ONCOMPASS™ Medicine

Precision Oncology Report

ID	[redacted]
NAME	[redacted]
PRINTED AT	[redacted]

PATIENT INFORMATION

Patient ID: [redacted]
Name: [redacted]
Year of birth: 1958

Primary Tumor Site: gastric
Histology Type: adenocarcinoma
Metastatic sites: liver

MEDICAL TEAM

Treating Physician: [redacted]
Molecular Pharmacologist: István Peták, MD PhD
Genetic Counselor: Júlia Déri, MSc
Molecular Biologist: Edit Várkondi, PhD
Consulting Physician: Gábor Pajkos, MD CSc
Case Coordinator: Réka Czető
Biochemical Engineer: Barbara Dudás, MSc
Info-bionics Engineer: Anna Dirner, MSc

PATHOLOGICAL AND MOLECULAR DIAGNOSTIC TESTS

Sample ID: [redacted] (histological sample)
Sample source: primary tumor
Sampling type: biopsy
Tumor type: gastric adenocarcinoma

Sample ID: [redacted]
Sample source: circulating cell-free DNA isolated from blood
Sampling type: liquid biopsy
Tumor type: gastric adenocarcinoma

Tests performed:
NGS - 591 genes [redacted]
Total variants identified: 7051
Variant count after filtering: 33
MSI test (NGS-based) - MSS (microsatellite stable) [redacted]
TMB - low: 3.05 mutations/Mb [redacted]

Previous tests performed:
IHC - MLH1 lack of expression (with G168-728 antibody); PMS2 lack of expression (with MRQ-28 antibody); MSH2 normal expression (with G219-1129 antibody); MSH6 normal expression (with SP93 antibody) (22H2478)
IHC - PD-L1 overexpression (CPS=2 with SP263 antibody) (22H2478)
IHC - ERBB2++ expression (22H2478)
FISH - ERBB2 amplification absence (HER2/neu signals: 1.3) (22H2478)

PREVIOUS THERAPIES

1st line: FOLFOX
2nd line: PEMBROLIZUMAB

SUMMARY

Oncompass Report of [redacted], **1958** diagnosed with **gastric adenocarcinoma** has been completed for digital drug assignment and treatment planning purposes using the Realtime Oncology Treatment Calculator.

SUMMARY

The following molecular tests were used for our analysis:
ONCOMPASS ONCODRIVER assay (NGS-591) was carried out from liquid biopsy [redacted]
Previous IHC (ERBB2, MLH1, MSH2, MSH6, PMS2, PD-L1) and HER2 FISH tests were performed on histological sample of the primary tumor (22H2478).

Tumor-agnostic biomarkers/immunotherapy-related biomarkers:
The tumor is **MSS**, **TMB-low (3.05 mutations/megabase)**, and **PD-L1 positive (CPS=2 with SP263 antibody)**.
IHC results of the tumor: **MLH1 (Lack of Expression, with G168-728 antibody), PMS2 (Lack of Expression, with MRQ-28 antibody)**
Loss of expression of PMS2 alone is indicative of a defect in the PMS2 gene. However, the combined loss of PMS2 and MLH1 suggests the defect lies in MLH1, as MLH1 is responsible for the stability of PMS2. According to clinical data, immunotherapies with PD-1 and PD-L1 inhibitors proved to be effective in **MMR deficient tumors**. Mutational signature analysis has been performed on the filtered variants of the NGS results and identified a significant fraction of the variants fit to **Signature 6** associated with defective DNA mismatch repair, which might support immunotherapy. In addition, in the present sample **1 frameshift mutation** was detected that is located **in an NMD-resistant position (KMT2D-P565fs*365)**, thus, the emergence of a 365-amino-acid neopeptide is likely, that could sensitize cells for immunotherapy via generating neoantigens.
NIVOLUMAB, in combination with chemotherapy, is approved as a first-line treatment for metastatic gastric, GEJ, and esophageal adenocarcinoma with PD-L1 overexpression combined positive score (CPS) 5 according to the EMA approval and irrespective of the PD-L1 expression according to the FDA-approval. In a phase Ib study **nivolumab + regorafenib** combinational therapy reached 44% response rate (11 /25) in heavily treated, microsatellite stable gastric cancer patients (KRAS status was not examined). In the EPOC1706 phase II trial, the combination of **lenvatinib** (multi tyrosine kinase inhibitor) **and pembrolizumab** showed anti-tumor activity in patients with advanced gastric cancer as a first- or second-line treatment. Objective response was observed in 20 (69%) of 29 patients (1 complete response (CR) and 19 partial responses (PR)), and stable disease was observed in 9 patients (31%), median PFS was 7.1 months. Response rates were 84% in patients with PD-L1 overexpression, and 40% in patients with normal PD-L1 expression. In a phase II trial, the efficacy and safety of lenvatinib plus pembrolizumab were evaluated in patients with advanced gastric cancer, who received at least 2 prior lines of therapy. PD-L1 positivity was detected in 71% of the patients. The ORR was 10%. One patient had CR (3%), two had a PR (6%) and 12 patients (39%) had stable disease (SD). Disease control rate (DCR) was 48%, median PFS was 2.5 months, median OS was 5.9 months.
NTRK fusions were not detected in the tested sample.
BRAF-V600E mutation was not detected during the molecular test.
The detected ERBB2-V842I mutation may cause resistance to immunotherapy.

Tumor-specific on-label biomarkers:
TRASTUZUMAB is registered in HER2-positive gastric and gastroesophageal junction (GEJ) tumors. TRASTUZUMAB DERUXTECAN is registered by the FDA in patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma after treatment with trastuzumab. Pembrolizumab was granted accelerated approval for use in combination with trastuzumab and fluoropyrimidine- and platinum-containing chemotherapy for the first-line treatment of patients with locally advanced unresectable or metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma.
The tumor is **HER2 IHC negative**. However, based on the NGS results, the tumor is **ERBB2-V842I** mutant and based on previous IHC it is **Her2I ow ++, FISH negative.**

Histology-based on-label treatments independent of the molecular profile:
Nivolumab is **FDA** approved in combination with fluoropyrimidine- and platinum-containing chemotherapy as a frontline treatment for patients with advanced or metastatic gastric cancer, GEJ cancer, and esophageal adenocarcinoma **independent of PD-L1 expression status.**
RAMUCIRUMAB is an approved VEGFR2 inhibitor with paclitaxel in gastric adenocarcinoma.
Lonsurf is a chemotherapeutic agent approved for gastric or GEJ adenocarcinoma patients, who have been previously treated with at least two prior systemic treatment regimens for advanced disease.

Based on the NGS results, the following additional results could be relevant as off-label treatments:
ERBB2-V842I driver (AEL: 525.65, AF/TR: 4.22%/NA) is a pathogenic alteration. It is an activating mutation in the kinase domain. In colorectal cancer cell lines, the variant caused resistance against cetuximab and panitumumab, but is was sensitive to neratinib or afatinib. The mutation was not sensitive to trastuzumab. HER2 inhibitors in clinical use are TRASTUZUMAB, PERTUZUMAB, LAPATINIB, T-DM1, AFATINIB, MARGETUXIMAB and NERATINIB. HER2 activation causes resistance against EGFR inhibitor monotherapies.

SUMMARY

According to the ClinVar database, **FGFR2-C382R driver (AEL: 67.40, AF/TR: 7.21%/NA)** is a likely pathogenic alteration. The mutation affects the transmembrane domain of the FGFR protein, resulting in gain of function that causes oncogenic transformation in cellular experimental systems and is sensitive to FGFR2 inhibition. Based on a case study, an intrahepatic cholangiocarcinoma patient carrying C382R mutation showed partial response to pemigatinib.

For gain of function FGFR mutations, FGFR inhibitors may be effective. Multi-tyrosine kinase inhibitors in clinical use that inhibit the FGFR signaling pathway include LENVATINIB, NINTEDANIB, PAZOPANIB, REGORAFENIB, and PONATINIB, and are less specific than SORAFENIB and SUNITINIB. The FDA-approved FGFR inhibitor in the indication of urothelial tumors is ERDAFITINIB.

The detected **ARID2-R1272* driver (AEL: 15.52, AF/TR: 2.96%/NA)** nonsense mutation alteration is listed as pathogenic in the ClinVar database, in association with Coffin-Siris syndrome. It is located in an NMD-resistant position. Preclinical results suggest that ARID2 deficiency sensitizes to PARP inhibition and to cisplatin and etoposide.

According to the ClinVar database, **DNMT3A-W297* driver (AEL: 11.26, AF/TR: 9.77%/NA)** is a likely pathogenic alteration. In the presence of this nonsense mutation, loss of function is highly likely. In the case of DNMT3A loss-offunction mutations, DOT1L target gene and pinometostat agent can be mentioned in positive association.

SMARCA4-R1077* driver (AEL: 10.76, AF/TR: 3.18%/NA) nonsense mutation t is a likely pathogenic alteration. In case of its loss-of-function alterations, indirect targets can be mentioned in positive association. According to preclinical data, SMARCA2 (BRM), EZH2, or AURKA inhibition might be effective in SMARCA4 mutant cancers.

KMT2D-P565fs*365 driver (AEL: 9.48, AF/TR: 3.33%/NA) frameshift mutation is located in an NMD-resistant position. According to preclinical evidence, KMT2D-deficiency sensitizes to the non-chemotherapeutic agent AICAR (aminoimidazole-carboxamideribonucleotide). AICAR is an AMP homolog, that inhibits angiogenesis and induces apoptosis through activating the protein AMPK, and thereby inhibits tumor growth. AICAR treatment proved to be effective in a clinical trial involving B-cell chronic lymphocytic leukemia patients.

ARID1A-Q766fs*67 driver (AEL: 7.62, AF/TR: 3.03%/NA) is not listed in the ClinVar database. Loss of function is highly likely in the presence of this frameshift alteration. According to a study, higher TMB values and higher PD-L1 expression was found in ARID1A mutant gastrointestinal (GI) tumors, than in ARID1A-wildtype GI cancers. PD-L1 inhibitors have been shown to be more efficient in ARID1A mutant mouse models than in wild-type ones. EZH2, YES1, PI3K/AKT, and PARP inhibitors are also in positive association with ARID1A inactivation. ARID1A loss is in synthetic lethal interaction with dasatinib, a compound in clinical use.

Several other alterations were identified and classified as non-driver or variant of unknown significance. The role and significance of these alterations are not clear, however, their contribution to tumorgenesis cannot be ruled out.

There was no relevant copy number variation detected in the examined sample.

Based on the histology, molecular profile, and DDA, the following treatments could be considered:

Pembrolizumab therapy is ongoing, which is supported by the previously detected MMRdeficiency status and CPS 2 score as well as ARID1A-Q766fs*67 stop mutations identified by NGS.

Pathogenic mutations were not identified in MLH1 or PMS2 genes. Based on the NGS it is not considered MSI-H. Germline testing for the MMR genes may still be warranted as a separate test to confirm or rule out Lynch syndrome.

Additional treatment option: **Trastuzumab deruxtecan** off-label due to the HER2-low status (++ designated in MSD) and **ERBB2-V842I** mutation could be considered (the identified ERBB2-V842I alteration and ++ HER2low status may not show sensitivity to trastuzumab).

Adding HER2 inhibitors, such as trastuzumab deruxtecan to pembrolizumab due to the HER2-low status (++ designated in MSD) and **ERBB2-V842I** mutation could be considered. Immunotherapy and ERBB2 inhibitors combined are synergistic.

