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Review

Prognostic versus predictive value of biomarkers in oncology

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ABSTRACT

Numerous options are currently available for tumour typing. This has raised intense interest in the elucidation of prognostic and predictive markers. A prognostic biomarker provides information about the patients overall cancer outcome, regardless of therapy, whilst a predictive biomarker gives information about the effect of a therapeutic intervention. A predictive biomarker can be a target for therapy. Amongst the genes that have proven to be of relevance are well-known markers such as ER, PR and HER2/neu in breast cancer, BCR–ABL fusion protein in chronic myeloid leukaemia, *c-KIT* mutations in GIST tumours and *EGFR1* mutations in NSCLC. Several reasons for the difficult elucidation of new markers will be addressed including the involvement of cellular pathways in tumour biology instead of single genes and interference in disease outcome as a result of anticancer therapies. Future perspectives for the development of prognostic and predictive markers will be given.

1. Introduction

With the availability and application of various treatment modalities, survival amongst cancer patients has improved over the past decades. However, there are still many patients who receive anticancer therapy from which they do not benefit whilst they do experience toxicity. In recent years, a widespread search for new, tumour biology driven therapeutics has started. This has raised intense interest in the elucidation of corresponding prognostic and predictive biomarkers in order to improve outcome by better patient selection for an anticancer treatment. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to a specified therapeutic intervention.[1] Biomarkers can be determined in numerous ways, for example, in easy obtainable body fluids serving as surrogate biological assay, like plasma, serum or urine. But also more invasive techniques requiring tumour tissue for immunohistochemistry as well as DNA and RNA analyses are widely used. A prognostic biomarker provides information about the patients overall cancer outcome, regardless of therapy. The presence or the absence of such a prognostic marker can be useful for the selection of patients for a certain treatment, but does not predict the response to this treatment. Prognostic biomarkers can be separated in two groups: biomarkers that give information on recurrence in patients who receive curative treatment and biomarkers that correlate with the duration of (progression free) survival in patients with metastatic disease. According to a NIH Consensus Conference, a clinical useful prognostic marker must be a proven independent, significant factor, that is easy to determine and interpret and has therapeutic consequences.[2] A biomarker with predictive value gives information on the effect of a therapeutic intervention in a patient. A predictive biomarker can also be a target for therapy. One can distinguish upfront and early predictive markers. The first can be used for patient selection and the second provides information early during therapy.

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The current interest in marker determination is boosted by the discovery of genes that have proven to be of clinical relevance such as the oestrogen receptor (ER), the progesterone receptor (PR) and HER2/neu in breast cancer, BCR–ABL fusion protein in chronic myeloid leukaemia, *c-KIT* mutations in gastrointestinal stromal tumours (GIST) and *epidermal growth factor receptor 1* (*EGFR1*) mutations in non-small cell lung cancer (NSCLC). These genes all seem to be key regulators of development, growth and proliferation in the respective tumour types. Euphoria is now somewhat tempered because the discovery of other the so-called promising markers translates rather slowly into clinical applicability. One reason for this is the fact that the course of most of malignancies is the consequence of a number of essential alterations in tumour cells rather than a single mutation.[3] In addition, the limited size of most studies and variable techniques used for marker determination plays a role. Often initially reported promising results are not reproducible. In an attempt to optimise biomarker studies, Hayes and colleagues proposed a tumour marker utility grading system (TMUGS). For each biomarker a grade of utility is assigned, accompanied by a level of evidence (LOE) that scores the quality of the research. The LOE categories range from I to V. Level V evidence is obtained from case reports and clinical experience and is considered weak, whilst level I evidence is derived either from at least one prospective randomised controlled trial specifically designed to test the marker or from a meta-analysis and/or overview of level II or III studies and is considered definitive.[4] In addition, a consortium of the National Cancer Institute-European Organisation for Research and Treatment of Cancer (NCI-EORTC) reported in several journals a guideline for reporting tumour marker prognostic studies (REMARK).[5]

In this review, the progress in the development of biomarkers in solid tumours will be addressed such as, involvement of cellular pathways in tumour biology instead of single genes and interference in disease outcome as a result of anticancer therapies. Examples of both well-known biomarkers and potential new discoveries (summarised in Table 1) will form the basis for a discussion below on the present knowledge and new avenues for the development of prognostic and predictive biomarkers.

