Target Therapy as a First Line Treatment for Advanced Colorectal Cancer a Prospective Phase Two Study |
| **Treatment** | FOLFOXIRI Protocol |
| **Location** | Egypt |

| NCT05327010 | PHASE2 |
|---|---|
| **Title** | Testing the Combination of the Anti-cancer Drugs ZEN003694 (ZEN-3694) and Talazoparib in Patients With Advanced Solid Tumors, The ComBET Trial |

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|---|---|
| **Treatment** | BET Bromodomain Inhibitor ZEN-3694 \| Biopsy Procedure \| Biospecimen Collection \| Diagnostic Imaging Testing \| Talazoparib |
| **Location** | United States |

| NCT06229340 | **PHASE2** |
|---|---|
| **Title** | Leflunomide or Combination of MEK Inhibitor and Hydroxychloroquine for Refractory Patients With RAS Mutations |
| **Treatment** | Leflunomide \| The combination of MEK inhibitor + hydroxychloroquine( plaquenil) ± bevacizumab |
| **Location** | Russia |

| NCT06520488 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | A Study of HRS-4642 in Combination With Antineoplastic Agents in Advanced Solid Tumors |
| **Treatment** | HRS-4642 |
| **Location** | China |

| NCT06949982 | **PHASE2** |
|---|---|
| **Title** | Efficacy And Safety Of Hydroxychloroquine Combined With Methotrexate, Capecitabine And Bevacizumab Vs. Regorafenib In Participants With Refractory Metastatic Colorectal Cancer With Mutations In RAS Genes |
| **Treatment** | experimental group x-MAP \| Regorafenib (BAY 73-4506) |
| **Location** | Russia |

| NCT06934538 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | First-in-human Trial of STC-1010, an Immunotherapy, in Patients With Unresectable Locally Advanced or Metastatic Colorectal Cancer |
| **Treatment** | STC-1010 + IS regimen + SOC therapy |
| **Location** | Belgium, France, United States |

| NCT05379985 | **PHASE1 \| PHASE2** |
|---|---|

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|---|---|
| **Title** | Study of RMC-6236 in Patients With Advanced Solid Tumors Harboring Specific Mutations in RAS |
| **Treatment** | RMC-6236 |
| **Location** | United States |

| NCT06634875 | **PHASE2** |
|---|---|
| **Title** | Isunakinra Alone and in Combination With Pembrolizumab in Patients With Colorectal Cancer (MSS) |
| **Treatment** | isunakinra |
| **Location** | United States |

| NCT05786924 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | Phase 1/2 Trial of S241656 in Selected RAS/MAPK Mutation- Positive Malignancies |
| **Treatment** | S241656 \| FOLFOX6/FOLFOX7 \| FOLFIRI \| Cetuximab \| Panitumumab \| Gemcitabine \| Nab-paclitaxel |
| **Location** | Denmark, Hong Kong, Japan, United Kingdom, United States |

| NCT06959615 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | A Phase I/IIa Study of JAB-23E73 in Patients With Advanced Solid Tumors Harboring KRAS Gene Alteration |
| **Treatment** | JAB-23E73 \| JAB-23E73 |
| **Location** | China |

| NCT07223047 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | A Study to Evaluate the Safety, Tolerability, and Efficacy of BMS-986523 Alone and in Combination With Anti-Cancer Agents in Participants With Advanced Solid Malignancies |
| **Treatment** | BMS-986523 \| Gemcitabine \| Nab-Paclitaxel \| Cetuximab \| Pembrolizumab |
| **Location** | Canada, Spain, United States |

| NCT06947811 | **PHASE1 \| PHASE2** |
|---|---|

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|---|---|
| **Title** | A Study of Almonertinib Combined With Palbociclib in Patients With Advanced Solid Tumors Harboring KRAS Mutations |
| **Treatment** | Almonertinib \| Palbociclib |
| **Location** | China |

| NCT06895031 | **PHASE2** |
|---|---|
| **Title** | Study of JYP0015 in Patients With Advanced Solid Tumors Harboring Specific Mutations in RAS |
| **Treatment** | JYP0015 |
| **Location** | China |

