8

Cytokeratin 18 (CK18) and Caspase-Cleaved CK18 (ccCK18) as Response Markers in Anticancer Therapy

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1. Introduction

Novel anticancer drugs are urgently needed for treatment of many types of tumors and, therefore, appropriate response markers are required that in preclinical studies and in human phase I and phase II clinical trials allow for a rapid evaluation of their efficacy and toxicity. The current drug development in oncology is hampered by a high discontinuation rate for new agents, partly due to a lack of appropriate preclinical studies that are capable of accurately predicting clinical suitability. Since newer targeted molecular therapeutics are often cytostatic, rather than cytotoxic, assessment of tumor shrinkage as an endpoint may not be suitable to estimate efficacy (Wittenburg & Gustafson, 2011). Better tumor response markers are expected to improve the drug development process in oncology by leading to a increased comprehension of the factors determining efficacy and toxicity, and, ultimately, to fewer failures. The present work reviews the conventional methods of the assessment of the activity of chemotherapeutics and discusses the newer response markers, particularly the use of CK18 and CK18 fragments for this purpose.

2. Assessment of tumor response to anticancer agents

2.1 Clinical parameters of tumor response

In oncology, a "clinical endpoint" is defined as a characteristic or variable that reflects how a patient feels, functions, or survives, while a "surrogate end point" is defined as a biomarker that is intended to substitute for a clinical end point. The process of proving a linkage between the biomarker and a clinical end point is termed "evaluation" in preference to validation. Assessment of tumor response to therapy is necessary for evaluation of the efficacy of novel anticancer drugs in clinical trials and, furthermore, response evaluation with high individual precision may allow for individualized therapy rather than standardized treatment. The definitive proof of the effectiveness of a therapy is improvement in clinical symptoms and survival (Table 1). However, overall survival (OS) as the ultimate end point of tumor therapies has the disadvantage of the requirement of a prolonged observation period depending on the respective tumor entity. The choice of surrogate and true end points has become a much debated and critical issue in oncology. Many recent randomized trials in solid tumor oncology have used progression-free survival

(PFS) as the primary end point because it is available earlier than OS and not influenced by second-line treatments (Saad et al., 2010). PFS is now undergoing validation as a surrogate end point in various malignancies; however, in advanced breast cancer validation of PFS as a surrogate for OS has so far been unsuccessful. In advanced colorectal cancer, in contrast, current evidence indicates that PFS is a valid surrogate for OS after first-line treatment with chemotherapy.

The activity of anticancer drugs is evaluated by measuring changes in tumor size in response to treatment (Therasse et al., 2000). In the early 1980s, the World Health Organization (WHO) developed recommendations in an attempt to standardize criteria for response assessment, and these were adopted as the standard method for evaluating tumor response. Tumor size has traditionally been estimated from bidimensional measurements (the product of the longest diameter and its longest perpendicular diameter for each tumor). However, measuring in two dimensions and then calculating their products and their sums is laborious and error-prone. The changes in one diameter only are now used since this parameter relates more closely to the fixed proportion of cells killed by a standard dose of chemotherapy than do changes in the bidimensional product. The response evaluation criteria in solid tumors (RECIST) categorises response to therapy as follows: complete response (CR), the disappearance of all target lesions; partial response (PR), at least a 30% decrease in the sum of the longest diameter of all target lesions; progressive disease (PD), at least a 20% increase in the sum of the longest diameter of all target lesions or the appearance of one or more new lesions; and stable disease (SD), neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD (Therasse et al., 2000). Appropriateness of RECIST criteria, for example, whether the change in tumor size is a proper end point for response assessment, has been widely discussed (Paules et al., 2011).

Traditional standards may not be appropriate to assess the efficacy of emerging numbers of cytostatic agents, which do not result in tumor regression to a point of PR or CR. Proposals of a general means of assessment of both cytotoxic and cystostatic effects must be developed (Gwyther & Schwartz, 2008). Since the natural growth rates of the tumors are not considered, a specific treatment that kills the same fraction of tumor cells in two different tumor types will give different results if the proliferation rates are different (Mehrara et al., 2011). A reduction of tumor size after therapy seems to indicate a better prognosis; however, this assumption is not necessarily correct when other tumor characteristics are unfavorable. Finally, drugs that result in stable disease without an objective response may retard tumor growth sufficiently to improve patient survival. Numerous studies have shown that the effect of treatment on tumors can be assessed by means of changes in tumor characteristics other than size, for example, estimated by positron emission tomography (PET) or magnetic resonance imaging or spectroscopy (Padhani & Miles, 2010). Moreover, tumor growth rate was demonstrated to constitute a valuable parameter for survival of patients and the change in tumor growth rate can serve as a surrogate end point for determination of therapy response (Mehrara et al., 2011).

2.2 Biomarkers for assessment of tumor response

Anticancer drug development remains slow, costly and unsuccessful in many cases. Suitable biomarkers are also seen as facilitating decision making during early discovery up to the point of preclinical evaluation (Carden et al., 2010). With the growing knowledge of the

human genome and genetic alterations in cancers, the development of new anticancer therapies has shifted from cytotoxic agents to mechanism-driven drugs (Zhao et al., 2009). As a result, tumor response to therapy may not be observed at the same magnitude or speed by conventional tumor imaging. Tumor-specific tracers and imaging techniques to visualize and quantify tumor changes at histologic and molecular levels aims for measuring tumor response more objectively.

Most anticancer drugs are effective only in subgroup of patients and currently accurate and reliable prediction which patient will benefit from a specific therapeutic regimen is restricted to special cases. Various techniques have, therefore, been developed for monitoring tumor response to therapy but so far measuring tumor shrinkage on computerized tomography (CT) represents the current standard (Weber, 2009). However, CT is inaccurate in differentiating viable tumor from necrotic or fibrotic tissue and limited in detecting responses in tumors that do not change in size during therapy. One way of addressing this might be the use of predictive biomarkers to select patients for Phase I/II trials. Such biomarkers, which predict response to molecular-targeted agents, have the potential to enrich the fraction of patients more likely to benefit. Forecasting of tumor response based on gene expression data is feasible in certain cases, for transcripts like HER2, KRAS, mutated EGFR and others, but gene signatures are only appropriate to give a probability of increased success or failure of specific treatment modalities (Mandrekar & Sargent, 2010; Goodison et al., 2010). A fundamental problem of using gene expression profiles or other molecular characteristics of tumor tissue to predict tumor response is the fact that malignant tumors are constantly changing and adapting to their environment. As a consequence, most responses to chemotherapy or targeted drugs are relatively short-lived and resistant cancer cells evolve quickly. Because of these inherent limitations in the current approaches for response prediction, the need for techniques to monitor tumor response to therapy well in advance of clinical parameters is obvious. Furthermore, the tests aim to identify nonresponding patients early, in order to stop ineffective therapies and avoid unnecessary exposure to side effects.

