Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Special Issue: *Global Perspectives on Esophageal Diseases*
PERSPECTIVE

TP53 is not a prognostic marker—clinical consequences of a generally disregarded fact

Tamara Braunschmid,[1] Irene Kührer,[2] Martina Mittlböck,[3] Maria Westerhoff,[4] Sonja Kappel-Latif,[5] Lindsay Brammen,[1] Kausilia K. Krishnadath,[6] Wayne A. Phillips,[7] Michael Gnant,[1] and Daniela Kandioler[1]

[1]Department of Surgery, Medical University of Vienna, Vienna, Austria. [2]Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria. [3]Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, Austria. [4]Department of Pathology, University of Michigan, Ann Arbor, Michigan. [5]Department of Surgery, Research Laboratories, Medical University of Vienna, Vienna, Austria. [6]Department of Translational Gastroenterology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. [7]Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Address for correspondence: Daniela Kandioler, M.D., Department of Surgery, Medical University of Vienna, Spitalgasse 23, A-1090 Vienna, Austria. daniela.kandioler@meduniwien.ac.at

Technological progress within the last 15–20 years has significantly increased our knowledge about the molecular basis of cancer development, tumor progression, and treatment response. As a consequence, a vast number of biomarkers have been proposed, but only a small fraction of them have found their way into clinical use. The aim of this paper is to describe the specific demands a clinically relevant biomarker should meet and how biomarkers can be tested stepwise. We name this procedure the "triple-R principle": robustness, reproducibility, and relevance. The usefulness of this principle is illustrated with the marker *TP53*. Since it is mutated in a broad spectrum of cancer entities, *TP53* can be considered a very promising marker. Thus, *TP53* has been studied in detail but there is still no explicit consensus about its clinical value. By considering our own experience and reviewing the literature, we demonstrate that a major problem of current biomarker research is disregard of whether the biomarker is prognostic or predictive. As an example, it is demonstrated that *TP53* is not a prognostic marker, but rather a purely predictive marker, and that disregard of this fact has made this otherwise strong biomarker appear as not being clinically useful so far.

Keywords: p53; cancer; prognostic biomarker; predictive biomarker

Introduction

"Clinically useful prognostic and predictive markers are those developed with a specific clinical context in mind and tested and validated within that clinical context."[1] In other words, to reach clinical utility, the clinical evaluation of a potential biomarker is necessary. Currently, many biomarkers are proposed, but only a few find their way into routine clinical use. Here, we demonstrate that even prominent biomarkers are not properly evaluated. The analysis as to whether a marker has prognostic or predictive qualities has previously not been recognized as crucial information thus far and therefore is often omitted.

TP53 is one of the most commonly mutated genes in cancer and was therefore considered a promising biomarker.[2,3] The presence of a *TP53* mutation is generally associated with poor prognosis for the clinical behavior of the cancer.[4] Nevertheless, no coherent explanation for this phenomenon can be found in the literature.[4,5] No clinically useful opinion has yet been formed about what a mutated *TP53* really means for a patient's course of disease or for its outcome.[4] As a consequence, many papers refer to *TP53* as a prognostic marker, while others label it a predictive marker.[6] The impression is given that these two terms are often used as synonyms, although, especially in clinical oncology, there is a significant difference.

doi: 10.1111/nyas.13947

Table 1. The triple-R principle for systematic clinical evaluation of a biomarker

		Questions to be answered	Study design
Phase I	Robustness	Specificity of the marker in populations with and without cancer Prevalence of marker positivity in cancer patients Hypothesis-finding	Retrospective studies
Phase II	Reproducibility	Marker type (diagnostic, prognostic, or predictive) Marker test (sensitivity and specificity, e.g., to detect a mutation)	Prospective studies
Phase III	Relevance	Confirm magnitude of effect(s) Confirm marker type Confirm effect is independent of known prognostic or predictive factors	Prospective studies (with treatment randomization for testing a predictive marker)

Note: A biomarker's robustness is determined by the specificity, the prevalence, and the hypothesis of the marker. Reproducibility is dependent on knowledge of the marker type—whether the marker is diagnostic, prognostic, or predictive—and the availability of standardized, sensitive marker tests. Relevance is assessed in prospective randomized phase III biomarker studies.

