News

Immunodiagnosis of Tumours

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INTRODUCTION

A CONSENSUS development workshop on Immunodiagnosis of Tumours was held within the framework of the European School of Oncology at Milan on 15–16 October 1992. The aim of this workshop was to address the present and the future role of circulating and cell membrane-bound substances collectively referred to as tumour markers.

An effort was made to reach general agreement on the scientific value and clinical relevance of these compounds and also on the laboratory or medical procedures to be employed for their measurement or demonstration. For this consensus workshop a panel of pre-clinical and clinical investigators actively working in the field were invited, who gave formal presentations to assess the present state of the art. The following consensus and recommendations are the outcome of these experts' views which were extensively discussed together with the participants* of the workshop.

Despite progress and improvement in diagnostic methodologies early diagnosis of a number of carcinomas of internal organs such as lung, pancreas, prostate or kidney is still a rare event, presumably a consequence of the anatomic site or the lack or late onset of characteristic clinical signs or symptoms. Therefore, the prognosis of certain of these tumours is traditionally very poor and an early diagnosis at a curative stage seems to be one of the best ways to improve the survival significantly.

Since there is a close relationship between tumour stage and prognosis, and since the latter dictates the therapeutic strategy, a precise staging is needed at first diagnosis.

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Consensus was reached that in this respect the measurement and determination of well chosen tumour markers, both circulating and cell-bound, can be of great help. Under the term of "tumour marker" are grouped substances, that are produced either eutopically or ectopically or the production of which is induced by the growth of malignant cells. Table 1 shows some of those tumour markers, which in the indicated tumour systems have been proven to reliably correlate with the tumour mass: significantly elevated marker levels are in general incompatible with an early tumour stage, while the reverse is not true, i.e. lack of marker elevations do not mean absence of tumour.

IN VITRO TESTS/METHODS

Circulating tumour markers

Mammary carcinomas. Among the most common tumours (lung, colon, prostate and breast), it is breast cancer that attracts the most scientific attention for the development of new markers. This is probably because breast cancer is so heterogeneous in respect to histology, progression, prognosis, and survival. A great need has been recognised to define subgroups of early stage breast cancer patients with a favourable or, what is clinically more important, poor prognosis which require intensive prophylactic or adjuvant therapy. This is probably why such a large array of tumour markers for breast carcinomas has been developed (Table 2). However, it is evident now, that none of the markers detects a breast tumour in a truly early stage (NO, MO). It was found that the CA 15/3 assay is more sensitive and correlates more accurately with the extent of the disease than carcinoembryonic antigen (CEA) [1, 2]. Recent studies have shown that the serum levels of the mucinous-like carcinoma-associated antigen MCA show a significant correlation with the CA 15/3 values, which is due to the fact, that the monoclonal antibody b12 detects both these glycoproteins, which have several similarities [3]. MCA, (also named TAG12) although it is expressed in about 96% of the mammary carcinomas, offers no advantage over CA 15/3 or CA 549, which is an acid glycoprotein characterised by two monoclonal antibodies, BC4 E549, and BC4N154, prepared against human milk fat globule membranes. A recent collaborative evaluation of this marker showed that CA 549 was elevated in less than 13% of patients with stage T 1 and T 2 carcinomas and in those with benign breast diseases, in 38% of women with stage T 3 disease, in 17% of patients without recurrence, but in 55% of patients with recurrence. This marker, like the others mentioned, was positive in > 80% of M1 patients with active disease [4]. Hence it becomes more a question of personal preference than of superior specificity, which marker to use for the surveillance, detection of recurrence or the evaluation of the therapeutic success, CA 15/3, MCA or CA 549 [5]. There was consensus, that it is not recommended to multiply marker tests because of the great physico-chemical similarity of the traced mucinous-like antigens in the three above-mentioned

Table 1. Some clinically used tumour markers

Abbreviation	Full name	Tumour system
AFP	α-Fetoprotein	Hepatomas, NSGCT, Teratocarcinomas, EST
CEA	Carcinoembryonic antigen	Colorectal-, breast-, stomach-, lung-, gynaecological carcinomas
βHCG	Human chorionic gonadotrophin hormon (β-subunit)	Throphoblastic and testis (NSGCT) tumours
PSA	Prostate specific antigen	Prostatic carcinoma
PAP	Prostatic acid phosphatase	
NSE	Neuron specific enolase	SCLC, phaeochromocytomas, carcinoids
SCC	Squamous cell carcinoma (antigen)	Squamous cell lung carcinomas, cervix-, oesophagous carcinoma
TG	Thyroglobulin	Thyroid carcinomas
CT	Calcitonin	Medullary thyroid carcinomas
MCA	Mammary carcinoma antigen	
EMA	Epithelial membrane antigen	Breast carcinomas
CA 15/3, CA 549		
CA 125		Ovarian carcinomas
CA 19/9		Pancreatic tumours, gastric
CA 195		carcinomas
CA 72/4 (TAG 72)		Gastric carcinomas
CA 50		
TPA/TPS		Proliferating tumours

