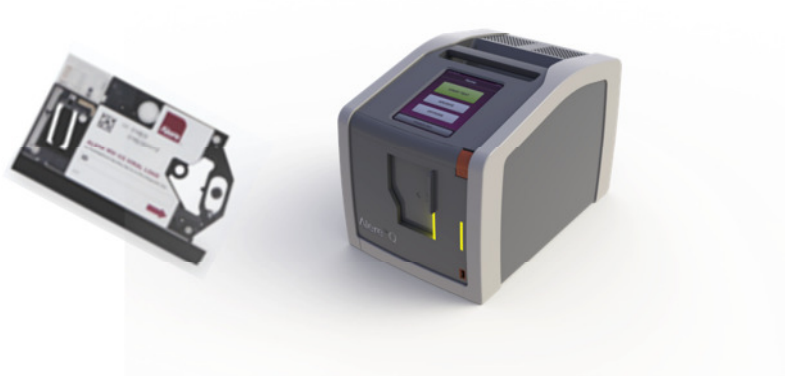
AdnaNews

News from the meeting on Advances in Circulating Tumor Cells (ACTC), Reythymnon, Crete, Greece October 8-11, 2014.

The ACTC meeting (www.actc2014.org) is together with the ISMRC meeting one of the most important meetings with regards to CTC in Europe. The 2014 meeting in Crete was organized by Evi Lianidou (University of Athens), Dimitris Mavroudis (University of Crete) and Klaus Pantel (UKE Hamburg). Besides focusing on the use of CTC diagnostics in a research setting, the conference also addressed the important issue on whether CTC diagnostics is mature enough to make its way into routine clinical settings as a liquid biopsy.

Alere™ q-Analyzer for CTC (Hubold et al. 2014)



AdnaGen/Alere was a platinum sponsor of the ACTC and presented the Alere™ q-Analyzer. The instrument is designed to provide an automated and normalized procedure for the AdnaTest Detect kit. The CTC test platform consists of the portable q-Analyzer instrument and a disposable test-specific cartridge, accommodating all required reagents for the processing of 100 µL of lysate of enriched CTCs from whole blood samples. After the lysate is loaded into the disposable cartridge, it is inserted into the instrument and all of the manual steps of the AdnaTest Detect kit system (mRNA isolation, reverse transcription and qPCR) are processed automatically and without any further manual intervention in about 90 minutes. The Alere™q also provides internal data storage and a printout of results.

Validation of the Alere™q was performed using a reaction cartridge for breast cancer CTC associated EpCAM (GA733.2), MUC1, HER2, ER and PR detection. Three sites (University of Athens, UKE Hamburg, and Dep. of Gynecology and Obstetrics, University Hospital Essen) participated in the validation by performing the manual AdnaTest BreastCancer Detection system while matched lysate samples were analyzed using the Alere™q. Briefly, three cells from two cell lines (T47D and SKBR-3) were spiked individually into peripheral blood. The recovery rates of T47D and SKBR-3 from spiked blood yielded 90.7% and 83.3%, respectively, for the Alere™ q platform and 86.3% and 81%, respectively, for the AdnaTest BreastCancerDetect. A higher sensitivity could be documented for HER2 (75.8%) and ER (46.1%) using the Alere™ q without losing specificity (97.7%). Comparative analysis considering lysates of T47D

and SKBR-3 cells spiked into blood and also directly in AdnaLysis/Binding buffer demonstrated an overall concordance of 98.7% (229/232) with high corresponding individual concordance for GA733.2 (96.1%), MUC1 (93.1%), HER2 (80.1%), ER (83.5%) and PR (99.6%). Using Cohen kappa statistics, excellent agreement was achieved for GA733.2 (=0.90; p=0.033), MUC1 (=0.86; p=0.033) and PGR (=0.99; p=0.009), whereas a substantial agreement for HER2 (=0.60; p=0.048) and ER (=0.61; p=0.056) was detected. The frequency of detecting replicative measurements of samples containing 3 spiked cell equivalents between three operators was 100% for both CTC detection systems. Further patient testing and subsequent follow up is ongoing to demonstrate the correlation with clinical parameters like therapy response, disease-free and overall survival.