FGFR2 aberrations are becoming important biomarkers in certain tumor types such as bladder and cholangiocarcinoma.

There is a pan-FGFRinhibitor local trial with immunotherapy in gastric cancer https://clinicaltrials.gov/ct2/show/NCT04604132 A Phase 1b/2 Study of Derazantinib as Monotherapy and Combination Therapy With Paclitaxel, Ramucirumab or Atezolizumab in Patients With HER2-negative Gastric Adenocarcinoma Expressing FGFR2 Genetic Aberrations (unfortunately, prior PD-L1 inhibitor use is not allowed.

Another interesting international clinical trial is with Bemarituzumab looking for FGFR2b overexpression in gastric cancer https://clinicaltrials.gov/ct2/show/NCT05052801

We cannot tell from this NGS if there is overexpression of FGFR2b (it would need additional IHC) but there is an FGFR2 activating mutation.

MOLECULAR TARGET ANALYSIS

MOLECULAR ALTERATIONS

PD-L1 protein overexpression driver (AEL: 731.66, AF/TR: NA/NA),
ERBB2-V842I driver (AEL: 525.65, AF/TR: 4.22%/NA),
MLH1 protein lack of expression driver (AEL: 190.54, AF/TR: NA/NA),
PMS2 protein lack of expression driver (AEL: 122.64, AF/TR: NA/NA),
FGFR2-C382R driver (AEL: 67.40, AF/TR: 7.21%/NA),
ARID2-R1272* driver (AEL: 15.52, AF/TR: 2.96%/NA),
DNMT3A-W297* driver (AEL: 11.26, AF/TR: 9.77%/NA),
SMARCA4-R1077* driver (AEL: 10.76, AF/TR: 3.18%/NA),
KMT2D-P565fs*365 driver (AEL: 9.48, AF/TR: 3.33%/NA),
ARID1A-Q766fs*67 driver (AEL: 7.62, AF/TR: 3.03%/NA),
FBXO11-I520fs*15 driver (AEL: 5.05, AF/TR: 2.93%/NA),
KMT2D-R3547C VUS in a driver gene (AEL: 4.48, AF/TR: 3.26%/NA),
KMT2D-P2363del VUS in a driver gene (AEL: 4.48, AF/TR: 3.88%/NA),
CHEK2-C324W VUS in a driver gene (AEL: 3.52, AF/TR: 50.75%/NA),
KMT2C-R254C VUS in a driver gene (AEL: 2.00, AF/TR: 3.89%/NA),
TET2-S1760del VUS in a driver gene (AEL: 1.79, AF/TR: 2.46%/NA),
BTK-N172I VUS in a driver gene (AEL: 1.08, AF/TR: 45.45%/NA),
AR-G326C VUS in a driver gene (AEL: 0.49, AF/TR: 2.6%/NA),
TNFAIP3-V19L VUS in a driver gene (AEL: 0.31, AF/TR: 49.39%/NA),
LRP1B-P3015L VUS in a driver gene (AEL: 0.22, AF/TR: 2.93%/NA),
PPARG-L171F VUS in a driver gene (AEL: 0.20, AF/TR: 50.64%/NA),
RECQL5-M512I VUS in a driver gene (AEL: 0.07, AF/TR: 45.92%/NA),
DMD-K3200R VUS in a driver gene (AEL: 0.07, AF/TR: 2.51%/NA),
DMD-R3436C VUS in a driver gene (AEL: 0.07, AF/TR: 2.62%/NA),
CSMD3-E13D VUS in a driver gene (AEL: 0.02, AF/TR: 48.33%/NA),
FAT3-R894Q variant of unknown significance (AEL: 0.00, AF/TR: 50.25%/NA),
RPTOR-A210T variant of unknown significance (AEL: 0.00, AF/TR: 50.46%/NA),
BCL6-E164D variant of unknown significance (AEL: 0.00, AF/TR: 48.55%/NA),
DCC-R201Q variant of unknown significance (AEL: 0.00, AF/TR: 2.86%/NA),
WDCP-H648R variant of unknown significance (AEL: 0.00, AF/TR: 45.28%/NA),
JUN-S37fs*69 variant of unknown significance (AEL: 0.00, AF/TR: 2.43%/NA),
CYP2D6-R380H variant of unknown significance (AEL: 0.00, AF/TR: 30.48%/NA),
CUBN-G599S variant of unknown significance (AEL: 0.00, AF/TR: 4.09%/NA),
ABCC2-S281N variant of unknown significance (AEL: 0.00, AF/TR: 47.97%/NA),
SLC22A2-V5M variant of unknown significance (AEL: 0.00, AF/TR: 50.78%/NA),
KIT-M541L non-driver (AEL: -20.20, AF/TR: 99.91%/NA)

TARGET GENES

ERBB2 wild-type (AEL: 1136.13),
- ERBB2-V842I driver (AEL: 525.65)

CD274 wild-type (AEL: 806.61),
- MLH1 protein lack of expression driver (AEL: 190.54) ;
- ERBB2-V842I driver (AEL: -525.65) ;
- ARID1A-Q766fs*67 driver (AEL: 7.62) ;
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- TET2-S1760del VUS in a driver (AEL: -1.79) ;
- PMS2 protein lack of expression driver (AEL: 122.64)

PD-1 wild-type (AEL: 610.30),
- ERBB2-V842I driver (AEL: -525.65) ;
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- PMS2 protein lack of expression driver (AEL: 122.64) ;
- MLH1 protein lack of expression driver (AEL: 190.54)

PARP1 wild-type (AEL: 54.08),
- CHEK2-C324W VUS in a driver (AEL: 3.52) ;
- ARID1A-Q766fs*67 driver (AEL: 7.62) ;
- ARID2-R1272* driver (AEL: 15.52) ;
- KMT2D-R3547C VUS in a driver (AEL: 4.48) ;
- RECQL5-M512I VUS in a driver (AEL: 0.07) ;
- KMT2D-P2363del VUS in a driver (AEL: 4.48) ;
- KMT2C-R254C VUS in a driver (AEL: 2.00) ;
- KMT2D-P565fs*365 driver (AEL: 9.48)

EZH2 wild-type (AEL: 23.87),
- SMARCA4-R1077* driver (AEL: 10.76) ;
- ARID1A-Q766fs*67 driver (AEL: 7.62)

KDM1A wild-type (AEL: 18.97),
- SMARCA4-R1077* driver (AEL: 10.76) ;
- ARID1A-Q766fs*67 driver (AEL: 7.62)

DOT1L wild-type (AEL: 13.16),
- DNMT3A-W297* driver (AEL: 11.26)

SMARCA2 wild-type (AEL: 11.65),
- SMARCA4-R1077* driver (AEL: 10.76)

PPARG wild-type (AEL: 8.68),
- PPARG-L171F VUS in a driver (AEL: 0.20)

AKT1 wild-type (AEL: 8.20),
- ARID1A-Q766fs*67 driver (AEL: 7.62)

YES1 wild-type (AEL: 8.14),
- ARID1A-Q766fs*67 driver (AEL: 7.62)

SOD1 wild-type (AEL: 4.67),
- CHEK2-C324W VUS in a driver (AEL: 3.52)

BRD4 wild-type (AEL: 4.14),
- KMT2C-R254C VUS in a driver (AEL: 2.00)

NFKB1 wild-type (AEL: 1.27)
- TNFAIP3-V19L VUS in a driver (AEL: 0.31)

DRUGS WITH THE HIGHEST AEL SCORES

DRUGS IN CLINICAL USE

PEMBROLIZUMAB (skin - Merkel cell carcinoma (MCC) [FDA]; all - mediastinal B-cell lymphoma [FDA]; breast - all [FDA+EMA]; lung - non-small cell carcinoma [FDA+EMA]; skin - squamous cell carcinoma [FDA]; all - Hodgkin lymphoma [FDA+EMA]; kidney - renal cell carcinoma [FDA+EMA]; all - malignant melanoma [FDA+EMA]; bile duct - all [EMA]; lung - adenocarcinoma [FDA+EMA]; cervix - all [FDA+EMA]; rectum - all [FDA+EMA]; gastroesophageal junction - adenocarcinoma [FDA+EMA]; all - endometrioid carcinoma [FDA+EMA]; head-neck - squamous cell carcinoma [FDA+EMA]; esophagus - carcinoma [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]; colon - all [FDA+EMA]; lung - squamous cell carcinoma [FDA+EMA]; biliary tract - all [EMA]; all - endometroid carcinoma [FDA+EMA]; all - cholangiocarcinoma [EMA]; esophagus - squamous cell carcinoma [FDA+EMA]; gastric - all [EMA]; all - urothelial carcinoma [FDA+EMA]; liver - hepatocellular carcinoma [FDA]; endometrium - all [FDA+EMA]; small intestine - all [EMA]) (AEL: 10178.32)
- PD-1 wild-type target (AEL: 610.30) ;
- PMS2 protein lack of expression driver (AEL: 122.64) ;
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- MLH1 protein lack of expression driver (AEL: 190.54)

NIVOLUMAB (all - urothelial carcinoma [FDA+EMA]; head-neck - squamous cell carcinoma [FDA+EMA]; lung - non-small cell carcinoma [FDA+EMA]; esophagus - squamous cell carcinoma [FDA+EMA]; bone marrow - Hodgkin lymphoma [FDA+EMA]; rectum - all [FDA+EMA]; liver - hepatocellular carcinoma [FDA]; kidney - renal cell carcinoma [FDA+EMA]; gastroesophageal junction - adenocarcinoma [FDA+EMA]; esophagus - adenocarcinoma [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]; all - malignant melanoma [FDA+EMA]; colon - all [FDA+EMA]; pleura - mesothelioma [FDA+EMA]) (AEL: 4028.22)
- MLH1 protein lack of expression driver (AEL: 190.54) ;
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- PD-1 wild-type target (AEL: 610.30)

ATEZOLIZUMAB (all - malignant melanoma [FDA]; breast - all [EMA]; soft tissue - alveolar soft part sarcoma (ASPS) [FDA]; lung - non-small cell carcinoma [FDA+EMA]; liver - hepatocellular carcinoma [FDA+EMA]; lung - small cell carcinoma [FDA+EMA]; all - urothelial carcinoma [EMA]) (AEL: 3538.09)
- CD274 wild-type target (AEL: 806.61) ;
- PD-L1 protein overexpression driver (AEL: 731.66)

AVELUMAB (kidney - renal cell carcinoma [FDA+EMA]; bladder - urothelial carcinoma [FDA+EMA]; ureter - all [FDA+EMA]; bladder - all [FDA+EMA]; skin - Merkel cell carcinoma (MCC) [FDA+EMA]) (AEL: 1780.69)
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- CD274 wild-type target (AEL: 806.61)

TRASTUZUMAB DERUXTECAN (gastroesophageal junction - adenocarcinoma [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]; lung - adenocarcinoma [FDA]; lung - non-small cell carcinoma [FDA]; breast - all [FDA+EMA]) (AEL: 1719.56)
- ERBB2 wild-type target (AEL: 1136.13) ;
- ERBB2-V842I driver (AEL: 525.65)

TRASTUZUMAB EMTANSINE (breast - all [FDA+EMA]) (AEL: 1703.30)
- ERBB2-V842I driver (AEL: 525.65) ;
- ERBB2 wild-type target (AEL: 1136.13)

