

National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers

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BACKGROUND: Updated National Academy of Clinical Biochemistry (NACB) Laboratory Medicine Practice Guidelines for the use of tumor markers in the clinic have been developed.

METHODS: Published reports relevant to use of tumor markers for 5 cancer sites—testicular, prostate, colorectal, breast, and ovarian—were critically reviewed.

RESULTS: For testicular cancer, α -fetoprotein, human chorionic gonadotropin, and lactate dehydrogenase are recommended for diagnosis/case finding, staging, prognosis determination, recurrence detection, and ther-

apy monitoring. α -Fetoprotein is also recommended for differential diagnosis of nonseminomatous and seminomatous germ cell tumors. Prostate-specific antigen (PSA) is not recommended for prostate cancer screening, but may be used for detecting disease recurrence and monitoring therapy. Free PSA measurement data are useful for distinguishing malignant from benign prostatic disease when total PSA is $<10 \mu\text{g/L}$. In colorectal cancer, carcinoembryonic antigen is recommended (with some caveats) for prognosis determination, postoperative surveillance, and therapy monitoring in advanced disease. Fecal occult blood testing may be used for

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screening asymptomatic adults 50 years or older. For breast cancer, estrogen and progesterone receptors are mandatory for predicting response to hormone therapy, human epidermal growth factor receptor-2 measurement is mandatory for predicting response to trastuzumab, and urokinase plasminogen activator/plasminogen activator inhibitor 1 may be used for determining prognosis in lymph node-negative patients. CA15-3/BR27-29 or carcinoembryonic antigen may be used for therapy monitoring in advanced disease. CA125 is recommended (with transvaginal ultrasound) for early detection of ovarian cancer in women at high risk for this disease. CA125 is also recommended for differential diagnosis of suspicious pelvic masses in postmenopausal women, as well as for detection of recurrence, monitoring of therapy, and determination of prognosis in women with ovarian cancer.

CONCLUSIONS: Implementation of these recommendations should encourage optimal use of tumor markers.

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We present here to clinical chemists, clinicians, and other practitioners of laboratory and clinical medicine the latest update of the National Academy of Clinical Biochemistry (NACB)²⁵ Laboratory Medicine Practice Guidelines for the use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. These guidelines are intended to encourage more appropriate use of tumor marker tests by primary care physicians, hospital physicians and surgeons, specialist oncologists, and other health professionals.

Clinical practice guidelines are systematically developed statements intended to assist practitioners and

patients in making decisions about appropriate health-care for specific clinical circumstances (1). An explanation of the methodology used when developing these guidelines is provided in an accompanying preamble (2). As might be expected, many of the NACB recommendations are similar to those made by other groups, as is made clear from the tabular comparisons presented for each malignancy (2). The disciplines of all authors and statements of conflicts of interest, declared according to NACB requirements, are provided in an online data supplement (Supplemental Data Disclosures Table that accompanies this Special Report at <http://www.clinchem.org/content/vol54/issue12>). The latter are also listed at the end of this manuscript. All comments received about these guidelines are also recorded in an online data supplement (Supplemental Data Comments Received Table), together with responses to these comments.

To prepare these guidelines, the literature relevant to the use of tumor markers was reviewed. Particular attention was given to reviews, including the few relevant systematic reviews, and to guidelines issued by expert panels. If possible, the consensus recommendations of the NACB Panels reported here were based on available evidence, i.e., were evidence based. An accompanying paper presents NACB recommendations relating to general quality requirements for tumor measurements and includes tabulation of important causes of false-positive tumor marker results that must also be taken into account (e.g., heterophilic antibody interference, high-dose “hooking,” etc.) (3).

Tumor Markers in Testicular Cancers^{26,27}

BACKGROUND

About 95% of all malignant testicular tumors are of germ-cell origin; most of the rest are lymphomas, Leydig or Sertoli cell tumors, and mesotheliomas. Germ cell tumors of adolescents and adults are classified into 2 main types, seminomas and nonseminomatous germ cell cancers of the testis (NSGCT). Testicular cancers represent about 1% of all malignancies in males, but they are the most common tumors in men age 15–35 years. Testicular cancers are a significant cause of death in this age group in spite of the fact that presently more than 90% of the cases are cured (4). Germ cell tumors may also originate in extragonadal sites, e.g.,

²⁵ Nonstandard abbreviations: NACB, National Academy of Clinical Biochemistry; NSGCT, nonseminomatous germ cell cancers of the testis; AFP, α -fetoprotein; hCG, human chorionic gonadotropin; LDH, lactate dehydrogenase; LOE, level of evidence; ITGCNU, intratubular germ cell neoplasia unclassified; MSI, microsatellite instability; PLAP, placental/germ cell alkaline phosphatase; SOR, strength of recommendation; PSA, prostate-specific antigen; NICE, United Kingdom National Institute for Health and Clinical Excellence; DRE, digital rectal examination; fPSA, free PSA; EGTM, European Group on Tumour Markers; cPSA, complexed PSA; ERSPC, European Randomized Screening for Prostate Cancer; SEER, Surveillance, Epidemiology and End Results; CTCs, circulating tumor cells; CRC, colorectal cancer; ASCO, American Society of Clinical Oncology; CEA, carcinoembryonic antigen; TIMP-1, tissue inhibitor of metalloproteinases type 1; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor 1; EGFR, epidermal growth-factor receptor; FOBT, fecal occult blood test; FIT, fecal immunochemical test; NCCN, National Comprehensive Cancer Network; ER, estrogen receptor; PR, progesterone receptors; IHC, immunohistochemical analysis or immunohistochemistry; MINDACT, Microarray for Node-Negative Disease Avoids Chemotherapy (trial); RS, recurrence score; TAILORx, Trial Assigning Individualized Options for Treatment; FIGO, International Federation of Gynecology and Obstetrics; GCG, Gynecologic Cancer Intergroup; TPA, tissue polypeptide antigen; LPA, lysophosphatidic acid; TATI, tumor-associated trypsin inhibitor; CASA, cancer-associated serum antigen; hCG β cf, hCG β -core fragment.

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²⁷ All comments received about the NACB Recommendations for Testicular Cancer are included in the online Data Supplement. Professor George Bosl, Professor Barry Hancock, Dr. Grahame Howard, and Professor Michael Seckl were invited expert reviewers.

the sacrococcygeal region, mediastinum, and pineal gland (5). Those of the sacrum are predominantly found in young males. Based on the histology, age of the patient at diagnosis, clinical behavior, and chromosomal constitution, these tumors can be subdivided into 3 distinct entities with different clinical and biological characteristics (6–9): (a) teratomas and yolk sac tumors of newborns and infants, (b) seminomas and nonseminomas of adolescents and young adults, and (c) spermatocytic seminoma of the elderly. Seminomas and nonseminomas in adolescence and adulthood were the focus of attention when developing these recommendations.

The incidence of testicular cancers varies considerably in different countries. In the US about 7200 new cases are diagnosed each year (4), and the age-adjusted incidence is 5.2 per 100 000. The incidence is about 4-fold higher in white than in black men. In Europe, the age-adjusted incidence is lowest in Lithuania (0.9 per 100 000), intermediate in Finland (2.5 per 100 000) and highest in Denmark (9.2 per 100 000) (10). The incidence in various European countries has increased by 2%–5% per year. In the US the incidence increased by 52% from the mid-1970s to the mid-1990s (11). The cause of germ cell tumors is unknown, but familial clustering has been observed, and cryptorchidism and Klinefelters syndrome are predisposing factors (4).

At presentation most patients have diffuse testicular swelling, hardness, and pain. At an early stage a painless testicular mass is a pathognomonic finding, but testicular masses are most often caused by infectious epididymitis or orchitis. The diagnosis can usually be confirmed by ultrasonography. If testicular cancer is suspected, the serum concentrations of α -fetoprotein (AFP), human chorionic gonadotropin (hCG), and lactate dehydrogenase (LDH) should be determined before therapy. As a rule, orchiectomy is performed before any further treatment, but may be delayed until after chemotherapy in individuals with life-threatening metastatic disease. After orchiectomy, additional therapy depends on the type and stage of the disease.

Surveillance is increasingly used for seminoma patients with stage I disease, but radiation to the retroperitoneal and ipsilateral pelvic lymph nodes, which is standard treatment for stage IIa and IIb disease, is also used, as is short (single)-course carboplatin (12). About 4%–10% of patients relapse, and more than 90% of patients who relapse are cured by chemotherapy. About 15%–20% of stage I seminoma patients under surveillance have a relapse and must be treated with chemotherapy. Patients with stage I nonseminomatous tumors are treated by orchiectomy. After this treatment, surveillance and nerve-sparing retroperitoneal

lymph-node dissection are accepted treatment options. About 20% of patients under surveillance will have a relapse and require chemotherapy. Patients with stage II nonseminomatous tumors are treated with either chemotherapy or retroperitoneal lymph node dissection. Testicular cancer patients with advanced disease are treated with chemotherapy (4).

Serum tumor markers have an important role in the management of patients with testicular cancer, contributing to diagnosis, staging and risk assessment, evaluation of response to therapy, and early detection of relapse. Increasing marker concentrations alone are sufficient findings for treatment initiation. AFP, hCG, and LDH are established serum markers. In most cases of NSGCT, serum levels of one or more of these markers are increased, and in seminomas LDH and hCG are useful indicators. Other markers have been evaluated but provide limited additional clinical information.

To prepare these guidelines, we reviewed the literature relevant to the use of tumor markers for testicular cancer. Particular attention was given to reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Only one relevant systematic review was identified. When possible, the consensus recommendations of the NACB panel were based on available evidence, i.e., were evidence based.

CURRENTLY AVAILABLE MARKERS FOR TESTICULAR CANCER

The most widely investigated tissue-based and serum-based tumor markers for testicular cancer are listed in Table 1. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use.

TUMOR MARKERS IN TESTICULAR CANCER: NACB RECOMMENDATIONS

A summary of recommendations from representative guidelines published on the use of tumor markers in testicular cancer is presented in Table 2. This table also summarizes the NACB guidelines for the use of markers in this malignancy. A number of groups have made detailed recommendations regarding the management of testicular cancer (13–21), with some of those relating to tumor marker use summarized in Table 3. Table 4 summarizes the prognostic significance of serum tumor markers in metastatic testicular cancer, according to the consensus statement of the International Germ Cell Consensus Group Classification, which remains the cornerstone for diagnosis and treatment of testicular germ cell tumors. Below, we briefly review the histological types of testicular cancer and present a more detailed discussion on the markers listed in these tables.

Table 1. Currently available serum and tissue markers for testicular tumors.