| NCT02029001 | **PHASE2** |
|---|---|
| **Title** | Adapting Treatment to the Tumor Molecular Alterations for Patients With Advanced Solid Tumors: MyOwnSpecificTreatment |
| **Treatment** | Nilotinib (400 mg BID) \| Everolimus (10 mg QD) \| Sorafenib (400 mg BID) \| Lapatinib (1500 mg QD) \| Pazopanib (800 mg QD) \| Olaparib (300 mg BID) \| Durvalumab + Tremelimumab |
| **Location** | France |

| NCT07148128 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | Phase 1/2a Study to Assess the Safety, Tolerability, Pharmacokinetics, and Preliminary Efficacy of WEF-001 as Monotherapy in Advanced KRAS-Mutant Solid Tumours. |
| **Treatment** | WEF-001 |
| **Location** | Canada, United Kingdom, United States |

| NCT06243354 | **PHASE1 \| PHASE2** |
|---|---|
| **Title** | Phase 1/2 Study of HYP-2090PTSA in Patients With Advanced Solid Tumors Harboring KRAS Mutation |
| **Treatment** | Test product: HYP-2090PTSA |
| **Location** | China |

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| NCT06026410 | PHASE1 |
|---|---|
| **Title** | KO-2806 Monotherapy and Combination Therapies in Advanced Solid Tumors |
| **Treatment** | Darlifarnib \| Cabozantinib \| Adagrasib |
| **Location** | France, Germany, Italy, Spain, United States |

| NCT06619587 | PHASE1 |
|---|---|
| **Title** | A Study to Evaluate Safety, Pharmacokinetics, and Activity of GDC-7035 as a Single Agent and in Combination in Patients With Advanced Solid Tumors |
| **Treatment** | Phase I Arm A \| Phase I Arm B |
| **Location** | Australia, Canada, France, Israel, Singapore, South Korea, Spain, United Kingdom, United States |

| NCT07094113 | PHASE1 |
|---|---|
| **Title** | AMG 410 Alone and in Combination With Other Agents in Participants With KRAS Altered Advanced or Metastatic Solid Tumors |
| **Treatment** | AMG 410 \| Pembrolizumab \| Panitumumab |
| **Location** | Australia, Belgium, Canada, China, Denmark, France, Germany, Italy, Japan, Netherlands, South Korea, Spain, United Kingdom, United States |

| NCT07207707 | PHASE1 |
|---|---|
| **Title** | A Study to Investigate the Safety and Efficacy of KQB548 in Participants With Advanced Solid Malignancies |
| **Treatment** | KQB548 |
| **Location** | United States |

| NCT05067283 | PHASE1 |
|---|---|
| **Title** | A Study of Calderasib (MK-1084) in KRAS Mutant Advanced Solid Tumors (MK-1084-001) |
| **Treatment** | Calderasib \| Pembrolizumab \| carboplatin \| pemetrexed \| cetuximab \| oxaliplatin \| leucovorin \| 5-fluorouracil |

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| | |
|---|---|
| **Location** | Argentina, Australia, Canada, Chile, China, Denmark, Israel, Italy, Japan, Lithuania, Malaysia, New Zealand, Panama, Poland, South Korea, Spain, Switzerland, Taiwan, Turkey (Türkiye), Ukraine, United States |

| NCT06962254 | **PHASE1** |
|---|---|
| **Title** | Imatinib and Trametinib for KRAS-mutated Solid Tumor |
| **Treatment** | Imatinib \| Trametinib |
| **Location** | Taiwan |

| NCT07300150 | **PHASE1** |
|---|---|
| **Title** | A Study of PT0511 in Participants With KRAS Mutated or Amplified Advanced Solid Tumors |
| **Treatment** | PT0511 \| Cetuximab |
| **Location** | South Korea, United States |

| NCT06484790 | **PHASE1** |
|---|---|
| **Title** | T Cell Receptor Gene-Engineered T Cell Therapy Targeting KRAS Mutations in the Treatment of Subjects With Advanced Solid Tumor |
| **Treatment** | NW-301V \| NW-301D |
| **Location** | China |

| NCT05438667 | **EARLY_PHASE1** |
|---|---|
| **Title** | TCR-T Cell Therapy on Advanced Solid Tumors |
| **Treatment** | TCR-T therapy |
| **Location** | China |

| NCT06336902 | **PHASE1** |
|---|---|
| **Title** | Botensilimab Plus Balstilimab and Fasting Mimicking Diet Plus Vitamin C for Patients With KRAS-Mutant Metastatic Colorectal Cancer |

