A breakthrough in biomarker research: In clinical studies, it has been possible to identify the tumour suppressor gene p53 as a comprehensive predictive marker.

- Predictive, not prognostic: For the first time it has been possible to determine a powerful interaction between the p53 marker and response to chemotherapy.
- Associated with survival: The interaction shown enables conclusions to be drawn on the probable efficacy of chemotherapy on the respective tumour tested.
- Clinical application: A highly sensitive test now creates the prerequisites for introducing the application of p53 as a predictive marker in cancer therapy.

It is has long been known that a genetic mutation of the p53 tumour suppressor gene (TP53) is not good news for cancer patients. Just why this so is the case has now been discovered at the Medical University of Vienna. The research group led by Prof. Dr. Daniela Kandioler has been able to prove, for the very first time, that a powerful interaction exists between the p53 gene and numerous chemotherapeutic substances.

With more than 600 cancer patients it has now been confirmed in several clinics that frequently applied chemotherapies are significantly more effective for patients with normal p53 gene status than was previously assumed, whilst the same chemotherapy has a negative impact on the survival of patients with mutated p53 gene status. It has been possible to prove the interaction in the case of oesophageal cancer, colorectal cancer and liver metastases - and should also be relevant for lung and breast cancer.

The hitherto unresolved question: prognostic or predictive?

Thanks to its unique biological function, the tumour suppressor gene p53 has always been attested as having great potential as a biomarker. The obstacle to be overcome was the fact that the marker type (prognostic or predictive) had not been known to date. Prognostic markers evaluate the likelihood of a relapse among cancer patients, without allowing for a medicinal therapy. Predictive markers evaluate the likelihood of a response to a cancer therapy. Therefore, both influence survival in different ways.

Until now it has been assumed that a mutated p53 gene in the tumour means a poor prognosis for the cancer patient per se, with the consequence that study results were misinterpreted. Another obstacle was also that numerous different p53 tests have been used to date, delivering starkly differing, contradictory and, in part, few exact results.

With the aid of a highly sensitive, standardised gene test (MARK53® analysis), it could now be reproducibly shown that a power interaction exists between the p53 gene and chemotherapeutic substances.

Yet what causes this interaction?

The p53 gene controls all key functions in the life and death of a cell and becomes especially active if damage occurs in the genetic information (DNA). By stopping the cell cycle, the p53 gene initially prevents the cell division and the transmission of damaged genetic information. If possible, the p53 gene then initiates the repair of the DNA or – in the case of massive damage – programmed cell death.

Many chemotherapeutics use precisely this mechanism of programmed cell death, in which numerous chemotherapeutic substances cause massive DNA damage that lead to cell death via an intact p53 gene.

Unfortunately, in cancer cells, the p53 gene itself is frequently affected by a genetic alteration (mutation) and is therefore unable to perform this function in approx. 50% of all malignant tumours. The consequence: resistance to chemotherapy and radiation. This means that if the p53 gene has mutated in the tumour cells, chemotherapy and radiation are ineffective but also cause the familiar side effects among patients.

In other words, depending upon the p53 status (normal or mutated p53 gene), the cancer therapy can have a positive or negative impact on the patient's survival. It is precisely this interaction between chemotherapy and p53 status that has been shown for the first time in the present clinical studies.

"The clinical application of the p53 marker is probably the most promising strategy for escalating the efficiency of cancer therapy and simultaneously reducing the risk for patients. The question as to which patient responds to a cancer therapy, and which doesn't, is a very crucial one," explained the scientific director, Prof. Kandioler.

Special sequencing to determine the marker status

For the precise determination of the marker status (mutated or normal p53 gene sequence)**, a new, highly sensitive process** (**MARK53®analysis)** has been developed for the studies, as well as being simplified and standardised for routine application. The process is based on the method of direct gene sequencing - in other words, complete clarification of the genetic code of the p53 gene.

1) Pilot study: Effect of the p53 status in the treatment of liver metastases

This clinical study examined whether the p53 gene concerned a prognostic or predictive marker. Participating in the study were 76 patients with colorectal liver metastases, who had undergone treatment at the Medical University of Vienna between 2001 and 2003. All patients were in the same operable tumour stadium. The therapy comprised either a pre-operative standard chemotherapy consisting of 5FU/oxaliplatin plus operation or a primary operation (surgery only). The marker status (mutated or normal p53 gene sequence) was ascertained by means of the MARK53® analysis. The mutation frequency amounted to 55 percent. The goal of the study was to evaluate whether a different marker status is accompanied by a different prognosis, or whether a different marker status only influences the response to a pre-operative chemotherapy and is thus predictive.