2.2.1 Serum tumor markers

The ultimate goal in drug development is to use tumor response as a surrogate for clinical benefit, because response is generally faster to assess and also less influenced by factors such as patient performance status or second-line therapy. The approach comprising serum tumor markers tries to measure specimens specifically secreted by cancer cells into the blood and their modulation by therapeutic interventions. The use of changes in serum markers as a measure of tumor response is appealing because it is noninvasive, can be repeated frequently and has a relatively low cost. Furthermore, it offers the opportunity to measure tumor response independently of the affected sites with a single parameter. To be useful the biomarker response evaluation must be in good agreement with data recorded by traditional anatomical methods. However, biomarkers that provide information about induction of tumor cell death will not necessarily correlate with clinical outcome (Linder & Alaiya, 2009).

Since the original description of carcinoembryonic antigen (CEA), a large number of tumor-associated proteins have been detected in sera from cancer patients. Clinically suitable markers to monitor disease progression include CEA, prostate-specific antigen (PSA), tissue

polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS), CA19–9 and CA15–3, among others (Mishra & Verma, 2010). However, results have to be checked carefully since, for example, a decline in PSA levels may be the result of dedifferentiation of the tumor and not of a successful therapeutic modality.

In addition, proteins that are not usually secreted by tumor cells may be detected in the circulation of cancer patients. Examples of such proteins are S100B (Ghanem et al., 2001), CA125 (Beck et al., 1998), high-mobility group box protein 1 (HMGB1, Candolfi et al., 2009; Gauley & Pisetsky, 2009), CKs (Stigbrand, 2001; Linder et al., 2004) and others. Nonsecreted intracellular proteins seem to reach the circulation upon cell death. In cases of enhanced cellular turnover and cell death, such as in cancer, the local clearance mechanisms through macrophages will be overcharged and apoptotic bodies will disintegrate in a process termed secondary necrosis (Kroemer et al., 1998).

Clinical parameters:
Overall survival (OS)
Progression free-survival (PFS)
Tumor imaging (RECIST)
Tumor metabolism (PET)
Biomarkers:
Tumor-associated antigens (CEA, PSA, CA125, CA19-1,....)
Circulating tumor cells (CTCs)
Release of intracellular antigens during apoptosis/necrosis
Circulating nucleic acids (nDNA, mRNA, miRNA,..)
Intracellular antigens (CKs, CA125, HMGB1, S100B,...)

Table 1. Methods for the assessment of tumor response

2.2.2 Circulating tumor cells

Circulating tumor cells (CTCs) can be released from the primary tumor into the bloodstream and may colonize distant organs giving rise to metastasis (Alunni-Fabbroni & Sandri, 2010). The presence of CTCs in the blood has been documented more than a century ago, and in the meanwhile various methods have been described for their detection and automated enumeration. Most of them require an initial enrichment step, since CTCs are a very rare event. CTCs can be detected in the blood of many patients with different types of early or advanced cancer using antibody-based assays or molecular methods (Mavroudis, 2010).

In many studies the detection and quantification of CTCs has been linked to unfavourable prognosis and CTC detection offers the opportunity for individualized risk assessment superior to TNM staging. However, discordant results have been reported when different methodologies for CTC detection were used (Pantel & Alix-Panabières, 2010). Therefore, well-standardized detection methods cross-validated between different laboratories are still needed. CTCs are a heterogeneous population of cells with biological characteristics often different from those of their respective primary tumor cells. CTCs have been reported to have high apoptotic indices. Pilot studies have shown that phenotyping of CTCs could be used to predict response to targeted therapies. In the era of biological therapeutics, CTC characterization at different time points during the course of disease may provide useful predictive information for the selection of the most appropriate treatment.

2.2.3 Circulating nucleic acids

DNA, mRNA and microRNA are released and circulate in the blood of cancer patients (Schwarzenbach et al., 2011). Since circulating cell-free nucleic acids (cfNAs) in cancer patients often bears similar genetic and epigenetic features to the related tumor DNA, there is evidence that some of the this genetic material originates from tumoral tissue. Changes in the levels of circulating nucleic acids have been associated with tumor burden and malignant progression. The cfNAs might be excellent blood cancer biomarkers, as they may be more informative, specific and accurate than protein biomarkers. However, to validate the actual clinical application of various cfDNA alterations as potential cancer biomarkers in practice for individual tumor types standardization of the described methods would be a prerequisite.

3. Circulating CKs and CK fragments as tumor biomarkers

3.1 Detection of CK expression in the diagnosis of tumors

Keratins have been recognized as tumor markers in the diagnosis of cancer for over 20 years (Weber et al., 1984; Sundstrom & Stigbrand, 1994). CKs are a family of more than 20 intermediate filament (IF) proteins expressed in cells of epithelial origin and endothelial cells (Ekman et al., 2007; Karantza, 2011). They are subdivided into two groups: CKs 1-8, the type II group comprising neutral to basic proteins of 53-68 kD and CKs 9–20, the type I group including acidic proteins of 40-56 kDa. CKs are composed of complexes of one type I and one type II CK protein that become organized into larger filamentous polymeric structures. The most abundant CKs are 8, 18 and 19, and a common example of the heteropolymer complex is the combination of CKs 8 and 18. Commonly, the tissue-specific CK expression profile is stable, even during malignant transformation and, therefore, it is utilized in pathology to distinguish different tumor entities. CK8, CK18 and CK19 are expressed by most types of carcinomas, including those of the breast, prostate, lung, colon and ovary. Under normal physiological conditions CKs are complexed in IF of epithelial cells and remain insoluble (Fuchs & Weber, 1994). Proliferating cancer cells also contain a substantial pool of soluble CKs (CK8, 18 and 19), which can increase in response to stress (Schutte et al., 2004). In the circulation CKs are present as large or small protein complexes or in form of partially degraded single-protein fragments (Ku et al., 1997). The levels of circulating CKs are significantly increased in patients with epithelia-derived malignancies (Linder, 2007). The exact mechanism of the release of the CKs may involve release of intact proteins from rapidly proliferating tumor cells or cell death (Linder et al., 2004). Most type I CKs display caspase cleavage sites allowing for the detection of apoptotic cell death through assessment of newly formed epitopes in the process of their specific degradation. During necrosis, mobilization of CK18 into the soluble pool occurs through remodelling of the IFs (Strnad et al., 2002), whereas during apoptosis IF proteins (including CK18) are targeted for rapid breakdown by activated caspases 3, 7 and 9 to facilitate the formation of apoptotic bodies (Kramer et al., 2004). Several monoclonal anti-CK antibodies are available that recognize the most abundant CKs, i.e. 8, 18 and 19 (Stigbrand et al., 1998). The three most commonly applied CK markers overall are TPA, TPS and CYFRA 21-1. TPA is a broad-spectrum test that measures CKs 8, 18 and 19, while TPS and CYFRA 21-1 measure CKs 18 and 19, respectively (Weber et al., 1984; Stieber et al., 1993; Sundström et al., 1994; Stigbrand et al., 1998; Barak et al., 2004).