The aim of this article is to point out possible pitfalls during the process of developing a putative molecular biomarker. For that purpose, the "triple-R principle" is presented. This concept is a standardized, stepwise process to evaluate a biomarker for its clinical usefulness. By considering our own experience and reviewing the literature, we demonstrate that *TP53*, contrary to opinion, is not a prognostic marker but a purely predictive marker. It can be seen that neglecting proper clinical evaluation of the biomarker *TP53* has generated a lot of confusion and prevented the routine clinical application of one of the most promising biomarkers known so far.

The triple-R principle

Biomarker studies evaluate the essential qualities of biomarkers. These evaluations need to be done stepwise in order to avoid misleading conclusions. The triple-R principle summarizes the qualities of a biomarker that have to be assessed stepwise in retrospective and prospective clinical studies. The studies have to be allocated to the three phases of biomarker evaluation. Results and conclusions from each phase are required to correctly progress to the next phase (Table 1).

Phase I: robustness of a biomarker

Most putative biomarkers arise out of the observation that a certain variant can be found in cancer patients. To clarify whether this variant represents a potential biomarker, three questions have to be answered upfront in retrospective analyses. First, Does this variant exclusively occur in cancer? (= specificity of the marker in cancer cells or patients); second, In which types of cancer and how frequently does this variant occur? (= prevalence of the biomarker in cancer patients or with cancer subtypes); and third, Does the biomarker status indicate cancer or affect the course of the disease, and if so, how? (= hypothesis, how the marker works). By answering the last of these questions, one can acquire an idea of whether the marker has diagnostic properties (= indicating cancer) or is influencing a patient's survival. Thus, robustness describes the basic characteristic features of a biomarker and gives an idea of possible implications of the marker status. Assessment of a marker's robustness can be done in retrospective phase I biomarker studies.

Robustness of *TP53*. It has been consistently demonstrated that *TP53* mutations can be found in all types of cancer, with an overall prevalence of 50%.[2] This represents the highest prevalence among currently established cancer biomarkers (e.g., *KRAS*, *BRAF*, *EGFR*, etc.).[4] *TP53* mutations are also highly specific for cancer, meaning that the mutations do not occur in normal cells. As the clinical value of a marker is directly related to its prevalence and its particular specificity, *TP53* appears as the most promising from that point of view. Finally, besides its diagnostic properties (not covered in this article), *TP53* has been recognized as the gatekeeper for many cellular processes, such as the cell cycle, apoptosis, and many more.[7] Therefore, numerous hypotheses describe that *TP53* most

likely affects the course of a malignant disease. How the marker actually works is still under discussion and in our view lacks proper investigation.

Phase II: reproducibility of a biomarker

A biomarker will be considered clinically useful if the biomarker evaluation delivers clinically reproducible results. We postulate that in biomarker research, reproducibility depends on two factors: knowledge of the marker type and the availability of a standardized, sensitive marker test. These issues have to be assessed in phase II biomarker studies, which should be carried out retro- or prospectively.

Knowledge of the marker type. If a marker appears to be associated with a cancer patient's survival, it is crucial to know why. The recognition of the biological activities of a marker provides initial ideas and is able to serve as a basis for the marker hypothesis. In principle, a marker can affect survival of a cancer patient because it is either prognostic or predictive. Not least because "predictive" and "prognostic" are frequently misused as synonyms, the difference between predictive and prognostic markers often remains unclear. The phrase that a marker "predicts a prognosis" demonstrates that there might also be a semantic problem involved.