AdnaTest EMT-2/StemCell and AdnaPanel BreastCancer results (Bredemeier et al. 2014)

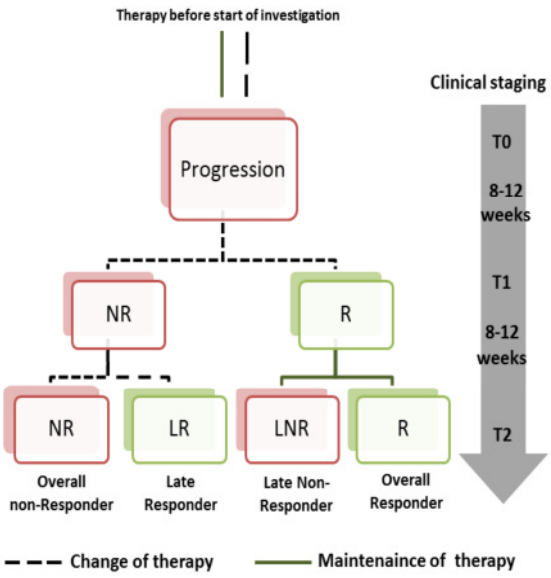


Fig 1: Study design

To better differentiate the molecular phenotype of CTCs in the blood of breast cancer patients, a nine gene qPCR panel, AdnaPanel BreastCancer, was developed. CTCs were captured using AdnaTest EMT-2/StemCellSelect comprised of a mix of immunomagnetic beads labelled with anti-EpCAM, anti-HER2 and anti-EGFR. This mixture of beads was developed to efficiently capture CTCs even if they lost their epithelial characteristics. The AdnaPanel BreastCancer Detection panel contained EpCAM, PI3KCA, AKT2, HER2, HER3, EGFR, ERCC1, AurkA and ALDH1. 45 patients with metastatic breast cancer were tested at 3 time-points T0, T1 and T2 according to the study design outlined in Fig 1.

Clear differences in gene expression frequency were observed for EpCAM, HER2, HER3 and AurkA when comparing the overall-responders (R; same therapy for entire course of treatment) and the overall-non-responders (NR; therapy change at T1). These results suggest that CTCs identified in the NR Group undergo a selective pressure under therapy. The increasing frequency of CTC-HER2 positive patients at T1/T2 might, therefore, indicate a potential effectiveness of HER2-targeted regimens for patients that were previously not indicated for such a therapy option.

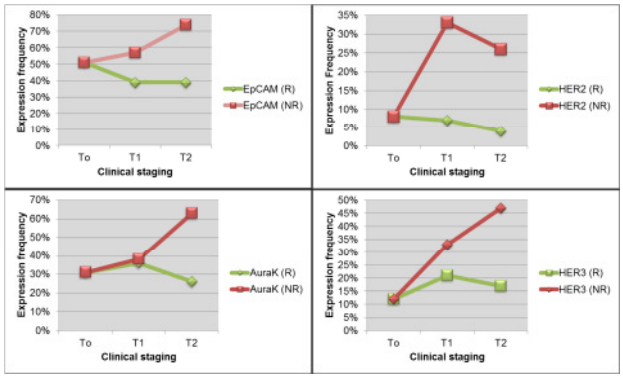


Fig 2: Overexpression observed in selected genes across all time points in non-responder (NR, LNR). Remarkingly, HER2 positive CTC were detected in almost 35% of these patients.

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AdnaTest in recent publications

Alere/AdnaTest OvarianCancer predictive for first line platinum therapy failure (Kuhlmann et al. 2014)

The results of a clinical trial using the AdnaTest OvarianCancer were published in Clinical Chemistry in October 2014. This test kit uses anti-EpCAM and anti-MUC1 labelled magnetic beads for CTC enrichment, followed by subsequent RT-PCR analysis of EpCAM, MUC1, CA125, and ERCC1 overexpression. In this study, blood samples of 147 patients were available at primary diagnosis. CTCs were detected in 14% of the patients and significantly predicted overall survival (OS; p=0,041). In addition, ERCC1-positive CTCs, found in 8% of the patients, significantly correlated with disease free survival (DFS: p=0,009) and overall survival (OS: p=0,026). Most importantly, it was clearly demonstrated in this study, that ERCC1-positive CTCs are an independent predictor of resistance to platinum based regimens (p=0,01). Surprisingly, this correlation was only found for the AdnaTest molecular CTC characterization, but not for IHC tissue staining using the antibody 8F1 commonly used for ERCC1 detection in tissues.