Marker	Proposed use	Phase of development	LOE ^a	References
Established serum markers				
AFP ^b	Diagnosis	Generally available	II	(4, 65, 73, 89)
	Prognosis/staging		I	
	Monitoring/surveillance		II	
hCG	Diagnosis	Generally available	II	(4, 89, 103)
	Prognosis/staging		I	
	Monitoring/surveillance		II	
LDH	Prognosis/staging	Generally available	I	(63, 109)
Potentially useful experimental serum markers				
hCG β	Diagnosis/monitoring	Experimental	IV	(96, 103)
LDH-1	Diagnosis/risk stratification	Experimental	IV	(109)
PLAP	Diagnosis	Experimental	IV	(111, 112)
Neuron-specific enolase	Diagnosis	Experimental	IV	(116, 117)
Established tissue markers				
PLAP	Histological typing ITGCNU	Antibodies for IHC generally available	II	(24)
c-KIT, stem cell factor receptor	Typing of seminoma and ITGCNU	Antibodies for IHC available	II	(28)
CD30	Embryonal carcinoma	Antibodies for IHC generally available	IV	(60, 519)
AFP	Typing of yolk sac tumors and embryonal carcinoma	Antibodies for IHC generally available	II	(24)
hCG	Typing of seminoma and choriocarcinoma	Antibodies for IHC generally available	II	(24)
Amplification of 12p	Diagnosis of extragonadal tumors	Limited availability	II	(107, 108)
Vascular invasion	Risk stratification	Limited availability	II	(54)
OCT3/4, POU5F1	Risk stratification	Experimental	IV	(58)

^a LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.

^b AFP, α -fetoprotein; hCG, human chorionic gonadotropin; hCG β , free β -subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; NSE, neuron specific enolase NSGCT, nonseminomatous germ cell tumors; PLAP, placental (germ cell) alkaline phosphatase.

HISTOLOGICAL TYPES OF TESTICULAR CANCER

In the most recent WHO-Mostofi classification (8, 22), testicular cancers are subdivided into 2 major types, seminomas and NSGCT, which differ with respect to both marker expression and treatment. The incidence of seminoma peaks in the fourth decade of life and that of NSGCT in the third. Seminomas can be either pure seminomas or the rare spermatocytic seminomas that occur in older age groups. Most NSGCTs are a mixture of histological types, i.e., embryonal carcinomas, choriocarcinomas, teratomas, and yolk sac tumors. About 10%–20% of the nonseminomas also contain a seminoma component. These are classified as combined tu-

mors according to the British classification (23), but as nonseminomas according to the WHO classification system (22). Teratomas are further subdivided as mature or immature. Somatic cancers of various types occasionally develop from a teratoma and are classified as nongerml cell malignancies. Metastases may contain any component occurring in the primary tumor, and occasionally components not detected in the primary tumor (22). Fewer than 10% of NSGCT contain a single tissue type, and all histological types of tissue should be described (24).

The precursor lesion of testicular seminomas and nonseminomas is carcinoma in situ (25), also re-

Table 2. Recommendations for use of tumor markers in testicular cancer by different expert groups.

	EAU ^a 2001 (14)	EGTM 1999 (13)	ESMO 2007 (17) and 2008 (21)	NACB 2002 (15)	NCCN 2007 (18)	NACB 2008	
						Recommendation	SOR ^b
AFP and hCG							
For screening	No	No	No	No	No	No	A
For diagnosis/case finding	Yes	Yes	Yes	Yes	Yes	Yes	B
For staging/prognosis	Yes	Yes	Yes	Yes	Yes	Yes	A
For detecting recurrence	Yes	Yes	Yes	Yes	Yes	Yes	A
For monitoring therapy	Yes	Yes	Yes	Yes	Yes	Yes	A
AFP							
For differential diagnosis of NSGCT	Yes	Yes	Yes	Yes	Yes	Yes	A
LDH							
For diagnosis/case finding	Yes	Yes	Yes	Yes	Yes	Yes	B
For staging/prognosis	Yes	Yes	Yes	Yes	Yes	Yes	A
For detecting recurrence	Yes	Yes	Yes	Yes	Yes	Yes	B
For monitoring therapy	Yes	Yes	Yes	Yes	Yes	Yes	B

^a EAU, European Association of Urologists; ESMO, European Society of Medical Oncology.
^b SOR (520), A, high (further research is very unlikely to change the panel's confidence in the estimate of effect); B, moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); C, low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate); D, very low (any estimate of effect is very uncertain).

ferred to as intratubular germ cell neoplasia unclassified (ITGCNU) and testicular intratubular neoplasia. Carcinoma in situ cells are found within the spermatogonial niche of the seminiferous tubule in the adult

testis in close proximity to the Sertoli cells, the nursing cells of spermatogenesis (26). The carcinoma in situ cells can be detected in the adjacent parenchyma of most invasive tumors and are more frequently associated with

Table 3. Recommended frequency of tumor marker measurements in the follow-up of testicular cancer patients (16).

	Frequency of tumor marker measurements per year					
	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6–10
Stage I seminoma after radiotherapy	4	3	3	2	2	
Stage I seminoma surveillance after chemotherapy	6	4	3	2	2	1
Stage I NSGCT surveillance	6 ^a	4 ^b	2	2	2	^c
Stage I NSGCT after RPLND or adjuvant chemotherapy	6	3	2	2	2	^c
Stage IIa-IIb seminoma after radiotherapy	6	4	3	2	2	1
Stage IIa-IIb NSGCT after RPLND ^d and chemotherapy or primary chemotherapy	4	2	2	2	2	1
Seminoma and NSGCT of advanced stage	12	6	4	3	2	1

^a Measurements every 2 months recommended; measurements every month for the first 6 months advisable.
^b Measurements every 3 months recommended; measurements every 2 months advisable.
^c Measurement once a year advisable.
^d RPLND, retroperitoneal lymph node dissection.

Table 4. Classification of metastatic germ cell tumors into various risk groups according to the International Germ Cell Consensus Classification (66).^a

Good prognosis	
Nonseminoma	Seminoma
Testis/retroperitoneal primary	Any primary site
and	and
No nonpulmonary visceral metastases	No nonpulmonary visceral metastases
and	and
Good markers AFP <1000 µg/L and hCG <5000 U/L (1000 µg/L) and LDH <1.5 × N ^b	Normal AFP, any hCG, any LDH
56% of nonseminomas	90% of seminomas
5-Year PFS 89%	5-Year PFS 82%
5-Year survival 92%	5 Year survival 86%
Intermediate prognosis	
Nonseminoma	Seminoma
Testis/retroperitoneal primary	Any primary site
and	and
No nonpulmonary visceral metastases	No nonpulmonary visceral metastases
and	and
Intermediate markers AFP >1000 and <10 000 µg/L or hCG >5000 U/L and <50 000 U/L or LDH >1.5 × N and <10 × N	Normal AFP, any hCG, any LDH
28% of nonseminomas	10% of seminomas
5-Year PFS 75%	5-Year PFS 67%
5-Year survival 80%	5-Year survival 72%
Poor prognosis	
Nonseminoma	Seminoma
Mediastinal primary	No patients classified as poor prognosis
or	
Nonpulmonary visceral metastases	
or	
Poor markers AFP >10 000 µg/L or hCG >50 000 U/L (10 000 µg/L or LDH >10 × N	
16% of nonseminomas	
5-Year PFS 41%	
5-Year survival 48%	

^a Table adapted from (66) and reproduced with permission.
^b N, upper limit of normal; PFS, progression-free survival.

NSGCTs than with seminomas (27). ITGCNU is considered to be the premalignant counterpart of an embryonic germ cell, most likely a primordial germ cell or gonocyte. This theory is supported by multiple findings, including epidemiology, morphology, im-

munohistochemistry, and molecular characterization (28, 29).

Recent data indicate that infertile men with bilateral microlithiasis have an increased risk (up to 20%) of developing testicular seminomas and NSGCTs

(30). Surgical biopsy to assess the presence of ITGCNU (31) is indicated in this condition.

Tissue Markers for Testicular Cancer

GENETIC ABERRATIONS

A gain of chromosomal 12p sequences is observed in germ cell tumors both of testicular and extragonadal origin, a finding that indicates that gain of 12p sequences may be of crucial importance for the development of this cancer. Indeed, this finding is used to diagnose germ cell tumors at extragonadal sites (32). The expression level of 12p sequences, however, does not correlate with stage of the disease or treatment sensitivity/resistance (33–35). The crucial determinant of response to cisplatin-based compounds appears to occur downstream of DNA binding in the intrinsic or extrinsic pathways of apoptosis or DNA repair (36–38).

Although the majority of germ cell tumors show an intact DNA mismatch-repair pathway, a defect leading to microsatellite instability (MSI) has been observed in tumors refractory to cisplatin (39–41). Other potentially relevant findings in the context of treatment sensitivity and resistance relate to a possible defect in caspase 9 function (42). All these factors might be important, and it is unlikely that a single factor determines treatment sensitivity or resistance. The multifactorial nature of treatment response is illustrated by the finding that mature teratomas are resistant to various DNA-damaging treatment protocols (38), possibly due to epigenetic changes occurring during somatic differentiation.

The majority of invasive seminomas and nonseminomas contain additional copies of the X chromosome (43). This finding is interesting, because during normal (female) development, X-chromosome inactivation can occur in these tumors, in which X (inactive)-specific transcript (non-protein coding) (*XIST*)²⁸ is the regulatory gene (6). Detection of unmethylated *XIST* DNA in plasma has been suggested to be useful for molecular diagnosis and the monitoring of testicular GCT-patients (44). This observation merits further investigation.

²⁸ Human genes: *XIST*, X (inactive)-specific transcript (non-protein coding); *RB1*, retinoblastoma 1; *APC*, adenomatous polyposis coli; *MLH1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*); *MSH2*, mutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*); *MSH6*, mutS homolog 6 (*E. coli*); *PMS2*, postmeiotic segregation increased 2 (*S. cerevisiae*); *HER-2* and *NEU*, aliases for *ERBB2* [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)]; *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; β -catenin; *PTEN*, phosphatase and tensin homolog; *MUC16*, mucin 16, cell surface associated; prostasin [alias for *PRSS8* (protease, serine, 8)]; *AKT2*, v-akt murine thymoma viral oncogene homolog 2; *RSF-1*, remodeling and spacing factor 1; *NAC-1* [alias for *NACC1* (nucleus accumbens associated 1, BEN and BTB (POZ) domain containing)]; *PCA3*, prostate cancer antigen 3 (non-protein coding).

A number of studies have linked the development of germ cell tumors to a deregulated G₁/S checkpoint, possibly related to the lack of a functional retinoblastoma [retinoblastoma 1 (*RB1*) gene] cell cycle regulator (45), and consequently no upregulation of p21 after induction of DNA damage. Cells without p21 show reduced cisplatin-induced DNA damage–repair capacity and increased sensitivity to cisplatin (46). The treatment-resistant mature teratomas show, in contrast to other invasive components, positive staining for multiple proteins potentially related to treatment resistance. In addition, they are positive for *RB1* and p21, allowing them to go into G₁/S cycle arrest (47, 48). These characteristics might explain the observation that residual mature teratoma is found in about 30%–40% of remnants of initial metastases after chemotherapy.