It is important to be aware that a prognostic marker predicts the course of a disease, that is, freedom or relapse of the disease. In contrast, a predictive marker predicts the efficacy of specific treatments, that is, response to a treatment or treatment failure. If the explicit definition of the marker type is missing, a marker may be used inappropriately, which will generate inconsistent results in clinical biomarker studies. At worst, due to the inconsistent results, the marker will be classified as clinically not useful.

What makes it difficult is that both types of markers, prognostic and predictive, may affect survival. Therefore, it is important to be aware that they still answer different questions in cancer cohorts. Indeed, the underlying scientific knowledge for the marker type is crucial for planning and interpreting clinical biomarker studies.[8]

A prognostic marker relates to the natural history of a disease and objectively predicts the patient's overall outcome.[9,10] Prognostic markers are used to estimate risk. They are independent of therapy and are therefore unable to predict response to therapy.[9,10] Thus, we find as a rule that a prognostic biomarker has to predict survival of cohorts in the absence of treatment. In contrast, a predictive marker is able to objectively predict the effect of a certain treatment and can be used to select a particular treatment over another.[9,10] Thus, we find as a rule that the clinical evaluation of a predictive marker has to be connected to a treatment.

The influence of the marker status on the treatment effect is usually described as an interaction, and both quantitative and qualitative interactions can be distinguished.[11] If all patients (both biomarker-negative and -positive) benefit from a certain treatment, but one marker group derives a greater benefit than the other, this phenomenon is referred to as a quantitative interaction.[9] If only biomarker-negative patients experience an advantage from a specific therapy while biomarker positive patients experience no advantage or even a disadvantage from this treatment, this is referred to as a qualitative interaction.[9] As a rule, a qualitative interaction gives rise to dramatically different treatment effects for marker-positive and -negative patients. Furthermore, the presence of an unrecognized qualitative interaction will always worsen the overall treatment effect in a clinical trial. Qualitative interactions have been rarely described in marker research so far.

While the prognostic marker type can be simply determined by analyzing the marker in the context of an untreated cohort (which might not always be feasible nowadays), determination of a predictive marker type is more difficult as it is connected to specific treatments. Additionally a biomarker might show its predictive ability for some treatments but not for others.

The marker type of *TP53*. Recently, two clinical studies have been published that qualify for determining the marker type of *TP53* because both studies included untreated control arms.[12,13] Both studies revealed that the *TP53* status did not affect survival in the untreated patients. This strongly suggests that *TP53* is not prognostic (Fig. 1B). Furthermore, in cohorts treated with certain chemotherapies, *TP53* was often found to be associated with survival, which indicates that *TP53* is a predictive marker.[12,14–17]

One of the studies was published by the University of Vienna p53 Research Group. Although the number of patients was quite small, the design