MOBOCERTINIB (lung - adenocarcinoma [FDA]) (AEL: 1661.88)
- ERBB2-V842I driver (AEL: 525.65) ;
- ERBB2 wild-type target (AEL: 1136.13)

DURVALUMAB (biliary tract - all [FDA+EMA]; lung - adenocarcinoma [FDA+EMA]; lung - small cell carcinoma [FDA+EMA]; liver - hepatocellular carcinoma [FDA+EMA]; lung - squamous cell carcinoma [FDA+EMA]; lung - non-small cell carcinoma [FDA+EMA]; all - cholangiocarcinoma [FDA]) (AEL: 1631.80)
- PD-L1 protein overexpression driver (AEL: 731.66) ;
- CD274 wild-type target (AEL: 806.61)

DOSTARLIMAB (all - endometrial carcinoma [FDA]; all - endometrioid carcinoma [EMA]; all - solid carcinoma [FDA]; endometrium - all [FDA+EMA]; all - solid [FDA]) (AEL: 963.48)
- PMS2 protein lack of expression driver (AEL: 122.64) ;
- MLH1 protein lack of expression driver (AEL: 190.54) ;
- PD-1 wild-type target (AEL: 610.30)

DRUGS WITH THE LOWEST AEL SCORES

DRUGS IN CLINICAL USE

PANITUMUMAB (rectum - all [FDA+EMA]; colon - all [FDA+EMA]) (AEL: -1184.83)
- ERBB2-V842I driver (AEL: -525.65) ;
- EGFR wild-type target (AEL: -601.26)

CETUXIMAB (head-neck - squamous cell carcinoma [FDA+EMA]; colon - all [FDA+EMA]; rectum - all [FDA+EMA]) (AEL: -1184.83)
- EGFR wild-type target (AEL: -601.26) ;
- ERBB2-V842I driver (AEL: -525.65)

ERLOTINIB (pancreas - all [FDA+EMA]; lung - adenocarcinoma [FDA+EMA]; lung - non-small cell carcinoma [FDA+EMA]; lung - squamous cell carcinoma [FDA+EMA]) (AEL: -1125.34)
- EGFR wild-type target (AEL: -601.26) ;
- ERBB2-V842I driver (AEL: -525.65)

TAMOXIFEN (breast - all [FDA]) (AEL: -673.37)
- ERBB2-V842I driver (AEL: -525.65) ;
- ESR1 wild-type target (AEL: -69.79) ;
- FGFR2-C382R driver (AEL: -67.40)

PALBOCICLIB (breast - all [FDA+EMA]) (AEL: -536.56)
- ERBB2-V842I driver (AEL: -525.65)

FULVESTRANT (breast - all [FDA+EMA]) (AEL: -536.32)
- ERBB2-V842I driver (AEL: -525.65)

INFIGRATINIB (all - cholangiocarcinoma [FDA]) (AEL: -228.64)
- FGFR2-C382R driver (AEL: -67.40) ;
- FGFR2 wild-type target (AEL: -158.97)

ZANUBRUTINIB (all - small lymphocytic lymphoma [FDA]; all - marginal zone lymphoma [FDA+EMA]; all - lymphoplasmacytic lymphoma [FDA+EMA]; all - chronic lymphocytic leukemia (CLL) [FDA+EMA]; all - mantle cell lymphoma [FDA]) (AEL: -205.73)
- BTK wild-type target (AEL: -207.07)

ACALABRUTINIB (all - chronic lymphocytic leukemia (CLL) [FDA+EMA]; all - mantle cell lymphoma [FDA]; all - small lymphocytic lymphoma [FDA]) (AEL: -205.32)
- BTK-N172I VUS in a driver (AEL: -1.08) ;
- BTK wild-type target (AEL: -207.07)

IBRUTINIB (all - mantle cell lymphoma [FDA+EMA]; all - lymphoplasmacytic lymphoma [FDA+EMA]; all - small lymphocytic lymphoma [FDA+EMA]; all - chronic lymphocytic leukemia (CLL) [FDA+EMA]; all - marginal zone lymphoma [FDA]) (AEL: -201.18)
- BTK wild-type target (AEL: -207.07) ;
- BTK-N172I VUS in a driver (AEL: -1.08)

DRUGS WITH THE HIGHEST AEL SCORES

TRASTUZUMAB (breast - all [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]; gastroesophageal junction - adenocarcinoma [FDA+EMA]) (AEL: 552.56)
- ERBB2 wild-type target (AEL: 1136.13) ;
- ERBB2-V842I driver (AEL: -525.65)

OLAPARIB (ovary - all [FDA+EMA]; peritoneum - all [FDA+EMA]; breast - all [FDA+EMA]; prostate - all [FDA+EMA]; pancreas - all [FDA+EMA]; fallopian tube - all [FDA+EMA]) (AEL: 106.45)
- CHEK2-C324W VUS in a driver (AEL: 3.52) ;
- KMT2C-R254C VUS in a driver (AEL: 2.00) ;
- PARP1 wild-type target (AEL: 54.08)

RAMUCIRUMAB (gastroesophageal junction - adenocarcinoma [FDA+EMA]; liver - hepatocellular carcinoma [FDA+EMA]; lung - adenocarcinoma [FDA+EMA]; rectum - all [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]; colon - all [FDA+EMA]) (AEL: 16.44)

TAS-102 (gastroesophageal junction - adenocarcinoma [FDA+EMA]; colon - all [FDA+EMA]; rectum - all [FDA+EMA]; gastric - adenocarcinoma [FDA+EMA]) (AEL: 9.89)

REGORAFENIB (gastroesophageal junction - gastrointestinal stromal tumor (GIST) [FDA+EMA]; gastric - gastrointestinal stromal tumor (GIST) [FDA+EMA]; rectum - all [FDA+EMA]; liver - hepatocellular carcinoma [FDA+EMA]; esophagus - gastrointestinal stromal tumor (GIST) [FDA+EMA]; colon - all [FDA+EMA]) (AEL: 5.97)

DRUGS WITH THE LOWEST AEL SCORES

DRUGS IN CLINICAL DEVELOPMENT

POZIOTINIB (AEL: 1689.84)
- ERBB2 wild-type target (AEL: 1136.13) ;
- ERBB2-V842I driver (AEL: 525.65)

TARLOXOTINIB (AEL: 1667.55)
- ERBB2-V842I driver (AEL: 525.65) ;
- ERBB2 wild-type target (AEL: 1136.13)

PYROTINIB (AEL: 1662.03)
- ERBB2 wild-type target (AEL: 1136.13) ;
- ERBB2-V842I driver (AEL: 525.65)

BINTRAFUSP ALFA (AEL: 1576.69)
- CD274 wild-type target (AEL: 806.61) ;
- PD-L1 protein overexpression driver (AEL: 731.66)

TORIPALIMAB (AEL: 1481.95)
- PD-1 wild-type target (AEL: 610.30) ;
- PD-L1 protein overexpression driver (AEL: 731.66)

SINTILIMAB (AEL: 1479.40)
- PD-1 wild-type target (AEL: 610.30) ;
- PD-L1 protein overexpression driver (AEL: 731.66)

SUGEMALIMAB (AEL: 806.87)
- CD274 wild-type target (AEL: 806.61)

PACMILIMAB (AEL: 806.61)
- CD274 wild-type target (AEL: 806.61)

TIRAGOLUMAB (AEL: 761.26)
- PD-L1 protein overexpression driver (AEL: 731.66)

TISLELIZUMAB (AEL: 611.44)
- PD-1 wild-type target (AEL: 610.30)

DRUGS IN CLINICAL DEVELOPMENT

BRILANESTRANT (AEL: -605.90)
- ERBB2-V842I driver (AEL: -525.65) ;
- ESR1 wild-type target (AEL: -69.79)

MEHD7945A (AEL: -601.26)
- EGFR wild-type target (AEL: -601.26)

NIMOTUZUMAB (AEL: -601.26)
- EGFR wild-type target (AEL: -601.26)

TESEVATINIB (AEL: -601.26)
- EGFR wild-type target (AEL: -601.26)

ROCILETINIB (AEL: -526.07)
- ERBB2-V842I driver (AEL: -525.65)

NAZARTINIB (AEL: -526.07)
- ERBB2-V842I driver (AEL: -525.65)

Debio1347 (AEL: -227.24)
- FGFR2 wild-type target (AEL: -158.97) ;
- FGFR2-C382R driver (AEL: -67.40)

DOVITINIB (AEL: -226.71)
- FGFR2-C382R driver (AEL: -67.40) ;
- FGFR2 wild-type target (AEL: -158.97)

DTRMWXHS-12 (AEL: -207.07)
- BTK wild-type target (AEL: -207.07)

GDC-0853 (AEL: -207.07)
- BTK wild-type target (AEL: -207.07)

Compound scores displayed represent aggregated evidence levels (AEL). AEL represents the number, scientific impact and clinical relevance of evidence relations in the system, connecting tumor types, molecular alterations, targets and compounds. Individual evidence relation scores are normalized and weighted according to the degree of similarity of the parameters to the parameters of the given patient case. Compound AELs are obtained by aggregating all relevant associations (and AELs) between the specific compound, tumor type, drivers and targets. Compounds are listed in descending order of their AELs.
(Abbreviations: AEL - aggregated evidence level, AF - allele frequency, TR: tumor ratio)

ANALYZED MOLECULAR PROFILE

MUTANT GENES

ABCC2-S281N, AR-G326C, ARID1A-Q766FS*67, ARID2-R1272*, BCL6-E164D, BTK-N172I, CHEK2-C324W, CSMD3-E13D, CUBN-G599S, CYP2D6-R380H, DCC-R201Q, DMD-K3200R, DMD-R3436C, DNMT3A-W297*, ERBB2-V842I, FAT3-R894Q, FBXO11-I520FS*15, FGFR2-C382R, JUN-

ANALYZED MOLECULAR PROFILE

S37FS*69, KIT-M541L, KMT2C-R254C, KMT2D-P2363DEL, KMT2D-P565FS*365, KMT2D-R3547C, LRP1B-P3015L, PPARG-L171F, RECQL5-M512I, RPTOR-A210T, SLC22A2-V5M, SMARCA4-R1077*, TET2-S1760DEL, TNFAIP3-V19L, WDCP-H648R