M30® and M65® (Peviva AB, Bromma, Sweden) are sandwich ELISA assays that determine different circulating forms of the CK18 in either plasma or serum and are proposed to be surrogate biomarkers of different mechanisms of cell death (Biven et al., 2003; Kramer et al., 2004). The M30 ELISA assay utilizes the M5 anti-CK antibody as a catcher and the M30 antibody to detect CK18 fragments that contain a neoepitope at positions 387–396 generated by the action of caspases 3, 7 and 9 activated during the early stages of apoptosis (Leers et al., 1999; Schutte et al., 2004). CK18 fragments detected by the M30 Apoptosense assay are frequently referred to as "M30" in the literature, which is incorrect since M30 is an antibody and not an antigen (Linder, 2011). The fragments can be referred to as "ccCK18" (caspase-cleaved CK18). Alternatively, since there is a second caspase-cleavage site in the CK18 molecule at Asp237, a more precise terminology would be "K18Asp396".

Thus, the M30 ELISA is proposed as a specific assay for apoptosis and immunological staining with the M30 (Cytodeath®) antibody has been shown to correlate with other apoptosis assays such as terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and presence of active caspase 3 (Carr, 2000; Duan et al., 2003). M65 also detects cleaved fragments; however, it uses a different detection antibody from M30 (namely M5) that does not distinguish between the full-length protein and its fragments (Kramer et al., 2004). Thus, M65 theoretically measures both caspase cleavage (apoptosis) and cellular release of intact CK18 (necrosis). None of the cell types that normally circulate in blood express CK18, neither do dividing and chemotherapy-sensitive cells of the bone marrow. Carcinoma cells may also contain fragmented CK molecules, presumably owing to increased levels of proteolytic enzymes in the cytosol of tumor cells. The CK18 material present in the circulation of cancer patients consists of higher-molecular-weight complexes between different types of CKs. In addition to such complexes, cells release shorter polypeptide fragments that have short half-lifes in the circulation and are not detected in patient serum (Linder, 2010). It is an advantage that CKs are present as complexes in the circulation, since such aggregates are relatively stable during storage of serum/plasma and survive multiple freeze–thawing cycles.

Both M30 and M65 assays have now been applied extensively in clinical trials as biomarkers of cell death induced by a variety of different cancer chemotherapeutic agents in a spectrum of different disease types (Biven et al., 2003; Ueno et al., 2003; Kramer et al., 2004, 2006; Demiray et al., 2006; Ulukaya et al., 2007). In some preliminary reports, the M30 assay has been claimed to be both predictive of drug response (Demiray et al., 2006) and prognostic of survival (Ulukaya et al., 2007). The two ELISAs have also been utilized as markers of host tissue toxicity in a number of different clinical conditions including trauma, sepsis (Roth et al., 2004), chronic liver disease (Yagmur et al., 2007), hepatitis C (Bantel et al., 2004) and in liver transplantation (Baskin-Bey et al., 2007).

3.2 CKs and tumor cell death

Within the era of molecularly targeted anticancer agents it has become increasingly important to provide proof of mechanism as early as possible. Selective activation of apoptosis is one of the major goals of cancer chemotherapy (Kepp et al., 2011). Serological assays utilising ELISA for detection of apoptotic events would have the advantage of sampling multiple time points. Potential biomarkers of apoptosis including CTCs, CKs and nuclear DNA (nDNA) are discussed at length (Ward et al., 2008). However, accepting that a single biomarker may not

have the power to predict proof of concept and patient outcome and technologies that can analyse panels of biomarkers in small volumes of samples will be a future necessity.

A hallmark of neoplasia is dysregulated apoptosis or programmed cell death (Hotchkiss et al., 2009). Dysregulation of apoptotic pathways leads to reduced responses to chemotherapeutic drugs or radiation and is a frequent contributor to therapeutic resistance in cancer. Virtually all methods for detecting apoptosis, including classic cytomorphologic evaluation, TUNEL assay, immunocytochemistry, and gene sequence analysis, may be applied to cytologic samples as well as tissue (Shtilbans et al., 2010). Early morphological features of apoptosis include blebbing of the cell surface, after which cell shrinkage, cytoskeletal rearrangements, chromatin condensation, and nuclear fragmentation occur. The duration of the apoptotic process is about 12–24 hours. As cells proceed to apoptosis, phosphatidylserine (PS), a lipid normally facing the cytoplasm, flips and faces the extracellular fluid. The protein annexin V binds PS strongly, specifically in a calcium-dependent fashion, which is a reflection of its biologic role as an anticoagulant. Attempts to image apoptotic tumor cells with help of fluorescent active Cy-annexin V and surface reflectance fluorescence to determine chemosensitivity were performed successfully in xenografts; however, clinical application is under development (Schellenberger et al., 2003). In addition to the reduced trauma to patients compared to obtaining surgical samples, the use of repeat cytologic sampling before and after cancer therapy to monitor therapy-induced apoptosis and rapidly predict therapeutic response, shows promise in several studies and may become increasingly valuable as cancer therapy becomes more individualized, based upon drug responsiveness of each individual patient's tumor. Thus, apoptotic products released into blood circulation are suggested as promising markers for the early cancer detection (Holdenrieder & Stieber, 2004, 2010). However, though sensitive assays are available, the lack of organ- and tumor-specificity limits the usefulness of most apoptotic parameters for screening purposes. Nevertheless, they seem to be valuable for the prognosis and the prediction of response to systemic chemo- or radiotherapy in cancer disease. Among those promising markers are ligands and receptors of the FAS-system, members of the intracellular caspase cascade, cleaved apoptosis substrates such as CK fragments, nDNA and apoptosis modulators like survivin.

3.3 Levels of circulating CKs and fragments in tumor patients

A number of groups have demonstrated elevated levels of CK18 and/or ccCK18 in serum/plasma from patients with various forms of carcinomas, including breast (Ueno et al., 2003; Demiray et al., 2006), colon (Koelink et al., 2009; Ausch et al., 2009a,b), lung (Hou et al., 2009; Ulukaya et al., 2007), prostate (Kramer et al., 2004, 2006), testicular (de Haas et al., 2008), head and neck (Ozturk et al., 2009), pancreatic (Dive et al., 2010) and gastrointestinal adenocarcinoma (Scott et al., 2009; Yaman et al., 2010). Increases of baseline ccCK18 were believed to mirror spontaneous apoptosis of tumor cells and appeared to be of clinical significance. Increased circulating amounts of ccCK18 were reported to be associated to the number of involved organs, performance status and shorter median survival (Ueno et al., 2003; Koelink et al., 2009; Ulukaya et al., 2007).