A predictive model for the histology of a residual retroperitoneal mass, based on primary tumor histology, prechemotherapy markers, mass size, and size reduction under chemotherapy, has been developed (49). Absence of teratoma elements or viable cancer cells in the primary tumor has been identified as the most powerful predictor for benign residual tissue (50). Caution is warranted, however, because small teratoma areas may be missed in the primary tumor, and absence of teratoma elements does not exclude occurrence of malignant cells in residual masses. These findings may again be related to the origin of these tumors (51), because *RB1* expression is not found in human fetal gonocytes or ITGCNU (52, 53).

VASCULAR INVASION

Particular attention must be paid to the presence or absence of vascular invasion as a predictor of metastatic spread and occult metastases (54). Distinguishing venous from lymphatic invasion does not add information as to the risk of occult metastasis. Besides vascular invasion, high proliferative activity (assessed with the monoclonal antibody MIB-1), and to a lesser extent the presence of embryonal carcinoma in the primary tumor and a high pathologic stage, have been reported to be predictors of systemic spread in clinical stage I NSGCT [for review, see (55)]. However, the predictive value of this model is limited, because the group defined as high risk in fact has a 50% risk of occult metastasis, and the low risk group a 16% risk.

Prospective assessment of risk factors for relapse in clinical stage I NSGCT also showed that vascular invasion was the strongest predictive factor (56). With the addition of 2 other risk parameters (MIB-1 score >70% and embryonal carcinoma \geq 50%), the positive predictive value increased to 63.6%. Thus, even with an optimal combination of prognostic factors and reference pathology, more than one-third of patients pre-

Table 5. Analytical requirements and potential interfering factors for established and experimental serum markers for germ cell tumors.

Marker	Sample type	Analytical requirements	Confounding factors
Established markers			
AFP ^a	Serum or plasma	Detection limit <1 µg/L	Hepatitis, heterophilic antibodies, drug-induced hepatic damage, hepatocellular cancer
hCG	Serum or plasma, urine to confirm results	Detection limit <2 U/L; cross-reaction with LH <2%, equimolar recognition of hCGβ (or use of separate assay for hCGβ)	Chemotherapy-induced elevation of hCG to >10 U/L, heterophilic antibodies, nontrophoblastic cancers producing hCGβ
LDH	Serum	Reference values are method dependent, clinical decision limits based on upper reference limit	Elevated values also caused by hemolysis, liver disease, muscle disease, myocardial infarction
Experimental markers			
hCGβ	Serum or plasma	Detection limit 0.5 pmol/L	Nontrophoblastic cancers
LDH-1	Serum	Reference values method dependent	Hemolysis, muscle disease, heart disease
PLAP	Serum	Reference values method dependent	Smokers may have 10-fold increased values
Neuron-specific enolase	Serum	Reference values method dependent	Hemolysis causes falsely elevated values

^a AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; hCGα, free α-subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; PLAP, placental (germ cell) alkaline phosphatase.

dicted to have pathologic stage II or a relapse during follow-up will not have metastatic disease and will be over-treated with adjuvant therapy. On the other hand, patients at low risk can be predicted with better accuracy (86.5%), suggesting that surveillance may be an option for highly compliant patients. Recently, cluster analysis has been used to identify prognostic subgroups in patients with embryonal carcinoma (57).

Serum Markers for Testicular Cancer

MARKER EXPRESSION AND TUMOR TYPE

Certain markers have been found to be informative for the classification of seminomas and NSGCT. Placental/germ cell alkaline phosphatase (PLAP) is detected in most seminomas and embryonal carcinomas and in 50% of yolk sac tumors and choriocarcinomas, but only rarely in teratomas. HCG is expressed by syncytiotrophoblasts, choriocarcinoma, and approximately 30% of seminomas. Of the other tissue markers, the stem cell factor receptor (c-KIT) has been used mainly to detect ITGCNU and seminoma, CD30 to detect embryonal carcinoma, and AFP to detect yolk sac tumors and a 10%–20% subset of embryonal carcinomas and teratomas. Recently, a potentially valuable marker, OCT3/4, also known as POU5F1, has been identified (58–61).

Although a large number of serum markers have been studied, only hCG, AFP, and LDH have thus far

been shown to have independent diagnostic and prognostic value (Tables 1 and 2). The clinical value of other markers remains to be established. Table 5 summarizes analytical limitations of the assays available for some of the most important established and experimental tumor markers. The implications of these limitations for tumor marker use in routine clinical practice are discussed in greater detail below.

CLINICAL APPLICATIONS OF SERUM TUMOR MARKERS IN TESTICULAR CANCER

Diagnosis. Patients with a testicular germ cell tumor may present with a painless testicular mass, while others also have symptoms caused by metastatic disease. The clinical workup comprises physical examination, ultrasound of the testis, and computerized tomographic scan of the pelvis, abdomen, and chest (62). Determination of hCG, AFP, and LDH in serum before therapy is mandatory in all patients. The marker concentration in serum is dependent on histological type and tumor load, i.e., stage. In a recent large collaborative study 64% of the tumors were NSGCT, and 36% were seminomas (63). Of the latter, 77% of patients presented with stage I disease, i.e., tumor localized to the testis, and 21% had increased serum levels of hCG. Of those with NSGCT, 52% had stage I disease and 79% had increased marker levels [both hCG and AFP increased in 44%, only AFP in 26%, and only hCG in 9%

(63)]. In seminoma patients, hCG concentrations are usually below 300 U/L. Levels >1000 U/L are mostly associated with NSGCT. Levels >10000 U/L are mainly seen in patients with pure choriocarcinoma but occasionally may occur in seminoma. LDH is increased in 40%–60% of patients with seminoma or NSGCT (64). The classification of a tumor is based on histological examination, but if serum AFP is increased, a tumor classified as a seminoma is reclassified as NSGCT and treated accordingly (4).

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 1:
TUMOR MARKERS IN THE DIAGNOSIS OF
TESTICULAR CANCER**

When testicular cancer is suspected, pretreatment determination of hCG, AFP, and LDH is mandatory (Table 2) [LOE, II; strength of recommendation (SOR), B].

STAGING, RISK STRATIFICATION, AND SELECTION OF THERAPY

Increased serum concentrations of AFP, hCG, and LDH are associated with adverse prognosis (65, 66). A high serum hCG concentration is a strong prognostic factor, and the risk of recurrence increases with increasing concentration (67). The International Germ Cell Cancer Collaborative Group has incorporated serum concentrations of hCG, AFP, and LDH in a scheme for classification of metastatic germ cell tumors (Table 4). Tumors are classified as having good, intermediate, or poor prognosis on the basis of marker levels, primary site of the tumor, and presence or absence of non-pulmonary visceral metastases (66).

The selection of treatment is based on tumor type and prognostic group. Stage I seminomas may be treated by orchiectomy alone, which leads to cure in 80%–85% of the cases. Orchiectomy in combination with radiotherapy of the abdominal lymph nodes leads to cure in 97%–99% of the cases, and this approach is routinely used in many centers. Without radiotherapy 15%–20% of the patients relapse, but most of these are cured by second line therapy. Therefore surveillance at increased frequency is an alternative to radiotherapy.

When treated by orchiectomy only, patients with stage I NSGCT have a 30% risk of relapse. The risk is higher (50%) if perivascular infiltration is present than if it is absent (risk 15%–20%). The relapse risk is very low if retroperitoneal lymph node dissection is performed in connection with primary therapy. This procedure is associated with morbidity and therefore surveillance is used as an alternative to retroperitoneal lymph node dissection. Chemotherapy is another alternative to retroperitoneal lymph node dissection but patients who undergo chemotherapy often have resid-

ual retroperitoneal tumors consisting of teratomas, which must be treated by surgery. If serum marker levels do not normalize or increase after retroperitoneal lymph node dissection, positive retroperitoneal lymph nodes or systemic disease requiring chemotherapy are most likely present (68, 69).

FURTHER RISK STRATIFICATION

Embryonal carcinoma is the most common cell type in NSGCT. Embryonal carcinoma is totipotent, and tumors with pure embryonal carcinoma are associated with early metastatic disease. Therefore, more accurate estimation of prognosis is needed for tumors containing this cell type. Cluster analysis of the serum markers AFP and hCG in combination with the tissue markers p53, Ki67, and apoptosis index suggest that a pattern with high Ki67, low apoptosis, and low p53 is associated with better survival than other patterns. Classification with this algorithm has been reported to be independent of the International Germ Cell Collaborative Group Classification (67). Confirmation of these results could provide a tool for more precise tailoring of therapy.

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 2:
TUMOR MARKERS IN THE STAGING OF TESTICULAR CANCER**
Measurement of hCG, AFP, and LDH is mandatory for staging and risk stratification according to the International Germ Cell Consensus Classification (Table 4) [LOE, I; SOR, A].

MONITORING OF RESPONSE TO THERAPY

If AFP or hCG in serum is increased before therapy, the rate of marker decline reflects the response to therapy. Persistent marker elevation after chemotherapy indicates residual disease and the need for further therapy (70, 71). Chemotherapy may induce a transient increase or surge in marker concentrations during the first week of treatment (72).

In the absence of residual disease after orchidectomy, the half-life of hCG is approximately 1.5 days and that of AFP 5 days (73, 74). During chemotherapy, half-lives >3.5 days for hCG or >7 days for AFP predict recurrence and adverse prognosis (75). Marker half-life is calculated from the slope of the logarithm of the marker concentration vs time. It is preferable to use marker concentrations from several time points and to calculate the half-life from the slope of the regression line (64). The half-life should be determined after the initial marker surge during 2 cycles of chemotherapy between days 7 and 56. A slow rate of marker decline is of potential use in poor-risk patients and may imply a need for more aggressive therapy (75).

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 3:
TUMOR MARKERS IN MONITORING RESPONSE TO
TREATMENT IN PATIENTS WITH TESTICULAR CANCER**

If raised before therapy, serum markers (AFP, hCG, and/or LDH) should be monitored weekly until concentrations are within the reference interval. Wherever possible, the marker half-life should be determined. Marker levels exceeding the upper reference limit after therapy suggest residual disease, which should be confirmed or excluded by other methods [LOE, II; SOR, A].

SURVEILLANCE

After successful primary therapy, all patients are monitored with physical examination, tumor marker determinations and computed tomographic scan. With such surveillance, relapse is in most cases detected before clinical symptoms appear. Most relapses occur within the first year and relapses after 2 years are rare but in some cases relapse may occur even after 10 years. The surveillance is tailored to take into account tumor type, stage, treatment, and likelihood of relapse (Table 3). Patients with low-risk disease treated with surgery alone are monitored most frequently, e.g., every 1–2 weeks during the first 6 months. Some centers recommend weekly monitoring to detect a relapse before the tumor grows to a size associated with adverse prognosis, as estimated by serum concentrations of AFP >500 kU/L and of hCG >1000 U/L (76). In all patients, monitoring is continued for 5 years (16).