AdnaTest ProstateCancer + AR-Variant7 predicts Arbiraterone and Enzalutamide failure (Antonarakis et al, 2014)

In a clinical trial at Johns Hopkins (Baltimore) the AdnaTest ProstateCancer was used to analyze the overexpression of androgen receptor variant 7 (AR-V7), a constitutively activated androgen receptor. It was discussed that AR-V7 is one of the reasons for Arbiraterone or Enzalutamide failure in the therapy of castration-resistant prostate cancer. In this study, 62 patients were either treated with Arbiraterone (n=31) or Enzalutamide (n=31) and analyzed for CTCs using the AdnaTest ProstateCancer for CTC enrichment and detection and an added qPCR test for AR-V7 developed by their lab. The AR-V7 positivity rate was 39% in the group receiving Enzalutamide and 19% in the Arbiraterone group, respectively. In patients with AR-V7-positive CTCs in both therapy-groups, they observed a lower PSA response rate [Enzalutamide group: 0% vs 53% (p=0,004); Arbiraterone group: 0% vs 68% (p=0,004), respectively]. AR-V7-positive CTCs were also significantly correlated with PSA progression free survival and radiological PFS (p=0,001 for all groups). Furthermore AR-V7 positive patients had a significantly shorter OS (Arbiraterone group: p=0,006; Enzalutamide group: p=0,002). It can be concluded that AdnaTest ProstateCancer in combination with molecular detection of AR-V7 might be useful in determining therapeutic strategies in prostate cancer.

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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 (CE)	EpCAM, Muc-1, CA-125, ERCC1
AdnaTest OvarianCancer-2 (non-CE)	EpCAM, Muc-1, CA-125, ERCC1
AdnaTest EMT-1/StemCell (non-CE)	ALDH1, Pi3K, Akt2, Twist
AdnaTest EMT-2/StemCell (non-CE)	ALDH1, Pi3K, Akt2, Twist + choice of Breast, Colon, Prostate or ovarian PCR

Papers & Posters

- Emmanuel S. Antonarakis, M.D., Jun Luo, Ph.D.et al. AR-V7 and Resistance to Enzalutamide and Arbiraterone in Prostate Cancer, NEJM 2014, 371, 1028-1038
- Kuhlmann JD, Wimberger P, Bankfalvi A, Keller T, Schöler S, Aktas B, Buderath P, Hauch S, Otterbach F, Kimmig R, Kasimir-Bauers S ERCC1-Positive Tumor Cells in the Blood of Ovarian Cancer Patients as a Predictive Biomarker for Platinum Resistance. Clin Chem. 2014 Oct;60(10):1282-9.
- Bredemeier M, Aktas B, Wagner J, Schellbach D, Kimmig, Kasimir-Bauer S Establishment of a new method for the selection and detection of circulating tumor cells in metastatic breast cancer patients, Poster at ACTC 2014, Crete
- Hubold S, Loncarevic IF, Hauch S, PlappertL, Schellbach D, Markou A, Strati A, Rasch J, Kirsch U, Hanssen A, Wikman H, Gorges T, Lianidou ES, Pantel K, Kasimir-Bauer S Comparative CTC analysis using the fully automated Alere™ q CTC Breast Detect and the manual AdnaTest BreastCancer Detect assays, Poster at ACTC 2014, Crete

Get the abstracts at: www.adnagen.com

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Conferences

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

- **The 2014 CTRC-AACR Breast Cancer Symposium in San Antonio, December 09-13, 2014:**
  "An international scientific symposium for interaction and exchange among basic scientists and clinicians in breast cancer."
  http://www.sabcs.org/

- **The AACR Annual Meeting 2015 in Philadelphia, April 18-22, 2015**
  http://www.aacr.org/Meetings/Pages/MeetingDetail.aspx?EventItemID=25&DetailItemID=154#.VHhFXsmImt4

AdnaGen an Alere company