The potential diagnostic and prognostic significance of circulating CK18 and ccCK18 was investigated in patients with non-small-cell lung cancer (NSCLC) in comparison with CYFRA 21.1, a fragment of cytokeratin 19 (De Petris et al., 2011). Subject cohorts consisted of

200 healthy blood donors, 113 patients with benign lung diseases and 179 NSCLC cases. Plasma levels of both ccCK18 and total CK18 were higher in the NSCLC group compared to the healthy controls and the cohort with benign diseases. The diagnostic accuracy of both CK18 forms to distinguish between NSCLC and nonmalignant control cases was 56%, whereas it was 94% for CYFRA 21.1. Multivariate survival analysis showed that total CK18 was a stronger prognostic factor than both ccCK18 and CYFRA 21.1., while ccCK18 was not of prognostic value, suggesting that tumor necrosis is of particular importance in this disease. Two studies were published evaluating CK18 in patients with colorectal cancer. The first studied pre- and postoperative serum levels of ccCK18 in 31 patients (Ausch et al., 2009b). Persisting levels of ccCK18 after apparently radical surgery on colon cancer patients correlated significantly with disease recurrence. The second study measured pre- and postoperative plasma levels of ccCK18 and total CK18 in 49 patients with colorectal cancer and correlated the levels with patient and tumor characteristics as well as OS (Koelink et al., 2009). The results showed that perioperative plasma levels of both ccCK18 and total CK18 were correlated with disease stage and were predictive of DFS independent of tumor stage. However, in none of these reports the determination of circulating CK18 reached sufficient accuracy to be proposed as a potential diagnostic assay. A positive predictive correlation between baseline circulating CK18 levels and patient outcome has been suggested in several other tumor diseases such as gastrointestinal adenocarcinomas (Brandt et al., 2010), testis tumors (de Haas et al., 2008), lung cancer (Hou et al., 2009; Ulukaya et al., 2007) and pancreatic cancer (Dive et al., 2010). In general, high pretreatment CK18 levels indicated a larger tumor burden and a less favourable prognosis.

An obvious concern in respect to the use of CK18 as a tumor biomarker is whether circulating CK18 is released from tumor and not from other cells. A number of observations from various studies suggested that CKs are indeed shed by tumor cells. For example, in patients with endometrial carcinoma, CK18 levels were higher in local tumor veins compared with peripheral blood in the same patients (Kramer et al., 2004) and CK18 levels generally decreased after surgical removal of tumors (Ausch et al., 2009a). It is, therefore, very likely that increased baseline levels of CK18 in patients serum/plasma is due to release by the tumor. Caspase activity has been detected in circulation in patients with malignancies (Linder et al., 2010). Therefore, a concern in the analysis of caspase-cleaved fragment in blood as a measure of cellular apoptosis is that cleavage of CK18 might occur in the circulation. Incubation of CK18-positive serum samples with 1,000 units/mL recombinant caspase-3 for 4 hours did not increase the levels of ccCK18 fragments. The association between CK18 markers and the number of CTCs observed by Hou et al. in patients treated with platinum-based therapy seems to provide another strong indication that plasma CK18 originates from the tumor (Hou et al., 2009). Furthermore, the association between CK18 increases and tumor response or patient outcome observed in various studies suggested that CK18 is derived from tumor cells disintegrated in response to cytotoxic therapy. It can, however, not be excluded that increased levels of circulating CK18 are due to higher degrees of drug exposure and may reflect higher toxicity (e.g., to liver tissue).

An expected relationship between plasma CK18 levels and signs of cell death was investigated using tumor histopathology slides of resected pancreatic cancers (Dive et al., 2010). On microscopic assessment one third of the patients exhibited significant areas of tumor necrosis (i.e. > 5%) but without any correlation with plasma M65 levels. Furthermore approximately half of the available cases showed positive staining for activated caspase-3,

again without any significant relation to the median plasma ccCK18/M65 ratio between positive and negative cases. This finding seems to implicate that additional factors other than intrinsic tumor biology have an important interfering effect on circulating M65 concentrations. Hepatocytes express CK18 and cancer therapeutics that induced liver toxicity may have evoked release of CK18. A marked correlation was seen between the concurrent bilirubin levels of the pancreatic cancer patients and circulating CK18 levels pointing to obstruction of the main bile duct as cause of cell death within the biliary epithelium and release of CKs in good accordance with findings in cholangitis and chronic liver disease (Yagmur et al., 2007). Endothelial cells, kidney and colon epithelia may shed CK18 and fragments in response to damaging agents due to side effects on normal tissues. Whether CK biomarkers are sufficiently specific for the assessment of apoptosis and necrosis of carcinoma cells to be useful for treatment decisions in routine clinical care is questionable.

3.4 Changes of circulating CK18 and ccCK18 in response to chemotherapeutic drugs

In regard to the potential use of CK18 as a serum biomarker for monitoring therapy efficacy in carcinoma patients supportive data have been reported by different investigators (Linder et al., 2010). A number of groups have observed increased levels of ccCK18 (M30) and/or CK18 (M65) in serum/plasma during cytotoxic therapy of cancer patients (Hou et al., 2009; Kramer et al., 2004; Demiray et al., 2006; Kramer et al., 2006, de Haas et al., 2008; Olofsson et al., 2007). Preclinical results in animal experimental studies discussed in the following raised the hope of successful validitation of these assays in clinical practise.

3.4.1 Animal experimental studies of CK18 fragments of tumor xenografts

Drug-induced increases of ccCK18/CK18 were observed in animal studies using human xenograft tumors (Olofsson et al., 2009; Cummings et al., 2008; Micha et al., 2008). Such increases are certain to originate from tumor cells, since the M30 and M5 antibodies utilized in the M30-Apoptosense and M65 assays detect human but not murine CK18 (Linder et al., 2010). The kinetics of increases in plasma ccCK18 was paralleled by increases in apoptosis in tumor tissue. The M30-Apoptosense therefore permits the specific determination of drug-induced apoptosis in human tumor xenografts in rodents using plasma samples, largely independently from host toxicity.

In detail, treatment of nontumor-bearing rats with the aurora kinase inhibitor AZD1152 produced no alterations in circulating baseline levels of ccCK18 and CK18 (Cummings et al., 2008). In treated tumor-bearing animals, the M30 and M65 assays detected a 2- to 3-fold increase in plasma ccCK18 but not CK18 levels by day 5 compared with controls. This correlated to a 3-fold increase in the number of apoptotic cells detected at the same time point in SW620 xenografts using immunohistochemistry. However, CK18 plasma levels correlated to changes in tumor growth in control animals. It was concluded that ccCK18 represents a pharmacodynamic biomarker of AZD1152-induced apoptosis in the SW620 xenograft model, whereas circulating CK18 is a biomarker of therapeutic response. Furthermore, cell death of SCLC xenografts in mice was measured with help of circulating CK18 in response to a proapoptotic dose of the BH-3 mimetic ABT-737 (Micha et al., 2008). Circulating CK18 levels correlated with tumor burden and ABT-737 caused apoptotic tumor regression in SCLC H146 xenografts indicated by a drug-specific and early increase in ccCK18 that subsequently declined (2 – 24 hrs and 15 days). In summary, in murine

xenograft models, in the absence of interference of cell death of normal epithelial tissues, CK18 and ccCK18 were valid markers of tumor mass and response, respectively.