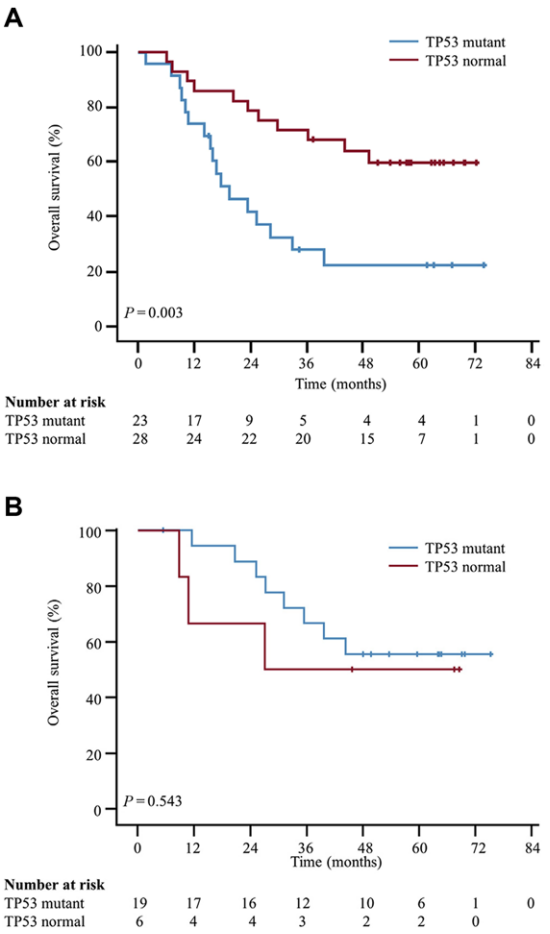

Figure 1. Phase II biomarker study design to assess the marker type of *TP53*. Prospective study randomized patients either received (A) neoadjuvant chemotherapy followed by surgery or (B) surgery alone for operable colorectal liver metastases. *TP53* analysis was done retrospectively from surgical specimens. (A) In the presence of neoadjuvant chemotherapy, a mutated *TP53* predicts a dramatic survival disadvantage (hazard ratio of 5.5), while a normal *TP53* predicts survival benefit. The opposite treatment effect is predicted by the respective marker status (mutated or normal), indicating the presence of a qualitative interaction between marker status and treatment effect. (B) In the absence of chemotherapy (surgery-only group), *TP53* status is not associated with overall survival, and thus is not prognostic. Originally published in Ref. 12.

of the study was qualified to demonstrate the absence of any prognostic but the presence of a strong predictive value of *TP53* in a single study for the first time.[12] In this study, a homogenous cohort of patients with operable colorectal liver metastases were treated either with chemotherapy (5-fluorouracil and oxaliplatin) followed by surgery or with surgery only. In the surgery-only patients, *TP53* marker status was not related to survival, whereas in the group of patients treated with preoperative chemotherapy and subsequent surgery, a dramatic survival difference associated with *TP53* status was noticed.[12] Strikingly, the chemotherapy effect was the opposite in the chemotherapy patients with and without *TP53* mutations. Overall, the *TP53*-mutated patients who received preoperative chemotherapy had the poorest outcome, even worse than the patients treated with surgery only (irrespective of their *TP53* status). *TP53*-normal patients treated with the respective preoperative chemotherapy had a significantly better outcome, which was also superior to the outcome of the surgery only patients (irrespective of their *TP53* status). Thus, a dramatically different treatment effect for *TP53*-mutated and *TP53*-normal patients was demonstrated, suggesting the presence of a qualitative interaction for the first time. The latter was supported by the demonstrated hazard ratio of 5.5 to the disadvantage of the chemotherapy-treated patients with *TP53* mutations (Fig. 1).

Standardized, sensitive marker tests. A clinically useful biomarker requires a reproducible method to assess the marker status (i.e., normal versus mutated gene status). In other words, a standardized marker test has to be available with adequate, proven sensitivity and specificity. If different tests are available, as for *TP53* worldwide, it has to be proven that they deliver identical results. In clinical practice, however, standardization of marker tests is only raised as a topic when a marker is considered as clinically useful. Thus, initially marker research is done with nonstandardized methods, limiting the comparability and significance of the research results.

Marker tests for *TP53*. p53 immunohistochemistry (IHC) is an ideal example to demonstrate the problems of marker tests. p53 IHC is still used as a common method to deduce the mutational status of *TP53*, even though it is well known that p53 IHC and *TP53* sequencing results can differ dramatically.[5,17–19] Besides the problem of false negative and false positive IHC results (= lack of specificity), the method is not standardized, meaning that different antibodies with varying degrees of sensitivity are in use.[9,13,17,20,21] Additionally, it is noteworthy that the concordance rates between IHC and sequencing differ between tumor entities,

Table 2. Systematic clinical evaluation of the biomarker *TP53*: the triple-R principle illustrated by 20 years of clinical research at the Medical University of Vienna p53 Research Group