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 4:
TUMOR MARKERS IN SURVEILLANCE OF PATIENTS WITH
TESTICULAR CANCER**

Serial monitoring with AFP, hCG, and LDH is recommended even when these are not raised before therapy, because marker expression can change during therapy. Frequency of measurement depends on the stage and pathology of disease but should be determined according to agreed protocols (e.g., as in Table 3). Because baseline levels are individual, increases are more important than absolute concentrations. A single increasing value must be confirmed with a second sample and the possibility of transient elevation due to nonspecific interference (e.g., iatrogenic hypogonadism) should be actively considered [LOE, II; SOR, A].

ANALYTICAL CONSIDERATIONS

Tumor marker measurements are mandatory in the management of testicular cancer patients. It is therefore appropriate to review analytical requirements for these important tests in more detail.

α-FETOPROTEIN

Biochemistry and biology. AFP is a homolog of albumin and is thought to act as a carrier protein in the fetus. During pregnancy, AFP is initially produced by the yolk sac and later by the fetal liver (77). Concentrations in fetal plasma reach levels of 3 g/L in the week 12–14 of pregnancy and decrease thereafter to 10–200 mg/L at term (78). After birth, circulating concentrations decrease, with a half-life of 5 days, falling to adult levels at 8–10 months of age (79, 80). The high values that are normal in early childhood must be remembered when using AFP as a marker for testicular yolk sac tumors, which are the most common testicular neoplasms in infants (81, 82).

Assay methods, standardization, and reference values. AFP is quantified by 2-site immunometric assays employing monoclonal antibodies or combinations of monoclonal and polyclonal antibodies. Results are generally comparable to those obtained with the competitive radioimmunoassay (RIA) format used previously. The WHO standard 72/225, in which 1 International Unit (U) of AFP corresponds to 1.21 ng, is used for calibration. Laboratories report values in mass units (ng/mL or μg/L) or kU/L. Reference values should be established for each assay to reflect differences in assay bias. Most centers quote an upper reference limit for AFP in the range of 10–15 μg/L. Circulating concentrations increase slightly with age; in one study the upper reference limit increased from 9.3 kU/L in patients younger than 40 years to 12.6 kU/L in those older than 40 years (83).

False-positive results. Rising levels of serum AFP indicate persistent germ cell tumor, even in the absence of radiographic evidence of disease, provided other possible causes can be excluded (see below) (4). Moderately increased AFP levels may persist even after chemotherapy, particularly when persistent disease has a large cystic component, serving as a reservoir leaking AFP into the circulation (84). Increased serum concentrations of AFP occur in most hepatocellular carcinomas and 10%–30% of other gastrointestinal cancers, but these diseases are rare in patients with testicular cancer. Increased AFP values may not reflect cancer, and it is therefore important to identify positive results caused by other diseases and by nonspecific interference. Benign liver disease, in particular hepatitis, and liver damage induced by chemotherapy are often associated with moderately increased serum AFP levels, and may result in misinterpretation especially if levels are rising (85, 86).

The carbohydrate compositions of AFP derived from the liver and the yolk sac are different (87). Lectin binding can differentiate increased levels caused by tes-

testicular cancer and liver disease (88), but such methods are not routinely used. Patients who initially have increased AFP levels may have normal levels during a relapse if therapy has eliminated AFP-producing elements but not all other components (89). Moderately increased values that remain stable do not usually indicate relapse (86).

NACB TESTICULAR CANCER PANEL RECOMMENDATION 5: ANALYTICAL REQUIREMENTS FOR MEASUREMENT OF AFP
 AFP methods should be calibrated against WHO Standard 72/225 and the units in which results are reported ($\mu\text{g/L}$ or kU/L) clearly stated. The detection limit for AFP assays should be $\leq 1 \mu\text{g/L}$ (i.e., $\leq 0.8 \text{kU/L}$). Reference values should be established to reflect method bias. AFP may be raised due to benign diseases, malignancies other than testicular cancer, or nonspecific interferences, and these possibilities must be considered when interpreting results [LOE, not applicable; SOR, A].

hCG AND hCG β

Biochemistry and biology. hCG is a member of the glycoprotein hormone family, which includes luteinizing hormone, follicle-stimulating hormone and thyroid stimulating hormone. All 4 contain a common α -subunit. The distinct β -subunits confer biological activity and display various degrees of homology, with that between the β -subunits of luteinizing hormone and hCG (hCG β) being about 80%. hCG β contains a 24-amino acid C-terminal extension not present in luteinizing hormone β , so antibodies to this part of the molecule are specific for hCG. Although the subunits lack hCG activity, hCG β has been shown to enhance the growth of tumor cells in culture by preventing apoptosis (90). hCG is expressed at very high concentrations by the placenta and trophoblastic tumors, including chorio-carcinoma of the testis. hCG is heavily glycosylated, hCG β containing 6 and hCG α 2 carbohydrate chains. The glycosylation of hCG secreted by tumors is often different from that of hCG found in pregnant women. An antibody, B152, detects only a hyperglycosylated variant of hCG. This form predominates in early pregnancy and is possibly more cancer-specific than “normal” hCG (91).

Nomenclature, assay methods, standardization, and reference values. Specific determination of hCG is based on antibodies reacting with hCG β (92). This practice has caused confusion in the nomenclature of hCG assays: the expressions “ β -hCG” or “hCG- β assay” may denote assays measuring both hCG and hCG β or only hCG β . According to the nomenclature recommended by the IFCC, hCG denotes the intact $\alpha\beta$ heterodimer,

hCG β the free β -subunit, and hCG α the free α -subunit (93). Assays should be defined according to what they measure, i.e., hCG and hCG β separately or hCG and hCG β together (64, 94).

Assays for hCG are currently calibrated against the Fourth International Standard (IS 75/589), in which concentrations are expressed in International Units (IU) based on bioactivity. It is difficult, however, to compare concentrations of hCG with those of hCG β and hCG α , which are expressed in different arbitrary units of the relevant International Standards (IS 75/551 and IRP 75/569, respectively). Recently established WHO Reference Reagents have values assigned in molar concentrations, which should facilitate direct comparison of hCG and hCG β concentrations in the future (93, 95).

Because seminomas may produce solely hCG β and not intact hCG, it is essential that both hCG and hCG β are measured when monitoring testicular cancer (14, 96) Recommendations about antibody combinations that recognize most important forms of hCG-related isoforms and are appropriate for use in oncology have been published (94). Assays recognizing both hCG and hCG β often use antibodies to epitopes on the C-terminal peptides of hCG β , but the relatively low affinities of these antibodies may limit assay sensitivity (94). Theoretically it should be possible to improve detection of testicular cancer by using separate assays for hCG and hCG β (64, 96) but this remains to be confirmed.

hCG is secreted at low levels by the pituitary, producing plasma levels that are measurable by sensitive methods. The serum concentrations may increase with patient age, particularly in women after menopause (97, 98). For most assays, the upper reference limit of hCG is stated to be 5–10 U/L. When determined by ultrasensitive methods, the upper limit in postmenopausal women is 5 U/L and in menstruating women is 3 U/L. The upper reference limit for men younger than 50 years is 0.7 U/L and for men older is 2.1 U/L (98). Cutoff values lower than the commonly used 5–10 U/L can be used to diagnose patients with testicular cancer. However, although most men with testicular cancer are young, their hCG levels may be increased due to testicular malfunction. Therefore diagnosis of active disease in a patient with a history of a germ cell tumor requires sequential determinations and rising values. The detection limit of most commercial assays does not allow reliable measurement of levels below 5 U/L and the utility of ultrasensitive assays and lower cutoff values needs to be determined (64). When expressed in molar concentrations, 5 U/L of hCG corresponds to 15 pmol/L. The upper reference limit for hCG β is 2 pmol/L and is independent of age and sex (98).

Specificity and confounding factors. It is important to note that chemotherapy often causes gonadal suppression that increases the hCG levels. Such hypogonadism can also be spontaneous. This can be confirmed by measurement of serum luteinizing hormone and follicle-stimulating hormone and, when necessary, suppression with testosterone replacement (99). Therefore, hCG levels increasing from below 2 up to 5–8 U/L during chemotherapy are often iatrogenic and do not necessarily indicate relapse. Moderately increased levels of hCG may be of pituitary origin, especially if accompanying serum levels of luteinizing hormone and follicle-stimulating hormone exceed 30–50 U/L, and are attributed to interrupted feedback inhibition from the gonads. This can be confirmed by short-term testosterone treatment, which suppresses pituitary secretion of hCG (100, 101).

Nontrophoblastic tumors may in extremely rare cases produce hCG, whereas hCG β is often expressed at moderate levels by a large variety of tumors, including ovarian, gastrointestinal, bladder, lung, and head and neck cancers (101). Some patients with such tumors will have increased hCG levels when measurement is carried out by an assay recognizing both hCG and hCG β .

Falsely increased results for serum hCG can be caused by heterophilic antibodies. This phenomenon has been reported only in women (102) but there is no reason why it should not also occur in men. False-positive results can be identified by analysis of hCG in urine or by repeating the assay after adding a blocking agent (e.g., nonimmune mouse IgG) to the sample to block the interference (64, 102).

Apparently false-negative results will be obtained with assays measuring only hCG if the tumor produces hCG β but not hCG. Although this situation is more common in seminoma patients (103), it may also occur in NSGCT patients (104).

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 6:
ANALYTICAL REQUIREMENTS FOR MEASUREMENT OF HCG**
It is essential that both hCG and hCG β be measured when using hCG to monitor testicular cancer patients, either using a method recognizing a broad spectrum of hCG-related isoforms or separate specific assays. hCG and hCG β should be recognized on an equimolar basis with a detection limit of ≤ 1 U/L. IFCC hCG nomenclature should be used to describe the method used. The possibility of interferences (e.g., from heterophilic antibodies) and transient increases (e.g., due to chemotherapy) must be considered when interpreting hCG results [LOE, not applicable; SOR, A].

LACTATE DEHYDROGENASE

Biochemistry and biology. LDH in the circulation exists as a tetramer that may contain various combinations of 2 subunits, LDH-A and LDH-B. The various subunits can combine in 5 isoenzymes, LDH-1 [consisting of 4 B subunits (B₄)], LDH-2 (B₃A₁), LDH-3 (B₂A₂), LDH-4 (B₁A₃), and LDH-5 (A₄). The gene encoding LDH-A is located on chromosome 11, whereas the gene for LDH-B is located on the short arm of chromosome 12 (i.e., 12p) (105). Interestingly, all invasive seminomas and NSGCTs show additional copies of this chromosomal arm (106), suggesting that it may play a role in disease progression. No gain of 12p is detected in ITGCNU (107, 108). A correlation between copy number of 12p, tumor invasiveness, and the serum level of LDH-1 has been reported, but thus far the relevant 12p-genes have not been identified (109). Although theoretically interesting, these findings need to be confirmed.

Specificity and confounding factors. Serum concentrations of LDH are measured enzymatically and the values are method-dependent. The degree of elevation is therefore most conveniently expressed relative to the upper reference limit. LDH-1 can be determined by zymography or by immunoprecipitation of the other isoenzymes and determination of residual catalytic activity. LDH is expressed in many tissues and increased levels may be caused by a wide variety of diseases. Despite its lack of specificity, LDH is a useful marker, especially for staging of seminoma and NSGCT (108). Hemolysis may cause falsely increased values and should be avoided.