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| | |
|---|---|
| **Treatment** | Balstilimab \| Biospecimen Collection \| Botensilimab \| Computed Tomography \| Dietary Intervention \| Magnetic Resonance Imaging \| Vitamin C |
| **Location** | United States |

| NCT07094204 | **PHASE1** |
|---|---|
| **Title** | A Study to Find a Suitable Dose of ASP5834 in Adults With Solid Tumors |
| **Treatment** | ASP5834 \| panitumumab |
| **Location** | France, Japan, Spain, United States |

| NCT06835569 | **PHASE1** |
|---|---|
| **Title** | A Study to Learn About Study Medicine ALTA3263 in Adults With Advanced Solid Tumors With KRAS Mutations |
| **Treatment** | ALTA3263 \| cetuximab \| mFOLFOX6 \| Pembrolizumab \| Pemetrexed + Cisplatin /Carboplatin \| mFOLFIRINOX \| GnP \| Midazolam |
| **Location** | United States |

| NCT06478251 | **PHASE1** |
|---|---|
| **Title** | T Cell Receptor Gene-Engineered T Cell Therapy Targeting KRAS Mutations in the Treatment of Subjects With Advanced Solid Tumor |
| **Treatment** | NW-301V \| NW-301D |
| **Location** | China |

| NCT06577532 | **EARLY_PHASE1** |
|---|---|
| **Title** | Study of KRAS Neoantigen mRNA Vaccine (ABO2102) in Patients With KRAS -Mutated Solid Tumors |
| **Treatment** | ABO2102 \| Toripalimab |
| **Location** | China |

| NCT06625775 | **PHASE1** |
|---|---|
| **Title** | Open-Label Study of BBO-10203 in Subjects With Advanced Solid Tumors |

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|---|---|
| **Treatment** | BBO-10203 \| Trastuzumab \| Fulvestrant \| Ribociclib \| FOLFOX \| Bevacizumab |
| **Location** | Australia, Brazil, France, Spain, United States |

| NCT07021898 | **PHASE1** |
|---|---|
| **Title** | A Study of ERAS-4001 in Patients With Advanced or Metastatic Solid Tumors. |
| **Treatment** | ERAS-4001 \| ERAS-4001 in combination |
| **Location** | United States |

| NCT06096974 | **PHASE1** |
|---|---|
| **Title** | Pan-RAS Inhibitor YL-17231 in Patients With Advanced Solid Tumors Harboring Mutations in KRAS, HRAS, or NRAS |
| **Treatment** | YL-17231 |
| **Location** | United States |

| NCT07204340 | **PHASE1** |
|---|---|
| **Title** | TLN-372 in Advanced KRAS Mutant Solid Tumors |
| **Treatment** | TLN-372 \| TLN-372 in combination with cetuximab \| TLN-372 in combination with pembrolizumab |
| **Location** | Australia, Canada, Spain, United States |

| NCT06403735 | **PHASE1** |
|---|---|
| **Title** | A Phase I Clinical Study of QLC1101 in Patients With Advanced Solid Tumors |
| **Treatment** | QLC1101 |
| **Location** | China |

| NCT06944457 | **PHASE1** |
|---|---|
| **Title** | Clinical Trial to Evaluate the Safety, Tolerability and Pharmacokinetics of MPD-1 in Patients With Advanced Solid Tumor |
| **Treatment** | MPD-1 |