Table 2. Circulating markers used or tried in breast carcinomas

CEA
CA 15/3
CA 549
MCA
BCM - retracted
TPA/TPS
SP-1
CA M26
CA M29

tests. If a second marker is wanted, CEA would be the best because of its different molecular nature.

For the detection of relapse attention must be paid to dynamic criteria, i.e. a steadily rising marker curve rather than single values [6]. If minimal changes occur, it must be remembered that benign biological factors and not tumour growth might be at the origin [7].

As concerns the prognostic value of the markers, it is the post-operative rather than the pre-operative one that is predictive. As is the case for other tumour systems (see below), marker levels that do not normalise post-operatively indicate a non-curative, i.e. incomplete tumour resection, probably in the form of (unknown) distant metastases. As more powerful prognostic parameters than tumour markers, factors, like the hormone receptor status, cathepsin D, or the number of invaded lymph nodes were tried, or the amplification of EGF-R, HER-2/*neu* or *myc*-oncogenes [8–10]. However, there is still controversy over their prognostic significance (Table 3). As has been shown by Ciocca *et al.* [11] the amplification of HER-2/*neu* correlates significantly with some other prognostic factors, like the oestrogen receptor- (ER-), progesterone receptor (PR)-negativity or positivity, low or high percentage of cells in S-phase and the DNA-ploidy, while no correlation was observed with tumour size, lymph node status, or patients' age.

Colorectal carcinomas. There was unanimous agreement that for colorectal carcinomas CEA was the optimal tumour marker. It can be used to improve the pre- and post-surgical staging, because the correlation between the tumour burden and the marker levels is very good in the sense that extremely high CEA levels are never seen in localised tumours. Therefore, the preoperative or postoperative serum values have a prognostic bearing: A failure of CEA to return to the normal range is indicative of incomplete tumour resection, suggesting that the patient is at risk from an early recurrency. A postoperative rise of the marker, which may occur with a lead-time of several weeks or months before the clinical manifestation of recurrence, is a reliable and sensitive signal for metastases formation. For this reason CEA assays are recommended for the surveillance of colorectal patients. The success of other therapeutic modalities can also be followed by CEA measurements. A marker curve that rises during radio- or chemotherapy indicates the non-responsiveness of the malignancy to the treatment.

There was agreement that other marker determinations are not needed in colorectal carcinoma, since none of those that had been proposed (e.g. CA 50, CA 19/9, CA 195) proved to be superior to CEA.

However, since the CEA test has been commercialised by a

Table 3. Potential prognostic factors in breast cancer

Histopathological factors	Histological type, histological grade, pT, tumour grade
Proliferative factors	Thymidine labelling index, bromodeoxyuridine labelling index, DNA flow cytometry, Ki67, PCNA
Hormone receptors	Oestrogen, progesterone, androgen, prolactin
Growth factor receptors	Insulin-like growth factor 1, somatostatin, epidermal growth factor
Oncogenes and products	*HER*-2,c-*myc*, *hst*, *int*-2, c-Ha-*ras*, p21-*ras*, c-*erb*B-2
Others	Cathepsin-D, tissue type plasminogen activator, β-2 microglobulin, pS2, ferritin, oncofetal ferritin-bearing lymphocytes, progesterone binding cyst protein, stress response proteins

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great number of firms it should be ensured that a given patient is always followed by the same test kit. To avoid inconsistencies in the longitudinal curves the origin of the test kit (firm) should be noted when reporting the results because of the various cut-off values of different kits.

It should be remembered that a CEA elevation, even in advanced tumour stages, is observed in only about 80% of the patients, although immunohistologically CEA can be demonstrated in over 90% of the tumours [12, 13]. Since the mechanism of the secretion of this cell membrane bound antigen has not been elucidated, it is unknown why in a certain percentage of cancer patients the antigen cannot be found in pathological concentrations in the circulation. It should be appreciated that CEA may be elevated (up to 10 ng/ml) in heavy smokers, and up to 15 ng/ml in other benign diseases and various non-cancerous conditions. These elevations overlap with those seen in early tumours so that the test cannot be employed for differential diagnostic or screening purposes [14].