3.4.2 Cytotoxic chemotherapy and circulating ccCK18 and CK18

As discussed above, the amount of ccCK18 which has accumulated in cells or tissue culture media during apoptosis is measured by the M30-ELISA and the M65-ELISA assay will detect all CK18 species that contain epitopes in the 300 to 390 amino acid region of the protein (Biven et al., 2003). Chemotherapeutic agents commonly induce apoptosis with slow kinetics over 24 hours. For example, cisplatin, etoposide and paclitaxel typically induce CK18 cleavage after more than 12 hours of incubation. During paclitaxel-induced apoptosis of human breast cancer cells, approximately 10^{-5} U of ccCK18 ccCK18 is generated per cell. This means that induction of apoptosis in 10% of the cells of a tumor containing 10^9 cells will generate approximately 1000 U ccCK18. In a plasma volume of 3 L, this will lead to a concentration of 330 U/L compared to a baseline level of approximately 150 U/L in normal subjects that would be easily detected (Biven et al., 2003). Bone marrow is one of the major organs affected by antineoplastic drugs, but since CK18 is only expressed in epithelial cells, apoptosis in bone marrow will not contribute to increases in M30-reactive material during chemotherapy. Selected studies on circulating CK18 and ccCK18 are listed in table 2.

In a study in 32 patients with recurrent breast cancer receiving chemotherapy with cyclophosphamide, epirubicin and 5-fluorouracil (5-FU) or docetaxel (Biven et al., 2003), an index was calculated for each patient based on the difference between the maximum ccCK18 level observed during treatment and the pretreatment level. Increases by 50% or more were observed in 57% of responders compared to 5.6% of the nonresponders. Release of the cytosolic pool of soluble CK18 during necrotic cell death and of ccCK18 during apoptosis, respectively, was described by Kramer et al. in two studies involving prostate cancer patients (Kramer et al., 2004, 2006). Circulating CKs in patients with hormone refractory prostate cancer receiving palliative chemotherapy showed significant increases in ccCK18, usually between days 5 and 7 of each treatment cycle (Kramer et al, 2006). During sequential treatment with either estramustine/vinorelbine or with estramustine/docetaxel, estramustine alone induced increases in serum CK18, but not in ccCK18, while docetaxel was able to induce significant levels of tumor apoptosis. The magnitude of docetaxel-induced increases in ccCK18 was associated with baseline prostate-specific antigen (PSA) and CK18 serum levels in these patients, providing potential evidence of a tumoral origin of caspase-cleaved fragments. However, toxicity accompanying administration of these combination chemotherapies was not reported. In another study, tumor cell apoptosis and antiapoptotic response was measured by determinations of ccCK18 and soluble Fas (sFas) in serial samples from 42 patients with different cancers under chemotherapy (Pichon et al., 2006). Baseline antiapoptotic sFas was higher in cancer patients than in normal subjects and increased with the number of previous chemotherapy cycles. The median baseline ccCK18 did not differ from normal subjects, but patients with a maximum increase >67.5% during chemotherapy had a better univariate OS. As measured by sFas concentration, chemotherapy induced an anti-apoptotic response of differing intensity according to tumor types and drugs, which had a prognostic value for survival. The effect of four cycles of anthracycline-based neoadjuvant chemotherapy on ccCK18 levels was investigated in a group of 42 patients with invasive breast carcinoma by Demiray et al. and ccCK18 serum concentrations at 24 and 48 hours after initiation of chemotherapy were found to be

AUTHORS	CHEMOTHERAPY	TUMOR ENTITY	# PTS	RESULT(S): CK18/ccCK18
Kramer et al. 2004	Estramustine/ vinorelbine	prostate cancer	25	M65+ upon cell death
Pichon et al. 2006	chemotherapy	epidermoid/ carcinoma	42	+ correlation with survival
Kramer et al. 2006	Estramustine-based	prostate cancer	82	ccCK18+ upon docetaxel therapy
Demiray et al. 2006	Anthracycline-based	breast cancer	42	d1 + d2 peaks in responders
Olofsson et al. 2007	Docetaxel/CEF	breast cancer	61	ccCK18+ in response to docetaxel M65+ in responders on CEF
Steele et al. 2008	Belinostat (HDAC inhibitor)	solid tumors	46	d2 +d8, in responders
de Haas EC et al. 2008	BEP	testicular cancer	34	d7 +, peaks similar in nonresponders
Scott et al. 2009	5-FU/platinum	gastrointestinal cancer	73	M65+ in responders
Hou et al. 2009	platinum/etoposide	SCLC	88	+ apoptotic CTC/ liver toxicity
Lickliter et al. 2010	CYT997 (microtubule inhibitor)	solid tumors/Phase 1	31	d1+, dose-dependent increase
Le Tourneau et al. 2010	Seleciclib (CDK inhibitor)	advanced tumor/Phase 1	56	+ dose-dependent increase + metastatic patients and toxicity d2
Brandt et al. 2010	5-FU/combination	gastrointestinal cancer	35	cycle end, ccCK18+ in responders
Ulukaya et al. 2011	FEC/ED	breast cancer	37	d1+d2, increase (also in 1 nonresponder)
Shin et al. 2011	TSU-68 (TK inhibitor) + S-1/oxaliplatin	mCRC/Phase 1	11	no significant changes upon therapy
Mahadevan et al. 2011	AT7519 (CDK inhibitor)	solid tumors/Phase 1	28	d5+, correlated with tumor apoptosis
Greystoke et al. 2011	ABVD/R-CVP/R-CHOP	lymphoma	49	d3+, correlated with epithelial toxicity
Gandhi et al. 2011	Navitoclax (Bcl2 inhibitor)	SCLC/carcinoid/ others	47	transient peak after 6 hrs
Dean et al. 2011	Obatoclax + carbo-platin/etoposide	SCLC Phase 1b-2	24	no ccCK18+ with Bcl2 inhibitor

Table 2. Overview of studies dealing with measurements of CK18 and/or ccCK18 in patients receiving conventional or targeted chemotherapy.

significantly higher than baseline in responders, while such increases were not observed in nonresponders (Demiray et al., 2006). Docetaxel induced increased levels of ccCK18 in serum from breast cancer patients, indicating apoptosis (Olofsson et al., 2007). Neoadjuvant cyclophosphamide/ epirubicin/ 5-fluorouracil (CEF) therapy led to increases predominantly in uncleaved CK18, indicating necrotic cell death. The increase in total CK18 at 24 h of the first treatment cycle correlated to the clinical response to CEF therapy.

Disseminated testicular germ cell cancer (TC) is a paradigm for a solid malignancy of epithelial origin chemosensitive to bleomycin/ etoposide/ cisplatin (BEP). The peaks of CK18 and ccCK18 observed after the start of each treatment cycle in good and intermediate-prognosis group patients indicated a drug-induced effect, which may have reflected tumor response (de Haas et al., 2008). However, the fact that these peaks are not observed in patients with poor prognosis was possibly related to high initial levels of CK18 and ccCK18. With this analysis in a small number of selected patients it could not be proven that CK18 and ccCk18 peaks were specific enough to prove chemotherapy-induced tumor cell death. Neither could it be excluded that these peaks (partially) reflected chemotherapy-induced toxicity to normal epithelial tissues. The four patients with TC who eventually did not respond to BEP chemotherapy after an initial decline in tumor markers showed patterns of circulating CK18 and ccCK18 comparable to responding patients. In case these peaks were tumor cell death-related they were interpreted as signs disintegration of chemotherapy-sensitive subpopulations of cells. Consequently, the presence of chemotherapy-induced peaks in CK18 and ccCK18 might not exclude future treatment failure.

