

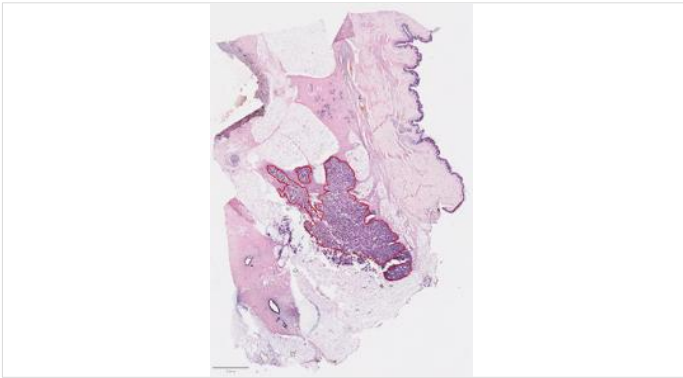
| PATIENT          | SAMPLE AND REPORT INFORMATION              |                   |
|------------------|--------------------------------------------|-------------------|
| Name:            | FFPE Block ID:                             |                   |
| Personal ID No.: | Sample Type:<br>(site - treatment Setting) | Breast – adjuvant |
| PHYSICIAN        | MDX Order ID:                              | MDX-PT-681        |
| Name:            | Sample receipt date:                       |                   |
| Institution:     | Report release date:                       |                   |
| Contact:         | Original or revision:                      | Original          |

This sample was tested with the Multiplex8+ test, which includes H&E and/or FISH-guided laser capture microdissection of tumor tissue, followed by next-generation sequencing (NGS) of total RNA from the microdissected tissue.

TEST RESULT

| Receptor | Gene  | NGS output (TPM) | NGS positivity threshold (TPM) | NGS result | RNA-FISH concordance |
|----------|-------|------------------|--------------------------------|------------|----------------------|
| ER       | ESR1  | 49.632           | ≥5.0                           | POSITIVE   | CONCORDANCE          |
| PR       | PGR   | 0.382            | ≥1.4                           | NEGATIVE   | CONCORDANCE          |
| HER2     | ERBB2 | 27.288           | ≥154.7                         | LOW        | CONCORDANCE          |
| Ki-67    | MKI67 | 30.173           | ≥18.9                          | POSITIVE   | CONCORDANCE          |

LASER CAPTURE MICRODISSECTION



Based on histological assessment and RNA-FISH biomarker expression one sample (**Sample A**) was laser capture microdissected for further analysis.

AUTHORISATION

| Reviewed | Approved | Released |
|----------|----------|----------|
| Name:    | Name:    | Name:    |

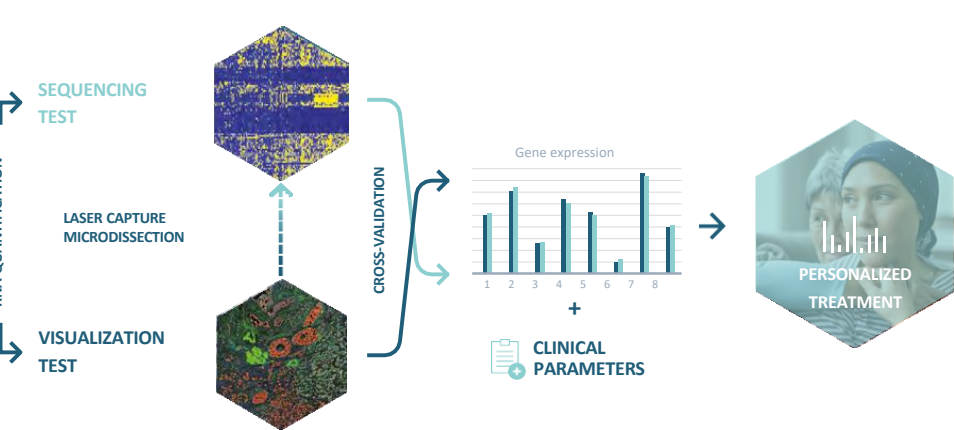
Patient Identifier:

Sample receipt date:

Report release date:

TEST DESCRIPTION

The **Multiplex8+** breast cancer test assesses RNA-based biomarkers by conducting a **VISUALIZATION TEST** that uses RNA fluorescent in situ hybridization (RNA-FISH) to visualize a panel of biomarkers. Based on the expression of these biomarkers and the tissue histology, laser capture microdissection is used to dissect out regions of interest. With these tumor-enriched samples, a **SEQUENCING TEST** that utilizes total RNA next generation sequencing to survey gene expression in a spatially resolved manner, is further carried out. Analytical validation of **Multiplex8+** was conducted on a large retrospective cohort of 1 082 breast tumors.



THE TEST PROVIDES INFORMATION ABOUT:

- 1. **RECEPTOR STATUS:** for RNA expression of the estrogen receptor, progesterone receptor, Her2 receptor, and Ki67 measured and cross-validated by the two tests.
- 2. **MOLECULAR SUBTYPE:** based on RNA gene expression tumor biology.
- 3. **GENE SIGNATURES:** personalized for patients' tumor biology and clinical status.

INTERPRETATION GUIDE

In the following report, each gene/gene signature is given a percentile score, which ranks the expression level in the context of the patients included in our retrospective cohort. For the four main breast cancer biomarkers, estrogen receptor (*ESR1*), progesterone receptor (*PGR*), Her2 receptor (*ERBB2*), and Ki67 (*MKI67*), these percentile rankings are in the context of all 1 013 eligible patients. For all other genes/gene signatures, the percentile rankings are in the context of other patients belonging to the same **MOLECULAR SUBTYPE**. For example, for patients classified as Luminal A, the genes and gene signature will receive a percentile score compared to all Luminal A samples in our retrospective validation. The percentile scores do not necessarily imply a given level of sensitivity or resistance to a therapy.

| Sample Percentile |
|-------------------|
| Low (1-33)        |
| Medium (<33-66)   |
| High (<66-100)    |

Percentile groups and ranges

| Subtype      | # of patients |
|--------------|---------------|
| Luminal A    | 432           |
| Luminal B    | 313           |
| HER2         | 87            |
| Basal-like   | 181           |
| All patients | 1 013         |

Number of patients in each molecular subtype and total retrospective cohort that are used to determine percentile rankings

|                     |                      |                      |
|---------------------|----------------------|----------------------|
| Patient Identifier: | Sample receipt date: | Report release date: |
|---------------------|----------------------|----------------------|

RESULTS SUMMARY

A SUMMARY IS PROVIDED BELOW AND ADDITIONAL DETAILS ARE PROVIDED IN THE FOLLOWING PAGES.

RECEPTOR STATUS

| Sample | ESR1 | PGR | ERBB2    | MKI67 |
|--------|------|-----|----------|-------|
| A      | +    | -   | -<br>low | +     |

MOLECULAR SUBTYPE

| Intrinsic subtype | TNBC subtype |
|-------------------|--------------|
| Luminal B         | -            |

| THERAPY                                     | KEY FINDINGS                                                  | PREDICTED CLINICAL BENEFIT*                                |
|---------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------|
| Endocrine therapy                           | Molecular subtype, gene expression, gene expression signature | Higher possibility of benefit                              |
| CDK4/6 inhibitors                           | Gene expression, gene expression signature                    | Higher possibility of benefit                              |
| Trastuzumab deruxtecan (Enhertu)            | Gene expression                                               | Higher possibility of benefit                              |
| Sacituzumab govitecan (Trodelvy)            | Gene expression                                               | Higher possibility of benefit                              |
| Datopotamab deruxtecan (Datroway)           | Gene expression                                               | Higher possibility of benefit                              |
| Gemcitabine and Capecitabine                | Gene expression                                               | Higher possibility of benefit                              |
| 5-fluorouracil (5-FU)                       | Gene expression                                               | Higher possibility of benefit                              |
| ADCs in clinical trials                     | Gene expression                                               | Higher possibility of benefit (off-label, clinical trials) |
| Anti-Her2                                   | Molecular subtype, gene expression, gene expression signature | Lower possibility of benefit                               |
| AKT/PI3K inhibitor (capivasertib/alpelisib) | Gene expression signature                                     | Lower possibility of benefit                               |
| Bevacizumab (Avastin)                       | Gene expression, gene expression signature                    | Lower possibility of benefit                               |
| Methotrexate                                | Gene expression                                               | Lower possibility of benefit                               |