Carcinomas of the lung. As shown in Table 4 a number of tumour markers are available, which may be used according to the histology of the pulmonary malignancy. At present two main groups of lung tumours are distinguished, i.e. small cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC). Although there are no specific markers for these neoplasias which facilitate an improvement in early diagnosis, the following markers have proved useful in the monitoring of the treated patients: In SCLC patients neuron-specific enolase (NSE) can help to monitor therapy resistance and/or progression of the tumour. Remissions are generally indicated by a drop in the marker values [15]. For NSCLC attention must be paid to the main histological type of the tumour, since CEA is a good marker only for adenocarcinomas, while squamous cell carcinoma antigen (SCC) is the optimal tumour marker for squamous cell carcinomas. Also immunoreactive calcitonin (ICT) has been suggested as a lung cancer marker. However, neither ICT nor any of the other markers is significantly elevated in early stage cancer, where such markers would be most needed. Consensus on the utility of NSE for staging SCLC could be reached, while the monitoring of lung tumour patients during the post-treatment period by tumour marker testing was judged controversial, mainly for two reasons: First, because of a too short and therefore unhelpful lead time and second, because in most cases effective salvage therapy is unavailable. However, it was stressed, that persistent elevations of a marker reflect the presence of a tumour cell population resistant to cytotoxic agents, which is a valuable indication that the therapy should be changed or interrupted. The prognostic significance of the presence of EGF-R requires larger studies. A promising new marker for lung cancers was described recently by Miyake *et al.* [16]. These authors reported that patients having lung carcinomas that stained positive for molecules ($H/Le^y/Le^b$) defined by the monoclonal antibody MIA 15/5 have a significantly shorter survival than those with MIA 15/5 negative cancers. If these study results were confirmed it would mean a true progress in the prognostic evaluation of these tumours.

Table 4. Tumour markers for lung tumours

Marker substance	Histology
NSE	SCLC
SCC	NSCLC, squamous
CEA	NSCLC, adenomatous
MIA 15/5	All, for prognosis
Cytokeratin	All

Pancreatic carcinomas. Since 90% of T1 tumours and still 77% of T2 tumours are resectable, a rate that drops to 40.9% and 17% in T3 and T4 tumours, it would be important to detect small carcinomas [17]. There seems to be, however, only one marker suitable for the management of this neoplasia and this is a sialylated Lewis a structure, CA 19/9. It has been shown to be elevated in about 60% of stage I pancreatic carcinomas. The other tested markers are less sensitive [17].

There is consensus that CA 19/9 is the best option for pancreatic neoplasia and that this marker is more sensitive (70–95%) and specific (72%) than CEA (40–60% and 70%, respectively). Combined measurements of both markers do not significantly augment the sensitivity of the test. The diagnostic value of CA 19/9 determinations is obscured by the fact that benign, e.g. acute or chronic pancreatitis, occlusive conditions like cholelithiasis etc. may also cause CA 19/9 elevations which, however, in most cases are seen intermittently and not as a steady rise. As indicated in Table 5 besides CA 19/9 other markers have been described, however, none of them equalled or surpassed CA 19/9 in sensitivity or specificity. CA 19/9 is therefore recommended for the follow-up of treated patients since the correlation between the clinical course of the disease

Table 5. Markers tried for pancreatic carcinomas

CA 19/9
S-PAN-1
CEA
DU-PAN-2
ELASTASE
CA 195
CA M43

and the marker values is very good [18]. Marker rises are almost regularly seen, i.e. in about 90% of the cases when there is tumour progression despite radio- or cytostatic therapy.

Gastric carcinomas. According to WHO statistics gastric cancer, especially in Mediterranean countries, remains a common cause of death. Early diagnostic measurements are needed because of an overlapping symptomatology with benign conditions and therefore a delayed diagnosis of the cancer.

Among the tumour markers currently used are CEA, CA 50 and CA 19/9 and a new marker, TAG 72. Several recent studies have shown that this marker is useful, especially in combination with either CEA or CA 19/9 [19, 20].

TAG 72 is a pan-carcinoma, high molecular weight (180 kD) mucin-like protein which is found in up to 40% of tumours of the gastrointestinal tract, lung, breast, prostate and stomach. However, despite this lack of organ specificity the measurement of TAG 72 has been found of particular help in monitoring postsurgical gastric cancer patients [21].

