



## **Patient Information**

**How are breast tumor cells circulating in the bloodstream detected and used?**



## AdnaTest Cancer

Test systems for the selection and detection of tumor cells out of human blood

### Does the detection of breast tumor cells circulating in the bloodstream allow a better therapy control?

#### AdnaGen has developed a test system for the detection of tumor cells circulating in the bloodstream of breast tumor patients

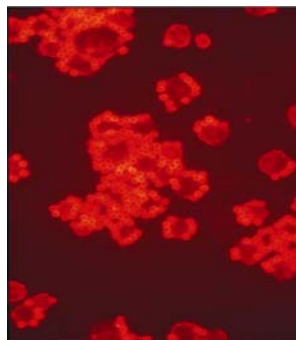
AdnaGen is active in different fields of molecular medicine. A special emphasis is put on the detection of rare cells in blood, which can be separated even in minuscule numbers from a cell mixture like blood. The cells can then be characterised by molecular genetic analysis. The advantage of AdnaGen's test systems in tumor diagnosis as compared to conventional analytical methods is the direct proof of the presence of tumor cells in patients' blood.

To start with, tumor cells are enriched from blood. This is followed by an analysis of tumor cell associated gene expression (metabolic activity of relevant genes) of separated tumor cells. Thus, conclusions about the success of a tumor therapy (surgery, chemotherapy, radiation) can be drawn at an early stage of therapy monitoring and treatment control. In this way also indications for a possible metastazation process can be obtained during aftercare.

Current studies will reveal the validity of the analysis of circulating tumor cells and its usefulness during therapy.

#### Tumor cell isolation

AdnaGen's test recognises one tumor cell amongst 10 million normal cells (this corresponds to one inhabitant of greater Rio de Janeiro, who is the only one wearing a red cap). About 0.01 percent of the circulating tumor cells are able to form metastases. Therefore, it is necessary to identify only one tumor cell in 1 to 5 ml blood in order to better judge on the development of the disease and on the potential of these tumor cells to metastasise.



Isolation of tumor cells with antibodies coupled to magnetic beads



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In a first step tumor cells are isolated from peripheral blood of tumor patients in the laboratory (*in vitro*) with antibody coated magnetic beads by the action of a magnet.

#### How does it work?

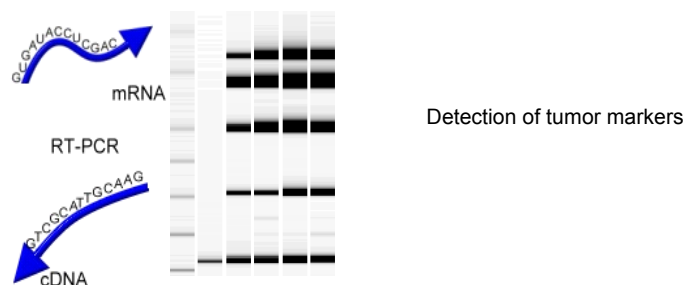
Cells carry certain type-related distinguishing marks on their surface. So-called antibodies have to be identified, which are proteins binding according to a key and lock principle to defined surface structures on tumor cells, thus distinguishing tumor cells from normal cells. AdnaGen has developed a special antibody mix for the recognition of various tumor cell types. The corresponding antibody cocktail is coupled to magnetic particles and mixed with patient blood. There is a special antibody cocktail for breast tumor cells. The tumor cells bound by the beads are then separated from the blood by magnetic force.

#### Molecular biological analysis

After the separation of benign and malign cells relevant metabolic activities of the separated tumor cells are investigated. This is a rather complicated procedure of modern gene technology:

A so-called mRNA (messenger RNA) is isolated from tumor cells. This is a "transcript" of the genetic information (DNA), which contains and passes on the "protocol" for the formation of certain proteins. Since this messenger RNA is very fragile it has to be artificially "copied" and transformed in the laboratory into a cDNA, which is more amenable for further investigation. The term cDNA implies that this DNA is a copy (c) of the corresponding mRNA. Only then it becomes possible to trace the gene dependent metabolic activity of tumor cells (in technical terms: tumor associated gene expression) by a highly sensitive method (multiplex-PCR).

Tumor cells vary extremely, even if they are coming from the same patient. Therefore, AdnaGen is considering in its test system the determination of various typical surface markers on tumor cells (by antibodies) and the analysis of several gene activities of these cells (by multiplex-PCR) as well. This assures that the different profiles of tumor cells as well as possible individual and therapeutically caused changes of the metabolic activities are taken care of.



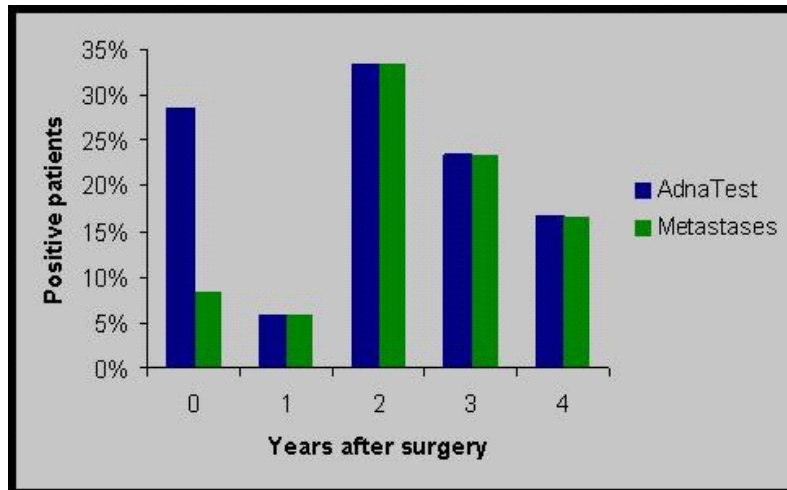


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### Study results

The diagnostic validity of the method developed by AdnaGen is being examined in several studies. A cross-sectional study of 180 breast cancer patients revealed, that there was a 100 percent agreement between the test results and the clinical diagnosis from one year after surgery onwards.



At the time of surgery, however, the AdnaGen test shows a significant higher positive rate of about 30 percent, which might suggest a worse prognosis for these patients. There was a high rate of "false positive" results with conventional biochemical serum tumor markers in comparison to the AdnaGen test.

### Planned clinical study with mamazone



mamazone - women and research against breast cancer - will organise in the near future a further clinical trial with the Klinikum Großhadern (Klinische Chemie, Dr. Petra Stieber 089/7095-3115) and AdnaGen AG.

Start of the study: mid-July 2003

Interested breast cancer patients may register for the clinical study in the office of mamazone,

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