Soluble plasma ccCK18 and CK18 were measured from 73 patients and 100 healthy volunteers with advanced gastrointestinal adenocarcinomas before treatment and during chemotherapy (Scott et al., 2009). The majority of the patients with gastric or oesophageal adenocarcinoma were treated with combination chemotherapy comprising epirubicin, cisplatin, and 5-FU and few patients with either cisplatin/5-FU or carboplatin/5-FU. CRC patients were treated with capecitabine monotherapy or in a few cases with 5-FU and folinic acid. Both ccCK18 and total CK18 plasma levels were significantly higher in patients compared with the healthy volunteers. The total CK18 baseline plasma levels before treatment were significantly higher in patients who developed progressive disease and the peak plasma levels of CK18 occurring in any cycle following treatment were also found to be associated with tumor response, but peak levels of ccCK18 did not reach significance. There was an overall sensitivity of 22% at a specificity of 90% for ccCK18 and an overall sensitivity of 19% at a specificity of 90% for CK18 baseline plasma levels in distinguishing between patients who subsequently progressed and those who had partial response/stable disease. Pooled data for all 73 patients demonstrated that ccCK18 has a sensitivity of 27% at a specificity of 90% and CK18 a sensitivity of 71% at a specificity of 90% in distinguishing patients with malignancy and healthy volunteers, respectively. Both markers may have limited use as a diagnostic marker. In the healthy volunteers both plasma ccCK18 and CK18 varied over a wide range. Alcohol intake is known to increase ccCK18 values in serum due to apoptosis of liver cells (Natori et al, 2001). Other studies have also shown that viral illness, chronic hepatitis, and sepsis will increase levels of ccCK18 detected by the M30 Apoptosense ELISA kit (Scott et al., 2009). Hou et al. compared measurements CK18 products in blood samples from 88 SCLC patients with levels of nDNA and numbers of CTCs (Hou et al., 2009). Before treatment, CK18 fragments were elevated in patients compared with controls and ccCK18 levels correlated with the percentage of apoptotic

CTCs. CTC number fell following chemotherapy with cisplatin/etoposide, whereas levels of serological cell death biomarkers peaked at 48 hours and fell by day 22, mirroring tumor response. A 48-hour rise in nDNA and ccCK18 levels was associated with early response and severe toxicity, respectively. Levels of nDNA did not correlate with M65 or ccCK18. Persistently elevated ccCk18 and CTC number at day 22 were adverse prognostic factors in univariate analysis. There was a significant association between an increase in nDNA at 48 hours and response compared with stable disease. Patients who developed toxicity requiring hospitalization had higher baseline levels of cell death biomarkers, potentially reflecting a higher disease burden and poorer performance status before therapy. The peak in ccCK18 at 48 hours was significantly higher in patients requiring admission for toxicity.

The putative association of serum CK-18 levels and clinical response was furthermore investigated in 35 patients with gastrointestinal cancers (Brandt et al., 2010). While both cleaved and total CK-18 levels were intrinsically elevated in tumor patients, they were further increased during 5-FU-based therapy. Cancer patients with a partial response or stable disease revealed a significantly higher increase of ccCK-18 during chemotherapy as compared to patients with progressive disease. Our own group assessed serum levels of ccCK18 in 10 CRC patients receiving combination chemotherapy with oxaliplatin/capecitabine and observed that increases in ccCK18 during chemotherapy did not correlate with tumor response (Ausch et al, 2009a). A breast cancer study of CK18 was perfomed by Ulukaya et al. employing 37 patients as well as 35 patients with benign breast disease and 34 healthy subjects for comparison (Ulukaya et al., 2011). Cancer patients received neoadjuvant chemotherapy consisting of either 5-FU, epirubicin, and cyclophosphamide (FEC) or epirubicin plus docetaxel (ED). Apoptosis was assessed before chemotherapy and 24 and 48 h after chemotherapy in the malignant group. It was found that the baseline apoptosis levels in either nonmetastatic malignant or benign group were not statistically different from that in the control group, but elevated in the metastatic cancer group. Following drug application, ccCK18 levels significantly increased about 3-fold in patients showing tumor regression at 24 h but not in nonresponders. However, the single progressive patient revealed an almost 5-fold increase in ccCK18. It is possible that these findings can be explained by therapeutic response, showing a stronger correlation to total cell death (apoptosis and necrosis) compared with apoptosis alone. Indeed, whether cytotoxic drugs kill tumor cells *in vivo* by apoptosis or necrosis is controversial (Kaufmann & Vaux, 2003; Zong & Thompson, 2006).

Circulating biomarkers of cell death such as nDNA, CK18 and circulating FLT3 ligand, a potential biomarker of myelosuppression, were assessed before and serially after standard chemotherapy in 49 patients with Hodgkin and non-Hodgkin lymphoma (Greystoke et al., 2011). CK18 is not expressed in lymphoma cells and thus represented a potential biomarker of epithelial toxicity in this setting. Decreases in nDNA levels were observed in the first week after chemotherapy; Circulating CK18 increased within 48 hours of chemotherapy and was significantly higher in patients experiencing epithelial toxicity graded >3 by Common Terminology for Classification of Adverse Events criteria (CTCAE).

3.4.3 Targeted chemotherapy and changes in circulating CK18 and ccCK18

Determinations of ccCK18 may also be applicable as a pharmacodynamic biomarker in phase I clinical trials of novel noncytotoxic molecularly targeted anticancer therapies, with which objective tumor responses, as determined by a reduction in tumor dimensions by

conventional imaging techniques, may not be observed. Several groups reported measurements of CK18 and CK18 fragments in clinical studies of such agents.

CYT997 is a novel microtubule inhibitor and vascular-disrupting agent with marked preclinical antitumor activity (Lickliter et al., 2010). Moreover, plasma levels of von Willebrand factor and ccC18 increased post-treatment at higher dose levels. Among 22 patients evaluable for response, 18 achieved stable disease for >2 cycles. Seventeen patients in this study were evaluable for the analysis of ccCK18 levels, which were observed to increase at 24 h after commencing CYT997 in a dose-dependent manner. In another study, 56 patients received a total of 218 cycles of the cyclin-dependent kinase (CDK) inhibitor seliciclib (Le Tourneau et al., 2010). Soluble CK18 fragments allowed detection of seliciclib-induced cell death in the blood of patients treated at doses above 800 mg/day. Another CDK inhibitor, namely AT7519, afforded stable disease for >6 months in four out of 28 patients and a prolonged partial response in one patient bearing solid tumors (Mahadevan et al., 2011). Inhibition of markers of CDK activity was observed across the dose range and manifested in antiproliferative activity. A consistent decrease in Ki67 levels and increase in both the cleaved and intact forms of CKs were observed in the majority of samples, suggesting that AT7519 was inducing tumor cell apoptosis.