The CA 72/4 test is commercially available, and is recommended for the monitoring of gastric carcinoma patients in the postoperative phase, although it is recognised, that it is not an optimal marker: it is only elevated in a little more than 60% in the late stage stomach cancers, however, this is a higher incidence than CEA (32%) or CA 19/9 (46%) [20]. CA 72/4 has not been shown to be useful for tumour staging. CEA, when measured in peritoneal washings can be a sensitive detector of an occult peritoneal dissemination and was found a reliable prognostic parameter [22].

Hepatocellular carcinomas. Primary liver cell carcinomas are infrequent malignancies in the western hemisphere. However, they were among the first cancers for which a truly valuable tumour marker was available in the form of alpha fetoprotein (AFP). As recommended for CEA in colorectal carcinomas, primary hepatomas do not need any other marker. AFP is optimal for monitoring therapeutic success. It correlates with the extension of the disease and is hence informative about tumour extension. It also has a diagnostic importance. If in a cirrhotic patient, AFP elevations are observed, it is an indication that a malignant transformation has probably taken place, i.e. the patient has to be considered as having a malignant disease [23]. AFP, which has been in clinical use for more than two decades now, is one of the markers, which has a restricted organ specificity and it is therefore also very helpful in the identification (and—as target antigen) in the localisation of an unknown primary (see below).

Trophoblastic/germ cell tumours. There is consensus that for this group of tumours only two markers are of clinical relevance. These are AFP and HCG for non-seminomatous germ cell tumours (NSGT) of the testis. HCG alone for trophoblast malignancies of the female and AFP + HCG for tumours with endodermal sinus components (EST) in the male. It was agreed that a combination of the two markers in NSGT is the only formal indication for the use of more than one marker, because it is known that germ cell tumours are mostly mixed tumours: during treatment one malignant cell population may be destroyed, which would result in the decline of one marker, while the other might be still present or even rising, indicating the proliferation of therapy resistent tumour cell clones [24]. HCG, if found in men is the only tumour marker with truly diagnostic properties and the only substance that deserves the name "tumour marker". In non-pregnant women the hormone also indicates a malignant tumour.

The differential diagnostic value of AFP is extremely high in testis tumours which are hard to classify, but in which the classification is of great importance, because of the therapeutical consequences: if AFP (or HCG) is found, the diagnosis of a pure seminoma is excluded and the prognosis is poor, because the mixed testis tumours are much more malignant than the pure seminomas. Unanimous consent was also reached for the usefulness of the marker combination to evaluate the stage of testis tumours, their prognosis and their sensitivity (or resistance) to the chosen treatment. The latter is almost impossible with other means than tumour marker measurements [25].

Carcinomas of the prostate. One of the few tumours that has a tissue specific marker is the carcinoma of the prostate: Prostate specific antigen (PSA), a serine protease, is one of the rare markers where a serum test for screening purposes is seriously considered. In men having PSA values > 10 ng/ml there is a fair chance of finding a cancer. Indeed, in a recent study [26] about two thirds of patients with serum levels above this value had biopsy-proven cancer. Since prostatic carcinoma is one of the most common malignancies in males, this test is of considerable interest, even though the PSA as a screening test is as yet not universally accepted.

There is common agreement, however, that for the early diagnosis of prostate carcinoma the rectal examination can no longer be regarded as the sole or even the most useful means for diagnosing a tumour, but in connection with the serum test in patients with symptoms it is a highly useful and inexpensive adjunct.

PSA tests, when used in conjunction with ultrasound and rectal examination, increased the prostate cancer detection rate by 72%: these three procedures were evaluated in a multicentre "National Prostate Cancer Detection Project" and were recommended for the detection of early and potentially curable tumours [27]. Since the 5-year relative survival rate for patients whose tumours are diagnosed while still localised is 84% (compared with only 30–73% in men with advanced disease), it seems that major progress in the defence of this common cancer has been made. PSA determinations can be used for monitoring patients with metastatic disease under therapy, and pretreatment values help to improve the staging, because PSA concentrations correlate with the tumour spread, i.e. high serum levels generally indicate distant metastases [28, 29].

An open question is, whether the determination of PAP (prostate acid phosphatase) should be continued in conjunction with the PSA test, or whether it can be eliminated. While the majority think that PSA is sufficient, others argued in favour of testing for both enzymes.

It has been concluded that PSA measurements in men suspected of having prostate cancer and in diagnosed patients are highly recommended. Test kits are commercially available and they should be routinely used.