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| | |
|---|---|
| **Location** | South Korea |

| NCT06487377 | PHASE1 |
|---|---|
| **Title** | IX001 TCR-T In the Treatment of Advanced Pancreatic Cancer and Colorectal Cancer Induced by KRAS Mutations |
| **Treatment** | IX001 TCR-T cells |
| **Location** | China |

| NCT06804824 | PHASE1 |
|---|---|
| **Title** | A First-in-Human (FIH) Study to Evaluate the Safety and Tolerability of VVD-159642 in Participants With Advanced Solid Tumors |
| **Treatment** | VVD-159642 \| Sotorasib \| Trametinib |
| **Location** | Australia, United States |

| NCT05382559 | PHASE1 |
|---|---|
| **Title** | A Study of ASP3082 in Adults With Advanced Solid Tumors |
| **Treatment** | Setidegrasib \| Cetuximab \| Leucovorin \| Oxaliplatin \| Fluorouracil \| Irinotecan \| Nanoparticle albumin-bound-paclitaxel \| Gemcitabine \| Docetaxel \| Pembrolizumab \| Cisplatin \| Carboplatin \| Pemetrexed \| Liposomal Irinotecan |
| **Location** | China, France, Japan, Puerto Rico, South Korea, Spain, United States |

| NCT06218914 | PHASE1 |
|---|---|
| **Title** | Phase 1 Study to Investigate TCRTs KRAS Mutation in Unresectable, Advanced, and/or Metastatic Solid Tumors |
| **Treatment** | NT-112: Autologous, engineered T Cells targeting KRAS G12D \| AZD0240: Autologous, engineered T Cells targeting KRAS G12D |
| **Location** | United States |

| NCT06917079 | PHASE1 |
|---|---|
| **Title** | BBO-11818 in Adult Subjects With KRAS Mutant Cancer |

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| | |
|---|---|
| **Treatment** | BBO-11818 \| Pembrolizumab \| Platinum chemotherapy (cisplatin or carboplatin) \| Pemetrexed \| Cetuximab \| mFOLFOX6 \| mFOLFOX6 \| BBO-10203 \| mFOLFIRINOX \| Gemcitabine \| Nab-paclitaxel |
| **Location** | Australia, United States |

| NCT06484556 | **PHASE1** |
|---|---|
| **Title** | T Cell Receptor Gene-Engineered T Cell Therapy Targeting KRAS Mutations in the Treatment of Subjects With Advanced Solid Tumor |
| **Treatment** | NW-301V \| NW-301D |
| **Location** | China |

| NCT05661201 | **PHASE1** |
|---|---|
| **Title** | NEROFE and Doxorubicin in KRAS-mutated ST2-positive Solid Tumors |
| **Treatment** | NEROFE \| Doxorubicin |
| **Location** | United States |

