

AdnaNews

Molecular Characterization: A differentiating factor of the Aleris™ CTC AdnaTest for companion diagnosis

The molecular characterization of CTCs will provide important information for identification of therapeutic targets for understanding resistance to therapies. It also should offer new insights into the biology of tumor dormancy and tumor cell dissemination. This will open new opportunities for the early detection of metastatic spread and its successful treatment.

CTCs have a central role in the metastatic cascade, tumor dissemination and progression. New experimental and clinical data suggest that the epithelial-to-mesenchymal transition plays an important role in the generation of CTCs and the acquisition of resistance to therapy. They are a heterogeneous population of cells with different phenotypes and biological value. Their detection could provide clinicians with an independent prognostic and predictive factor as well as an opportunity for an improved personalized anticancer treatment. CTCs and their molecular expression profile better reflect the effectiveness of a targeted therapy and the outcome of pharmacodynamic studies can be monitored in real-time.

Overview of Kits available:

AdnaTest BreastCancer (CE)	Muc-1, Her2, EpCAM, ER/PR
AdnaTest ProstateCancer (CE)	PSA, PSMa, EGFR, AR
AdnaTest ColonCancer (CE)	EpCAM, EGFR, CEA
AdnaTest OvarianCancer (non-CE)	EpCAM, Muc-1, Her2, CA-125
AdnaTest Melanoma (non-CE)	MELAN A, TYR, CSPG, MITF, SILV
AdnaTest EMT-1/StemCell (non-CE)	ALDH1, Pi3K, Akt2, Twist

Latest Research

Breast Cancer

In the breast cancer field, there is increasing focus on the molecular characterization of CTCs rather than just their enumeration. It has been shown that the molecular detection of CTCs using the AdnaTest BreastCancer correlates well with the enumeration method provided by CellSearch™ (Andreopoulou et al. 2011). However, recent publications demonstrate the added utility of AdnaTests for further characterization of CTCs that are undergoing epithelial to mesenchymal transition (EMT), adopting stemness characteristics or phenotypic shifts reflected by changes in the receptor levels of ER, PR and Her2. Analysis of CTCs obtained from 502 patients with primary

breast cancer showed that there is a subset of circulating tumor cells that cannot be detected using epithelial marker based methods but that is positive for EMT or tumor stemness markers as characterized with the AdnaTest EMT-1/StemCell (Kasimir-Bauer et al. 2012). The expression profiles of ER and PR in metastatic breast cancer samples at baseline were addressed in a trial including 193 patients using the AdnaTest ER/PR, an add-on test for the AdnaTest BreastCancer (Aktas et al. 2011). They found that in comparison to the primary tumor tissue, the steroid receptor expression was strongly down-regulated in CTCs, which may indicate their resistance to Tamoxifen treatment. In the context of the DETECT trial, it was shown that Her2 over-expression in CTCs in metastatic breast cancer corresponded significantly to Her2 phenotype in metastatic tissue, which indicates the potential utility of the AdnaTest BreastCancer as “liquid biopsy” for targeted Trastuzumab treatment (Aktas et al. 2012).

One of the key benefits of the AdnaTest approach in research is its design as an open platform for the analyses of expression profiles that extend well beyond what is routinely offered in the kits. This opportunity is nicely demonstrated by the analysis of CTCs in a 31 gene expression panel using the Fluidigm qPCR platform following the use of AdnaTest BreastCancer (Kolostova et al. 2011). They found significant differences in the expression profiles of CTCs obtained from primary compared to metastatic breast cancer patients, as well as in a subgroup of CTCs with low EpCAM expression level, again indicating an EMT like phenotype change. They concluded that this subgroup, which was in parallel over-expressing KRT19 and AURKA, may represent a group of high risk patients with potential resistance to Paclitaxel treatment. Another example of AdnaTest as an open platform is demonstrated by the gene expression measurement of BMI1 and CD44, in addition to the ALDH1 routinely measured by the AdnaTest EMT-1/StemCell kits, as stemness markers in CTCs in a cohort of 61 primary breast cancer patients (Barriere et al. 2012). They stated that the detection rate of 39% in this cohort using EMT and stemness markers rather than epithelial markers was very high, indicating the presence of CTCs which have lost their epithelial characteristics.