Gynaecological carcinomas. This group of neoplasias comprises carcinomas of the ovary, endometrium, and cervix, but only for the first tumour is a valuable marker available, CA 125. Serum levels are reliably related to tumour burden (i.e. stage) and high levels in the postoperative phase indicate recurrence [30]. Serum determinations could, therefore, help to avoid unnecessary explorative second look operations: if the marker is highly elevated or rising, a tumour is present in the vast majority of cases. It was agreed that CA 125, which belongs to the mucinous-like marker family, is the optimal marker for the most common, the serous type of ovarian carcinomas, while CEA may be helpful in the rarer mucinous variant of ovarian tumours. Ovarian germ cell tumours may be managed with the same markers as the testicular germ cell tumours.

Choriocarcinomas can be diagnosed and monitored with βHCG measurements which are highly specific and of great diagnostic value in non-pregnant women. Rising serum levels of this hormone indicate tumour progression or resistance to therapy, and they are therefore unanimously recommended for the surveillance of patients with trophoblastic malignancies.

There are no good markers that could be recommended for cancer of the endometrium. For cervical lesions cytological techniques are still the best approach for the initial diagnosis, SCC is still under trial as a marker.

Carcinomas of the thyroid. Thyroglobulin (Tg) is an iodoprotein synthesised and excreted by thyroid tissue as well as by differentiated carcinomas. In thyroid carcinoma, total ablation of the gland (by surgery and radioiodine) is a commonly accepted therapeutic modality. As a consequence, normally Tg drops to undectable values. A persistent elevation or an increase from zero values is highly suspicious for viable or recurrent tumour.

In a multicentre study [31] including about 4 000 patients, the sensitivity of Tg-determinations under hormone replacement was determined to be 86% and increased to 95%, when the patients were off the hormone therapy. The high specificity was independent from therapeutic administration of thyroid hormones.

The use of Tg in the clinical follow-up of thyroid cancer patients can be considered as a well established procedure. Tg can replace radioiodine scans in many cases. For the rare medullary carcinomas of the thyroid, immune-reactive calcitonin in conjunction with CEA has proven helpful.

New markers. In Table 6 a list of new markers is shown which need to be critically evaluated. A final judgement on their clinical relevance can be made only after comparative studies have been completed on sufficient numbers of patients. For some of the markers early results indicate their usefulness, however, sufficient time should be taken before commercial distribution to avoid the need to retract the marker later from the market as has happened for some markers in the past. This had a harmful impact on the acceptance of the other useful tumour markers by the medical public.

The new tumour markers which may have a promising future will probably include some cytokines like interleukin-1α, interleukin-1β, interleukin-6 and TNF-α. A recent study suggested that high preoperative TNF-α levels in patients with primary hepatomas and to a certain degree in patients with gastrointestinal carcinomas indicated earlier recurrencies than did elevations of other tumour markers such as AFP or CEA. These serum elevations were seen before the tumour detection by computed tomography or ultrasonography. The differences in TNF-α, IL-1α and IL-1β elevations in patients with or without recurrence were highly significant [32].

IL-6 was described in 1992 as a prognostic marker for M1 renal cell carcinomas in the sense that patients without serum IL-6 serum levels have a longer survival (16 vs. 8 months) when 75 pg/ml is taken as a cut-off value. Since the same study also showed that the IL-6 concentrations are predictive for the success of an IL-2 treatment, it may be that the measurement of this interleukin will be interesting to select patients for an immunotherapy [33].

Tumours without proper markers. There are still a number of malignancies which lack marker substances and which cannot be monitored by serum tests. Among them are renal and urothelial carcinomas, sarcomas, pure seminomas, and melanomas. A melanoma marker would be especially useful in this highly malignant disease.

Tissue tumour markers

The development of new staining techniques and especially of well characterised polyclonal and monoclonal antibodies initiated great progress in the use of immunohistochemistry for the identification of tumour tissues and cells by tumour characteristic substances. The availability of a vast array of immune sera to various intracellular structures such as intermediate filament, vimentin, the cytokeratins, S 100, desmin etc. helped enormously to study tumour-typical changes of their expression in malignant as compared with normal cells. In special cases the analysis of the staining pattern of these cytostructural proteins can assist in the histological differential diagnosis and classification of various tumours (Table 7), and also in the distinction of benign vs. malignant cell growth. However, it is recognised that in only about 10% of cases are immunohistological stainings needed to establish a diagnosis of a primary tumour or to finalise its classification. Examples are the so called blue infant tumours or testicular carcinomas, where the differential diagnosis between pure seminomas and mixed tumours with entodermal sinus tumour (EST) cell structures may often only be diagnosed when the presence of AFP and/or HCG is immunohistologically demonstrated. Also, the biopsy specimens of undifferentiated secondaries, or of so called "unknown primaries" can sometimes only be identified by an immune staining using for instance an antibody against S 100 for amelanotic melanomas, PSA for prostatic or EMA for breast carcinomas.