\*Predicted Clinical Benefit is based on inferences from various data sources. The relationship of Multiplex8+ results to actual Clinical benefit has not been validated.

|                     |                      |                      |
|---------------------|----------------------|----------------------|
| Patient Identifier: | Sample receipt date: | Report release date: |
|---------------------|----------------------|----------------------|

MOLECULAR SUBTYPE

| Intrinsic subtype | TNBC subtype <sup>2-4</sup> |
|-------------------|-----------------------------|
| Luminal B         | -                           |

RECEPTOR STATUS

| Sample | ESR1 | PGR | ERBB2    | MKI67 |
|--------|------|-----|----------|-------|
| A      | +    | -   | -<br>Low | +     |

Based on the [SEQUENCING TEST](#), we used a consensus subtyping approach consisting of our proprietary 293 gene molecular subtyping signature, a research-based PAM50 test and the AIMS method to classify the intrinsic molecular subtype <sup>1</sup>. TNBC subtype, if applicable, was classified according to Lehmann <sup>2-4</sup>.

Receptor status was determined using both the [VISUALIZATION TEST](#) and [SEQUENCING TEST](#): the table shows results after cross-validation.

INTERPRETATION

- The results from the Multiplex8+ are concordant with the immunohistochemistry findings.

INTERPRETATION

- The biology of the Luminal B tumor type is consistent with the immunohistochemical and clinical designation.
- Luminal B tumors are generally ER+, but may have variable ER/PR expression, and they can be either Her2+ or Her2-. Relative to luminal A tumors, they may have higher grade, proliferative activity, poorer outcomes, and consequently may require chemotherapy in addition to endocrine therapy.

GENE SIGNATURE

- Based on the assigned molecular subtype, and TNBC subtype (if applicable), we evaluated several individual genes and gene signatures that demonstrate prognostic and predictive potential in early and advanced/metastatic settings.

| Treatment type/ Pathway | Gene signature              | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Sample A Percentile |  |
|-------------------------|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|--|
| Prognosis               | HR+_<br>Prognostic          | The prognostic signature is derived from a consensus of three research-based prognostic signatures, including the 21-gene signature GENE21 <sup>5</sup> , the 70-gene GENE70 signature <sup>6</sup> , and the 50-gene risk of relapse based on subtype alone (ROR-S) signature <sup>7</sup> . The prognostic signatures are intended for early-stage breast cancer patients with ER+/Her2- IHC, lymph node-negative, or 1-3 positive lymph nodes. The score is reported as high, intermediate, or low. Patients with high signature scores are at a greater risk of relapse and may benefit from adjuvant chemotherapy, while patients with low scores have lower risk of relapse and may not benefit from adjuvant chemotherapy. | High risk           |  |
|                         | TNBC_<br>Prognostic_<br>MDX | MultiplexDX (MDX) 60-gene signature for recurrence-free survival risk prediction for TNBC patients. The signature was derived from the MDX-BRCA retrospective cohort <sup>1</sup> , comprising 159 TNBC patients and validated on 508 TNBC patients from two large international cohorts spanning Asia, Europe, and the USA.                                                                                                                                                                                                                                                                                                                                                                                                      | High risk           |  |

Patient Identifier:

Sample receipt date:

Report release date:

| Treatment type/ Pathway | Gene signature         | Description                                                                                                                                                                                                                                                                                                                                                                 | Sample A Percentile |  |
|-------------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|--|
| Luminal signatures      | ESR1                   | The ESR1 and PGR genes encode for the estrogen (ER) and progesterone (PR) hormone receptors, respectively, which are involved in growth, metabolism, and reproductive functions. High ER/PR is predictive of endocrine therapies and low or negative ER/PR is associated with poor prognosis <sup>8</sup> .                                                                 | Medium (48)         |  |
|                         | PGR                    |                                                                                                                                                                                                                                                                                                                                                                             | Low (16)            |  |
|                         | ESR1_PGR average       | The average gene expression of ESR1 and PGR. Higher levels of hormone receptors are predictive markers for endocrine therapies.                                                                                                                                                                                                                                             | Low (33)            |  |
|                         | E2F4_score             | This gene signature assesses activity of the E2F4 transcription factor and its targets. A high E2F4 signature is associated with endocrine resistance to aromatase inhibitors and may predict sensitivity to CDK4/6 inhibitors <sup>9</sup> .                                                                                                                               | High (83)           |  |
| Her2                    | ERBB2                  | The ERBB2 gene is translated into Her2, a receptor tyrosine kinase involved in cell growth/proliferation and is both a prognostic marker and predictive of response to Her2 targeted therapies <sup>8</sup> .                                                                                                                                                               | Low (21)            |  |
|                         | MUC4                   | Mucin 4 (MUC4) is a glycoprotein that is implicated in resistance to trastuzumab through interactions with the Her2 receptor. High MUC4 is associated with reduced sensitivity to trastuzumab <sup>10</sup> .                                                                                                                                                               | Low (5)             |  |
|                         | NRG1                   | NRG1 codes for neuregulin 1, a ligand of the Her3 receptor. In the phase II NeoSphere trial, high NRG1 gene expression was associated with reduced response to neoadjuvant trastuzumab, but not combination trastuzumab-pertuzumab <sup>11</sup> .                                                                                                                          | Low (1)             |  |
|                         | pSTAT3-GS              | A signature that predicts phosphorylation of STAT3 and was found to be predictive of trastuzumab resistance in the FinHer study <sup>12</sup> .                                                                                                                                                                                                                             | Low (26)            |  |
|                         | Her2 amplicon_MDX      | Proprietary MDX 43-gene signature used to assess Her2 status.                                                                                                                                                                                                                                                                                                               | Low (0)             |  |
|                         | Module7_ERBB2          | Her2-signaling signature predictive of response to multiple anti-Her2 treatments in the I-SPY2 trial <sup>13</sup> .                                                                                                                                                                                                                                                        | Low (4)             |  |
|                         | T-DM1_pred             | The trastuzumab emtansine (T-DM1) predictive signature is a Research Use Only classifier that combines 19 genes/gene signature involved in the mechanism of action of T-DM1 and was shown to predict response in the T-DM1 arm of the I-SPY2 trial ( <a href="https://www.nature.com/articles/s41467-024-55583-2">https://www.nature.com/articles/s41467-024-55583-2</a> ). | Low (20)            |  |
| Proliferation           | AURKA                  | Aurora Kinase A (AURKA) is a protein coding gene involved in cell proliferation and is an independent prognostic marker in breast cancer.                                                                                                                                                                                                                                   | Medium (56)         |  |
|                         | MKI67                  | MKI67 codes for the marker of proliferation Ki67 protein, a marker of poor prognosis in ER+/Her2- tumors, but not Her2+ or TNBC tumors. Ki67 levels are also predictive of sensitivity to neoadjuvant endocrine and chemotherapies <sup>8</sup> .                                                                                                                           | High (72)           |  |
|                         | Module11_proliferation | Proliferation index used in I-SPY2 trial broadly predictive of pathological complete response in hormone receptor positive patients <sup>4</sup> .                                                                                                                                                                                                                          | High (72)           |  |
|                         | Proliferation_MDX      | Proprietary MDX 7-gene signature used to assess cellular proliferation and cross-validate MKI67 expression levels.                                                                                                                                                                                                                                                          | High (75)           |  |
| CDK4/6 inhibitors       | CDK4                   | Cyclin-dependent kinases 4 and 6 (CDK4 and CDK6) are important proteins that regulate cell cycle progression from G1 to S phases. They are the main targets of CDK4/6 inhibitors such as palbociclib (Ibrance), ribociclib (Kisqali), and abemaciclib (Verzenio); however, it is unclear whether their expression level predicts CDK4/6 inhibitor sensitivity.              | Low (1)             |  |
|                         | CDK6                   |                                                                                                                                                                                                                                                                                                                                                                             | Low (10)            |  |
|                         | CCNE1                  | Elevated expression of the G1/S cell cycle regulators, CCNE1, CCND3, and CDKN2D, was associated with resistance to palbociclib (Ibrance) in the single-arm phase II neoadjuvant trial (NeoPalAna) <sup>14</sup> .                                                                                                                                                           | Low (12)            |  |
|                         | CCND3                  |                                                                                                                                                                                                                                                                                                                                                                             | Low (0)             |  |
|                         | CDKN2D                 |                                                                                                                                                                                                                                                                                                                                                                             | Medium (57)         |  |
| PIK3CA mutations        | PIK3CA-GS              | A gene signature that is predictive of mutations in the PIK3CA gene and consequently the PI3K inhibitor alpelisib (Piqray). A high PIK3CA-GS score is also associated with activation of the PI3K/AKT pathway and loss of mTORC1 signaling, which may be relevant for response to mTOR inhibitors (e.g., everolimus) <sup>15</sup> .                                        | Low (22)            |  |