2. ER/PR in breast cancer

ER and/or PR expression is an independent prognostic factor in breast cancer. Patients with ER and/or PR positive tumours have a better survival than hormone receptor negative tumours, with a 5-year overall survival (all stages) of 83% in the ER+/PR+ group versus 69% in the double negatives (LOE III).[6] High cellular expression of ER and PR predicts benefit from endocrine therapy in the adjuvant and metastatic setting (LOE I).[7] Tumour hormone receptor status is, therefore, routinely assessed in breast cancer. It now also becomes clear that hormone receptor status in a patient can change during the course of the disease and may differ across lesions. For example, the ER status of metastatic disease is different from the primary tumour in about 20% of cases.[8,9] In addition, PR expression is lost in 40% of previous positive tumours when they metastasise. Therefore, recently revised guidelines of the American Society of Clinical Oncology recommend measurement of both ER and PR in metastatic lesions if these results might influence treatment planning.[10] A search for non-invasive techniques to predict response to treatment is ongoing. Studies with positron emission tomography (PET) for whole body ER imaging with [^{18}F]fluoroestradiol (FES) suggest feasibility of such an approach.[11]

3. HER2/neu in breast cancer

Another relevant biomarker in breast cancer patients is HER2/neu. The *HER2/neu* gene amplification leads to overexpression of its receptor on the cell membrane. This results in increased proliferation and angiogenesis, and inhibition of apoptosis. HER2/neu positive tumours are more aggressive and have, therefore, a worse prognosis compared to negative tumours. In this respect, HER2/neu in node positive breast cancer is of prognostic value (LOE II). For the node negative, HER2/neu positive group of patients this is less clear.[12–17] HER2/neu is

Table 1 – Biomarkers of interest: an overview of prognostic and predictive value

Biomarker	Tumour type	Prognostic value		Predictive value		
		LOE		LOE		Therapy
ER/PR	BRCA	Yes	III	Yes	I	Endocrine therapy
HER2/neu	BRCA	Yes	II	Yes	II	Trastuzumab
c-KIT	GIST	Yes, subgroup[a]	II	Yes, subgroup[b]	II	Imatinib
EGFR1	NSCLC	No	III	Yes, subgroup[c]	II	Gefitinib, erlotinib
	CRC	No	III	Yes	IV	Cetuximab, panitumumab
Mutated K-ras	NSCLC	Yes	II	Yes	III	Gefitinib, erlotinib
	CRC	No	III	Yes	IV	Cetuximab, panitumumab
TRAIL receptors	CRC	Yes	II	NK	–	RhTRAIL; TRAIL receptor antibodies
VEGF	RCC	Yes	II	No	II	Angiogenesis inhibitors

LOE: level of evidence; ER: oestrogen receptor; PR: progesterone receptor; BRCA: breast cancer; GIST: gastrointestinal stromal tumours; EGFR1: epidermal growth factor receptor 1; NSCLC: non-small cell lung cancer; CRC: colorectal cancer; TRAIL: tumour necrosis factor (TNF)-related apoptosis-inducing ligand; NK: not known; VEGF: vascular endothelial growth factor; RCC: renal cell carcinoma.

a c-KIT exon 11 mutation.

b c-KIT exon 9 mutation.

c EGFR1 exon 18, 19 or 21 mutation.

the target for the monoclonal antibody trastuzumab and the EGFR1 and HER2 dual tyrosine kinase inhibitor (TKI) lapatinib. Patients with HER2/neu overexpressing tumours benefit from treatment with trastuzumab in the metastatic as well as in the adjuvant setting (LOE II).[18–20] Interestingly, HER2/neu positive patients receiving adjuvant chemotherapy plus trastuzumab showed the same recurrence-free survival as HER2/neu negative patients treated with chemotherapy alone.[21] Thus, the prognostic value of HER2/neu overexpression is neutralised in this study by targeting the prognostic biomarker.