Efforts to improve the detection of tumour cells in bone marrow specimens, especially of early stage breast cancer patients, are currently made to evaluate the prognostic significance of this approach. Antibodies against the epithelial membrane antigen (EMA) are used in conjunction with antibodies against cytokeratins (to guarantee the specificity of the staining for tumour cells) and it seems, indeed, that by this immunohistochemical procedure one can identify patients at risk for early relapse [34, 35].

There was a consensus that immunohistochemical techniques are highly sensitive and suitable methods to resolve certain differential diagnostic problems and to help to subtype certain tumours for which an exact classification is of great importance

Table 6. Some reported new tumour markers

Designation	Suggested for	Literature
TAF (Tumour antigen factor)	Cervical cancer	Taccone *et al. J Nucl Allied Sci* 1990, **34**, 63.
	Oesophageal carcinoma	Taccone *et al. Int J Biol Markers* 1991, **6**, 173.
TGFα (Transforming growth factor)	NSCLC	Ming Liu, *et al. Tumor Biol* 1992, **13**, 224.
CA 27.29	Breast cancer	Correale *et al. Int J Biol Markers* 1992, **7**, 43.
CYFRA 21-1 (Cytokeratin 19 Fraction)	Lung cancer (NSCLC) Cervix	Rastel D, Ramaioli A. IV. *Int. Symp. on Biol. and Clin. Usefulness of Tumor Markers*, Barcelona, Spain 1993, Feb. 4–6. Molina R. dto.
CA 242	Pancreatic cancer	Röthlin *et al. Cancer* 1993, **71**, 701.
TNFα (Tumour necrosis factor)	Hepatoma	Nakazaki H. *Cancer* 1992, **70**, 709.
UGP (Urinary gonadotropin peptide)	Gynaecological cancers	Cole *et al. Yale J Biol Med* 1989, **62**, 367.

Table 7. Use of immunohistochemistry in morphological tumour diagnosis

1.	Classification of childhood small round blue cell tumours
2.	Classification of undifferentiated tumours: carcinomas, sarcomas, melanomas, lymphomas
3.	Subclassification of carcinomas, sarcomas, lymphomas
4.	Subclassification of germ cell tumours
5.	Subclassification of endocrine tumours
6.	Subclassification of malignant haematopoietic cells

because having severe therapeutic consequences. It was agreed that this method needs to be employed only in very selected cases and that in most common tumours classical pathological or cytological methods are sufficient. However, it should not be forgotten that immunohistochemistry paved the way for the immuno tumour localisation by showing that most circulating antigens are also cell bound and hence can serve as target antigens for the radioimmunodetection (RID) of those tumours.

Conclusions and general recommendations on in vitro *tests*

Serological tests.

- At present a large number of tumour marker test kits is available providing for almost every common tumour type one marker. Therefore great care should be taken to select the marker with the most optimal clinical utility and not to rely on multi-marker testing. There are only very few exceptions where the determination of more than one marker is obligatory, because it truly brings an increase in clinical information. An example of such an exception is for instance the management of men with (embryonal) testicular tumours.
- The individual cut-off values of the various commercial tumour marker kits should be taken into consideration when a patient is monitored over a long period of time, because any changes in test kits may bring about "spikes" in the marker curves which might be misleading. Therefore the origin, i.e. name of the firm of the marker kit, should be reported together with the results, so that the treating physician might be aware of any such changes.
- Since it is the kinetics of a curve only which allows conclusions on the evolution of the malignant disease, multiple serial marker measurements are obligatory to warrant conclusions.
- Cut-off values, however, should not be taken as absolute values, because each patient is his own control: steadily rising marker values, even within the normal range, have to be taken as a serious sign for a change in the tumour cell behaviour in the sense of tumour progression, beginning therapy resistance etc. Single marker measurements should be avoided.

It should be kept in mind that marker determinations are very sensitive, non-invasive and low cost measurements, that if performed in experienced hands are of high clinical value for the patient in respect to improving his management, the diagnosis, staging, prognosis, and posttherapeutic surveillance for the earlier detection of recurrencies.