**NACB TESTICULAR CANCER PANEL RECOMMENDATION 7:
ANALYTICAL REQUIREMENTS FOR MEASUREMENT OF LDH**
Because LDH is measured enzymatically and the values are method dependent, the degree of elevation should be expressed relative to the appropriate upper reference limit. Care must be taken to avoid hemolysis, which may cause falsely increased values [LOE, not applicable; SOR, A].

PLACENTAL ALKALINE PHOSPHATASE

Biochemistry and biology. A tumor-associated isoenzyme of alkaline phosphatase was first described in a patient with lung cancer and later detected in serum of patients with other cancers and identified as placental alkaline phosphatase (PLAP) (110). In fact, 2 genes encode the proteins detected as PLAP activity, i.e., the placental (PLAP) and germ cell enzymes. Both genes map to chromosome 2 and the proteins cannot be distinguished from each other using routine enzymatic or

Table 6. NACB recommendations for the clinical use of PSA serum markers in the management of prostate cancer.

Marker	Application	NACB recommendations (2008)	LOE ^a	SOR ^b	References
PSA	Screening	No	III	B	(136, 183, 521, 522)
	Early detection (with DRE)	Yes	III	B	(136, 183, 521, 522)
	Early detection, age-specific reference ranges	No	Expert opinion	B	(146)
	Staging/prognosis	Yes	III	B	(193, 201, 205, 206, 523–526)
	Surveillance/monitoring	Yes	III	B	(527, 528)
%fPSA	Differentiation of prostate cancer from benign prostatic disease when total PSA is 2–10 µg/L	Yes	III	B	(160, 529)

^a LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.

^b Strength of recommendation (520): A = High [Further research is very unlikely to change the Panel's confidence in the estimate of effect]; B = Moderate [Further research is likely to have an important impact on the Panel's confidence in the estimate of effect and is likely to change the estimate]; C = Low [Further research is very likely to have an important effect on the Panel's confidence in the estimate of effect and is likely to change the estimate]; D = Very low [Any estimate of effect is very uncertain.]

immunohistochemical methods (111). PLAP is increased most frequently in patients with seminoma (60%–70%) (112, 113) and less frequently in those with other germ cell tumors, including ITGCNU (24). An enzymatic method can be used to detect ITGCNU cells in frozen tissue sections (114).

Assay methods, standardization, and reference values. PLAP has usually been determined by zymography but it can be also be measured by immunoassay or enzymatically after immunocapture (113). The result should be compared with locally determined reference values. Because of homology with other alkaline phosphatase isoenzymes, antibody selection is critical. However, the antibodies available so far cannot distinguish between the PLAP and germ cell alkaline phosphatase isoenzymes. Therefore, PLAP denotes both of these isoenzymes.

Specificity and confounding factors. Serum concentrations of PLAP are increased up to 10-fold in smokers and its measurement is therefore of little value in this group (113). This fact and the paucity of commercial assays limit its clinical application, and serum assays for PLAP are not routinely included in the diagnostic workup of testicular cancer patients.

OTHER MARKERS

Although pregnancy-specific β -1 glycoprotein and hCG are both expressed in trophoblastic cells, hCG is the superior marker (115). Consequently, pregnancy-specific β -1 glycoprotein is not routinely measured. Neuron-specific enolase is increased in about 30%–50% of

patients with seminomas and less often in NSGCT patients (16, 116, 117), but in spite of these promising results the use of neuron-specific enolase is limited.

KEY POINTS: TUMOR MARKERS IN TESTICULAR CANCER

Tumor markers are of central importance in the diagnosis, staging, risk assessment, and monitoring of patients with testicular cancer. Several serum markers have been described but only AFP, hCG, and LDH have been thoroughly validated and shown to have independent prognostic value. Several tissue markers may prove to be clinically important in the diagnosis and classification of testicular germ cell tumors. Germ cell tumors also display typical chromosomal abnormalities and amplification of 12p is sufficiently characteristic to be useful in the clinic to identify extratesticular germ cell tumors. Developments in DNA-based diagnostics have revealed a number of changes that may in the future enable more accurate stratification of prognosis.

Tumor Markers in Prostate Cancer^{29,30}

BACKGROUND

Prostate cancer is the most common tumor in men in the US. In 2007, 218 890 new cases and 27 050 deaths

²⁹ NACB Prostate Cancer Sub-Committee members: Hans Lilja, Chair; Richard Babaian; Barry Dowell; George Klee; Harry Rittenhouse; Axel Semjonow; Paul Sibley; Lori Sokoll; and Carsten Stephen.

³⁰ All comments received about the NACB Recommendations for Prostate Cancer are included in the online Data Supplement. Prasad Bollina, Professor Fritz Schröder, and Professor Hein von Poppel were invited expert reviewers.

Table 7. Recommendations by different expert groups for use of PSA and %fPSA as tumor markers for prostate cancer.

Marker	Application	ACS ^a (138)	ACP (530)	ASTRO (527)	AUA (528)	EAU (537)	EGTM (148)	ESMO (532)	NACB/EGTM 2002 (15)	NCCN (533)	USPSTF (534)	NICE 2008 (121, 139)	NACB 2008 ^b
PSA	Screening (with DRE)	Yes	No ^c	None published	Yes	Yes	No ^c	No ^d	Yes (NACB)	Yes	Insufficient evidence available for men <75 years of age. Screening for men 75 years or older not recommended (535)	Insufficient evidence available	No at present
	Early detection: Age-specific reference ranges	None published	None published	None published	None published	None published	No	None published	Yes (NACB)	None published	None published	None published	No
	Early detection: PSA velocity	None published	None published	None published	None published	None published	None published	None published	None published	Yes	None published	Yes	Yes
	Staging/Prognosis	None published	None published	None published	Yes	Yes ^e	None published	Yes	None published	Yes ^e	None published	Yes	Yes ^e
	Follow-up negative biopsy (with DRE)	None published	None published	None published	None published	None published	None published	None published	None published	Yes	None published	Yes	Yes
	Surveillance/monitoring	None published	None published	Yes ^f	Yes	Yes	Yes	Yes	None published	Yes	None published	Yes	Yes
%fPSA ^g	Differentiation of Pca and benign prostatic disease when total PSA is between 2–10 µg/L	None published	None published	None published	None published	None published	Yes	None published	Yes	Yes	None published	None published	Yes
	Follow-up negative biopsy (with DRE) or patients with increased biopsy risk	None published	None published	None published	None published	None published	None published	None published	None published	Yes	None published	None published	Yes

^a ACS, American Cancer Society; ACP, American College of Physicians; ASTRO, American Society for Therapeutic Radiology and Oncology; AUA, American Urological Association; ESMO, European Society for Medical Oncology; Pca, Prostate cancer; USPSTF, US Preventive Services Task Force.

^b For SORs see Table 6.

^c Not routinely, individual decision.

^d Except in men with urinary symptoms.

^e As part of nomograms with DRE and biopsy Gleason grade (Partin tables).

^f Following radiation therapy.

^g In men with a total PSA of 4–10 µg/L and a negative DRE.

were predicted. Although prostate cancer is unequivocally lethal in some patients, most men die with rather than of their cancer (118). Autopsy data suggest that 42% of men older than 50 years old have cancerous foci in their prostates but prostate cancer will be diagnosed in only approximately 16% of men during their lifetime and only a quarter of these will die from it. Many more men die with than of prostate cancer (119). Current incidence rates of clinical disease are 15-fold higher in the US than in Japan despite similar frequencies of histological cancer. Hence, the far greater prevalence of histological than symptomatic cancer has been cited to support a conservative, noninterventionist approach to this disease. However, once prostate cancer reaches advanced stages either locally or systemically with bone metastases, or becomes refractory to hormone therapy, little if any therapeutic means for cure are available.

The optimal management of patients with prostate cancer requires the use of the tumor marker prostate-specific antigen (PSA) in all instances and disease states. The use of PSA-related isoforms is appropriate in certain specific circumstances. Here we present new National Academy of Clinical Biochemistry guidelines on the use of these and other serum-based tumor markers in prostate cancer. A summary of relevant guidelines published by other expert panels on this topic is also provided.

To prepare these guidelines, the literature relevant to the use of tumor markers in prostate cancer was reviewed. Particular attention was given to reviews (including systematic reviews), prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence based.

CURRENTLY AVAILABLE MARKERS FOR PROSTATE CANCER

Commercially available PSA markers cleared by the FDA for use in the management of patients with prostate cancer are listed in Table 6, together with the phase of development for each marker as well as the LOE for their clinical use (120).

TUMOR MARKERS IN PROSTATE CANCER: NACB RECOMMENDATIONS

Table 7 summarizes the NACB guidelines for the use of PSA markers in prostate cancer together with recommendations from other representative guidelines published on the use of tumor markers in prostate cancer, including recently published recommendations issued by the United Kingdom National Institute for Health and Clinical Excellence (NICE), which has undertaken a systematic review of best available evidence (121). Although other markers have been investigated (Table

8), based on currently available evidence only the use of PSA and its isoforms can be recommended in prostate cancer. Below we present a more detailed discussion of the use of these measurements.

PSA Markers in Patient Management

PSA MARKERS IN THE SCREENING AND EARLY DETECTION OF PROSTATE CANCER

The widespread measurement of serum PSA is largely responsible for the increased incidence of prostate cancer in the US during the past 2 decades. As demonstrated by epidemiological data showing both a marked increase in the number of men diagnosed with prostate cancer and a profound migration toward earlier stage disease at the time of diagnosis (122), there is strong evidence supporting the growing concern that such “stage migration” causes overdiagnosis and overtreatment of men with indolent cancer, a condition that may pose little threat to the life or health of the patient (123). The usefulness of PSA screening has also been questioned owing to poor specificity when serum concentrations are modestly increased (124). Although extensive evidence shows that elevations of PSA in serum are exclusively associated with disease conditions in the prostate, such findings are not cancer specific, occurring also in other conditions such as benign prostatic hyperplasia and prostatitis. This well-documented lack of specificity of the conventional PSA test even prompted researchers to question whether any association exists between serum PSA levels and prostate cancer (125). In contrast, reports from many other investigators have shown that there is very strong evidence of a very significant association between serum PSA levels and presence or outcome of prostate cancer (126–130). Also, the lack in specificity of the PSA test is less critical in monitoring patients with a prostate cancer diagnosis, for whom PSA is the most important marker in evaluating response to therapeutic interventions and in detecting tumor relapse. Although potentially valuable as part of multivariate panels to identify aggressive cancers and/or cancer recurrence, measurement of prostatic acid phosphatase alone does not provide any clinically useful information additional to PSA measurement (131, 132), and therefore is not recommended by the NACB.

NACB PROSTATE CANCER PANEL RECOMMENDATION 1: CHOICE OF TUMOR MARKER FOR MANAGEMENT OF PATIENTS WITH PROSTATE CANCER

PSA is currently the most useful serum tumor marker in management of prostate cancer patients and is required in all stages of the disease [LOE, III; SOR, A].