Press here for a live search of clinical trials for KRAS

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## 8. Appendix

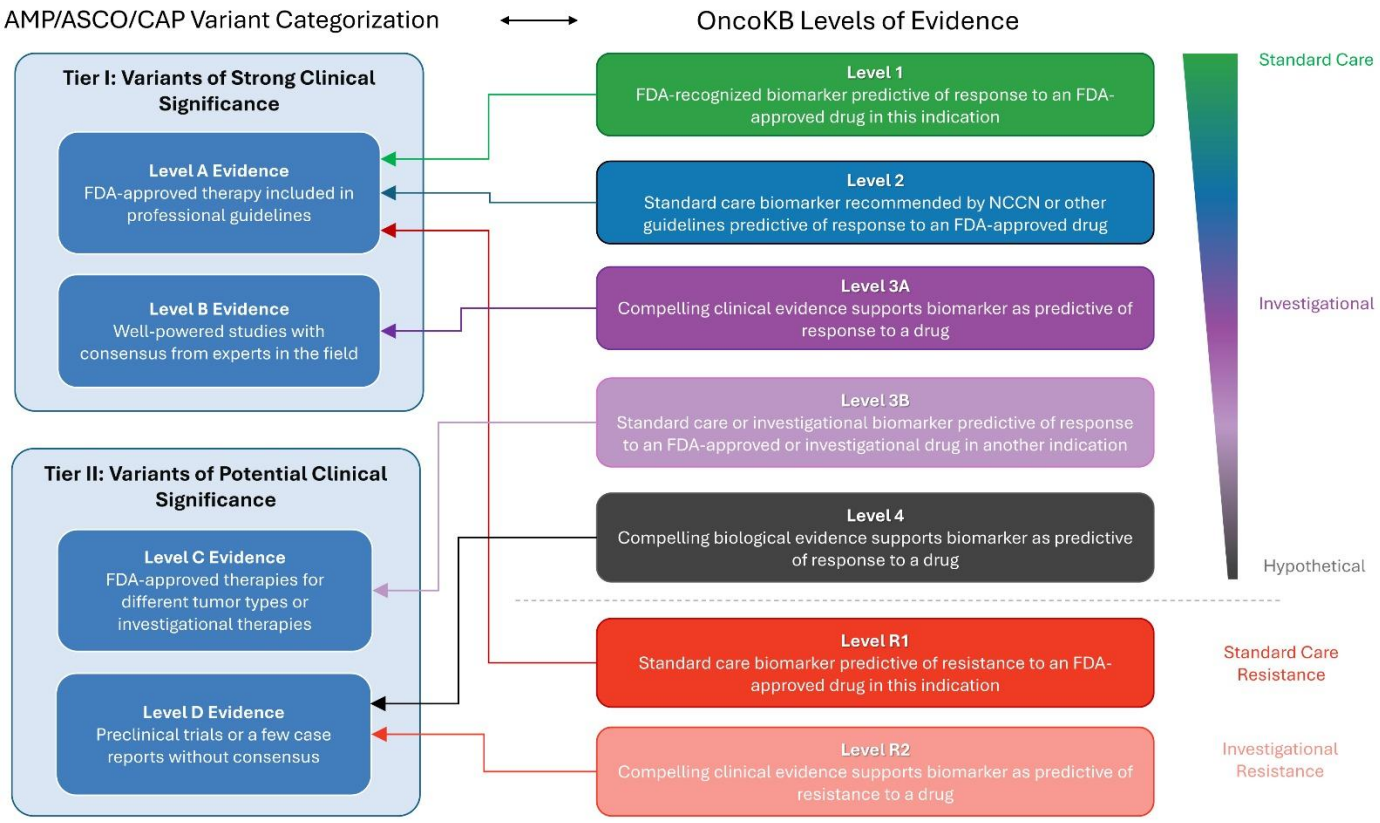


**Figure 1.** Joint consensus recommendation of AMP, ACMG, ASCO and CAP for reporting genetic variants in cancer. [1-2]

1. Li MM, Datto M, Duncavage EJ, et al. J Mol Diagn. 2017 Jan;19(1):4-23.
2. Leichsenring J, Horak P, Kreutzfeldt S, et al. Int J Cancer. 2019 Dec 1;145(11):2996-3010.

## ERBB2 (HER2) expression by IHC

ERBB2, a receptor tyrosine kinase, is altered by mutation, amplification and/or overexpression in various cancer types, most frequently in breast, esophagogastric and endometrial cancers. FDA has granted approval to several anti-ERRB2 regimens, either as single or combination therapy for various cancer types. In addition, recently FDA granted accelerated approval to fam-trastuzumab deruxtecan-nxki, for adult patients with unresectable or metastatic HER2-positive (IHC 3+) solid tumors who have received prior systemic treatment and have no satisfactory alternative treatment options.