Patient Identifier:

Sample receipt date:

Report release date:

| Treatment type/<br>Pathway | Gene signature                                      | Description                                                                                                                                                                                                                                         | Sample A<br>Percentile |  |
|----------------------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|--|
| Chemotherapy               | TOP1                                                | The gene encoding DNA topoisomerase I, an enzyme critical for DNA transcription, is a target for anticancer drugs.                                                                                                                                  | High (99)              |  |
|                            | TOP2A                                               | The gene encoding DNA topoisomerase IIa, an enzyme critical for DNA transcription, is a target for anticancer drugs.                                                                                                                                | Medium (38)            |  |
|                            | RAD51                                               | The DNA repair protein RAD51 homolog 1 (RAD51) is involved in the repair of damaged DNA and is associated with resistance to chemotherapy.                                                                                                          | High (93)              |  |
|                            | ERCC1                                               | The DNA excision repair protein ERCC-1 (ERCC1) is involved in the repair of DNA damage and is associated with resistance to chemotherapy.                                                                                                           | Medium (37)            |  |
|                            | TYMS                                                | The Thymidylate Synthetase (TYMS) gene encodes a protein involved in DNA biosynthesis and is the target of the antimetabolite chemotherapy, 5-Fluorouracil <sup>16</sup> .                                                                          | High (69)              |  |
|                            | SLC29A1                                             | SLC29A1 codes for the equilibrative nucleoside transporter 1 (ENT1) protein, which is a nucleoside transporter that is involved in transporting gemcitabine and capecitabine <sup>17</sup> .                                                        | Medium (54)            |  |
|                            | DHFR                                                | Dihydrofolate reductase is an enzyme coded by the DHFR gene and is involved in folate metabolism and cell growth. It is the target of the antimetabolite chemotherapy, methotrexate <sup>18</sup> .                                                 | High (80)              |  |
|                            | SLC19A1                                             | SLC19A1 codes for the reduced folate carrier 1 (RFC1) protein, which transports methotrexate into the cell <sup>18</sup> .                                                                                                                          | Low (25)               |  |
|                            | CDK12                                               | The protein product of the Cyclin Dependent Kinase 12 (CDK12) gene regulates transcription, DNA repair pathways, and cell cycle <sup>19</sup> .                                                                                                     | Low (1)                |  |
|                            | Chemotherapy_<br>MDX                                | MultiplexDX (MDX) 45-gene signature predicting pathological complete response (pCR) to neoadjuvant chemotherapy for TNBC patients, developed and validated on 938 TNBC patients from 20 diverse cohorts, including randomized Phase 2 and 3 trials. | pCR negative           |  |
| Immune system              | PDCD1                                               | PDCD1 codes for the immune checkpoint marker PD-1. PD-1 is the target of pembrolizumab (Keytruda), an immunotherapy approved for the first-line treatment of metastatic TNBC.                                                                       | Medium (51)            |  |
|                            | CD274                                               | CD274 codes for the immune checkpoint marker PD-L1. PD-L1 is the target of atezolizumab (Tecentriq), an immunotherapy approved for the first-line treatment of metastatic TNBC.                                                                     | Low (14)               |  |
|                            | CTLA4                                               | Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is an immune checkpoint marker.                                                                                                                                                                 | Medium (38)            |  |
|                            | Immunotherapy_<br>_MDX                              | MultiplexDX (MDX) 5-gene signature predicting pathological complete response (pCR) to neoadjuvant immunotherapy for TNBC patients, developed and validated on 60 TNBC patients from the I-SPY2 pembrolizumab and durvalumab arms.                   | pCR negative           |  |
| DNA damage and<br>repair   | VCpred_TN                                           | DNA damage repair / immune signature predictive of response to veliparib (PARP inhibitor) and carboplatin (I-SPY2 trial) <sup>13</sup> .                                                                                                            | Low (24)               |  |