Table 8. Biomarkers currently being explored for prostate cancer.^a

	Proposed use or uses and comments	Phase of development	LOE	References
Circulating biomarkers				
PSA subfractions: complexed PSA, proPSA, intact PSA, benign PSA	Absolute concentrations in serum and percentage relative to total PSA may help discriminate between malignancy and benign conditions.	Undergoing evaluation (clinical assays in development)	IV, V	(536–538)
Human kallikrein 2 (hK2)	Shares 80% amino acid sequence with PSA and is produced in prostatic epithelium at concentrations 50–100 times less than PSA. Generally elevated in prostate cancer vs BPH, ^b and is more sensitive than PSA at detecting extracapsular extension.	Undergoing evaluation	IV, V	(538, 539)
Insulin-like growth factor (IGF-1), IGF binding protein (IGFBP-3)	High serum IGF-1 concentrations associated with increased risk for prostate cancer. IGFBP-3 can be detected in tissue with ProstaScint; serum concentrations elevated in prostate cancer; discriminates between cancer and BPH or no disease; also being investigated as a therapeutic target.	Undergoing evaluation	IV, V	(540, 541)
Molecular urine/tissue markers				
PCA3	Prostate cancer antigen 3 (non-protein coding) (PCA3) (prostate-specific gene) highly expressed in prostate cancer compared to other genitourinary tissues and nonneoplastic prostatic tissues. Urine assays measure PCA3 mRNA following an attentive DRE; the mRNA is noncoding, no protein products are made.	Undergoing evaluation	IV, V	(542, 543)
Alpha-methylacyl-CoA racemase (AMACR)	Mitochondrial and peroxisomal enzyme involved in oxidation; overexpressed in prostate cancer; detected in tissue by IHC, and in conjunction with loss of basal cell markers (e.g. basal cytokeratins, p63) can help establish diagnosis of cancer on prostate needle biopsy. Assays to detect a humoral response may supplement PSA screening in identifying significant tumors.	Undergoing evaluation (urine and tissue)	IV, V	(544–548)
Glutathione S-transferase-pi (GSTPi)	Protects cells from oxidative damage; reduced expression in prostate cancer due to hypermethylation of its promoter region; distinguishes between BPH and cancer; methylation status of GSTPi gene promoter quantified in prostatic tissue, cells derived from serum, urine and seminal plasma by PCR.	Undergoing evaluation in a clinical trial	IV, V	(549, 550)
Methylation panel	Hypermethylation of a panel of markers in combination with histology may aid in prostate cancer diagnosis; aberrant methylation profiles in prostate tissue samples correlated with clinicopathological features of poor prognosis.	Undergoing evaluation	IV, V	(551, 552)
Telomerase activity	Telomerase activity is detectable in the vast majority of prostate cancers but not in benign prostate tissues. Improved methods of telomerase detection may make this marker useful for early detection of prostate cancer in tissue samples or in urine.	Undergoing evaluation	IV, V	(553, 554)

Continued on page e27

Table 8. Biomarkers currently being explored for prostate cancer. (Continued from page e26)^a

	Proposed use or uses and comments	Phase of development	LOE	References
Cell/gene tests				
Circulating prostate cells RT-PCR gene targets PSA, hK2, and PSMA mRNAs	Measurements of the frequency in the shedding of circulating prostate/tumor cells in blood using RT-PCR assays for PSA-, hK2-, and/or PSMA-mRNAs as a means to define invasive and/or systemic disease stage.	Undergoing evaluation in a clinical trial	IV, V	(536, 555)
PTEN	A lipid phosphatase that functions as a tumor suppressor by inhibiting the phosphatidylinositol 3-kinase/protein kinase B (P13K/Akt) signaling pathway. Gene somatically deleted or mutated in some prostate cancers. Protein can be detected by IHC and decreased levels are associated with higher grade and stage.	Undergoing evaluation	IV, V	(556, 557)
CDKN1B (P27)	Cyclin-dependent kinase inhibitor. Protein decreased in prostate tumor cells and levels correlated with worse outcome.	Undergoing evaluation	IV, V	(558, 559)
Ki-67	Marker of cellular proliferation. Fractions of cells staining positive by IHC associated with worse outcome.	Undergoing evaluation	IV, V	(560)
Chromosome 8p22 loss and 8q24 (C-MYC) gain	Bq24 overrepresentation, especially in combination with loss of 8q22 using a FISH assay, is associated with prostate cancer progression in men with stage pT2N0M0, pT3N0M0 and pT2N1–3M0 prostate cancers.	Undergoing evaluation	IV, V	(561)
Prostate stem-cell antigen (PSCA)	Cell surface protein found primarily in the prostate; increased expression in many higher-grade prostate cancers and most metastatic lesions; correlated with late-stage disease; detection in prostatic tissue via FISH, PCR, IHC.	Undergoing evaluation	IV, V	(562)

^a Based on Table 3 of the Prostate Cancer Foundation *Report to the Nation on Prostate Cancer* (563).
^b BPH, benign prostatic hyperplasia; LOE as defined in Table 6; PSMA, prostate-specific membrane antigen; RT, reverse transcription; ASR, analyte-specific reagent.

Population-based median levels are $<0.6 \mu\text{g/L}$ for men ≤ 50 years, the vast majority of whom have yet to develop any signs or symptoms of prostate cancer or benign enlargement of the gland (130, 133, 134). The 80th centile is close to $1 \mu\text{g/L}$, and the 90th centile is about $1.25 \mu\text{g/L}$ (130). An upper limit of normal according to the 95th percentile for men ≤ 50 years has never been implemented in clinical practice, but would correspond to a PSA level of about $1.5 \mu\text{g/L}$. A modest increase in PSA levels in older men reflects a higher frequency of benign prostate conditions at higher age. Population-based demographics of PSA levels for men 50–70 years old show that 8%–9% of these men have PSA levels $\geq 4.0 \mu\text{g/L}$, while 11%–12% have PSA levels $\geq 3.0 \mu\text{g/L}$, and as many as 20% of all men have serum PSA levels $\geq 2.0 \mu\text{g/L}$ (135).

In men who present with modestly increased levels of PSA in serum (i.e., $4\text{--}10 \mu\text{g/L}$), there is extensive evidence showing that histopathologic examination of

tissue harvested by systematic prostate biopsies confirms presence of prostate cancer in 25%–35% of these men (136, 137). When serum PSA levels rise above $10 \mu\text{g/L}$, the cancer specificity of the test is 40%–50% or higher. Current recommendations in the US suggest that most men older than 50 years should have annual prostate cancer screening with PSA and digital rectal examination (DRE), and that men should be advised to have biopsies when the DRE is abnormal or when the PSA level in serum is $\geq 4.0 \mu\text{g/L}$ (138). The NICE guidelines conclude that the serum PSA level alone is a poor predictor of the presence of prostate cancer and should not automatically lead to a prostate biopsy, particularly because many cancers diagnosed on this basis alone will be of low risk, causing little or no impact on life expectancy (121, 139).

These recommendations all have some limitations, as has recently been discussed (140). The PSA cutoff of $\geq 4.0 \mu\text{g/L}$ represents a clinical decision limit that was introduced on the basis of a single report eval-

uating the optimal combination of sensitivity and specificity of the PSA test in a study cohort, and the distribution of values observed in this original study may no longer apply (141). It is debatable whether a PSA cut-point lower than 4 $\mu\text{g/L}$ should be recommended. Also debatable is whether decisions to recommend prostate biopsy should be based solely on a single PSA cut-point value (e.g., $\geq 4 \mu\text{g/L}$). Lower PSA cutoffs increase the cancer detection rate at the expense of increasing the number of men advised to undergo biopsy. It has also been clearly demonstrated, however, that 20% or more of all men who have PSA levels from 2.0 (or 3.0) up to 4.0 $\mu\text{g/L}$ are found to have prostate cancer at biopsy (142, 143). This finding was confirmed in a recent study, in which prostate cancer was diagnosed by biopsy in as many as 15.2% of all 2950 biopsied men with PSA values $< 4.0 \mu\text{g/L}$ (128). This study showed that the prevalence of prostate cancer in men 62–91 years old increased from 6.6% in men with PSA of 0–0.5 $\mu\text{g/L}$, 10% with PSA 0.6–1.0 $\mu\text{g/L}$, 17% with PSA 1–2 $\mu\text{g/L}$, up to 23.9% with PSA 2.1–3.0 $\mu\text{g/L}$, and 26.9% with PSA 3.1–4.0 $\mu\text{g/L}$ (128). Also, the prevalence of high-grade prostate cancer increased with increasing PSA values. Hence, the positive predictive value of the PSA test in terms of biopsy-proven (histological) prostate cancer is similar for men with a PSA value between 2–4 $\mu\text{g/L}$ and those with a PSA value between 4–10 $\mu\text{g/L}$ (136, 144).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 2:
CLINICAL DECISION LIMITS**

Given the controversy regarding the use of PSA to detect very small tumors, reported benefits arising from lowering the clinical decision limit for biopsy below 4 $\mu\text{g/L}$ are too uncertain to mandate any general recommendation. Cut-points lower than the commonly used 4 $\mu\text{g/L}$ limit will increase sensitivity with a concomitant decrease in specificity unless other adjunctive tests or measures are employed to increase specificity. Conversely, use of clinical decision limits for PSA higher than 4.0 $\mu\text{g/L}$ decreases the sensitivity, which results in the missed diagnoses of clinically significant tumors in men who might potentially benefit from early treatment [LOE, not applicable; SOR, B].

The across-the-board recommendation of annual PSA testing for men older than 50 years (138) is overly simplistic and fails to alter testing frequency based on the individualized risk imparted by previously determined PSA levels. For example, a 55-year-old man with a baseline PSA of 0.4 $\mu\text{g/L}$ is much less likely to develop prostate cancer in the future than a similarly aged man

with a baseline PSA of 3.3 $\mu\text{g/L}$. Stenman et al. (126) used frozen serum samples and information from a health examination survey in Finland, and Gann et al. (145) used information from the Physicians' Health Study to examine the usefulness of PSA for identifying men in whom prostate cancer subsequently was or was not clinically diagnosed. The data of Gann et al. suggest that men with PSA levels between 2.0 and 3.0 $\mu\text{g/L}$ have 5.5-fold higher relative risk for diagnosis of prostate cancer than men with PSA levels $< 1.0 \mu\text{g/L}$. In the former group, serum PSA levels reached 2–3 $\mu\text{g/L}$ on average more than 5 years before the cancer was detected by DRE. Recently, Lilja et al. (130) demonstrated a very strong association between PSA levels in blood collected more than 20 years before prostate cancer diagnosis and the likelihood of that diagnosis in a large representative population of Swedish men age 44–50 years who had not previously undergone PSA testing. These data and those reported from others (129) suggest that risk stratification during early middle age may be important to consider in refining current imperfect early cancer detection strategies. Several additional issues particularly relevant to screening programs are discussed below.