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**Table S1.** ERBB2 Biomarkers associated with treatment response (LoE)

| Cancer Type | 3+ IHC | 2+ IHC & ≥2 FISH | ERBB2 amplification by NGS | IHC 1+ or IHC 2+/ISH -ve |
|---|---|---|---|---|
| **Breast Cancer** | Trastuzumab Deruxtecan (A)<br>Trastuzumab (A)<br>Ado-Trastuzumab Emtansine (A)<br>Lapatinib + Capecitabine (A)<br>Lapatinib + Letrozole (A)<br>Margetuximab + Chemotherapy (A)<br>Neratinib (A)<br>Neratinib + Capecitabine (A)<br>Trastuzumab + Tucatinib + Capecitabine (A)<br>Trastuzumab + Chemotherapy (A)<br>Trastuzumab + Pertuzumab + Chemotherapy (A) | Trastuzumab Deruxtecan (A)<br>Ado-Trastuzumab Emtansine (A)<br>Lapatinib + Capecitabine (A)<br>Lapatinib + Letrozole (A)<br>Margetuximab + Chemotherapy (A)<br>Neratinib (A)<br>Neratinib + Capecitabine (A)<br>Trastuzumab + Tucatinib + Capecitabine (A)<br>Trastuzumab + Chemotherapy (A)<br>Trastuzumab + Pertuzumab + Chemotherapy (A)<br>Trastuzumab (C) | Trastuzumab Deruxtecan (C)<br>Trastuzumab (C)<br>Ado-Trastuzumab Emtansine (C)<br>Lapatinib + Capecitabine (C)<br>Lapatinib + Letrozole (C)<br>Margetuximab + Chemotherapy (C)<br>Neratinib (C)<br>Neratinib + Capecitabine (C)<br>Trastuzumab + Tucatinib + Capecitabine (C)<br>Trastuzumab + Chemotherapy (C)<br>Trastuzumab + Pertuzumab + Chemotherapy (C) | Trastuzumab Deruxtecan (A) |
| **Colorectal Cancer** | Trastuzumab Deruxtecan (A)<br>Tucatinib + Trastuzumab (A)<br>Lapatinib + Trastuzumab (A)<br>Trastuzumab + Pertuzumab (A) | Tucatinib + Trastuzumab (A)<br>Lapatinib + Trastuzumab (A)<br>Trastuzumab + Pertuzumab (A)<br>Trastuzumab Deruxtecan (C) | Tucatinib + Trastuzumab (A) *(RAS/BRAF wild type)*<br>Trastuzumab Deruxtecan (C)<br>Lapatinib + Trastuzumab (C) *(RAS/BRAF wild type)*<br>Trastuzumab + Pertuzumab (C) *(RAS/BRAF wild type)* | N/A |
| **Gastric/GEJ** | Trastuzumab Deruxtecan (A)<br>Trastuzumab + Chemotherapy (A)<br>Pembrolizumab + Trastuzumab + Chemotherapy (A) *(PD-L1 with CPS>1% required)* | Pembrolizumab+ Trastuzumab + Chemotherapy (A) *(PD-L1 with CPS>1% required)*<br>Trastuzumab + Chemotherapy (A)<br>Trastuzumab Deruxtecan (A) | Pembrolizumab+ Trastuzumab + Chemotherapy (C) *(PD-L1 with CPS>1% required)*<br>Trastuzumab + Chemotherapy (C)<br>Trastuzumab Deruxtecan (C) | N/A |
| **Billiary Tract** | Trastuzumab Deruxtecan (A)<br>Zanidatamab-hrii (A)<br>Trastuzumab + Pertuzumab (A)<br>Tucatinib + Trastuzumab (A) | Trastuzumab Deruxtecan (C)<br>Zanidatamab-hrii (C)<br>Trastuzumab + Pertuzumab (A)<br>Tucatinib + Trastuzumab (A) | Trastuzumab Deruxtecan (C)<br>Zanidatamab-hrii (C)<br>Trastuzumab + Pertuzumab (C)<br>Tucatinib + Trastuzumab (C) | N/A |
| **Uterine Serous Carcinoma/ Papillary Serous** | Trastuzumab Deruxtecan (A)<br>Trastuzumab + Carboplatin-Taxol Regimen (A) | Trastuzumab Deruxtecan (C)<br>Trastuzumab + Carboplatin-Taxol Regimen (C) | Trastuzumab Deruxtecan (C)<br>Trastuzumab + Carboplatin-Taxol Regimen (C) | N/A |
| **Lung Cancer** | Trastuzumab Deruxtecan (A) | Trastuzumab Deruxtecan (C) | Trastuzumab Deruxtecan (C) | N/A |
| **All tumors** | Trastuzumab Deruxtecan (A) | Trastuzumab Deruxtecan (C) | Trastuzumab Deruxtecan (C) | N/A |