Like ER expression, HER2/neu expression can change over time and can vary between lesions within a patient. Several reports suggest a conversion from negative into positive HER2/neu status when the disease did recur, although depending on technique, for a varying percentage.[22–25] Instead of serial biopsies, SPECT or PET whole body radiolabelled trastuzumab scintigraphy would be more attractive. This approach was capable to detect HER2/neu expression in tumour lesions in the patients.[26,27]

Both primary and acquired resistance to trastuzumab occurs. In addition to absence of the receptor, there are a number of other factors that can potentially explain resistance. For example, the presence of multiple truncated HER2/neu receptors at the tumour cell surface might play a role. The truncated p95HER2/neu receptor lacks the extracellular binding domain for trastuzumab, but has tyrosine kinase activity. Therefore, trastuzumab resistant tumours that express p95HER2/neu might benefit from treatment with lapatinib.[28]

4. Prognostic biomarkers for the relapse of breast cancer

Decision making about adjuvant systemic treatment for breast cancer is based on nodal status, tumour grade, tumour size, tumour hormone receptor and HER2/neu status, age and co-morbidity. Prognostic biomarkers that could provide better information on risk of relapse could spare many patients chemotherapy toxicity without compromising survival. Amongst several initiatives Buyse and colleagues validated a 70-gene signature for node negative breast cancer patients that has independent prognostic value additive to clinicopathologic parameters.[29] This approach is now tested in a prospective European study (MINDACT).

5. c-KIT in GIST

Several features are evaluated over the last few years to determine malignant behaviour of GISTs. Of known relevance are tumour size and mitotic index, which are used to classify the biologic behaviour of GIST.[30] The majority of GISTs are characterised by mutations in either the proto-oncogene *c-KIT* or the *platelet-derived growth factor receptor alpha* (*PDGFRα*). Interestingly, patients with mutation in the *c-KIT*-gene in exon 11 have a better prognosis as compared to those who lack a mutation or have another mutation (LOE II).[31–33] With the introduction of imatinib and sunitinib the outcome of GIST patients improved dramatically.[34,35] Imatinib and sunitinib are small molecule TKIs, which block signalling via c-KIT and PDGFRα. In 50–55% of the patients with advanced disease imatinib results in a durable objective response, whilst another 25–30% have stable disease according to RECIST.[36] During the course of treatment, however, most patients develop resistance to imatinib. A subgroup of these patients with progressive disease within a few months of imatinib treatment was characterised by bearing exon 9 activating mutations in *c-KIT*. In the two studies comparing an imatinib dose of 400 and 800 mg daily, the only difference was a better progression-free survival at the highest dose in patients with a tumour harbouring an exon 9 mutation (LOE II).[32] Exon 9 mutational status is, therefore, a negative predictive factor for response to imatinib and a positive predictive factor for benefit of 800 mg imatinib.[37] Progression after an initial response or stable disease for at least 3 months is caused by secondary *c-KIT* mutations in exon 13, 14, 17 or 18 in 50–70% of these patients.[38] Secondary mutations can differ across lesions in an individual patient. Several studies explored the value of different mutations in *c-KIT* and *PDGFRα* in the light of predicting response to TKIs.[39]