WILD TYPE GENES

ABCB1, ABL1, ABL2, ABRAXAS1, ACVR1B, ACVRL1, ADGRB3, AGTRAP, AIP, AKAP9, AKT1, AKT2, AKT3, ALK, AMER1, AMPH, APC, APEX1, ARAF, ARFRP1, ARID1B, ASXL1, ATM, ATP11B, ATP4A, ATP6V0D2, ATR, ATRX, AURKA, AURKB, AXIN1, AXIN2, AXL, B2M, BAP1, BARD1, BAX, BAZ2B, BCL2, BCL2L1, BCL2L11, BCL2L2, BCL9, BCOR, BCORL1, BCR, BIRC2, BIRC3, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BTG1, BUB1B, CARD11, CASP8, CASR, CBFB, CBL, CBLB, CBLC, CCDC178, CCDC6, CCN6, CCND1, CCND2, CCND3, CCNE1, CD274, CD74, CD79A, CD79B, CDA, CDC27, CDC73, CDH1, CDK12, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CEP57, CHD1, CHD2, CHD4, CHD7, CHEK1, CHIC2, CIC, CIT, CREBBP, CRKL, CRLF2, CSF1R, CSNK2A1, CTCF, CTNNA1, CTNNB1, CUL3, CYLD, CYP19A1, CYP2A6, CYP2B6, CYP2C19, CYP2C9, DAXX, DCUN1D1, DDB2, DDR1, DDR2, DDX11, DDX3X, DICER1, DIS3L2, DOT1L, DPYD, DSE, ECT2L, EED, EGFR, ELMO1, EML4, EMSY, EP300, EPCAM, EPHA2, EPHA3, EPHA5, EPHA7, EPHB1, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ERRFI1, ESR1, ESR2, ESRP1, ETV6, EXOC2, EXT1, EXT2, EZH2, EZR, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FAT1, FBXO32, FBXW7, FGF10, FGF14, FGF19, FGF23, FGF3, FGF4, FGF5, FGF6, FGF9, FGFR1, FGFR3, FGFR4, FH, FLCN, FLT1, FLT3, FLT4, FN1, FOXA1, FOXL2, FOXO1, FOXP1, FRS2, FSTL5, FUBP1, FZD3, G6PD, GABRA6, GALNT17, GAS6, GATA1, GATA2, GATA3, GATA4, GATA6, GEN1, GID4, GLI1, GNA11, GNA13, GNAI2, GNAQ, GNAS, GNAT2, GOPC, GPC3, GPR78, GREM1, GRIN2A, GRM3, GRM8, GSK3B, GSTP1, GXYLT1, H3F3A, HGF, HIST1H3B, HNF1A, HOXB13, HRAS, HSD3B1, HSP90AA1, HSPH1, IDH1, IDH2, IFITM1, IFITM3, IGF1R, IGF2, IGF2R, IGSF10, IKBKE, IKZF1, IKZF4, IL2RA, IL2RB, IL2RG, IL6, IL6ST, IL7R, INHBA, INPP4B, IRAK4, IRF2, IRF4, IRS2, ITCH, JAK1, JAK2, JAK3, KAT6A, KDM4B, KDM5A, KDM5C, KDM6A, KDR, KEAP1, KEL, KIAA1549, KIF5B, KLF6, KLHL6, KMT2A, KNSTRN, KRAS, KREMEN1, LAMA2, LCK, LMO1, LPAR2, LRRK2, LTK, LYN, LZTR1, MAGI2, MAGI3, MAGOH, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MAP3K4, MAP4K3, MAP7, MAPK1, MAPK3, MAS1L, MAX, MCL1, MDM2, MDM4, MED12, MED13, MEF2B, MEN1, MET, MIER3, MITF, MLH1, MLLT3, MPL, MRE11, MSH2, MSH3, MSH6, MST1R, MTOR, MUC16, MUTYH, MYC, MYCL, MYCN, MYD88, MYO18A, MYO1B, NBN, NCOA2, NCOR1, NEK2, NELL2, NF1, NF2, NFE2L2, NFKBIA, NIPA2, NKX2-1, NKX2-8, NKX3-1, NOTCH1, NOTCH2, NOTCH3, NPM1, NRAS, NRCAM, NRG1, NSD1, NT5C2, NTRK1, NTRK2, NTRK3, NUP93, OR5L1, OTOP1, PAK3, PALB2, PAX3, PAX5, PAX7, PBRM1, PCBP1, PCGF2, PDCD1LG2, PDGFRA, PDGFRB, PDK1, PDZRN3, PHF6, PHOX2B, PIK3C2B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PLCG2, PMS1, PMS2, PNP, POLD1, POLE, POT1, PPM1L, PPP2R1A, PPP2R2A, PRDM1, PREX2, PRF1, PRKAR1A, PRKCI, PRKDC, PRKN, PRPF40B, PRSS8, PSMB1, PSMB2, PSMB5, PSMD1, PSMD2, PTCH1, PTEN, PTGFR, PTPN11, PTPN12, PTPRD, QKI, RAC1, RAC2, RAD21, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD54L, RAF1, RANBP2, RARA, RARB, RARG, RB1, RBM10, RECQL4, RET, RHBDF2, RHEB, RHOA, RICTOR, RIT1, RNF43, ROS1, RPS6KB1, RUNX1, RUNX1T1, RXRA, RXRB, RXRG, S1PR2, SAMD9L, SBDS, SCN11A, SDC4, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEC16A, SEPT9, SETBP1, SETD2, SF1, SF3A1, SF3B1, SH2B3, SHH, SHOC2, SLC22A1, SLC31A1, SLC34A2, SLC45A3, SLC7A8, SLC9A9, SLCO1B1, SLIT2, SLX4, SMAD2, SMAD3, SMAD4, SMARCB1, SMARCE1, SMC1A, SMC3, SMO, SNCAIP, SOCS1, SOS1, SOX10, SOX2, SOX9, SPEG, SPEN, SPOP, SPRED1, SPTA1, SRC, SRSF2, SSTR1, STAG2, STAT3, STAT4, STK11, SUFU, SUZ12, SYK, SYNE3, TACC3, TAF1, TAS2R38, TBX20, TBX3, TCERG1, TCF7L2, TENT5C, TERC, TERT, TFG, TGFBR2, THSD7B, TIAF1, TMEM127, TMPRSS2, TNFRSF14, TOP1, TOP2A, TP53, TP53BP1, TP63, TPM3, TPM4, TPMT, TRAF5, TRIO, TRRAP, TSC1, TSC2, TSHR, TYK2, U2AF1, U2AF2, UBR3, UGT1A1, USP16, USP25, VCL, VEGFA, VHL, WEE1, WNK2, WRN, WT1, WWP1, XPA, XPC, XPO1, XRCC2, YAP1, YES1, ZBED4, ZBTB2, ZFHX3, ZIC3, ZMYM3, ZNF2, ZNF217, ZNF226, ZNF473, ZNF595, ZNF703, ZRSR2

FISH/CNA/IHC POSITIVE GENES

MLH1 PROTEIN LACK OF EXPRESSION, PD-L1 PROTEIN OVEREXPRESSION, PMS2 PROTEIN LACK OF EXPRESSION

FISH/CNA/IHC NEGATIVE GENES

ABL1 TRANSLOCATION ABSENCE, ALK TRANSLOCATION ABSENCE, BCR TRANSLOCATION ABSENCE, BRAF TRANSLOCATION ABSENCE, BRD4 TRANSLOCATION ABSENCE, CD74 TRANSLOCATION ABSENCE, EGFR TRANSLOCATION ABSENCE, ERBB2 PROTEIN NORMAL, FGFR1 TRANSLOCATION ABSENCE, FGFR2 TRANSLOCATION ABSENCE, FGFR3 TRANSLOCATION ABSENCE, KIF5B TRANSLOCATION ABSENCE, MET TRANSLOCATION ABSENCE, MSH2 PROTEIN NORMAL, MSH6 PROTEIN NORMAL, NRG1 TRANSLOCATION ABSENCE, NTRK1 TRANSLOCATION ABSENCE, NTRK2 TRANSLOCATION ABSENCE, NTRK3 TRANSLOCATION ABSENCE, RAF1 TRANSLOCATION ABSENCE, RARA TRANSLOCATION ABSENCE, RET TRANSLOCATION ABSENCE, ROS1 TRANSLOCATION ABSENCE, TACC1 TRANSLOCATION ABSENCE, TACC3 TRANSLOCATION ABSENCE

MICROSATELLITE INSTABILITY

MSS

BIOMEDICAL INTERPRETATION

Liquid biopsy

Using liquid biopsy, circulating cell-free plasma DNA fragments can be analyzed to detect genomic changes. Tumor DNA constitutes only a small proportion of the circulating DNA, with the majority representing germline DNA derived from ruptured leukocytes or other benign cells. Currently , it cannot be determined to what extent a blood sample contains tumor DNA.

The amount of circulating tumor DNA depends on many factors, including stage and tumor mass – circulating tumor DNA levels are low in case of early stage, non-metastatic disease or small tumor volume. It can happen in any stage of the disease that the amount of circulating cell-free DNA in a blood sample does not reach the limit of detection with next-generation sequencing (1). Therefore, it is possible that driver mutations present in the histology sample are not detected with DNA analysis of liquid biopsy.

References:

(1) Luke JJ et al., Cell Free DNA Working Group. Realizing the potential of plasma genotyping in an age of genotype-directed therapies. J Natl Cancer Inst. 2014 Aug 8;106(8). PubMed PMID: 25106647

Result of the tumor mutational burden (TMB) analysis

BIOMEDICAL INTERPRETATION

The tumor mutational burden (TMB) value is 3.05 mutations/megabase. The calculation is based on the NGS analysis. Based on our database of calculated TMB values (n=1174), 67% of our cases had lower TMB values.

PEMBROLIZUMAB is approved by the FDA for the treatment of adult and pediatric patients with unresectable or metastatic TMB-high solid tumors.
The approval was based on the prospectively-planned retrospective analysis of the KEYNOTE-158 phase II trial (NCT02628067). According to study results, tissue TMB-high status (defined as 10 mutations/mb) was associated with improved outcomes with pembrolizumab monotherapy in previously treated, advanced solid tumor patients (n=790, 10 tumor types). The objective response rate was 29% (30/102) in the TMB-high group, 28% (23/81) in the TMB-high group excluding patients with high or unknown MSI status, and 6% in (43/688) in the TMB-low group. As of data cutoff with a median follow-up of 37.1 months, the median duration of response had not been reached in the TMB-high group and was 33.1 months in the TMB-low group. In this study, 13% of the tested patient were classified to be TMB-high and 87% to be TMB-low (1). In this study, 13% of the tested patients were classified as TMB-high and 87% as TMB-low (1). Because the numerical value of TMB is dependent on the applied NGS panel, we defined TMB-high status as TMB values higher than 87% of all samples previously tested.

Immunotherapy-treated patients (n=151) with various tumor types (n=17) were analyzed in a study. High TMB was defined as 20 mutations/mb. The RR (response rate) for patients with high vs. low/intermediate TMB was 22/38 (58%) vs. 23/113 (20%). Results were similar when anti-PD-1/PD-L1 monotherapy was analyzed (n=102 patients), with a positive correlation between higher TMB and more favorable outcome (2). A similar benefit was obtained upon analyzing microsatellite stable (MSS), high versus low/intermediate TMB samples from 60 patients (14 different histologies) treated with anti-PD-1/PD-L1 monotherapy, the median progression-free survival was 26.8 and 4.3 months (3).
Survival data of 1662 immunotherapy treated cancer patients were analyzed in a study. The top 20% of the TMB values were considered high in every histology group. Overall survival was significantly higher in the TMB-high group. Survival benefit was shown to be increasing with the level of TMB (4).

References:
(1) Marabelle A et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020 Sep 10:S1470-2045(20)30445-9. Epub ahead of print. PMID: 32919526
(2) Goodman AM et al., Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Mol Cancer Ther. 2017 Nov;16(11):2598-2608. Epub 2017 Aug 23. PMID: 28835386
(3) Goodman AM et al., Microsatellite-Stable Tumors with High Mutational Burden Benefit from Immunotherapy. Cancer Immunol Res. 2019 Oct;7(10):1570-1573. Epub 2019 Aug 12. PMID: 31405947
(4) Samstein RM, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019 Feb;51(2):202-206. Epub 2019 Jan 14. PMID: 30643254

The result of the MSI analysis
The tumor is microsatellite stable (MSS), microsatellite instability indicating mismatch-repair (MMR) deficiency was not detected. The result was determined by an NGS-based MSI detection method, that classifies MSI status based on the calculated MSI score.
The MSI score is determined by the ratio of unstable loci detected among total microsatellite loci analyzed (MSI score = N(unstable loci) / N(total loci)). Loci with insufficient coverage for instability calling are excluded from total loci. MSI status of the tumor is interpreted based on using a stability cutoff value of 0.2 for the MSI score. An MSI score lower than the cutoff value (MSI score < 0.2) is classified as MSS, while an MSI score greater than or equal to the cutoff (MSI score >= 0.2) is classified as MSI-HIGH.
In this analysis, the MSI score is below 0.2, so the sample is classified as MSS.