| Angiogenesis/<br>hypoxia   | VEGFA                                               | A gene coding for vascular endothelial growth factor, a protein involved in angiogenesis, vasodilation, and endothelial cell growth. VEGF is the target of the drug bevacizumab (Avastin).                                                          | Low (7)                |  |
|                            | Hypoxia /<br>Angiogenesis /<br>Inflammatory_<br>MDX | Proprietary MDX 7-gene signature used to assess hypoxia, angiogenesis, and inflammation. Signature includes genes known to be predictive of response to bevacizumab (Avastin) in the neoadjuvant GeparQuinto trial <sup>22</sup> .                  | Low (12)               |  |

| Patient Identifier:                   |                | Sample receipt date:                                                                                                                                                                                                                                                                                                                                                                     | Report release date:   |  |
|---------------------------------------|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|--|
| Treatment type/<br>Pathway            | Gene signature | Description                                                                                                                                                                                                                                                                                                                                                                              | Sample A<br>Percentile |  |
| ADC (antibody-drug conjugate) targets | ERBB2          | ERBB2 codes for the protein receptor Her2, which is a target for classical anti-Her2 treatments. Low and ultralow levels of Her2 can be eligible for treatment with the antibody-drug conjugate, trastuzumab deruxtecan (Enhertu) <sup>23</sup> .                                                                                                                                        | Low (21)               |  |
|                                       | TACSTD2        | TACSTD2 codes for Tumor-associated calcium signal transducer 2, also called Trop-2, which is the target of sacituzumab govitecan (Trodelyv), an antibody-drug conjugate approved for metastatic HR+/HER2- or TNBC <sup>24</sup> and also the drug datopotamab deruxtecan (Datroway), an ADC being investigated in clinical trials for metastatic HR+/HER2- breast cancer <sup>25</sup> . | Medium (40)            |  |
|                                       | NECTIN4        | Nectin Cell Adhesion Molecule 4 (NECTIN4) is a cell adhesion molecule that is a target for antibody-drug conjugates in clinical trials for breast cancer.                                                                                                                                                                                                                                | Medium (41)            |  |
|                                       | ERBB3          | ERBB3 codes for a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. It is under investigation in clinical trials for the antibody-drug conjugate patritumab deruxtecan.                                                                                                                                                                         | Low (31)               |  |
|                                       | FOLR1          | FOLR1 encodes the protein Folate Receptor Alpha, which is an antibody-drug conjugate target under investigation in several phase 1 and 2 clinical trials for breast cancer.                                                                                                                                                                                                              | Medium (34)            |  |
|                                       | F3             | F3 codes for tissue factor, coagulation factor III a target of several antibody-drug conjugates in phase 1 and 2 clinical trials.                                                                                                                                                                                                                                                        | Medium (36)            |  |
|                                       | SLC39A6        | The SLC39A6 genes encodes for the zinc transporter LIV-1, which is highly expressed in luminal breast cancers and is under investigation in several phase 1 and 2 clinical trials.                                                                                                                                                                                                       | High (92)              |  |
|                                       | CD276          | This gene codes for an immune checkpoint marker called CD276 (also known as B7-H3). It is the target of the antibody-drug conjugate (Mirzotamab clezutoclax (ABBV-155) that is in a phase 1 and 2 clinical trial for advanced solid cancers, including breast cancer.                                                                                                                    | Low (21)               |  |
|                                       | VTCN1          | V-Set Domain Containing T Cell Activation Inhibitor 1 (VTCN1 also called B7-H4) is an immune checkpoint marker and the target of the antibody-drug conjugate, SGN-B7H4V, which is under investigation in a phase 1 and 2 clinical trial for advanced solid cancers, including breast cancer.                                                                                             | Low (5)                |  |