6. EGFR1 and K-ras in NSCLC and colorectal cancer (CRC)

In NSCLC and CRC, biomarkers of interest are EGFR1 and the *K-ras* oncogene. EGFR1 is overexpressed in multiple cancer types and is one of the targets in the treatment of NSCLC and metastatic CRC. The EGFR pathway plays a role in several cellular functions, including regulation of cell proliferation, migration and differentiation (Fig. 1). The prognostic value of EGFR1 protein expression is extensively studied in NSCLC and CRC patients but no definitive association between EGFR1 expression and prognosis was found.[40,41]

The *K-ras* oncogene controls cell growth via regulation of signal transduction pathways. *K-ras* mutation results in malignant transformation. In a meta-analysis including 28 studies assessing the correlation between *K-ras* mutation and survival in NSCLC patients, *K-ras* mutation appeared to be a biomarker of poor prognosis.[42] A multivariate analysis including 3439 CRC patients failed to prove an association between mutant *K-ras* and disease outcome.[43]

In recent years, two small-molecule EGFR1 TKIs (gefitinib and erlotinib) and two anti-EGFR1 monoclonal antibodies (cetuximab and panitumumab) were introduced in the clinic. Four phase III trials in previously untreated patients with advanced NSCLC combining two different chemotherapy regimens with and without gefitinib or erlotinib demonstrated no survival benefit. Subgroup analysis, however, identified four characteristics associated with benefit for the patient namely adenocarcinoma, female sex, Asian ethnicity and non-smoking.[44] Over the last years, it became clear that a subgroup of NSCLC patients, especially consisting of non-smokers, have mutations in the tyrosine kinase domain (exons 18, 19, and 21) of *EGFR1*. These mutations are predictive for response to either gefitinib or erlotinib (LOE II).[45–48] In contrast to *EGFR1* mutations being a predictive biomarker for a beneficial effect, mutations in *K-ras* are most commonly observed in heavy smokers predicting for treatment failure on EGFR1 TKIs.[49,50]