Year	Reference	Title		
Phase I: robustness				
1994	29	Carcinogen-specific mutations in the p53 tumor suppressor gene in lung cancer	Prevalence	Retrospective
2004	30	p53 analysis in gallbladder cancer: comparison of gene analysis versus immunohistochemistry	Prevalence	Retrospective
1996	31	Molecular genetic differentiation between primary lung cancers and lung metastases of other tumors	Specificity	Retrospective
2006	32	Genetic detection of lymph node micrometastases: a selection criterion for liver transplantation in patients with liver metastases after colorectal cancer	Specificity	Retrospective
1999	33	*TP53* genotype but not immunohistochemical result is predictive of response to cisplatin-based neoadjuvant therapy in stage III nonsmall cell lung cancer	Hypothesis/marker test	Retrospective
2000	34	*TP53* mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients	Hypothesis/marker test	Retrospective
2002	17	*TP53* genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer	Hypothesis/marker test	Retrospective
Phase II: reproducibility				
2008	35	Growing clinical evidence for the interaction of the p53 genotype and response to induction chemotherapy in advanced nonsmall cell lung cancer	Marker type/marker test	Prospective/retrospective
2014	16	Biomarker *TP53* divides patients with neoadjuvantly treated esophageal cancer into two subgroups with markedly different outcomes. A p53 research group study	Marker type/marker test	Prospective/retrospective
2015	15	*TP53* mutational status and prediction of benefit from adjuvant 5-fluorouracil in stage III colon cancer patients	Marker type/marker test	Prospective/retrospective
2015	12	Assessing *TP53* marker type in patients treated with or without neoadjuvant chemotherapy for resectable colorectal liver metastases: a p53 Research Group study	Marker type/marker test	Prospective/retrospective
Phase III: relevance				
2010	36	PART1—p53 adapted preoperative radiotherapy for T2 and T3 rectal cancer. A study of the p53 research group	Magnitude of effect/marker test	Prospective
2018	28	Pancho trial (p53-adapted neoadjuvant chemotherapy for resectable esophageal cancer) completed—mutation rate of the marker higher than expected	Magnitude of effect/marker test	Prospective randomized

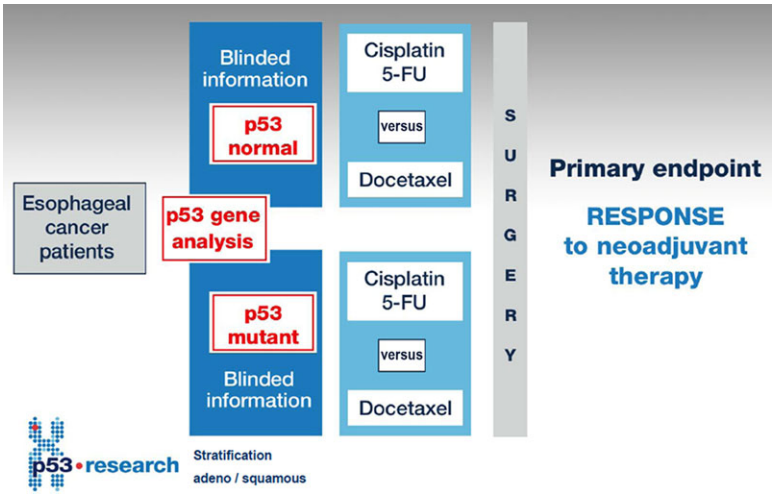

Figure 2. Phase III biomarker trial for clinical validation of biomarker *TP53*: the PANCHO trial (p53-Adjusted Neoadjuvant Chemotherapy for Potentially Resectable Esophageal Cancer; ClinicalTrials.gov identifier: NCT00525200) uses the Marker by Treatment Interaction clinical trial design. Originally published in Ref. 28.

which might derive from different *TP53* mutation patterns present in different tumor entities. This demonstrates that IHC is also detecting different *TP53* mutations unequally.[22,23] However, for a predictive marker, the highest levels of sensitivity and specificity of the marker test are required because a predictive marker is used to select an appropriate treatment.