AGE-SPECIFIC REFERENCE INTERVALS FOR PSA

Because serum PSA levels gradually increase with age in men older than 40 years, age-specific reference intervals have been proposed with the expectation that their implementation would increase cancer detection rates in younger men by lowering the cut-point and would increase specificity in older men by raising the cut-point (146). Although there is no consensus, many experts—including a majority of opinion of the National Comprehensive Cancer Network—favor the use of clinical decision limits lower than 4.0 $\mu\text{g/L}$ for serum PSA in younger men. The NACB, however, is not yet convinced of the net benefit of this protocol in the absence of additional test(s) that could significantly increase diagnostic specificity (i.e., reduce unnecessary biopsies). At the same time the NACB advises caution in increasing the decision limit above 4.0 $\mu\text{g/L}$, because this could result in failure to diagnose clinically significant tumors in men who might potentially benefit from early treatment (147). Hence, contrary to previously issued recommendations (148), the NACB does not endorse the use of age-specific reference ranges.

**NACB PROSTATE CANCER PANEL RECOMMENDATION 3:
AGE-SPECIFIC REFERENCE INTERVALS FOR PSA**
Age-specific reference intervals should not be used for PSA [LOE, expert opinion; SOR, B].

INCREASING PSA SPECIFICITY IN SCREENING FOR PROSTATE CANCER

The total PSA in circulation roughly corresponds to the sum of circulating free PSA (fPSA) and PSA bound as a stable complex to α -1-antichymotrypsin. The free fraction constitutes from 5% up to more than 40% of the total (149). Free and bound forms may be selectively detected by commercially available assays without any significant interfering cross-reaction (150). Several composite measures have been proposed to improve the specificity of a single serum total PSA concentration for the early detection of prostate cancer. PSA density (151–153), PSA velocity (154), PSA doubling time (155, 156), and percentage of free PSA (%fPSA) (157–161) have all been evaluated in this context, but only %fPSA has been widely validated and implemented in clinical practice. Men with benign disease generally present with higher %fPSA than men with prostate cancer (and no benign enlargement). Unfortunately, concurrent benign prostatic enlargement and prostate cancer complicates interpretation of %fPSA data (162). Nevertheless, in a systematic review carried out in 2005, the use of %fPSA was suggested as a means of decreasing the number of unnecessary biopsies, particularly for men with PSA levels of 4–10 $\mu\text{g/L}$ (163). In accord with the conclusions of a recent meta-analysis (164), the current NACB Panel and the European Group on Tumor Markers (EGTM) (148) both recommend the use of %fPSA as an aid in distinguishing men with prostate cancer from men with benign disease in selected high-risk groups, e.g., when total PSA is $<10 \mu\text{g/L}$ and DRE is negative. In particular, %fPSA may be useful in identifying men who have prostate cancer despite initial negative biopsy findings. In men with low %fPSA suspected to indicate a high risk of harboring malignant disease, a cancer diagnosis may become evident after a repeat biopsy. This recommendation is tempered by the need to validate the medical decision limit for each fPSA and total PSA commercial assay combination (165).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 4:
USE OF %fPSA IN DIAGNOSIS**

The use of %fPSA is recommended as an aid in distinguishing men with prostate cancer from men with benign prostatic hypertrophy when the total PSA level in serum is within the range of 4–10 $\mu\text{g/L}$ and DRE is negative, most frequently in men undergoing repeat biopsy, in selected high-risk groups and particularly in identifying men who have prostate cancer despite initial negative biopsy findings. The clinical decision limit must be properly validated for each combination of free and total PSA assays [LOE, I; SOR, A].

More than 95% of the immunodetectable complexed PSA (cPSA) fraction is bound to α -1-antichymotrypsin with $<5\%$ bound to other complex ligands, e.g., α -1-protease inhibitor (157, 166–168). PSA bound to α -2-macroglobulin is not detected by current immunoassays for PSA. Levels of cPSA in blood can be determined either directly using assays for PSA bound as a stable complex to α -1-antichymotrypsin (157, 158, 169), which first block access to fPSA and then measure levels of cPSA (170), or indirectly by subtracting fPSA from total PSA levels (171) using 2 assays designed to work together and standardized appropriately. Measurement of cPSA alone provides comparable cancer detection to total PSA but appears to give somewhat better specificity in a narrow concentration range (172). However, cPSA levels alone cannot achieve specificity similar to that of %fPSA (170).

GUIDELINES FOR THE EARLY DETECTION OF PROSTATE CANCER

The American Cancer Society has issued guidelines related to the early detection of prostate cancer. These guidelines recommend an annual screening with DRE and serum PSA measurement beginning at the age of 50 in men at average risk with at least 10 years of life expectancy (138). Although PSA is considered the best biochemical test currently available to detect prostate cancer, a DRE should also be included whenever possible, according to the American Cancer Society. Screening at earlier age (45 years or even 40 years) is warranted in men at increased risk, including those of African-American descent and those with one or more first-degree relatives with prostate cancer. Both of these groups often develop prostate cancer several years earlier than the general population and also tend to present with a more aggressive type of cancer (173).

The recommended follow-up testing of high-risk individuals initially screened at 40 years of age depends on the PSA result. Those with PSA levels $<1 \mu\text{g/L}$ would resume testing at 45 years of age, those with levels >1 but $<2.5 \mu\text{g/L}$ would be tested annually, and those with levels $\geq 2.5 \mu\text{g/L}$ would be evaluated further and considered for biopsy (138).

These guidelines do not endorse a general recommendation for mass screening, but support the notion that individual men should be informed of the benefits and limitations of prostate cancer screening before making their decision, as for example is recommended in the United Kingdom through the Prostate Cancer Risk Management Program (174) and by NICE (121, 139, 174). Much greater emphasis than previously is being placed on informed decision-making by the individual. This topic has recently been the subject of a systematic review in which PSA decision aids and evaluations were identified and appraised (175). The authors concluded that PSA decision aids improve

knowledge about PSA testing, at least in the short term. There are many issues to consider, including the disparity between incidence and mortality associated with prostate cancer, because many more men are diagnosed with prostate cancer than eventually die from it. However, early detection affords the opportunity to detect organ-confined disease when curative treatment is possible. Metastatic disease now constitutes only about 5% of initial diagnoses in the US, a dramatic fall from the 50% incidence rate of the pre-PSA era (122). Nevertheless there are still many uncertainties concerning treatment of early stage disease, including the preferred treatment for clinically localized prostate cancer.

MERITS OF EARLY DETECTION OF PROSTATE CANCER

Because of the uncertainties regarding prostate cancer treatment, considerable debate is ongoing regarding the merits of early detection of prostate cancer, and not all physician organizations advocate routine screening (176). Although the American Urological Association endorses the American Cancer Society policy statement on the early detection of prostate cancer, recommendations of other organizations differ regarding the benefit of prostate cancer screening (177, 178). Arguments against screening are based on the fact that there is no conclusive evidence from any randomized trials that early detection and treatment influence overall mortality, whereas the standard treatments for organ-confined prostate cancer are associated with a significant frequency of side effects. Currently, the US Preventive Task Force, the American Academy of Family Physicians, the American College of Physicians, the National Cancer Institute, and the EGTM do not recommend population-based prostate cancer screening (177, 178). The overriding concern is that current screening modalities result in overdiagnosis and overtreatment of early stage disease that may not be clinically significant, as has recently been reviewed (179).

The NACB and the EGTM recommend that widespread implementation of screening for prostate cancer in the general population should await the final outcome of ongoing prospective randomized studies, in particular the European Randomized Screening for Prostate Cancer (ERSPC) trial (180), which are sufficiently powered to establish whether early detection and treatment decreases prostate cancer mortality. The ERSPC trial has been underway for 10 years, with results expected in 2010 (181). Long-term multicenter trials to determine the impact of prostate cancer screening on survival are also ongoing in the US under the aegis of the National Cancer Institute and the US Public Health Service (182).

With no clear-cut evidence as yet that prostate cancer screening is of net benefit, proponents of

screening have pointed to the association of PSA testing with earlier cancer stage at detection and reduced mortality arising from prostate cancer. Registry data from heavily and sparsely screened male populations in Austria provide a case in point. The expected death rate from prostate cancer (183) declined much more in the Tyrol, a heavily screened section of the country, than in less intensely screened areas (184). The decrease in observed mortality was associated with a shift toward a more favorable stage at diagnosis, in particular an increase in the proportion of organ-confined disease. The inference is that early detection and availability of effective treatment resulted in a corresponding improvement in disease-specific survival. A similar trend has been observed in data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program, from a study conducted in Olmsted County, Minnesota (185), and from a comparison of prostate cancer mortality in the US and the United Kingdom between 1975 and 2004 (186).

Although recent data suggest that the apparent stage shift to early stage disease and subsequent treatment of localized prostate cancer detected with PSA have positively influenced mortality rates, it is still an open question whether early detection and therapeutic intervention alters the natural history of the disease, as observed benefits may be the result of selection or lead-time bias(es) (187). The stage at diagnosis may be more dependent on the biological behavior of the tumor (aggressiveness) than on delay in presentation, and early detection may not have a significant impact on mortality. An increase in the proportion of localized prostate cancers that are being treated may account for some of the change in the mortality statistics (181).

Currently there is insufficient evidence either to support or refute the routine use of mass, selective or opportunistic PSA-based screening, and it is equally unclear whether to advise against the use of PSA-based screening, for which success in reducing prostate cancer mortality has yet to be demonstrated. Currently, no robust evidence from randomized controlled trials is available regarding the impact of screening on quality of life, the disadvantages of screening, or its economic value. Results from 2 ongoing large-scale multicenter randomized controlled trials that will be available in the next several years are required for evidence-based decision-making regarding prostate cancer screening (188).

NACB PROSTATE CANCER PANEL RECOMMENDATION 5: PROSTATE CANCER SCREENING

A decision as to whether widespread implementation of PSA screening for prostate cancer in the general population can be recommended must await the outcome of ongoing prospective randomized

screening studies (e.g., the European Randomized Screening for Prostate Cancer trial in Europe) which are due to be completed by 2010 [LOE, III; SOR, A].

PSA IN PATIENT MANAGEMENT

The optimal treatment of early stage prostate cancer has yet to be established. Treatment options include expectant management (active surveillance or watchful waiting), radical prostatectomy, or radiation therapy (external beam radiation or brachytherapy) (139). Alternative treatment modalities (e.g., cryosurgery or high-intensity focused ultrasound) await evaluation of their long-term results. Patients with advanced (metastatic) disease are typically offered hormonal therapy to deprive the prostate of androgen stimulation. PSA synthesis by differentiated prostate cells is greatly impaired by such treatment and the PSA levels in blood reflect tumor burden differently from before androgen deprivation. When the disease becomes refractory to either first- or second-line androgen deprivation, patients may be entered into chemotherapy or experimental protocols with various agents (e.g., Taxotere). The assessment of PSA levels in the blood plays a cardinal role in all aspects of the management of prostate cancer from surveillance to selection of optimal treatment to estimation of prognosis to posttherapeutic monitoring. fPSA measurement has not been shown to offer any advantages over total PSA during the follow-up of prostate cancer (189).