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## Microsatellite Instability (MSI)

Microsatellite instability (MSI) is a condition of genetic hypermutability resulting from impaired DNA mismatch repair (MMR) mechanisms. It is characterized by the accumulation of mutations in short repetitive DNA sequences known as microsatellites. MSI-H is found across multiple cancer types, most notably in colorectal, endometrial, gastric, and ovarian cancer. It can arise sporadically or as part of Lynch syndrome, a hereditary condition caused by germline mutations in MMR genes (MLH1, MSH2, MSH6, PMS2).

The results of MSI are divided into three types: MSI-H, which means microsatellites are highly unstable; MSS, which means microsatellites are stable; MSI-U, which means that the sample does not meet the MSI evaluation conditions (tissues or pleural fluid samples may not have passed the MSI indicator calculation quality control due to the low DNA and/or content of tumor cells).

Pembrolizumab has received full FDA approval for adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors((PMID: 35680043, 33264544) — representing the first full approval for an immunotherapy based on a predictive biomarker, regardless of solid tumor type. This approval applies to patients who have progressed after prior treatment and have no satisfactory alternative options. Beyond the tumor-agnostic indication, specific approvals have been granted for tumor types such as endometrial and colorectal cancer.

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## 9. Biomarker findings across all Levels of evidence (A-D)

| Biomarker | Result | Therapies with strong clinical significance | | Therapies with potential significance | | Therapies with potential resistance |
|---|---|---|---|---|---|---|
| | | Approved/ Standard of care therapies (Level A) | Therapies in well powered studies (Level B) | Off label/ Investigational therapies (Level C) | Therapies in pre-clinical trials (Level D) | |
| **KRAS** | Exon 2 c.35G>A (p.G12D) VAF: 9% | - | - | Avutometinib+Defactinib | Daraxonrasib<br>MRTX1133<br>Setidegrasib<br>Trametinib<br>Cobimetinib<br>Binimetinib | Cetuximab<br>Tucatinib+Trastuzumab<br>Panitumumab |

## 10. Treatment Table for Colon and Rectal non metastatic setting based on the NCCN guidelines (acc. June 2025)

| **Rectal Cancer** | | |
|---|---|---|
| **Phase** | **Indication** | **Treatment** |
| **Neoadjuvant** | dMMR/MSI-H or POLE/POLD1 mutation with ultra hypermutated phenotype (e.g., TMB > 50 mut/Mb):<br>- T3, N any<br>- T1-2, N1-2<br>- T4, N any<br>- Locally unresectable or medically inoperable | Checkpoint inhibitor immunotherapy for up to 6 months:<br>- Dostarlimab-gxly<br>- Nivolumab<br>- Pembrolizumab |
| **Adjuvant** | Stage II-III with PIK3CA mutation | Add aspirin 100-162 mg PO daily for 3 years following surgery |
| **Colon Cancer** | | |
| **Phase** | **Indication** | **Treatment** |
| **Neoadjuvant** | dMMR/MSI-H or POLE/POLD1 mutation with ultra hypermutated phenotype (e.g., TMB > 50 mut/Mb):<br>- Clinical T4b or bulky nodal disease | Consider checkpoint inhibitor immunotherapy (preferred) |
| | - Locally unresectable or medically inoperable | Checkpoint inhibitor immunotherapy (preferred). Evaluate for complete response or conversion to resectability |
| **Adjuvant** | dMMR/MSI-H or POLE/POLD1 mutation with ultra hypermutated phenotype (e.g., TMB > 50 mut/Mb):<br>- Tis, T1-4a, N0, M0 (Stage 0-IIB) | Observation |
| | - T4b, N0, M0 (Stage IIC) | Observation or consider adjuvant chemotherapy as for low-risk stage III disease |
| | Stage II-III with PIK3CA mutation | Add aspirin 100-162 mg PO daily for 3 years |

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