Disease stabilisation was associated with ccCK18 plasma levels in patients with advanced solid tumors treated in a phase 1 clinical trial of the novel hydroxamate histone deacetylase (HDAC) inhibitor, belinostat (Steele et al, 2008). Of the 24 patients treated at the maximum tolerated dose, 50% achieved stable disease and ccCK18 levels were significantly increased on days 2 and 8 in patients with stable disease but showed no significant elevation in patients with progressive disease. Consistent with this epitope being derived from tumor and not normal cells undergoing therapy-induced apoptosis, patients who did not have epithelial cancer showed no change in level of ccCK18 during treatment. No significant mean changes of ccCK18 after administration of each of several cycles of chemotherapy was observed in a phase I study evaluating the safety and pharmacokinetic of TSU-68, when used with S-1 (combination of tegafur, gimeracil and oteracil) and oxaliplatin (SOX) in mCRC patients (Shin et al., 2011). TSU-68 is a novel multiple tyrosine kinase inhibitor that inhibits VEGFR-2, FGF and PDGF receptors.

SCLC is an aggressive disease in which, after initial sensitivity to platinum/etoposide chemotherapy, patients frequently relapse with drug-resistant disease. Deregulation of the Bcl-2 pathway is implicated in the pathogenesis of SCLC, and early phase studies of Bcl-2 inhibitors were initiated in SCLC. A phase I study of navitoclax, a novel inhibitor of Bcl-2 family proteins, was conducted (Gandhi et al., 2011). Forty-seven patients, including 29 with SCLC or pulmonary carcinoid, were enrolled. A dose-dependent transient increase after 6 hours in circulating ccCK18 was observed in higher dose cohorts. Obatoclax represents another small-molecule drug designed to target the antiapoptotic Bcl-2 family members to a proapoptotic effect (Dean et al., 2011). In vitro, obatoclax was synergistic with cisplatin and etoposide, and priming of cells with obatoclax before the cytotoxics maximized tumor cell death. Peak levels of apoptosis, reflected by ccCK18 levels and caspase activity, occurred 24 hours after obatoclax treatment. A phase 1b-2 trial of obatoclax administered using two infusion regimens in combination with carboplatin and etoposide was completed in previously untreated patients with extensive-stage SCLC. All SCLC patients classified as responders after two cycles of treatment showed significantly increased levels of full-length

and cleaved CK18 on day 3 of the study. However, in difference to the preclinical data a peak in circulating ccCK18 was not detectable in trial patients and explained by a suboptimal timing of blood sampling.

Dulanermin (rhApo2L/TRAIL) induces apoptosis by binding to death receptors DR4 and DR5, leading to caspase activation and subsequent cell death (Pan et al., 2011). A preclinical study using Colo205 xenografts revealed a transient increase of ccCK18 in response to dulanermin that peaked around 24 hours and gradually declined thereafter. A Phase 1a trial evaluated the safety and tolerability of dulanermin in patients with advanced tumors. In two out of seven NSCLC patients with evaluable biomarker measurements at baseline, a significant increase of cell-death biomarkers was observed 5 hours after dulanermin administration. In CRC patients increases in cleaved CK18 at 24 hours post dulanermin treatment relative to baseline were also observed. Circulating caspase 3/7 increased in a statistically significant manner in CRC and sarcoma patients treated with dulanermin. Remarkably, in contrast to the preclinical results, the clinical analysis showed little correlation between cell-death markers and tumor response.

4. Acute side effects of chemotherapy and relation to tumor response

Chemotherapeutic drugs act on rapidly multiplying tumor and normal cells accounting for both response and the toxic side effects. Therefore, a significant correlation may exist between the effects of the agents and the toxicity produced. The time course of various toxicities depends on the drug, its dose and frequency of administration, intrinsic characteristics of the affected tissue of interest and other factors. Mucosal toxicities of pain, erythema, ulceration etc. occur 3–10 days after the administration of respective drugs and bone marrow effects are detectable between days 7–14 after initiation of treatment. The recovery of normal cellular function is ongoing 4–5 days after culmination of these toxic effects. Cytotoxic drugs, in particular 5-FU, impair replacement of intestinal epithelia and induce flattening of the villi, leading to increased exposure of luminal contents to crypts and increased absorption (Melichar & Zezulova, 2011). Intestinal permeability changes correlated with clinical manifestations, including diarrhea, mucositis, neutropenic enterocolitis and systemic infections. After chemotherapy apoptosis increased sevenfold in intestinal crypts at one day, and villus area, crypt length, mitotic count per crypt, and enterocyte height decreased at three days followed by recovery after 16 days (Keefe et al., 2000).

Several studies reported correlations between toxic side effects and tumor responses induced by chemotherapeutic drugs. Dahl et al. described a correlation between an adverse reaction of normal bowel and radiation sensitivity of presumably operable rectal adenocarcinomas derived from the same tissue (Dahl et al., 1994). Patients experiencing severe side effects including necessary medication had significantly smaller tumors at subsequent surgery. These patients had less recurrence and better disease-specific survival rates than did patients without high-grade acute side effects. In 303 patients with advanced CRC the relationship between chemotherapy-associated adverse events and treatment efficacy was investigated using toxicity, objective response and survival data (Schuell et al., 2005). The results of this study suggested that the frequency of side effects during chemotherapy in advanced CRC was an independent and reliable prognostic indicator for response and survival.

During neoadjuvant chemotherapy for breast cancer responders revealed significant toxicity while nonresponders did not (Chintamani et al., 2004). The chemotherapy-induced toxicity

was concluded to be a cost-effective and reliable predictor of response to this type of chemotherapy. The putative association between acute organ toxicity with treatment outcome was investigated in patients with locally advanced head and neck squamous cell carcinoma (HNSCC) during adjuvant radiation and chemotherapy (Wolff et al., 2011). Patients varied in their disposition to side effects during therapy, independently of treatment regimen and high-grade acute organ toxicity during radiation and chemotherapy was associated with better outcomes. Treatment response was variable among patients and tumor types most likely due to individual differences in cellular sensitivity. These data point to a similar behaviour of normal and tumor tissues with respect to treatment response. Finally, Bonner et al. showed that in patients with locally advanced HNSCC treated with radiation therapy and cetuximab, acute toxicity (rash and skin toxicity) could also predict tumor response (Bonner et al., 2010). The correlation of skin rashes with a significantly better OS in lung cancer patients receiving anti-EGF-receptor therapy was reviewed by Perez-Soler (Perez-Soler, 2006).