Immunohistological techniques. It must be emphasised that the use of immunoreagents demands an extensive experience with regard to specificity testing of the immune sera in order to avoid cross-reactions leading to false positive results. Also an appropriate knowledge of tissue fixation techniques is required to avoid false negative reactions. Immunohistology as a highly sensitive method is full of pitfalls in as much as many tumours are not only of the mixed type, but most cellular structures are common to normal, benignly changed or malignantly transformed cells and tissues. Immunohistochemical techniques are helpful only if employed by experienced pathologists. It was

agreed that this methodology can be used only in conjunction with classical staining procedures and that it is recommended only in a restricted number of problem cases because the technology is still relatively work-intensive and time-consuming.

IN VIVO METHODS

Radioimmunodetection (RID) using murine monoclonal antibodies has been employed for over a decade now in clinical oncology. Although within this time RID made a huge progress, some questions remain to be answered:

(1) Has this 'new' imaging technique (RID) lived up to the great promise it held 10 years ago?
(2) What was achieved with the immunolocalisation that could not have been shown by conventional means?
(3) What are the difficulties preventing the technique from becoming a 100% success?
(4) Is it realistic to believe that immunoscintigraphy will ever play a major role in oncology?

(1) The initial (exaggerated) optimism raised by the successful animal model studies was somewhat diminished when larger clinical trials were published by various centres particularly in Europe. It is now a fact that RID does enrich the diagnosis of primary and secondary tumours. It is generally recognised as a useful technique for the detection of occult and recurrent tumours. It has its definite place among the numerous other imaging techniques, in restricted areas only, however. Based on 10 years of clinical experience they have been defined as follows:

RID is not a universally applicable method but must be used only when a definite clinical indication and appropriate antibodies/target antigens are given (e.g. in colorectal or ovarian carcinomas, melanomas) [36–38].

The usefulness of RID for the detection of some primary tumours is still debated; for general tumour screening the technique has been shown not to be useful. However, it is the most sensitive method to disclose occult metastases with an accuracy often greater than that of other conventional methods such as X-rays, magnetic resonance imaging (MRI) or computed tomography (CT) [39, 40].

Since RID is a safe procedure with almost no harmful side effects for the patient—given cautions monitoring for human anti-mouse antibodies (HAMA) formation—it can be considered to have fulfilled its promise of a decade ago.

(2) The recent employment of antibody F (ab') fragments and the use of short lived radionucleids such as Technetium 99m results in excellent images obtainable within 24 h. This permits the use of RID for preoperative staging or the detection of unknown metastases. A further advantage is that it allows the use of SPECT (single photon emission computerised tomography) and high-resolution (HR) SPECT with multiple rotating detectors for a better exploration of the pelvic (for colorectal and ovarian cancer) and thoracic regions (for mediastinal lymphnode metastases) [41]. The role positron emission tomography (PET) might play in tumour detection is still in an explorative phase [42].

Radiommunodetection is the only imaging technique which permits a distinction between vital and scarified, fibrotic or calcified tumour tissues. This is also possible intraoperatively, using a particular gamma sensitive probe. RID, therefore, is helpful in the evaluation of the success (or failure) of a chemotherapeutic regimen. It facilitates the early demonstration of loco-regional or (non-hepatic) distant recurrencies.

(3) Despite more than a decade of employment of RID some (known) technical and biological handicaps of this methods are still not resolved.

One of the main obstacles for the full success of the technique is the lasting lack of a truly tumour specific antigen. New targets and monoclonal antibodies (Mab) must be generated, for instance antibodies against growth factor receptors, e.g. as targets for CNS-tumours or oncogene products, e.g. EGF-Rs. Since the blood pool background makes the interpretation of small hot spots difficult, the ratio of specific vs. unspecific uptake needs to be improved. Actually only 0.001% of the injected activity binds to tumour cells. Several possibilities for improvement are under study such as local hyperthermia, three-labelling techniques, pretreatment of the patient with IFN-α or IFN-γ, computerised background reduction techniques, or the use of a second antibody [43, 44]. Depending on the quality of the Mab employed the use of F(ab')2 might be advantageous as compared to F(ab'), which is sometimes too rapidly cleared [45].

Although the antigen–antibody complex formation does not prevent tumour targeting, the penetration of labelled antibodies into the tumors is not yet optimal. The mixing of antibodies with different specificities has not sensibly improved the images.

Another unresolved problem is the antigenic heterogeneity of the carcinomas and their metastases which are often subject to an antigenic modulation due to an immunological or therapeutic pressure. Human antimouse antibody (HAMA) formation, however, has been excluded as a major obstacle for the successful employment of this technique [46, 47].