According to the scientific literature in the case of microsatellite unstable tumors, the efficacy of immunotherapies is higher compared to microsatellite stable tumors (1, 2).

References:
(1) Le DT et al., PD-1 blockade in mismatch repair deficient non-colorectal gastrointestinal cancers. J Clin Oncol 34, 2016 (suppl 4S; abstr 195)
(2) Le DT et al., PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015 Jun 25;372(26):2509-20. PubMed PMID: 26028255

PD-L1 overexpression - targets
There is correlation in several tumor-types between PD-L1 overexpression and the efficacy of PD-1 and PD-L1 inhibitory immunotherapies (1, 2).

BIOMEDICAL INTERPRETATION

PD-1 and PD-L1 inhibitors in clinical use are NIVOLUMAB, PEMBROLIZUMAB, AVELUMAB, ATEZOLIZUMAB, DURVALUMAB, CEMIPLIMAB, and DOSTARLIMAB.

References:

(1) Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther. 2015 Apr;14(4):847-56. doi: 10.1158/1535-7163.MCT-14-0983. Epub 2015 Feb 18. Review. PubMed PMID: 25695955.

(2) Herbst RS et al., Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014 Nov 27;515(7528): 563-7. doi: 10.1038/nature14011. PubMed PMID: 25428504

MMR deficiency

The analyzed genes are components of the DNA mismatch repair system (MMR). Loss of expression of PMS2 alone is indicative of a defect in the PMS2 gene. However, combined loss of PMS2 and MLH1 suggests the defect lies in MLH1, as MLH1 is responsible for the stability of PMS2. A similar situation is seen with MSH6 and MSH2, with loss of MSH6 only indicating defective MSH6, whereas loss of expression of both proteins would indicate the defect is within MSH2 (1).

According to clinical data, immunotherapies with PD-1 and PD-L1 inhibitors proved to be effective in MMR deficient tumors (2).

PEMBROLIZUMAB is a human PD-1-blocking antibody approved by the FDA indicated for the treatment of microsatellite instability-high or MMR deficient solid cancer progressing following standard treatment, the EMA label includes tumor type restrictions. DOSTARLIMAB (PD-1 inhibitor) is approved by the FDA for the treatment of patients with MMR deficient recurrent or advanced solid tumors progressing on or after prior therapy. DOSTARLIMAB is also approved for the treatment of patients with recurrent or advanced MSI-H (EMA inclusion only) or dMMR endometrial cancer who have progressed on or following platinum-based chemotherapy.

In case of MMR deficiency, resistance has been observed during treatment with chemotherapeutic agents, like 5-FU, cisplatin and carboplatin. However, there was no decrease observed in the efficacy of oxaliplatin (3).

According to a retrospective study, pretreatment lung immune prognostic index (LIPI) might identify fast-progressors to immune checkpoint inhibitory (ICI) treatment among MSI-High or MMR deficient patients (n=151, 66% gastrointestinal, 22% gynecological). In response to ICI therapy, 24-month OS rates were 71.1%, 54.2%, and 14.3%, and median PFS values were 20.9, 9.9, and 2.3 months for good, intermediate and poor LIPI risk groups, respectively (4).

In cohort F of the phase I GARNET trial, dostarlimab (anti-PD-1 antibody) resulted in an objective response rate (ORR) of 38.7% (41/106) among non-endometrial solid tumor patients with dMMR or POLE mutated status (5).

In the Z1D subprotocol arm of the phase II NCI-MATCH trial, the efficacy of nivolumab (PD-1 inhibitor) was investigated among patients with noncolorectal MMR-deficient solid tumors. The ORR was 36% (15/42), the disease control rate (DCR) was 57% (24/42), the estimated 12-month PFS rate was 46.2%, the median OS was 17.3 months (6).

References:

(1) Richman S. Deficient mismatch repair: Read all about it (Review). Int J Oncol. 2015 Oct;47(4):1189-202. Epub 2015 Aug 12. Review. PMID: 26315971

(2) Viale G et al., Mismatch Repair Deficiency as a Predictive Biomarker for Immunotherapy Efficacy. Biomed Res Int. 2017;2017:4719194. Epub 2017 Jul 10. Review. PMID: 28770222

(3) Devaud N, Gallinger S. Chemotherapy of MMR-deficient colorectal cancer. Fam Cancer. 2013 Jun;12(2):301-6. Review. PMID: 23539382

(4) Auclin E et al., 2P Lung immune prognostic index (LIPI) can identify the fast-progressor to immune checkpoints inhibitors (ICI) in microsatellite instability (MSI) or mismatch repair deficient (dMMR) tumours. Annals of Oncology. 2020;31(suppl_7):S1417-S1424. doi: 10.1016/j.annonc. 2020.10.487.

(5) Andre T et al., Safety and efficacy of anti–PD-1 antibody dostarlimab in patients (pts) with mismatch repair-deficient (dMMR) solid cancers: Results from GARNET study. Journal of Clinical Oncology. 2021;39(3_suppl):9-9. doi: 10.1200/JCO.2021.39.3_suppl.9.

(6) Azad NS et al., Nivolumab Is Effective in Mismatch Repair-Deficient Noncolorectal Cancers: Results From Arm Z1D-A Subprotocol of the NCI-MATCH (EAY131) Study. J Clin Oncol. 2020 Jan 20;38(3):214-222. Epub 2019 Nov 25. PMID: 31765263

Mutational Signatures Associated with Defective DNA Mismatch Repair (Signature 6)

Mutational signature analysis (1-3) has been performed on the filtered variants of the NGS results. A significant fraction of the variants fits to signatures associated with defective DNA mismatch repair (MMR-D): signatures 6, 15, 20, and 26 (Defective DNA MMR / MSI (small INDELs)). Immune checkpoint inhibition therapies are in positive association with MMR-D (4-6).

References:

BIOMEDICAL INTERPRETATION

(1) Alexandrov LB et al., Signatures of mutational processes in human cancer. Nature. 2013 Aug 22;500(7463):415-21. doi: 10.1038/nature12477. Epub 2013 Aug 14. PMID: 23945592

(2) Alexandrov LB et al., The repertoire of mutational signatures in human cancer. Nature. 2020 Feb;578(7793):94-101. doi: 10.1038/s41586-020-1943-3. Epub 2020 Feb 5. PMID: 32025018; PMCID: PMC7054213.

(3) cancer.sanger.ac.uk/cosmic/signatures

(4) Le DT et al., PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015 Jun 25;372(26):2509-20. doi: 10.1056/NEJMoa1500596. PMID: 26028255; PMCID: PMC4481136.

(5) Bouffet E et al., Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency. J Clin Oncol. 2016 Jul 1;34(19):2206-11. doi: 10.1200/JCO.2016.66.6552. Epub 2016 Mar 21. PMID: 27001570.

(6) Le DT et al., Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017 Jul 28;357(6349):409-413. doi: 10.1126/science.aan6733. PMID: 28596308; PMCID: PMC5576142.

Immunotherapies in gastric and gastroesophageal junction (GEJ) cancer

Higher PD-L1 expression is correlated with worse prognosis in gastric cancer (1).

PEMBROLIZUMAB is approved in combination with platinum- and fluoropyrimidine-based chemotherapy for the treatment of locally advanced unresectable or metastatic esophageal and GEJ cancer (EMA additional condition: HER2 negative, PD-L1 with a CPS 10) based on the results of the KEYNOTE-590 trial.

In the KEYNOTE-062 phase III clinical trial treating PD-L1 positive gastric or GEJ cancer patients, first line pembrolizumab monotherapy was noninferior to chemotherapy for median overall survival (OS) in patients with CPS of 1 or greater (10.6 vs 11.1 months), though it prolonged OS in patients with CPS of 10 or greater (17.4 vs 10.8 months) (2).

NIVOLUMAB, in combination with chemotherapy, is approved as a first-line treatment for metastatic gastric, GEJ, and esophageal adenocarcinoma with PD-L1 overexpression combined positive score (CPS) 5 according to the EMA approval and irrespective of the PD-L1 expression according to the FDA-approval. In the CheckMate 649 phase III clinical trial, in patients with PD-L1 positive (CPS >=5%) gastric, GEJ or esophageal adenocarcinoma, first line nivolumab + chemotherapy combination treatment resulted in a statistically significant improvement in OS and PFS compared with chemotherapy alone (median OS: 14.4 and 11.1 months; median PFS: 7.7 and 6.1 months) (3).

In a phase III placebo-controlled trial, the PD-1 blocker nivolumab resulted in significantly longer OS, PFS and higher response rate compared to placebo in non-selected pre-treated gastric or GEJ cancer patients (4). No data has been published regarding the relevance of PD-L1 expression in the efficacy of nivolumab. In the phase III CheckMate 577 trial, adjuvant nivolumab treatment improved disease-free survival (DFS) in resected esophageal or GEJ cancer following neoadjuvant chemoradiation therapy compared with placebo. According to the interim analysis, median DFS was 22.4 versus 11.0 months with nivolumab (n=532) and placebo (n=262), respectively (5). Based on the trial results, NIVOLUMAB was granted approval for the adjuvant treatment of patients with resected esophageal or GEJ cancer who have received neoadjuvant chemoradiotherapy.

In a phase III clinical trial (ATTRACTION-4) in HER2-negative gastric and GEJ cancer patients, first line nivolumab + chemotherapy combination therapy resulted in a statistically significant improvement in PFS compared with chemotherapy (median PFS: 10.45 and 8.34 months), but did not result in a statistically significant improvement in OS (median OS: 17.45 and 17.15 months) (6).

In a phase Ib study nivolumab + regorafenib combinational therapy reached 44% response rate (11/25) in heavily treated, microsatellite stable gastric cancer patients (KRAS status was not examined) (7).

According to a phase I/II trial, nivolumab + paclitaxel + ramucirumab demonstrated promising antitumor activity as the second-line treatment for advanced gastric cancer patients (8). The objective response rate (ORR) was 37.2%, the median PFS was 5.1 months.

In the EPOC1706 phase II trial, the combination of lenvatinib (multi tyrosine kinase inhibitor) and pembrolizumab showed anti-tumor activity in patients with advanced gastric cancer as a first- or second-line treatment. Objective response was observed in 20 (69%) of 29 patients (1 complete response (CR) and 19 partial responses (PR)), and stable disease was observed in 9 patients (31%), median PFS was 7.1 months. Respon se rates were 84% in patients with PD-L1 overexpression, and 40% in patients with normal PD-L1 expression (9).

In a phase II trial, the efficacy and safety of lenvatinib plus pembrolizumab were evaluated in patients with advanced gastric cancer, who received at least 2 prior lines of therapy. PD-L1 positivity was detected in 71% of the patients. The ORR was 10%. One patient had CR (3%), two had a PR (6%) and 12 patients (39%) had stable disease (SD). Disease control rate (DCR) was 48%, median PFS was 2.5 months, median OS was 5.9 months (10).