The most important results:

- Without chemotherapy (surgery only), patient survival was the same - despite a different marker status.
- With a pre-operative chemotherapy, there were distinct anomalies in the survival of patients with different marker statuses. Patients with a mutated p53 marker status had a fivefold increased risk of dying than patients with a normal p53 marker status.
- The 5-years survival rate of these patients only amounted to 22 percent, whilst the survival rate for patients with a normal p53 marker status lay at 60 percent.

- In the case of patients with a mutated p53 marker status, the chemotherapeutic treatment even resulted in a poorer survival rate then a purely surgical treatment.

"For the first time, this study has been able to show that the p53 marker only predicts the response to chemotherapy and is therewith purely predictive. The results also mean that, in the case of patients with liver metastases and a mutated p53 marker, the pre-operative chemotherapy with 5FU/axaliplatin causes considerable harm," said Kandioler.

2) Pilot study: TP53 as a predictive marker in oesophageal cancer

The following clinical study examined the predictive power of the p53 marker. Between 2001 and 2007, 36 pilot patients participated prospectively in the study at MUV and the Clinic of Sankt Pölten and were followed-up over a period of seven years. The goal was to evaluate whether, with the help of the MARK53 analysis, the response to pre-operative chemotherapy could also be predicted in the case of oesophageal cancer. All oesophageal cancer patients received a pre-operative standard chemotherapy with cisplatin/fluorouracil and were then operated on. The response of the tumours to chemotherapy could be precisely histologically determined with the OP-compound. The mutation frequency amounted to 50 percent.

The most important results:

- Patients with a normal p53 marker status survived significantly longer – by 1.5 years in the median – than patients with a mutated p53 marker status.
- In the case of the normal p53 marker status, the pre-operative standard chemotherapy used proved to be surprisingly effective, whilst the same therapy among patients with a mutated p53 status was ineffective or even counter-productive.

"The results show that patients benefit significantly from a pre-operative chemotherapy, if the tumour has a normal p53 gene. However, if the p53 gene is mutated, patients with pre-operative chemotherapy live significantly shorter than patients that are only operated on – thus, without chemotherapy. Here too is confirmation that the standard chemotherapy is clearly counter-productive in the case of a mutated marker status," said Kandioler.

3) TP53 influences the effect of standard chemotherapy in the case of colorectal cancer

This study among colorectal carcinoma patients is the largest series to date, with which the described interaction between marker and response on chemotherapy has been confirmed. For the purpose of the study, 389 colorectal patients with lymph node involvement were post-operatively treated with the active ingredient fluorouracil. The treatment was conducted between 1991 and 1999, within the framework of a study by the *Austrian Breast and Colorectal Cancer Study Group*. The patients were observed over a ten-year period. The marker status was retrospectively ascertained from the archived tumour material of these patients, with the aid of the MARK53® analysis. The mutation frequency amounted to 33 percent. The goal was to evaluate whether a different marker status had an impact on the chemotherapy.

The most important results:

- In the case of low-risk patients (patients with less than four positive lymph nodes) with a normal p53 marker status, the post-operative chemotherapy showed itself to be more effective than previously assumed. The 5-year survival rate of these patients lay at 81 percent.

- In the case of low-risk patients with a mutated p53 marker status, the survival with post-operative chemotherapy was – at 62 percent - significantly poorer. The risk of dying was twice as high for these patients.
- In the case of high-risk patients (more than 7 positive lymph nodes), the marker did not influence survival.

Internationally, fluorouracil alone is viewed as being too weak, or rather, ineffective a therapy for high-risk colorectal patients. This is reinforced by the fact that there is no interaction to be seen between fluorouracil and the p53 marker in the high-risk group.

Marker status is more significant than the TNM system

In this retrospective study it was possible to show that the marker had a stronger impact on the survival of low-risk colorectal patients with post-operative chemotherapy, than the traditional prognostic categories of the TNM system, such as e.g. tumour size or lymph node involvement.