## Multiplex8+ - INTERPRETATION\*

### INTERPRETATION\*

- Classification as Luminal B and positive ESR1 expression suggest that the patient may benefit from endocrine therapy, such as tamoxifen and aromatase inhibitors. However, the high E2F4 score suggests potential resistance to aromatase inhibitors.
- A high E2F4 score suggests potential resistance to aromatase inhibitors. A retrospective analysis of the Pre-Operative Palbociclib trial showed that palbociclib reduced the E2F4 signature score, suggesting that these patients may respond to CDK4/6 inhibitors either as monotherapy or in combination with endocrine therapy. The potential benefit from CDK4/6 inhibitors is further supported by low expression of markers associated with CDK4/6 inhibitor resistance (CCNE1 and CCND3) and medium-to-high expression of proliferation markers (AURKA, MKI67, Module11\_proliferation, and Proliferation\_MDX).
- As the sample is classified as ERBB2-low, the patient may potentially benefit from the antibody-drug conjugate (ADC) trastuzumab deruxtecan (Enhertu), which is approved for patients with metastatic breast cancer with low HER2 expression. The cytotoxic payload of this ADC, deruxtecan, targets TOP1, which is highly expressed in this sample. The expression of both the ADC target and the target of its cytotoxic payload suggests that trastuzumab deruxtecan may be effective in the advanced/metastatic setting.

\*Supporting literature can be found in the relevant gene description above. This report and its citation coverage are currently being revised.

|                     |                      |                      |
|---------------------|----------------------|----------------------|
| Patient Identifier: | Sample receipt date: | Report release date: |
|---------------------|----------------------|----------------------|