In a phase Ib study, the combination of AK104 (PD-1/CTLA-4 bispecific antibody) plus mXELOX (oxaliplatin and capecitabine) was investigated in untreated patients with advanced gastric and gastroesophageal junction (G/GEJ) adenocarcinoma, regardless of PD-L1 status. Of 24 patients evaluable for antitumor activity, ORR was 66.7% including 2 CRs and 14 PRs. The DCR was 95.8%. Response was seen regardless of PD-L1 status. At a median follow-up of 8.6 months, the 6-months PFS rate was 69.5% (11).

BIOMEDICAL INTERPRETATION

The FDA granted orphan drug designation to APX005M, a monoclonal antibody that stimulates the antitumor immune response, for the treatment of patients with gastroesophageal junction cancer.

The FDA granted orphan drug designation to the anti-CLDN18.2 autologous CAR T-cell agent, CT041, for the treatment of patients with gastric and gastroesophageal junction (GEJ) adenocarcinoma. In a phase I clinical trial patients with CLDN18.2 positive advanced gastric or pancreatic adenocarcinoma received CT041 treatment. Among the 11 patients, 1 achieved a CR (gastric adenocarcinoma), 3 had PRs (2 gastric adenocarcinomas and 1 pancreatic adenocarcinoma), 5 had SD and 2 had progression of disease. The ORR was 33.3%, with a DCR of 75%. Median PFS was 130 days (12).

In a phase III clinical trial patients with locally advanced or metastatic gastric or GEJ adenocarcinoma received sintilimab in combination with chemotherapy (S+C) or placebo plus chemotherapy (P+C) as first-line treatment. S+C showed a significant improvement in OS vs P+C in patients with CPS5 (median 18.4 vs 12.9 months) and all patients (median 15.2 vs 12.3 months). PFS was superior with S+C vs P+C in patients with CPS5 and all patients. The ORR was 72.8% in the S+C arm and 59.6% in the P+C arm in patients with CPS5 and 65.1% (S+C) vs 58.7% (P+C) in all patients (13).

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Molecular alterations and mechanisms associated with resistance / reduced efficacy of immunotherapies

BIOMEDICAL INTERPRETATION

Based on preclinical and clinical evidence, genetic alterations that may result in decreased efficacy or resistance to immunotherapies are loss of function mutations in the B2M (1), CBLB (2), JAK1/2 (3-6), NSD1 (7), PTEN (8, 9), CDKN2A (10) and STK11 (11-13) genes as well as deletion of TET2 (14), and the activation of the WNT/beta-catenin signalling pathway (15). IDO expression (16) and IFNGR1 gene loss (6) may induce resistance to CTLA-4 targeting immunotherapies. Furthermore, immunotherapies were shown to be ineffective in case of non-small cell lung cancer (NSCLC) tumors harboring EGFR (17, 18), or HER2 mutations (19), ROS1 translocations (19) and MET exon 14 skipping mutations (20). Immunotherapies were also ineffective in case of medullary thyroid carcinoma (MTC) and NSCLC tumors with RET fusions, and mutations (21, 22). Mutations in RB1 have also been associated with resistance to immunotherapies (23, 24), but further studies are needed to confirm this observation. Poor clinical outcome and hyperprogression have been reported in patients with MDM2, MDM4 or MYC amplifications after receiving immunotherapy (18, 25, 26). NTRK1 overexpression may also contribute to the development of resistance to immune checkpoint inhibitors (27).

Epigenetic processes can also contribute to immunotherapy resistance. Epidrugs can restore sensitivity to immunotherapies (28). In a murine melanoma model the combination of panobinostat, a HDAC inhibitor and an anti-PD-1 agent B16-F10 yielded better response rates than those obtained with either drug alone (29). Combination of HDAC inhibitors and anti-PD-1 drugs proved to be safe in phase I and II clinical trials (30-32). There are several ongoing clinical trials using this combination (vorinostat + pembrolizumab: NCT02638090, NCT02538510, NCT02909452, NCT02437136, NCT04357873, entinostat + pembrolizumab: NCT02453620, vorinostat + (pembrolizumab or nivolumab): NCT01928576, NCT02437136, belinostat + nivolumab: NCT04315155, mocatnostat + pembrolizumab: NCT03220477, NCT02954991, mocetinostat + durvalumab: NCT02805660, NCT02993991, panobinostat + spartalizumab: NCT02890069, citarinostat + nivolumab: NCT02635061, NCT02718066). Preliminary results from a randomized phase II trial comparing the combination of vorinostat with pembrolizumab versus pembrolizumab alone in metastatic non-small cell lung cancer patients having PD-L1 expression > 1% showed a higher ORR in the combination arm (48% versus 25%, *P* = 0.026) (31). The ENCORE 601 phase II study evaluated the combination of entinostat and pembrolizumab in melanoma patients pretreated with anti PD-1 drugs. The ORR was 19% with a median duration of response of 12.5 months (33).

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Result of the copy number variation (CNV) analysis

CNV analysis was performed within the NGS test. Copy number variation means, that the detected copy number is different from the normal copy number (n=2). With NGS-based technology only approximation of copy number variations is feasible.

There weren't any relevant copy number changes in the examined genes.

Results of the next generation sequencing (NGS)

The variants listed in the molecular profile were selected via bioinformatic and functional filtering.

These variants have been uploaded into the Realtime Oncology Calculator for further biomedical functional interpretation and medical decision support.

The following filters of the QIAGEN Clinical Insight Interpret software were used:

- CONFIDENCE: Filtering is based on variant call quality (QUAL), read depth (DP), allele fraction (computed from AD), upstream filter (PASS) and genotype quality (GQ). If the presence of a variant was uncertain based on the sequencing quality scores, the alteration was filtered out.
- COMMON VARIANTS: The filter is used to exclude variants that are commonly observed in the healthy population. If the frequency of a certain variant is at least 10% in the population according to the 1000 Genomes Project, the ExAC or the NHLBI ESP exomes database, it was excluded from further analysis.

BIOMEDICAL INTERPRETATION

- PREDICTED DELETERIOUS: The filter was used to identify variants in a dataset that have either predicted or observed evidence suggesting they could disrupt gene function or expression. The alterations, which are "benign" or "likely benign" according to the ACMG guideline were filtered out.
- CANCER DRIVER VARIANTS: The filter can be used to identify variants within a dataset that have predicted or established association with driving tumorigenesis or metastasis. Variants, which are related to cancer pathways, cell cycle regulation or cellular processes according to the scientific literature were selected. Alterations, which have been mentioned in the scientific literature related to cancer indication were also selected.

Other filtering methods used besides the Variant Analysis:
- Non-exonic alterations were excluded
- Further bioinformatic filtering was used considering other sequencing quality scores

The filtered variants are listed in the molecular profile of the patient.

Databases used for the interpretation of the detected alterations:

NCBI dbSNP (National Center for Biotechnology Information, Single Nucleotide Polymorphism database): Database dbSNP serves as a central repository for both single base nucleotide substitutions and short deletion and insertion polymorphisms detected as germline variants in either healthy population or in patients with various diseases (including, but not only cancer patients).
NCBI ClinVar: It is a publicly available archive of relations between human variations and phenotypes (clinical significance), with supporting evidence. It is not restricted to cancer diseases.
SNPEffect: This database contains the clinical relevance of single nucleotide mutations/polymorphisms based on OMIM and other databases and in silico predictions.
IARC (International Agency for Research on Cancer) TP53 Database: The IARC TP53 Database compiles various types of data and information on human TP53 gene variations related to cancer. Data is compiled from peer-reviewed literature and generalized databases. Functional classification of the mutations based on the overall transcriptional activity on 8 different promoters can also be found in the database.
BRCA Exchange: BRCA Exchange contains functional information about and classification of BRCA1 and BRCA2 mutations.
UniProt: UniProt is a knowledgebase of protein sequences and their function.

Functional interpretation of the detected alterations:

The detected genetic alterations were classified into the following categories by the Molecular Treatment Calculator (MTC), based on their functional consequences and their contribution to tumor formation (gains selective growth advantage compared to healthy cells): driver, variant of unknown significance in a driver gene (VUS, driver gene), non-confirmed driver, biomarker, variant of unknown significance (VUS), non-driver.

The algorithm calculates with positive score, in case of scientific evidence describing that a mutation or a gene contributes to cancer formation. It calculates with negative score, in case of scientific evidence describing that a mutation or a gene does not contribute to cancer formation.
The classification of a given variant is based on evidence describing the given alteration, the mutant gene or other specific mutations of the same gene as driver alterations. The algorithm summarizes and biases the related evidence and calculates the aggregated evidence level (AEL).

Driver: The algorithm classifies variants as drivers if there is available matching evidence in the database (describing the detected alteration) and it has a positive AEL.

Variant of unknown significance in a driver gene (VUS in a driver gene): In case of these variants there is no available matching evidence. The classification is based on evidence describing the mutant gene or other specific mutations of the same gene as drivers.

VUS (variant of unknown significance): There is no available evidence regarding the given alteration, the mutant gene or other specific mutations of the same gene.

Biomarker: These alterations are associated with the efficacy of a targeted drug based on matching scientific evidence (describing the detected alteration), but it does not fulfill the criteria to be a driver.

Conflicting driver: In case of these variants the number and level of the available matching evidence describing the detected alteration as a driver is limited.

BIOMEDICAL INTERPRETATION

Non-driver: The AEL values of these variants are negative.

ERBB2-V842I

According to the ClinVar database, this is a pathogenic alteration. It is an activating mutation in the kinase domain. In colorectal cancer cell lines, the variant caused resistance against cetuximab and panitumumab, but is was sensitive to neratinib or afatinib. The mutation was not sensitive to trastuzumab (1). The variant showed sensitivity to neratinib or lapatinib in a breast cell line (2). A breast cancer patient with ERBB2-V842I and ERBB2-S310F mutations had a stable disease longer than 30 weeks on neratinib treatment (3).

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ERBB2 (HER2) mutant gene - targets

HER2 inhibitors can be beneficial in HER2 mutant tumors (1). HER2 inhibitors in clinical use are TRASTUZUMAB, PERTUZUMAB, LAPATINIB, T-DM1, AFATINIB, MARGETUXIMAB, NERATINIB, TUCATINIB, and the anti-HER2 and topoisomerase-I inhibitor antibody-drug conjugate, TRASTUZUMAB DERUXTECAN.

HER2 activation causes resistance against EGFR inhibitor monotherapies and endocrine therapies.

In a phase II trial, TRASTUZUMAB DERUXTECAN showed efficacy in patients with central nervous system metastases (CNS subgroup: ORR: 58.3%, mPFS: 18.1 months) (2).

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ERBB2 (HER2) mutant gastric cancer

In a current clinical trial, neratinib is tested in HER2 mutation-positive or EGFR-amplified solid tumors (NCT01953926).

TRASTUZUMAB is registered in HER2-positive gastric and gastroesophageal junction (GEJ) tumors. TRASTUZUMAB DERUXTECAN is registered by the FDA in patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma after treatment with trastuzumab. Other HER2 inhibitors have been registered in the indication for breast and lung cancer.

Molecular alterations and mechanisms associated with resistance / reduced efficacy in case of HER2 inhibition

Based on preclinical and clinical evidence, decreased efficacy of, or resistance to HER2 inhibitors may arise due to various genetic alterations and mechanisms.

Different mutations in the HER2 gene might reduce the efficacy of different HER2 inhibitors or result in resistance (1-4).