The TNM classification was developed in the 50's and serves for the staging of malignant tumours. The classification is based upon statistical studies, which define the anticipated behaviour of tumours on account of the tumour size and lymph node involvement. The staging of a tumour in accordance with the categories of the TNM system enables the evaluation of a prognosis and, to this day, determines the further therapy.

From the hitherto available studies, Kandioler is able to identify the consequence that the p53 marker status is to be taken into account in future clinical studies. If the p53 marker status is not taken into account in the study planning, this can lead to an unequal distribution of patients with normal and mutated marker status and, as a result, to an incorrect assessment of the actual effectiveness of a chemotherapy.

Prospective randomised PANCHO study

On account of the findings already in existence, a pan-Austrian study has been initiated, one which – for the first time - is making a prospective randomised study into the predictive value of the p53 marker. In thirteen centres in Austria, 168 patients with oesophageal cancer have participated in the PANCHO study (p53 adapted neoadjuvant chemotherapy with operable oesophagus carcinoma). The study is among the largest oesophageal cancer studies worldwide and will be published soon.

For Kandioler it is now a matter of bringing the p53 marker into wide-scale clinical application in order to select those patients who actually benefit from a pre-operative chemotherapy. Thanks to the MARK53® analysis, a standardised and clinically proven test is available. The cost of the gene test amounts to 900 euro, including a research cost contribution, which it is intended will further guarantee research that is independent of the pharmaceutical industry.

For further information:

Christina Aumayr-Hajek, press spokesperson

Dorotheergasse 7, A-1010 Vienna

E-mail: c.aumayr@freistil-pr.at, Tel: +43/ (0) 676/427 3788

Further Information: www.p53.at , www.kandioler.at and www.mark53.com

Personal Details

Since 2002, **Univ. Prof. Dr. Daniela Kandioler** has been professor of general surgery at the Medical University of Vienna (MUV). She has been working as senior physician at the Surgical University Clinic (Vienna General Hospital) for more than ten years and is additionally a medical specialist for vascular surgery and thoracic surgery. The focus of her activities at the Medical University of Vienna is the application of genetic markers for the improved efficacy of cancer therapy.

For some twenty years, the university professor has been concerning herself with clinical marker studies as a foundation for individualised and more efficient cancer therapy.

Focal areas: biotechnology, translational research and marker studies

The oncology surgeon's scientific focus lies in the areas of biotechnology, translational research, as well as in the evaluation of genetic markers in clinical studies of the following types of cancer: colorectal cancer, breast cancer, oesophageal cancer, stomach cancer, pancreatic cancer, liver metastases, lung cancer, ovarian cancer, familial cancer syndrome;

Molecular genetic research unit at MUV

In 1994, Kandioler established a molecular genetic research unit in association with the surgical research laboratory of MUV – representing a first in surgery at the time – and, since then, has been in charge of the priority research into p53 (www.p53.at) at the Surgical University Clinic in Vienna. The research group has so far produced publications with a journal impact factor of 217 and has already been able to submit two patents. To date it has been possible to raise more than one million euro in research funds for the priority research into p53.

1999 saw Kandioler being awarded the Theodor Billroth Prize by the Austrian Surgical Society, with the thesis entitled: *TP53 genotype but not immunohistochemistry is predictive for response to cisplatinum based neoadjuvant therapy in stage III NSCLC.* Since 2004, in her capacity as academic sponsor, the oncology surgeon has been leading clinical studies, the central orientation of which lies in researching the prediction of therapy response with the aid of the genetic tumour marker p53. Examples of application areas are bronchial carcinoma, oesophageal cancer and colonic carcinoma.

Kandioler is a member of the board of *Austrian Society of Surgical Oncology (ACO-ASSO),* the *European Federation of Colorectal Cancer* (EFR) and a member of the *TP53 International Collaborative Group*. In 2009, in her capacity as president, she organised the ACO-ASSO meeting on the topic *Individualised Cancer Therapy*. As editor-in-chief, Kandioler has published the journal *Interdisziplinäre Onkologie* (English: Interdisciplinary Oncology) for several years and is board member and reviewer for a series of scientific journals.

For further information: www.p53.at and www.kandioler.at