Prostate Cancer

It has recently been shown that the AdnaTest ProstateCancer is able to predict therapeutic response in metastatic prostate cancer (Todenhöfer et al. 2012). In a cohort of 16 patients with castrate resistant prostate cancer, they found that CTC positivity at baseline as well as during treatment predicted the therapy response towards a Docetaxel regimen.

New Products

Ovarian cancer

The clinical usefulness of CTC detection in Ovarian cancer has been recently demonstrated using an AdnaTest Prototype (Aktas et al. 2011). They analyzed CTCs in a cohort of 122 ovarian cancer patients and found that their presence at baseline (19%) as well as after chemotherapy (27%) is a significant predictor of shorter overall survival. Based on these results the AdnaTest OvarianCancer utilizes EpCAM, Muc1, Her2 and CA125 as molecular markers for the identification of CTCs. The test will be CE certified in December 2012.

Papers & Posters

- Kasimir-Bauer S, Hoffmann O, Wallwiener D, Kimmig R, Fehm T, Expression of stem cell and epithelial-mesenchymal transition markers in primary breast cancer patients with circulating tumor cells. Breast Cancer Research, 2012
- Banys M, Krawczyk N, Becker S, Jakubowska J, Staebler A, Wallwiener D, Fehm T, Rothmund R, The influence of removal of primary tumor on incidence and phenotype of circulating tumor cells in primary breast cancer. Breast Cancer Research, 2012
- G. Barriere, A. Riouallon, J. Renaudie, M. Tartary, Prof. M. Rigaud, Mesenchymal and stemness circulating tumor cells in early breast cancer diagnosis. BMC Cancer 2012, March 2012
- K. Kolostova, M. Romzova, V. Bobek, M. Kubista, S. Kasimir-Bauer, High throughput expression profiling of circulating tumor cells from breast cancer patients as potential therapy decision indicator. AACR, 2012
- T. Todenhöfer, J. Hennenlotter, S. Feyerabend, S. Aufderklamm, J. Mischinger, U.Kühs, V. Gerber, J. Fetisch, D. Schilling, S. Hauch, A. Stenzl and C. Schwentner, Preliminary Experience on the Use of the Adnatest® System for Detection of Circulating Tumor Cells in Prostate Cancer Patients, Anticancer Res. 2012 Aug
- B. Aktas, MD, S. Kasimir-Bauer, M. Heubner, R. Kimmig, and P. Wimberger, Molecular Profiling and Prognostic Relevance of Circulating Tumor Cells in the Blood of Ovarian Cancer Patients at Primary Diagnosis and After Platinum-Based Chemotherapy International Journal of Gynecological Cancer, 2011

Get the abstracts at: www.adnagen.com

Conferences

You will be able to find our company represented at the following conferences:

- ACTC Meeting, September 2012 in Athens
<http://www.actc2012.org/>

Advances in Circulating Tumour Cells (ACTC)
from Basic Research to Clinical Practice
September 26-29, 2012

- The 2012 CTCRC-AACR Breast Cancer Symposium in San Antonio:
"An international scientific symposium for interaction and
exchange among basic scientists and clinicians in breast cancer."
<http://www.sabcs.org/>



- Molecular Med TriCon, February 2013 in San Francisco
<http://www.triconference.com/>



- AACR Annual Meeting, April 2013 in
Washington DC: <http://www.aacr.org>



Troubleshooting

In our new troubleshooting section online you will find practical hints for the test procedure and for the correct interpretation of test results:

- Low Actin?
- Weak Positive Control sample?
- What are critical steps during the CTC enrichment?
- Clotting samples
- Beads are "slippery" during mRNA purification
- Are the Oligo-dT beads included in the PCR reaction?
- Bands in the RT- or negative control samples
- In the AdnaTest EMT-1/StemCell the fragment sizes of the markers are false
- Can TAQ-Polymerase or Reverse Transcriptase be different from what is recommended in the manual?
- How pure are the CTC enriched from blood samples using the AdnaTest?

Go to: www.adnagen.com and download our troubleshooting guide in the product section.

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