Activating mutations in the following genes can be mentioned in a negative association with the efficacy of HER2 inhibitors: PIK3CA (1-3, 5, 6), AKT1 (3), PIK3R1 (3), and KRAS (7, 8).

The amplification and/or overexpression of the following genes might induce resistance to HER2 inhibitors: HER3 (1-3), EGFR (2, 3, 5), FGFR1 (6), FGF3/4/19 (6), PIK3CA (3), AKT2 (3, 9), IGF1R (2, 10, 11), MET (1, 3, 12), CDK12 (13, 14), CCND1 (15), MUC4 (1-3, 16), MIR4728 (17), PD-L1 (5). The increased activity of the CYP3A4 metabolizing enzyme (18), or the overexpression of the ABCB1 transporter protein (19) may also confer resistance to HER2 inhibitors. The activation of the PI3K-AKT pathway (1-3, 6, 7), MEK (2), MAPK (2), mTOR (2, 7), FGFR (6), or SRC (20) pathways may also result in reduced efficacy of HER2 inhibition.

Furthermore, loss-of-function alterations or lack of protein expression in the following genes, may also confer resistance to HER2 inhibitors: PTEN (1-3, 5, 19), INPP4B (3), CCNB1 (5), SLC46A3 (5, 19), HER2 (5, 19), PCGF2 (21), FOXO1 (22).

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FGFR2-C382R

According to the ClinVar database, it is a likely pathogenic alteration. This alteration has been detected in endometrial cancer (1), lung squamous cell carcinoma and cervical carcinoma (2). The mutation affects the transmembrane domain of the FGFR protein, resulting in gain of function that causes oncogenic transformation in cellular experimental systems and is sensitive to FGFR2 inhibition (2-4). An intrahepatic cholangiocarcinoma patient carrying C382R mutation showed partial response to pemigatinib (5).

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FGFR2 mutant gene – targets

FGFR2 (fibroblast growth factor receptor 2) is a member of the FGFR receptor tyrosine kinase gene family, and the FGFR2 protein it encodes acts as a receptor for fibroblast growth factor (FGF). The FGF ligand is bound by the extracellular region of the protein, which activates signaling pathways that regulate cell division through activation of cytoplasmic tyrosine kinase.

For gain of function FGFR mutations, FGFR inhibitors may be effective (1). Multi-tyrosine kinase inhibitors in clinical use that inhibit the FGFR signaling pathway include LENVATINIB, NINTEDANIB, PAZOPANIB, REGORAFENIB, and PONATINIB, and are less specific than SORAFENIB and SUNITINIB. The FDA-approved FGFR inhibitor in the indication of urothelial tumors is ERDAFITINIB.

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Molecular alterations and mechanisms associated with resistance / reduced efficacy in case of FGFR inhibition

Based on preclinical and clinical evidence, decreased efficacy of or resistance to FGFR inhibitors may arise due to various genetic alterations and mechanisms.

FGFR gatekeeper mutations (1-4) and secondary FGFR2 kinase domain mutations (5, 6) have been described in a negative association with the efficacy of FGFR inhibitors.

The amplification and/or overexpression of the following genes might induce resistance to FGFR inhibitors: NRAS (7), MET (7, 8), ABCG2 (9).

The activation of the STAT3 (10), EPHB3 (11), HER2/3 (12), EGFR (13), PI3K-AKT (14) or RAS-MAPK pathways (15) may also result in reduced efficacy of FGFR inhibition.

The presence of fusions, or translocations of the BRAF (16) or FGFR2 (17) genes may also lead to the decreased efficacy of FGFR inhibition.

Furthermore, loss-of-function alterations (mutations, gene loss), downregulation or lack of protein expression in the following genes, may also confer resistance to FGFR inhibitors: DUSP6 (7), RASA1 (9), PTEN (18), PHLDA1 (19), GSK3beta (20).

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ARID2-R1272*

This alteration is listed as pathogenic in the ClinVar database, in association with Coffin-Siris syndrome. This nonsense mutation hits a position of the ARID2 gene, which is resistant to nonsense-mediated decay (NMD), thus it most probably does not trigger degradation of the mutant mRNA (1). By affecting a long protein-coding exon of the gene (rank: 15/21), the mutation leads to the expression of a transcript variant encoding a truncated protein variant compared with the wild-type protein (1272 vs 1835 amino acids). Thus functional loss is highly likely.

References:

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ARID2 mutant gene – targets

The ARID2 tumor suppressor protein plays a role in the DNA damage response (DDR) (1, 2). Preclinical results suggest that ARID2 deficiency sensitizes to PARP inhibition and to cisplatin and etoposide (2).

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DNMT3A-W297*

According to the ClinVar database, it is a likely pathogenic alteration. Due to the premature STOP codon (nonsense mutation) in the DNMT3A gene, a variant encoding a substantially shorter protein version is generated, thus loss of function is highly likely.

DNMT3A mutant gene - targets

DNMT3A is a DNA methyltransferase protein. It has oncogenic and tumor suppressor functions as well (1). In the case of DNMT3A loss-of-function mutations, DOT1L target gene and pinometostat agent can be mentioned in positive association (2).

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SMARCA4-R1077*

According to the ClinVar database, it is a likely pathogenic alteration. Due to the premature STOP codon (nonsense mutation) in the SMARCA4 gene, a variant encoding a substantially shorter protein version is generated, thus loss of function is highly likely.

SMARCA4 mutant gene - targets

SMARCA4 (BRG1) is a tumor suppressor gene, encoding the SMARCA4 protein, a key component of the SWI/SNF chromatin remodeling complex. SMARCA4 is frequently inactivated in different cancer types (1).
In case of its loss-of-function alterations indirect targets can be mentioned in positive association. According to preclinical data, SMARCA2 (BRM) (2), EZH2 (3, 4), or AURKA (5) inhibition might be effective in SMARCA4 mutant cancers.

TAZEMETOSTAT is an FDA approved EZH2 inhibitor for the treatment of follicular lymphoma and epitheloid sarcoma. In a phase I trial, tazemetostat showed efficacy in solid tumors patients with SMARCB1 or SMARCA4 loss, disease control was observed in 5 (3 rhabdoid tumors, 2 epitheloid sarcoma) of 13 patients (6). Tazemetostat is currently tested in phase II trial for the treatment of cancers with EZH2, SMARCB1, or SMARCA4 gene mutations (NCT03213665).
In non-small cell lung cancer (NSCLC) preclinical models, in the presence of SMARCA4 loss-of-function mutations, the activity of AURKA was demonstrated to be essential, and the AURKA inhibitor VX-680 showed anti-tumor activity (5).

According to clinical data, loss of SMARCA4 expression is associated with increased efficacy of adjuvant cisplatin-based chemotherapy in NSCLC patients (7).
In a study analyzing breast cancer patient samples and performing in vitro experiments, the loss of SWI/SNF complex was indicated as a resistance mechanism to topoisomerase II inhibitors (8).

In a study, patients with lung adenocarcinoma, treated with immunotherapy, carrying coexisting mutations in at least two genes among KEAP1, STK11, PBRM1 and SMARCA4, had significantly shorter survival compared to the wild-type (WT) group. Furthermore, patients with co-mutations harbored higher TMB than the WT group (9).

References:
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KMT2D-P565fs*365

BIOMEDICAL INTERPRETATION

This variant is not listed in the ClinVar database. This frameshift mutation hits a position of the KMT2D gene, which is resistant to nonsense-mediated decay (NMD), thus it most probably does not trigger degradation of the mutant mRNA (1). By affecting a long protein-coding exon of the gene (rank: 11/55), the mutation leads to the expression of a transcript variant encoding a truncated protein variant compared with the wild-type protein (931 vs 5537 amino acids, and possessing an altered 365 amino acid long C-terminal sequence. Thus functional loss is highly likely.

References:

(1) Litchfield K et al., Escape from nonsense-mediated decay associates with anti-tumor immunogenicity. Nat Commun. 2020 Jul 30;11(1):3800. PMID: 32733040

KMT2D mutant gene - targets

KMT2D/MLL2 is a histone methyltransferase that regulates transcription. Its role in tumorigenesis is controversial: some sources associate loss-of-function mutations of KMT2D with decreased cell proliferation and migration (1-3), while other sources point to the tumor suppressor function of the gene stating that KMT2D-deficiency increases tumor growth (e.g. via inducing genomic instability) (4, 5).
It has also been reported that the MLL2 protein is part of an ER-alpha coactivatior complex. Inhibition (loss of function) of MLL2 decreased the estrogen-induced expression of ER-alpha target genes, and reduced tumor cell growth (6).
According to a study using a multivariate Cox regression model, KMT2D mutation was one of the most significant prognostic factors in NSCLC. The KMT2D mutation rate was 17.5% in NSCLC. Patients with mutant KMT2D had significantly lower median OS (9.97 vs. 30.2 months; P < .0001) and median PFS (8.46 vs. 24.1 months; P = .0004) compared with patients with wild-type KMT2D (7).
According to preclinical evidence, KMT2D-deficiency sensitizes to the non-chemotherapeutic agent AICAR (aminoimidazole-carboxamide-ribonucleotide) (8). AICAR is an AMP homolog, that inhibits angiogenesis and induces apoptosis through activating the protein AMPK, and thereby inhibits tumor growth (9). AICAR treatment proved to be effective in a clinical trial involving B-cell chronic lymphocytic leukemia patients (10, 11).

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Frameshift mutations

Frameshift mutations, resulting from short insertions and deletions, can cause premature termination codons (PTCs) and are susceptible to degradation at the mRNA level through the process of nonsense-mediated decay (NMD). NMD normally functions as a surveillance pathway to protect eukaryotic cells from the toxic accumulation of truncated proteins, but a subset of frameshift mutations may escape NMD degradation (1) and create alternative open reading frames (ORFs) with novel tumor-specific sequences (neoantigens), that are distinct from wild-type encoding antigens (2). These neoantigens may contribute substantially to directing anti-tumor immunity in low-TMB patients (1, 3), and could be targeted by immunotherapy. Suggesting that frameshift mutations could be of significance despite their overall low frequency compared to single nucleotide variations (SNVs) (4, 5).

BIOMEDICAL INTERPRETATION

NMD efficiency is reduced in the last exon of the genes, in the penultimate exon within 50 nucleotides of the 3' exon junction, in the first 150 nucleotides of exon 1, and in exons longer than 400 nucleotids (3). Allele-specific frameshift indels (fs-indels) detection in paired DNA and RNA sequencing data (n=453, TCGA) revealed that expressed fs-indels are enriched in genomic positions predicted to escape NMD, and associated with higher protein expression, consistent with NMD escape rules (3).
Analysis of TCGA demonstrated that frameshift-derived neoantigens were present in every cancer type (4), with the highest prevalence in renal cell, breast invasive lobular and colorectal carcinomas (6).
Compared with non-synonymous SNV (nsSNV) mutations, frameshift mutations were observed to generate higher load of high-binding-affinity neoantigens in several cancer types, including malignant melanoma, renal cell carcinoma, head and neck squamous cell carcinoma and lung cancer (4, 5, 6), and had been associated with increased infiltration of cytotoxic T-cells and better responses to immune checkpoint inhibitors (ICIs) (3, 4, 6, 7). In malignant melanoma cohorts, the number of expressed frameshift mutations were found to be a stronger predictor for ICI response than nsSNVs (1, 3).
A subset of frameshift mutations, with highly elongated neoORFs, were found to be significantly enriched for immunogenic reactivity (1, 3).