In recent years, DNA-based marker tests have gained increasing importance. We like to assume that a gene mutation detected at the DNA level is either present or not and that this fact should not change, no matter when or how often the test is run. However, there are various different DNA sequencing technologies on the market and their strategic emphases are different; most often, the focus is on high throughput rather than on gene-specific sensitivity.

Some technologies—like next-generation sequencing—are known to generate numerous sequencing artifacts,[24,25] which are considered to be identified by multiple testing. Such platforms offer the simultaneous testing of hundreds of genes or the detection of whole genome mutations of a patient. But, who is able to handle the vast amount of data properly? How will we correlate thousands of results with a certain cancer type, a certain cancer stage, a certain course of disease, or a certain response to a certain treatment? We will possibly never have enough data for meaningful statistical correlation of so many parameters.[26]

Phase III: relevance of a biomarker

As soon as the marker type is clarified and a sensitive, standardized marker test is established, both as a result of phase II biomarker studies, the therapeutic relevance of a biomarker can be finally assessed. The magnitude of the effect of a biomarker needs to be evaluated in a phase III biomarker study comparing standard treatment versus biomarker-adapted treatment in a prospective randomized design.[11] For the implementation of the biomarker in the trial, knowledge of the marker type is crucial (i.e., whether the biomarker is prognostic or predictive).

For the clinical validation of a prognostic marker, the marker should ideally be assessed in an untreated cohort; if not, then in a cohort treated with surgery only. A clinically relevant prognostic biomarker will split the cohort into two or more groups with significantly different outcomes. A prognostic marker is of clinical relevance if it can be demonstrated that its effect is independent of other prognostic parameters already established (using a multivariate analysis). As a consequence, stratification for the status of the validated, independent prognostic marker is mandatory for upcoming clinical trials or for reanalysis of published trials.

For the clinical validation of a predictive marker, an interaction between the marker and the effect of

a certain treatment has to be demonstrated. For that purpose, the Marker by Treatment Interaction Design has been suggested by Sargent *et al.*[11] This design splits the study population into two groups depending on the particular marker status (marker-negative and marker-positive patients). Subsequently, each group is randomized into two different treatment arms (standard versus experimental) and analyzed separately. The advantage of this design is that the superiority of the specific treatment as well as a possible interaction between the marker status and the treatment allocation can be tested.[11] However, this design is not ideal for testing a panel of markers.[11]

A predictive marker is of clinical relevance if a significant interaction between the marker status and the effect (preferably on survival) of the treatment can be demonstrated. As a consequence, certain treatments can be preferably or even exclusively applied to patients with the appropriate marker status. Conversely, if it is demonstrated that a certain marker status interacts with a certain treatment in a disadvantageous way, this treatment can be avoided.

Relevance of TP53. For the biomarker *TP53*, the first prospective randomized biomarker trial has been conducted by the University of Vienna p53 Research Group.[27] The aim of the p53-Adjusted Neoadjuvant Chemotherapy for Potentially Resectable Esophageal Cancer (PANCHO) trial (ClinicalTrials.gov identifier: NCT00525200) is to validate the biomarker *TP53* as potentially predicting the effect of neoadjuvant chemotherapy in esophageal cancer patients. The trial has been designed according to the Marker by Treatment Interaction design (Fig. 2). A standardized marker test (Mark53® test; Mark53 Ltd, Vienna, Austria), which has been previously assessed in phase II biomarker trials (Table 2), is used and validated in that trial. The trial is completed and first results have been recently published.[28]

Summary

Only a few biomarkers find their way into clinical use. In this paper, the lack of systematic evaluation is identified as a major issue for biomarkers. As an answer to this problem, the "triple-R principle" (robustness, reproducibility, and relevance) is introduced as a systematic procedure for clinical biomarker evaluation. The importance of this systematic procedure is demonstrated with the well-known marker *TP53*, for which clinical research has so far failed to demonstrate clinical utility. Besides its diagnostic properties, there is still no consensus as to whether the marker is useful to prognosticate the course of disease or to predict treatment response in cancer patients.