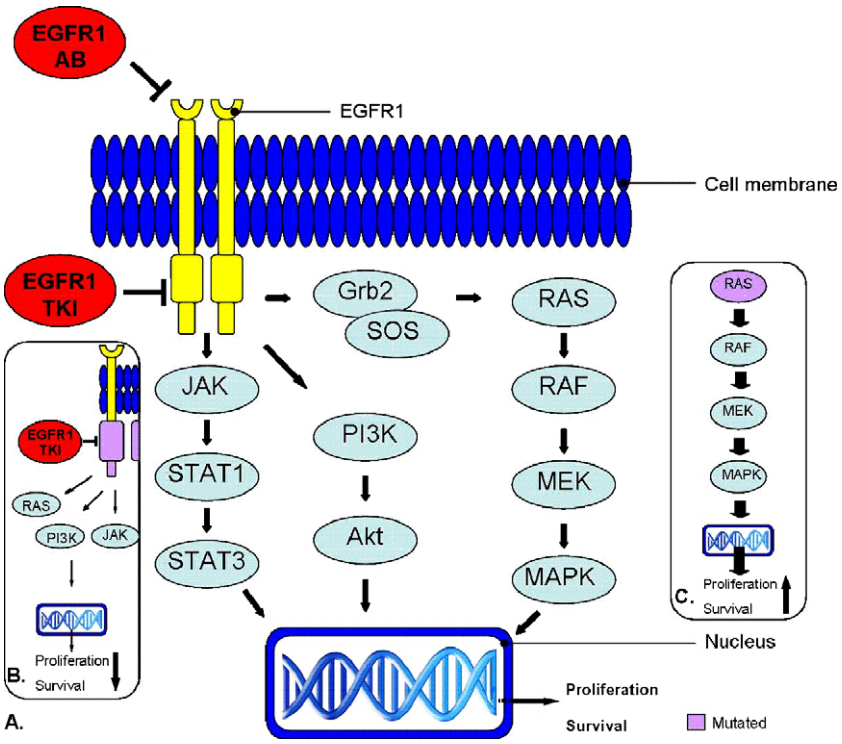

Fig. 1 – (A) EGFR1 pathway. EGFR1 forms homodimers after binding by its growth factor ligands. This results in stimulation of intrinsic tyrosine kinase (TK) activity. Subsequently, several downstream signal transduction pathways are initiated resulting in the cell proliferation and the cell survival. EGFR1 antibodies and TK inhibitors can block signalling by binding to the extracellular domain and the intracellular TK domain, respectively. AB: antibody. (B) Mutations in the tyrosine kinase domain (exons 18, 19 and 21) of EGFR1 result in a better response to EGFR1 TKIs. (C) Mutated RAS results in continuous signalling, independent of EGFR1 and EGFR1 targeting agents.

In metastatic CRC, a subgroup of patients benefits of EGFR1 directed antibody treatment. However, *EGFR1* mutations are rare in CRC patients and do not predict benefit from anti-EGFR1 therapy. In contrast, *EGFR1* gene amplification appears to be a predictive factor for response to anti-EGFR1 antibody treatment in CRC, although the studied series are small and retrospective (LOE IV).[40] In CRC, there is also increasing evidence that mutations in *K-ras* are predictive of non-response to cetuximab and/or panitumumab.[40,51,52]

7. TRAIL receptors

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL or Apo2L) induces apoptosis in a wide variety of tumour cell lines without causing toxicity to normal cells and is, therefore, a potential attractive agent. TRAIL binds the death receptors TRAIL-R1 (DR4) and TRAIl-R2 (DR5) and initiates the apoptotic pathway. DR4 and DR5 are expressed on most tumour cells. In contrast to, e.g., HER2 and EGFR1, this receptor has to be activated and not inhibited in order for cells to go into apoptosis.

Several studies addressed the prognostic role of DR expression in malignancies. In 376 stage III CRC patients receiving adjuvant chemotherapy as part of a randomised study, high DR4 expression was associated with a worse disease-free and overall survival (LOE II).[53] RhTRAIL and several agonistic antibodies targeting the TRAIL receptors are currently evaluated in the clinic.[54] So far, very little is known about possible predictive factors, and only pre-clinical data are available. DR4 and/or DR5 have to be available at the tumour cell surface to initiate the apoptotic pathway, but just their presence is not sufficient to predict response to TRAIL receptor targeting agents. Several downstream factors in the TRAIL signalling pathway, for example, defects in caspase 8 or loss of function of the pro-apoptotic proteins BAK or BAX determine apoptotic response to TRAIL.[55] In addition, mutations in *DR5* are responsible for the inhibition of apoptosis by blocking the signal after TRAIL binding.[56] An interesting biomarker is the *O*-glycosylation status of DRs. O-linked glycans regulate biochemical and functional properties of cell surface proteins, including apoptosis. *O*-Glycosyltransferase mRNA levels correlated with rhTRAIL sensitivity in several cancer cell lines and DR *O*-glycosylation resulted in the activation of caspase 8 via TRAIL induced clustering of DR4 and DR5.[57] In an attempt to predict which patients might benefit TRAIL receptors targeting therapy, a SPECT imaging study with the radioactively labelled DR4 agonistic antibody mapatumumab is initiated.

8. VEGF and renal cell carcinoma (RCC)

Even very small tumours require angiogenesis to provide nutrients and oxygen for survival. There is a close interaction

between tumour cells that produce pro-angiogenic growth factors, like vascular endothelial growth factor (VEGF) and PDGF, and endothelial cells expressing growth factor receptors. The stimulation of endothelial cells results in proliferation and migration and eventually in the formation of new vessels. Clear cell RCC provides a unique model for studying angiogenesis because of frequent somatic inactivation of the *Von Hippel Lindau* (*VHL*) gene. The *VHL* gene plays a key role in regulation of the oxygen-sensing pathway by targeting the hypoxia-inducible factor (HIF) for degradation in the proteasome. Impaired VHL function, therefore, results in high expression of pro-angiogenic growth factors. Targeting the VEGF pathway with TKI sunitinib and sorafenib, the mTOR inhibitor temsirolimus and with the VEGF targeting monoclonal antibody bevacizumab prolongs progression-free survival in metastatic clear cell RCC.[58–62]