In the present sample 1 frameshift mutation was detected that is located in an NMD-resistant position (KMT2D-P565fs*365), thus, the emergence of a 365-amino-acid neopeptide is likely.

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ARID1A-Q766fs*67
This variant is not listed in the ClinVar database. Due to the frameshift mutation in the ARID1A gene, a variant encoding a substantially shorter protein version is generated, thus loss of function is highly likely.

ARID1A mutant gene - targets
ARID1A inactivation leads to decreased mismatch repair. ARID1A deficiency correlated with microsatellite instability in a preclinical study (1), and in gastric-, and colorectal cancer patients (2). According to a study, higher TMB values and higher PD-L1 expression was found in ARID1A mutant gastrointestinal (GI) tumors, than in ARID1A-wildtype GI cancers (3). PD-L1 inhibitors have been shown to be more efficient in ARID1A mutant mouse models than in wild-type ones (1). EZH2 (4), YES1 (5), PI3K/AKT (6), and PARP (7) inhibitors are also in positive association with ARID1A inactivation. ARID1A loss is in synthetic lethal interaction with dasatinib, a compound in clinical use (5). TAZEMETOSTAT is an FDA approved EZH2 inhibitor. PD-L1 inhibitors in clinical use are AVELUMAB, ATEZOLIZUMAB, and DURVALUMAB. A YES1 inhibitor in clinical use is DASATINIB. PI3K inhibitors in clinical use are IDEALISIB, COPANLISIB (FDA only), ALPELISIB, and DUVELISIB. PARP inhibitors in clinical use are OLAPARIB, RUCAPARIB, TALAZOPARIB, and NIRAPARIB.
According to a case study, PEMBPROLIZUMAB monotherapy has been shown to be effective in a patient with lung adenocarcinoma, adrenal metastasis, where PDL-1 overexpression, high TMB and ARID1A mutation have been identified. After 5 months, PET/CT images showed an important reduction of uptake and dimensions of the lung lesion and complete response of adrenal mass (8).
In another case study, an ARID1A mutant, PD-L1 negative, MSS, TMB-Low ovarian tumor patient achieved complete remission with 9 cycles of PEMBPROLIZUMAB and BEVACIZUMAB. The patient received prior chemotherapy (9).

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Molecular alterations and mechanisms associated with resistance / reduced efficacy in case of PARP inhibition

Based on preclinical and clinical evidence, decreased efficacy of, or resistance to PARP inhibitors may arise due to various genetic alterations and mechanisms.

Secondary mutations in the BRCA1, BRCA2 (1-7), RAD51C, RAD51D (5-7), and PALB2 (5) genes, which restore protein function, may reduce the efficacy of different PARP inhibitors or result in resistance.

Mutations affecting the RING and BRCT domains of the BRCA1 gene, the BRCA1-11q splice variant, as well as fusions of the BRCA1 gene, may also contribute to the decrease in the efficacy of PARP inhibition (3-7).

Activating mutations in the HRAS, KRAS, NRAS genes can be mentioned in a negative association with the efficacy of PARP inhibitors (8). However, in the case of activated KRAS-MAPK signaling, MEK plus PARP inhibition resulted in a synergistic effect in several cell lines (8-10), as PARP and MEK inhibitors mutually block adaptive responses to the other drug, resulting in synthetic lethality. Thus, the combination of MEK and PARP inhibitors is currently being tested in several clinical trials (NCT03162627, NCT03637491).

The amplification and/or overexpression of the following genes might induce resistance to PARP inhibitors: HOXA9 (1, 4), MET (4), EHMT1/2 (6), MIR622 (4, 6, 7), MIR493 (6, 7), FANCD2 (7), RAD51 (11), CCNE1 (12-14), CDK12 (15), CDK18 (7, 16), NBN (17).

The overexpression of the ABCB1 transporter protein (1-7), the activation of the PI3K/AKT/mTOR (4, 18), or the Wnt/beta-catenin (6, 7) pathways, furthermore the activation of RPS6 (1, 4) or ATR (7) may also confer resistance to PARP inhibitors. In the case of activated PI3K-AKT-mTOR signaling, PI3K/mTOR plus PARP inhibition resulted in a synergistic effect in several cell lines (10, 19, 20), and the combination of PI3K plus PARP inhibition has also been shown to be effective in a phase I clinical trial (21). The combination of PI3K/AKT/mTOR and PARP inhibitors is currently being tested in several clinical trials (NCT03154281, NCT02208375, NCT03586661).

Furthermore, loss-of-function alterations or lack of protein expression in several genes, may also confer resistance to PARP inhibitors.

Loss of function of TP53BP1 (1-7), RIF1 (3, 5, 7), or genes encoding the proteins of the Shieldin complex (MAD2L2, SHLD1, SHLD2, SHLD3 (1, 5-7)), as well as PAXIP1, DCLRE1C (5), or CTC1, STN1, TEN1 (5) which form the CST complex, furthermore the loss of HELB (5), DYNLL1 (6), EMI1 (6, 7) may contribute to the restoration of homologous recombination repair, resulting in reduced efficacy of PARP inhibition.

Loss-function alterations of the PAXIP1 (4-7), CHD4 (5, 7), EZH2 (5-7), MUS81 (5-7), SMARCAL1 (5-7), E2F7 (6), RADX (7) genes can also cause resistance to PARP inhibitors through the stabilization of the replication fork.

PARP1 (1, 2, 5-7), PARG (5, 7), or ADPRS (ARH3) gene loss (22), as well as PARP1 loss-of-function mutations (5-7) may also contribute to the development of PARP inhibitor resistance.

Furthermore, loss-of-function alterations in the SLFN11 (5, 6), MUTYH (23), OGG1 (23), and JMJD1C (24) genes can also be associated with decreased efficacy of PARP inhibition.

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FBXO11-I520fs*15

This variant is not listed in the ClinVar database. Due to the frameshift mutation in the FBXO11 gene, a variant encoding a substantially shorter protein version is generated, thus loss of function is highly likely. Loss of FBXO11 function has no known role in tumorigenesis.

JUN-S37fs*69

This variant is not listed in the ClinVar database. Due to the frameshift mutation in the JUN gene, a variant encoding a substantially shorter protein version is generated, thus loss of function is highly likely.

CYP2D6-R380H

Its effect on enzymatic activity of CYP2D6 is unknown.

BIOMEDICAL INTERPRETATION

KIT-M541L

It is described as a polymorphism without a tumorigenic effect in the literature (1, 2) and in the SNPEffect and ClinVar databases. However, it is associated with pediatric mastocytosis. Based on preclinical data, cells harboring KIT-M541L have approximately 2-fold enhanced sensitivity to the KIT inhibitor, imatinib mesylate as wild type cells (3).

Somatic KIT-M541L substitution was found in 4 out of 5 chronic eosinophilic leukemia patients. All patients were treated with low dose imatinib (100 mg daily orally), achieving complete and persistent clinical and hematological remission (median follow-up 74 months). All 5 of the patients appeared to be negative for the BCR/ABL1, FIP1L1/PDGFRalpha fusion transcripts and for JAK2 mutations, which alterations sensitize to imatinib treatment (4).

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Targeted therapies in gastric cancer regardless of the molecular profile

RAMUCIRUMAB is an approved VEGFR2 inhibitor in gastric adenocarcinoma.

A placebo-controlled, double-blind, phase III trial was conducted to evaluate the efficacy and safety of paclitaxel +/- ramucirumab in patients with metastatic gastroesophageal junction (GEJ) or gastric adenocarcinoma. Median overall survival (OS) was 9.6 months for ramucirumab + paclitaxel and 7.4 months for paclitaxel (1). In another phase III trial 355 patients were assigned to receive ramucirumab (n=238) or placebo (n=117) and best supportive care (BSC). Median OS was 5.2 and 3.8 months in the two groups, respectively (2).

According to a real-world study, apatinib (multi tyrosine kinase inhibitor) therapy showed promising efficacy in patients with gastric cancer (n=1000) administered in different lines (3). Apatinib was granted orphan drug designation from the EMA and the FDA in gastric cancer indication.

Lonsurf is a chemotherapeutic agent approved for gastric or GEJ adenocarcinoma patients, who have been previously treated with at least two prior systemic treatment regimens for advanced disease. In a phase III trial, Lonsurf treatment resulted in 5.7 months median overall survival in heavily pretreated gastric cancer patients compared to 3.6 months in the placebo group (4).

The FDA has granted an orphan drug designation to BOLD-100 (ruthenium-based, small molecule that selectively inhibits stress-induced upregulation of GRP78) for the treatment of patients with gastric cancer.

The FDA has granted an orphan drug designation to TST001, an anti-CLDN18.2 monoclonal antibody, for the treatment of gastric cancer or gastroesophageal junction cancer (GEJ). TST001 is currently being evaluated in two phase I studies of advanced or metastatic solid tumors (NCT04495296, NCT04396821).

In a phase II clinical trial patients with locally advanced gastric cancer received apatinib combined with S-1 plus oxaliplatin as a neoadjuvant treatment. The pathological response rate was 54.2% (26/48 patients) and the pathological complete response rate was 6.3% (3/48 patients) (5).

The FDA has granted an orphan drug designation to ceclazepide (gastrin/CCK2 receptor antagonist) for the treatment of patients with gastro-entero-pancreatic neuroendocrine tumors.

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Molecular alterations and mechanisms associated with resistance / reduced efficacy in case of angiogenesis inhibitors

Based on preclinical and clinical evidence, decreased efficacy of or resistance to angiogenesis inhibitors may arise due to various genetic alterations and mechanisms.

Amplification or overexpression of angiogenic and lymphangiogenic mediators such as FGF1/2, VEGF, PDGF, PIGF, EFNA1/2, IL8, ANGPT1/2, EGF, G-CSF, HGF, IGF1, SDF-1, TGF can be mentioned in a negative association with the efficacy of angiogenesis inhibitors (1, 2).

Molecular alterations in general that may also cause resistance to each antiangiogenic compound or reduce their efficacy include the following growth factor receptors, such as VEGFR, FGFR, EGFR, PDGFR, IGF1R, MET, and alterations that activate their downstream signaling pathways, such as PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, JAK/STAT, as well as activation of the following genes or signaling pathways: AXL, EPHA2, HIF-1a /2a, JNK, SRC, NF-kB, NOTCH1, TGF-a/b, BCLAF1, CCR2, CCR7, FOXF1, MDM2, NRF2, PIN1, POLR1D, PSMD10, RIT1, TBX5, XPO1, YAP, YB1, and PD-1/PD-L1 overexpression. Furthermore, PTEN inactivation and DUSP6, FBXW7, KEAP1, MED12, IFNG, IFNG, IFNGR, PTPRD, PTPRT loss-of-function, as well as certain polymorphisms in the ABCB1, CYP3A5, IL8, PXR genes, or also ABCB1, CYP3A4, MDR1 overexpression may reduce the efficacy of certain angiogenesis inhibitors (3, 4). In the report, alterations associated with reduced efficacy are calculated with a negative score in the aggregated evidence level (AEL) of each antiangiogenic compound.

According to a preclinical study, loss of TP53 function may result in reduced efficacy of VEGFR2 inhibition (5). However, conflicting results were obtained in several clinical trials, in which TP53 mutant status (vs. wild type) associated with longer survival in case of bevacizumab- or pazopanib-containing treatments (6-8). In two other trials no significant association was found between bevacizumab- or ramucirumab-containing therapies and TP53 expression or mutant status (9, 10).

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