Twenty years of systematic clinical evaluation of the marker *TP53* by the Medical University of Vienna p53 Research Group has culminated in the conclusion that *TP53* is not a prognostic but a predictive marker. We believe that the community's disregard of the marker type of *TP53* has generated a lot of confusion in clinical p53 research, which has prevented the marker from clinical use so far. As a consequence, the development and validation of standardized marker tests was not tackled.

We contend that the reproducibility of results in clinical biomarker research crucially depends on the knowledge of the marker type of a biomarker. We consider the omission of initial determination of the marker type as a serious mistake in clinical biomarker research.

Author contribution

T.B. and D.K. contributed to the conception, design, acquisition, and interpretation of data. All authors revised the article and approved the final version.

Competing interests

D.K. is an uncompensated consultant and holds leadership position at MARK53 Ltd, Vienna, Austria.

References

1. Kim, C., Y. Taniyama & S. Paik. 2009. Gene expression-based prognostic and predictive markers for breast cancer: a primer for practicing pathologists. *Arch. Pathol. Lab. Med.* **133:** 855–859.
2. International Agency for Research on Cancer, W.H.O. Somatic mutations. Accessed September 1, 2017. http://p53.iarc.fr/TP53SomaticMutations.aspx.
3. Hollstein, M. *et al.* 1991. p53 mutations in human cancers. *Science* **253:** 49–53.
4. Munro, A.J., S. Lain & D.P. Lane. 2005. P53 abnormalities and outcomes in colorectal cancer: a systematic review. *Br. J. Cancer* **92:** 434–444.
5. Kressner, U. *et al.* 1999. Prognostic value of p53 genetic changes in colorectal cancer. *J. Clin. Oncol.* **17:** 593–599.