In conclusion, damage to normal tissues is suggested to be associated with increased tumor response in several carcinomas and for specific chemotherapeutic regimens. Therefore, it cannot be excluded that increases in circulating CK18 and ccCK18 in short-term reaction to chemotherapeutic agents are due to toxic side effects and that the larger release of CKs in responding patients is only indirectly correlated with tumor cell death. Elevations of CK fragments in lymphoma patients with CK-negative hematopoietic tumor cells treated with anthracyclines, alkylating agents and Vinca alkaloids clearly indicated cell death of epithelial normal cells and release due to toxic side effects within 2 days of start of chemotherapy (Greystoke et al., 2011). Thus, an idealized course of CK18 during chemotherapeutic treatment is shown in Figure 1. Patients with carcinomas exhibit elevated circulating CK18 and fragments compared to healthy controls (approximately 150 U/L CK18).

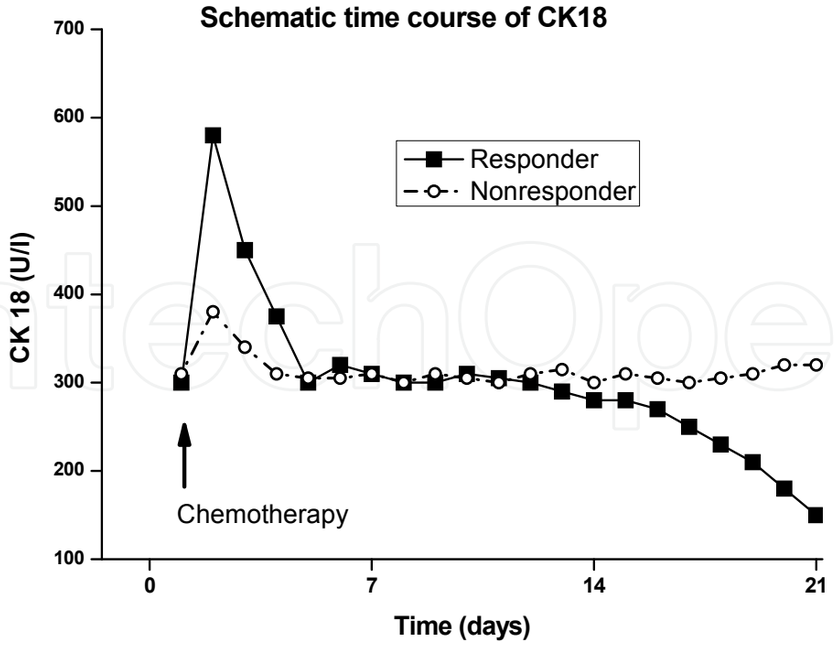

Fig. 1. Schematic course of circulating CK18 typically observed in patients in response to chemotherapy. The two curves summarize results of CK18 in responding and nonresponding patients described in studies shown in table 2.

In responding patients peaks of CK18 within 5 - 48 hours after initiation of treatment are typically observed that seem to coincide with the maximal plasma concentrations of the drugs. The sources of the circulating CKs in this situation seem to be damaged normal tissues like hepatocytes and epithelial cells of the kidney and the gastrointestinal tract as well as tumor cells. In clinical practise, the CK18 assays are not expected to be suitable to discriminate the toxic side effects on normal epithelia and anticancer activity of the drugs. However, residual tumor burden after therapy is indicated by a lack of normalization of circulating CK18 few weeks after start of therapy, well past to the recovery of normal tissues. Nonresponders fail to exhibit drug-induced peaks most likely due to high baseline values of the circulating CKs, that indicate a larger tumor burden, and a possible reduced individual sensitivity to the agents applied.

5. Conclusions

Advances in genomics, proteomics and molecular pathology have generated many candidate biomarkers of potential clinical value (Ludwig & Weinstein, 2005). Their use for cancer staging and personalization of therapy at the time of diagnosis could improve patient care. However, translation from bench to bedside outside of the research setting has proved more difficult than might have been expected. Understanding how and when biomarkers can be integrated into clinical care is crucial if we want to translate the promise into reality. Biomarkers of tumor response to chemotherapy could provide the means to get data on anticancer activity of the agents in preclinical reseach and in clinical setting very rapidly and well in advance of other morphological and clinical data. Released cellular constituents that under normal conditions reside intracellularly could constitute indicators of abnormal cell proliferation or cell death in the peripheral circulation. Different CKs and a caspase-cleaved CK fragments have been employed to detect tumor load, proliferation and tumor necrosis/apoptosis in patients bearing epithelial cancers. While in general a good correlation exists between elevated levels of circulating CK18/ccCK18 and tumor burden of untreated patients, the influence of anticancer treatment with various agents on the release of CKs by tumor cells is less clear. The blood concentration of these CKs has predictive power in various tumor entities since larger tumor have a poorer prognosis in the majority of carcinomas. Measurements of circulating CK18 and ccCK18 before and after chemotherapy using conventional as well as experimental drugs revealed significant increases usually occurring within several hours or a few days within application of the respective agents. Although the levels of these CKs were variable, dependent on tumor entity and drug used, most studies reported higher peak values in responding patients versus nonresponders or those with stable disease. The putative sources of the circulating CK18s are spontaneously dying or drug-damaged tumor cells as well as normal epithelial tissues affected by toxic drug side effects. A study involving lymphoma patients with obviously CK-negative tumor cells demonstrated increased CK levels stemming from toxic effects to normal epithelia in response to chemotherapy and this finding is corroborated by other studies correlating release of CKs with liver toxicity and other severe adverse drug effects. Nevertheless, the positive correlation of increased CK18 levels with clinical response may be partially explained by successful therapeutic intervention in those patients who exhibited more severe toxicity. In summary, the complex mode of the release of CK18s by tumors and normal epithelia in response to various stimuli complicates the interpretation of the results and seems to preclude their use as acute response biomarkers in oncology. However, tumor burden may be assessed by pre- and posttreatment determinations of circulating CK18.

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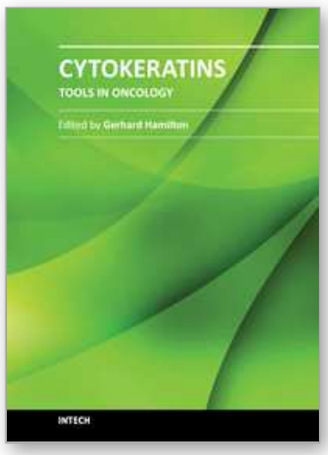

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The first chapters of the volume "Cytokeratins - Tools in Oncology" discuss multiple functions of cytokeratins in organization of the intermediary filaments in normal intestine and liver as well as microfold L cells and the usability of cytokeratins 7, 8 and 20 in tumor diagnosis in detail. Epithelial to mesenchymal transition as a mechanism important in pathogenesis is touched in another chapter, followed by several articles dealing with the role of cytokeratins for detection of disseminated tumor cells and as response markers during chemotherapy. This book is therefore destined to all cancer researchers and therapists who want to understand the diagnostic application of cytokeratins in histology and, especially, the use of anti-cytokeratin antibodies to identify viable residual tumor cells accounting for a higher risk of tumor recurrence or cancer cells responding to chemotherapy, respectively.

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