However, if anti-idiotype HAMA are generated, the antibody binding site on the tumour cells may be blocked thus preventing successful imaging especially of small lesions [48]. The molecular engineering of antibodies may have the greatest impact on the improvement of the RID methodologies and the future use of PET. Since allergic reactions were seen only in 0.35% (n = 1700 patients) when used diagnostically, and only limited reactions in 47% of patients when antibodies were given therapeutically [49], the RID method can be recommended as safe. Repeated application can, however, lead to a HAMA complex formation which consequently can lead to an altered biodistribution. It is known now that chimeric antibodies also induce HAMA but

Table 8. Selected clinically tested monoclonal antibodies

Monoclonal antibody	Target/tumour	Label
Anti-CEA Mab 35	Colon carcinoma	Indium
Anti-CEA BW 431/26	Colon carcinoma	Technetium
Anti-CEA Immu-4	Colon carcinoma	Technetium
PR1A3	Colon carcinoma	Technetium
17-1A	Colon carcinoma	Technetium
B 72.3	Colon carcinoma	Indium
OC 125	Ovarian carcinoma	Indium
MOV 18	Ovarian carcinoma	Technetium
B 43.13	Ovarian carcinoma	Technetium
OVTL 3	Ovarian carcinoma	Technetium
225.28S	Melanoma	Technetium
BW 494/32	Pancreas carcinoma	Technetium
Lym-1	B-cell lymphoma	Technetium
T 101	T-cell lymphoma	Indium
Anti-AFP	AFP	Technetium
B 80.3	PSA	Technetium
174 H 64	SCC	Technetium

they seem to have a therapeutic effect. These findings need confirmation [50].

A major drawback for the development of the RID technique for tumour imaging is the fact, that only few of the antibodies tried clinically (Table 8) have been officially authorised for use in Europe. This hampers large international cooperative studies necessary for the evaluation of the usefulness of this technique in new tumour systems, especially of rarer tumour types.

(4) The unanimous opinion of the experts specialising in oncological nuclear medicine was that RID paved the way for radioimmunotherapy trials and has an important role to play in routine tumour diagnosis by imaging techniques. However, radioimmunoscintigraphy, though a simple inoffensive technique should be used only by experienced hands in specialised centres. RID can be fully recommended for the following fields:

RID is useful for the detection of unknown secondary lesions, especially soft tissue lesions and it is indicated for:

- — The differential diagnosis of benign vs. malignant lesions.
- — The pre- and (post-)surgical evaluation of the extent of the tumour spread (staging) prior to further therapy.
- — The preoperative confirmation of the diagnosis if surgery of a single metastasis is envisaged.
- — The localisation of recurrences indicated by a rise in tumour marker levels.
- — The disclosure of proliferative (vital) tumour tissues after therapy.

General comments and recommendations

The *in vivo* RID technique needs to have an extended array of commercialised Mabs. Efforts should be made to advance radioimmunotherapy as a new treatment modality. If those tumour systems or areas where RID has not been shown to be useful are excluded, this technique can be recommended to the critical oncologist as being highly specific, non-invasive, safe, and if the diagnostic benefit it gives to the patient is calculated, of low cost. Indications should be derived by experts in oncology and nuclear medicine. The same holds true for the immunodiagnostic *in vitro* methods, i.e. immunohistology and tumour marker measurements in sera and other body fluids.

All participants of the workshop express their strong wish that health authorities take notice of the great usefulness of tumour marker assays, which are specially advantageous in their cost/benefit ratio compared to other diagnostic, staging or surveillance procedures: tumour marker determinations, besides being also a valuable prognostic parameter are practically the only low cost mean for the evaluation of the efficiency of a therapeutic measurement or the evolution of a malignant disease. Marker elevations often occur with lead times which then allow an earlier therapeutic intervention of the treating physician.

For the optimal use of *in vitro* and *in vivo* immunodiagnostic technologies several important rules need to be respected:

- — For the immunohistological demonstration of tumour-characteristic antigens never trust "shelf reagents". Test their specificity and crossreactivity before use.
- — For serum tests never change the kit of one firm for that of another during the surveillance of a given patient.
- — Only use a tumour marker curve made up of repeated serum levels to cast a judgement, because not a single value but the slope of the curve is informative for the evolution of the disease.
- — Never forget that a negative (or low) tumour marker value does not necessarily rule out the presence of tumour.

Finally, it should always be remembered that tumour marker tests are adjuncts, i.e. they should be used in addition and in conjunction with other clinico-diagnostic parameters.

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