6. Duffy, M.J. *et al.* 2007. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *Eur. J. Cancer* **43:** 1348–1360.
7. Levine, A.J. & M. Oren. 2009 The first 30 years of p53: growing ever more complex. *Nat. Rev. Cancer* **9:** 749–758.
8. Gasparini, G. & D.F. Hayes. 2008. *Biomarkers in Breast Cancer—Molecular Diagnostics for Predicting and Monitoring Therapeutic Effect.* Springer.
9. Mehta, S. *et al.* 2010. Predictive and prognostic molecular markers for cancer medicine. *Ther. Adv. Med. Oncol.* **2:** 125–148.
10. Ballman, K.V. 2015. Biomarker: predictive or prognostic? *J. Clin. Oncol.* **33:** 3968–3971.
11. Sargent, D.J. *et al.* 2005. Clinical trial designs for predictive marker validation in cancer treatment trials. *J. Clin. Oncol.* **23:** 2020–2027.
12. Pilat, N. *et al.* 2015. Assessing the TP53 marker type in patients treated with or without neoadjuvant chemotherapy for resectable colorectal liver metastases: a p53 Research Group study. *Eur. J. Surg. Oncol.* **41:** 683–689.
13. Ma, X. *et al.* 2014. Significance of TP53 mutations as predictive markers of adjuvant cisplatin-based chemotherapy in completely resected non-small-cell lung cancer. *Mol. Oncol.* **8:** 555–564.
14. Kandioler, D. 2008. Personalized medicine—p53 gene analysis for prediction of response to neoadjuvant therapy in esophageal cancer. *Magazine Eur. Med. Oncol.* **1:** 137–142.
15. Kandioler, D. *et al.* 2015. TP53 mutational status and prediction of benefit from adjuvant 5-fluorouracil in stage III colon cancer patients. *EBioMedicine* **2:** 825–830.
16. Kandioler, D. *et al.* 2014. The biomarker TP53 divides patients with neoadjuvantly treated esophageal cancer into 2 subgroups with markedly different outcomes. A p53 Research Group study. *J. Thorac. Cardiovasc. Surg.* **148:** 2280–2286.
17. Kandioler, D. *et al.* 2002. TP53 genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer. *Ann. Surg.* **235:** 493–498.
18. Scartozzi, M. *et al.* 2009. Toward molecularly selected chemotherapy for advanced gastric cancer: state of the art and future perspectives. *Cancer Treat. Rev.* **35:** 451–462.
19. Kaserer, K. *et al.* 2000. Staining patterns of p53 immunohistochemistry and their biological significance in colorectal cancer. *J. Pathol.* **190:** 450–456.
20. Pan, Y. *et al.* 2017. P53 and Ki-67 as prognostic markers in triple-negative breast cancer patients. *PLoS One* **12:** e0172324.
21. Babinska, A. *et al.* 2017. Diagnostic and prognostic role of SF1, IGF2, Ki67, p53, adiponectin, and leptin receptors in human adrenal cortical tumors. *J. Surg. Oncol.* **116:** 427–433.
22. Rajendra, S. *et al.* 2017. Active human papillomavirus involvement in Barrett's dysplasia and oesophageal adenocarcinoma is characterized by wild-type p53 and aberrations of the retinoblastoma protein pathway. *Int. J. Cancer* **141:** 2037–2049.
23. Kobel, M. *et al.* 2016. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. *J. Pathol. Clin. Res.* **2:** 247–258.
24. Lassmann, T., Y. Hayashizaki & C.O. Daub. 2009. TagDust—a program to eliminate artifacts from next generation sequencing data. *Bioinformatics* **25:** 2839–2840.
25. Star, B. *et al.* 2014. Palindromic sequence artifacts generated during next generation sequencing library preparation from historic and ancient DNA. *PLoS One* **9:** e89676.
26. Kandioler, D. & R. Jakesz. 2006. P53 as a prognostic and predictive indicator. In *Biomarkers in Breast Cancer. Molecular Diagnostics for Predicting and Monitoring Therapeutic Effect.* G. Gasparini & D.F. Hayes, Eds.: 193–209. Humana Press.
27. Kappel, S.B., C. Wolf, B. Gacic, *et al.* 2008. Turning the tables on surgical oncology: the Pancho trial unplugged. *Eur. Surg.* **40:** 277–283.
28. Kappel-Latif, S. *et al.* 2018. Pancho trial (p53-adapted neoadjuvant chemotherapy for resectable esophageal cancer) completed—mutation rate of the marker higher than expected. *Eur. Surg.* **50:** 160–166.
29. Kandioler, D. *et al.* 1994. Carcinogen-specific mutations in the p53 tumor suppressor gene in lung cancer. *J. Thorac. Cardiovasc. Surg.* **107:** 1095–1098.
30. Puhalla, H. *et al.* 2004. p53 analysis in gallbladder cancer: comparison of gene analysis versus immunohistochemistry. *Anticancer Res.* **24:** 1201–1206.
31. Kandioler, D. *et al.* 1996. Molecular genetic differentiation between primary lung cancers and lung metastases of other tumors. *J. Thorac. Cardiovasc. Surg.* **111:** 827–831; discussion 832.
32. Kappel, S. *et al.* 2006. Genetic detection of lymph node micrometastases: a selection criterion for liver transplantation in patients with liver metastases after colorectal cancer. *Transplantation* **81:** 64–70.
33. Kandioler-Eckersberger, D. *et al.* 1999. The TP53 genotype but not immunohistochemical result is predictive of response to cisplatin-based neoadjuvant therapy in stage III non-small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* **117:** 744–750.
34. Kandioler-Eckersberger, D. *et al.* 2000. TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. *Clin. Cancer Res.* **6:** 50–56.
35. Kandioler, D. *et al.* 2008. Growing clinical evidence for the interaction of the p53 genotype and response to induction chemotherapy in advanced non-small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* **135:** 1036–1041.
36. Wolf, B. *et al.* 2010. PART 1—p53 adapted preoperative radiotherapy for T2 and T3 rectal cancer. A study of the p53 research group. *Eur. Surg.* **42:** 18–23.