INTERPRETATION\*

- Medium to high TACSTD2 expression predicts sensitivity to the Trop-2-directed ADC sacituzumab govitecan (Trodelyv). High TOP1 expression, the target of the cytotoxic payload SN-38, further supports the expected benefit from this treatment.
- Medium to high TACSTD2 expression predicts sensitivity to the Trop-2-directed ADC datopotamab deruxtecan (Datroway). High TOP1 expression, the target of the cytotoxic payload of datopotamab deruxtecan, further supports the expected benefit from this treatment.
- Expression levels of nucleoside transporters, such as SLC29A1, are associated with sensitivity to gemcitabine and capecitabine, with medium-to-high expression levels predicting sensitivity.
- High TYMS expression may predict response to 5-fluorouracil (5-FU) and to chemotherapy agents that are metabolized to 5-FU, such as capecitabine.
- The sample shows high expression of SLC39A6, which is a potential target of antibody-drug conjugates currently being investigated in clinical trials for HR-positive, HER2-negative breast cancer.
- Low ERBB2 expression (ERBB2-low) suggests that HER2-targeted therapies that rely on HER2 overexpression and/or amplification, such as trastuzumab, pertuzumab, trastuzumab emtansine, tucatinib, lapatinib, and neratinib, may not provide a benefit. This is further supported by the presence of several markers associated with resistance to anti-HER2 therapy, including classification as the Luminal B molecular subtype, positive ESR1/PGR expression, and low expression of Her2 amplicon\_MDX, Module7\_ERBB2, and T-DM1\_pred.
- A PIK3CA-GS value below the 90th percentile does not predict the presence of mutations in the AKT1 or PIK3CA genes, suggesting that treatment with the serine/threonine kinase (AKT) inhibitor capivasertib or the PI3K inhibitor alpelisib, both administered in combination with fulvestrant, is not expected to provide a benefit.
- Low expression of angiogenesis-related markers, including VEGFA and the Hypoxia/Angiogenesis/Inflammatory\_MDX signature, suggests that the patient is unlikely to benefit from bevacizumab (Avastin).
- Low CDK12 expression suggests that the patient is unlikely to benefit from methotrexate.

\*Supporting literature can be found in the relevant gene description above. This report and its citation coverage are currently being revised.

AUTHORISATION

|          |          |          |
|----------|----------|----------|
| Reviewed | Approved | Released |
| Name:    | Name:    | Name:    |

REFERENCES

1. Gendoo, D.M.A. et al. Bioinformatics 32(7): 1097–1099 (2016). 2. Lehmann, B. D. et al. J Clin Invest 121: 2750–2767 (2011). 3. Lehmann, B. D. et al. PLoS One 11: e0157368 (2016). 4. Bareche, Y. et al. Ann Oncol 29: 895–902 (2018). 5. Paik, S. et al. N Engl J Med 351(27): 2817–2826 (2004). 6. van’t Veer, L.J. et al. Nature 415(6871): 530–536 (2002). 7. Parker, J.S. et al. J Clin Oncol 27(8): 1160–1167 (2009). 8. Cardoso, F. et al. Ann Oncol 30(8): 1194–1220 (2019). 9. Guerrero-Zotano, A.L. et al. Clin Cancer Res 24(11): 2517–2529 (2018). 10. Mercogliano, M.F. et al. Clin Cancer Res 23(3): 636–648 (2017). 11. Guardia, C. et al., Clin Cancer Res 27(18): 5096–5108 (2021). 12. Sonnenblick, A. et al. BMC Med 13:177 (2015). 13. Wolf, D. M. et al. Cancer Cell 40: 609–623.e6 (2022). 14. Ma, C.X. et al. Clin Cancer Res 23(15): 4055–4065 (2017). 15. Loi, S. et al. PNAS 107(22): 10208–10213 (2010). 16. Foekens, J.A. et al. Cancer Res. 61: 1421–1425 (2001). 17. Mackey, J.R. et al. Clin Cancer Res. 8(1): 110–116 (2002). 18. Yang, V. et al. RSC Med Chem. 11(6): 646–664 (2020). 19. Filippone, M.G. et al. Nat Commun. 13(1): 2642 (2022). 20. Rodrigues-Ferreira, S. et al. Proc Natl Acad Sci USA 116(47): 23691–23697 (2019). 21. Hatzis, C. et al. JAMA 305(18):1873–81 (2011). 22. Karn, T. et al. Clin Cancer Res 26: 1896–1904 (2020). 23. Modi, S. et al. N Engl J Med 387: 9–20 (2022). 24. Michaleas, S. et al. ESMO Open 7 (2022). 25. Bardia, A. et al. J Clin Oncol 43(3): 285–296 (2025).