Simple clinical parameters like performance score and number of metastatic sites are known powerful prognostic factors in cancer. Motzer and colleagues developed a scoring system for metastatic RCC patients consisting of five clinical parameters: performance score, time between diagnosis and metastasis, haemoglobin, serum calcium and lactate dehydrogenase (LDH).[63] With this system, developed retrospectively in patients receiving interferon, patients can be classified as having good, intermediate or poor prognosis (LOE II).[4] This classification has been used to design and stratify medical intervention studies and as a consequence is now widely used to guide therapy.

Several other parameters like C-reactive protein, platelet count and HIF expression also have prognostic value in RCC but do not have a role in clinical decision making.

A high baseline serum VEGF level is associated with shorter progression free and overall survival in two prospective studies.[4,64] In a phase III study of sorafenib versus placebo in advanced RCC, baseline VEGF level is an independent prognostic factor for overall survival (LOE II). Baseline serum VEGF in 2 cytokine studies in RCC patients also found VEGF to be an independent prognostic factor for survival (LOE III). No predictive biomarkers have been found so far which predict patients' benefit from angiogenesis inhibitors.[65,66] VEGF mRNA and protein levels in serum, plasma and tumour have been investigated extensively with disappointing results. Even in RCC, the role model for angiogenesis, a high serum VEGF level or a change after starting therapy does not predict response to anti-angiogenic treatment. In the sorafenib study, both patients with high and patients with low baseline serum VEGF benefited from sorafenib.[64] There is a growing list of candidate markers from pre-clinical studies, and multiple clinical trials are underway to assess predictive biomarkers for angiogenesis inhibition. Single molecular markers may not be able to predict the benefit because of the complexity of signalling routes and because of the cross talk between different signalling pathways.

Functional imaging with MRI, CT and PET scans for the assessment of tumour vascularity and metabolic activity is under investigation for its ability to predict response to angiogenesis inhibitors earlier. *In vivo* imaging of VEGF by radiolabelled bevacizumab has been successful in a human ovarian tumour xenograft and is an interesting concept for early response prediction.[67]

9. Drug induced toxicity as a predictive biomarker

Interestingly a number of studies showed that the effect of a drug on normal tissues can be used as a biomarker. In both a phase II and a phase III study evaluating the antitumour activity of cetuximab in metastatic CRC, skin rash was strongly related to response and survival.[68,69] Similar results were found for erlotinib in NSCLC.[70] Toxicity might thus be used to titrate drugs to effective doses as is done in the EVEREST study in CRC patients. Patients with no or mild skin toxicity after 22 days of treatment with cetuximab and irinotecan were randomised between standard and escalating doses of cetuximab until the development of grade 3 toxicity. Preliminary data show that dose escalation improves tumour response to a rate comparable to the group with initial moderate to severe skin toxicity at the standard dose.[71]

In a small series of 40 metastatic RCC patients treated with sunitinib, grade 3 hypertension was associated with a higher objective response.[72]

10. Discussion

A confusing mix-up exists of the terms prognostic and predictive biomarkers. This is partially due to the fact that predictive and prognostic biomarkers are frequently exchanged. In addition, during therapy or as a result of therapy initial factors can vary in their presence and actual levels, e.g. a strong prognostic factor can be neutralised as a consequence of treatment (HER2/neu).

Despite a growing number of publications about biomarkers that give information on disease outcome, the best prognostic factors are still simple clinical parameters like performance status, number of metastatic sites, tumour grade and LDH level. Prognostic biomarkers might especially be useful for hypothesis testing for their relevance as predictive markers, as targets for therapy and for the selection of patients for adjuvant treatment.

What we need is predictive biomarkers that can guide patient tailored therapy as with our increasing knowledge of biologic behaviour of malignancies it becomes more and more evident that great heterogeneity amongst tumours exists. Together with the development of new anticancer biologicals an explosive search for effective predictive biomarkers has been initiated. Most studies only contribute low levels of evidence due to retrospective data and small sample size. In addition, many reports lack sufficient information to be compared to other studies, and it is therefore difficult to form an opinion about usefulness of such markers in daily practice. A predictive factor is used upfront to predict response to therapy or is monitored during treatment to define the effectiveness of this treatment. When a biomarker is used repeatedly to evaluate response, it is important that it can be measured non-invasively and gives information on all tumour lesions. In this perspective, and also for the evaluation of biomarker conversion during the course of the disease, there might be a role for imaging techniques to quantify levels of biomarkers over time for certain therapies.

Different tumour types can be treated by blocking the same pathway. Predictive biomarkers may be shared between tumour types, like the negative predictive value of *K-ras* mutations in CRC and NSCLC for benefit from EGFR1 inhibition. However, *EGFR1* mutations do predict benefit from EGFR1 directed therapy in NSCLC but not in CRC.

Response to c-KIT and EGFR1 targeting agents in GIST and NSCLC cannot be predicted by the expression of their respective receptors, only by analysing specific mutations in the genes encoding for these receptors. This research finally might pay off as it will allow specific selection of patients that will benefit from the TKIs at a certain dose-level.

For EGFR targeting agents and angiogenesis inhibitors we presented studies that indicate that apart from the tumour also drug effects on normal tissues can be used as a predictive factor.[68–70,72] Preliminary data in CRC patients in which the dose of cetuximab was titrated to skin rash suggests the improvement of tumour response rate.[71]

These findings show that toxicity caused by the drug can be an early predictive factor for response. This is of great interest, because such clinical phenomena are much cheaper, always available and may be easier to exploit than the previously discussed genes or their products.

Biomarkers are in general based on single markers. However, given the fact that tumour biology is often dictated by several essential cellular alterations it may be idle to think that single factors will be enough as predictive or prognostic factors in oncology. Solutions are now sought by analysing multiple factors with multiple reverse transcriptase-polymerase chain reactions (RT-PCRs), RNA microarrays and tissue microarrays. Significant contributions have already been made in the area of breast cancer research. Paik and colleagues tested whether the results of a RT-PCR assay of 21 prospectively selected genes in paraffin-embedded tumour tissue would correlate with the likelihood of distant recurrence in patients with node-negative, tamoxifen-treated breast cancer who were enrolled in the National Surgical Adjuvant Breast and Bowel trial B-14. RT-PCR of the selected genes was significant in predicting recurrence and overall survival.[73] Other microarray studies in breast cancer identified independent sets of genes that might have prognostic value.[29,74–76]

Until recently, analysis was directed at identifying major differences in the expression of separate genes. Currently, there is increasing interest in minor changes in related genes that are involved in particular signalling pathways. Elucidation of pathways that are dysregulated in a specific tumour may lead to rational treatment selection.[77]

In conclusion, prognostic biomarkers for relapse after local treatment are needed for better patient selection for adjuvant treatment strategies. Discovery of prognostic factors in the metastatic setting may identify new therapeutic targets and new predictive factors. The need for more upfront predictive biomarkers to select patients for tailored therapy is clear. Clinical observations during treatment can contribute to the identification of upfront predictive biomarkers, like EGFR1-mutations in non-smoking NSCLC patients. In addition, early predictive markers might be useful in dose selection and early response measurement, because classical response measurement by RECIST criteria underestimates the clinical benefit of the new biological agents. Progress is made, but there is still an urgent need for prospective data to validate all the small, hypotheses generating studies and it is therefore of great importance that biomarker analyses are incorporated in randomised clinical trials as a separate objective.

Conflict of interest statement

None declared.

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