The treatment selected after detection of prostate cancer depends critically on whether the disease is confined to the prostate. Radical prostatectomy is primarily an option for patients with organ-confined disease, although patients with extracapsular disease may also benefit from radical surgery (190). However, the extent of disease is difficult to predict accurately. PSA alone is not informative (191), but in combination with the clinical stage and Gleason score predicts reasonably well the pathological stage of localized prostate cancer. Predictive tables that incorporate these parameters have been published (192–194) and are used by physicians to estimate the probability of organ-confined disease and to determine whether radical prostatectomy is indicated. It is recommended by NICE that urological multidisciplinary teams should assign a risk category to all men with newly diagnosed localized prostate cancer, taking these parameters into account (121, 139).

Assessment of changes of PSA levels with time (PSA velocity or PSA doubling time) was first introduced in 1992 (154), with a rapid increase indicating a higher risk for subsequent development of prostate cancer. Results of several studies further suggested that a more rapid rise in PSA before treatment is correlated

with aggressive disease and early recurrence after treatment. In more recent studies reported by D'Amico et al. (195, 196) a PSA velocity of more than $2.0 \mu\text{g/L/year}$ measured during the year before diagnosis was shown to be significantly associated with prostate cancer-specific mortality. Recently, Carter et al. reported evidence that total PSA velocity could also be used to predict life-threatening prostate cancer up to 15 years before diagnosis (197). However, to demonstrate that PSA velocity has important clinical value, it must also be unequivocally shown that a multivariable model that incorporates both PSA and PSA velocity (e.g., addition of PSA velocity to a model that includes total PSA, age, and date of diagnosis) is superior to the model that uses PSA alone. This LOE appears still to be lacking, even in the most recently reported studies on this subject.

Following successful surgery, PSA should decrease to undetectable levels (198, 199). Persistently increased PSA provides evidence of residual disease. However, the converse does not always hold, namely that undetectable PSA postoperatively indicates a surgical cure. Considerable time may elapse before residual disease becomes evident through detectable PSA. Most commonly, residual disease will become evident within 3 years of surgery. Up to 20%–30% of the men who undergo radical prostatectomy present with residual disease during the first 10 years after surgery.

A rising PSA level after radical prostatectomy is a biochemical sign of recurrent disease that typically predates other signs of progression by many years. However, not all patients with biochemical recurrence will progress to symptoms of clinical disease and metastatic spread in their lifetimes and require treatment (200, 201). Factors reported to predict the time course to the development of metastatic disease include time to biochemical recurrence, tumor grade (Gleason score), and PSA doubling time (156, 161). These parameters can be used to estimate the likelihood of patients remaining free of overt metastatic disease and allow physicians to stratify patients into low-risk and high-risk categories and to make better treatment decisions.

Monitoring response after initial treatment and evaluating outcome during subsequent therapy are significant clinical applications of PSA determinations. Measurement of PSA provides essential information about the efficacy of surgery or radiation therapy, helps establish the possibility of residual disease (local or distant), signals recurrent metastatic disease before it can be detected by other conventional diagnostic procedures, and provides a useful adjunct in the evaluation of therapeutic response.

PSA may provide the earliest measure of treatment efficacy or disease recurrence, and as such influence the patient's perception of well-being. For some patients, it may be most appropriate to stop measuring PSA, particularly if effective alternative treatments to counter adverse findings are not available (148).

PSA MARKERS IN THE POSTTREATMENT MONITORING OF PROSTATE CANCER

Following treatment, it is the Panel's view that a single PSA measurement at or near the lower detection limit of the assay is not sufficient to diagnose recurrence of prostate cancer. Rising PSA levels demonstrated by repeat or serial measurements provide much more reliable evidence (121, 139, 202). Following radical prostatectomy, circulating PSA declines to undetectable levels if the prostate cancer was organ-confined and all residual prostate tissue surgically excised. Sustained detection of PSA suggests either incomplete resection or metastatic deposits. If ultrasensitive PSA assays are used in this setting, the functional detection limit of the assay should be established and should correspond to the lower reporting limit.

At present, evidence is equivocal regarding the clinical benefit of reporting biochemical recurrence of prostate cancer at PSA levels below 0.4 $\mu\text{g/L}$ (200). Recently, however, salvage radiation therapy following prostatectomy has been shown to yield best results when PSA levels are still very low ($\leq 0.5 \mu\text{g/L}$) (203). The recurrence limit is less clear following radiation therapy because of the typically slower decline in circulating PSA concentration. The American Society for Therapeutic Radiation and Oncology has defined biochemical recurrence as a rise of 2 $\mu\text{g/L}$ or more above the nadir PSA, after external beam radiotherapy with or without hormonal therapy (204).

Monitoring with PSA after treatment for prostate cancer is a mainstay of clinical practice, although the clinical utility of PSA is variable and depends on the disease stage of the individual patient. As has recently been observed, the lack of high quality information and paucity of clinical trials hampers development of guideline recommendations for prostate cancer, but implementation of available guidelines are likely to improve prostate cancer outcomes while reducing unnecessary, ineffective, and costly care (140). PSA has high sensitivity for detecting recurrence after radical prostatectomy but is less sensitive in detecting recurrence following radiation therapy. For monitoring hormonal treatment, PSA provides a sensitive tool with which to verify treatment response and detect tumor growth (recurrence). However, in patients with advanced disease who suffer recurrence during androgen depriva-

tion therapy, PSA has only limited usefulness for predicting survival outcome.

NACB PROSTATE CANCER PANEL RECOMMENDATION 6: USE OF PSA IN THE POSTTREATMENT MONITORING OF PROSTATE CANCER

PSA is recommended for management of patients with prostate cancer to monitor disease status following treatment [LOE, III; SOR, A].

USE OF NOMOGRAMS INCORPORATING PSA TO MANAGE PROSTATE CANCER

Nomograms incorporating one or more factors provide the most accurate means of individualizing therapy and predicting outcome, and reflect the most recent advances in patient management (205). Rather than relying on physician experience or general risk assessments of patient populations with similar characteristics, the nomograms assess treatment options or prognosis based on computerized models of Cox proportional hazards regression analysis. Predictive outcomes provided by computer models are not perfect, but nomograms can be extremely useful in assisting with treatment decisions. On occasion, it may be difficult to select the best nomogram when several competing versions apply to the same clinical decision. Kattan and coworkers (205, 206) have developed pre- and postoperative nomograms, incorporating PSA together with Gleason score and other variables, to predict disease recurrence following radical prostatectomy.

PREANALYTICAL, ANALYTICAL, AND POSTANALYTICAL CONSIDERATIONS

A number of factors in the preanalytical, analytical and postanalytical stages can affect the clinical interpretation of PSA results and must be carefully considered. A number of these factors were the subject of a systematic review carried out in 2001 (207).

PREANALYTICAL SPECIMEN PROCESSING AND STORAGE

It is desirable to collect blood before any manipulation of the prostate by DRE, cystoscopy, or prostate biopsy (166). If prior collection is not possible, then it is prudent to delay several days after DRE before drawing blood for PSA, although in most men DRE does not cause a clinically relevant change in circulating PSA concentration (166). Following prostate biopsy or surgery, the recommended delay is several weeks to permit sufficient time for the PSA bound as a stable complex to α -1-antichymotrypsin to be eliminated from the blood circulation, although the kidneys rapidly clear from the blood any fPSA that was liberated from the prostate by the procedure (208, 209).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 7:
PREANALYTICAL REQUIREMENTS FOR PSA—
PROSTATE MANIPULATION**

Blood should be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis [LOE, not applicable; SOR, A].

To eliminate in vitro artifacts, blood should be centrifuged within 3 h of collection to isolate the serum or plasma (210). Serum and plasma may be kept at refrigerated temperatures for up to 24 h without loss of PSA. If analysis is delayed longer, then it is vital to store specimens frozen, preferably at or below -30°C to avoid the eutectic point. Long-term storage at temperatures of at least -70°C is desirable. Data show that fPSA is more susceptible to loss of immunoreactivity than cPSA (166, 211), and that for fPSA this is slower in plasma than in serum (210).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 8:
PREANALYTICAL REQUIREMENTS FOR PSA—
SAMPLE HANDLING**

Samples should be centrifuged and refrigerated within 3 h of phlebotomy; this recommendation is particularly relevant for fPSA, which is more labile than total PSA. Samples may be stored at refrigerated temperatures for up to 24 h, but samples that will not be analyzed within 24 h of collection should be stored frozen (at least at -20°C and preferably at -30°C or lower). For long-term storage, samples should be frozen at -70°C or lower [LOE, not applicable; SOR, B].

PSA ASSAY STANDARDIZATION

Two reference standards currently are commonly used for PSA assays: those traceable to the WHO International Standards and those traceable to the Hybritech, Inc. standard. Most clinicians assume that all PSA assays give similar test values and that changes in these test values probably are related to pathophysiological changes in prostate glands. It is assumed that PSA measurements are consistent between laboratories and between assay manufacturers, but this is not necessarily the case (212). Although practice guidelines and disease management strategies vary in terms of what “number” should be used to follow up specific types of patients, these guidelines seldom contain subcategories for various analytical methods.

In practice there are considerable differences between PSA assays. Historically, the Hybritech Tandem-R PSA assay (Hybritech) was the first widely used FDA-cleared commercial assay. This assay was standardized using the absorptivity for PSA of 1.42 mL/

mg/cm reported by Graves et al. in 1990 (213). The Hybritech assay was well adopted by the medical community and provided the basis for the traditional 4.0 $\mu\text{g/L}$ upper reference limit (141). The second widely used commercial assay, the Abbott IMx (Abbott Laboratories), was standardized to harmonize with this initial Hybritech assay, and other assays also were closely aligned with these assays (214). However, in 1995, Stamey et al. reported that the mean (SD) true absorptivity for PSA is 1.84 (0.04) mL/mg/cm, based on quantitative amino acid analysis (215). It was suggested that the error in the initial gravimetric analysis was caused by the presence of bound water, salt, or carbohydrate in the lyophilized preparations. The net result of this error is that the initial Hybritech PSA values are about 20% higher than the WHO First International Standard for PSA (IRR 96/670) (216).

The First International Standards for PSA (IRR 96/670) and Free PSA (IRR 96/688) were established in 1999 using the correct absorptivity. The 2 standards contain PSA derived from seminal plasma. IRR 96/670 is a mixture of PSA and ACT in a 90:10 ratio selected to mimic circulating PSA, and IRR 96/688 contains solely free (unbound) PSA. An editorial that accompanied the standardization report, *WHO First International Standards for Prostate-Specific Antigen: The Beginning of the End for Assay Discrepancies*, concluded that this standard would lead to greater consistency of PSA as manufacturers began to use this material to calibrate PSA assays (217). It is now recommended that PSA assays used in the United Kingdom National Health Service must be accurately calibrated against the appropriate International Standard and must be equimolar (218), with formal arrangements in place for independent annual confirmation of satisfactory performance. Although several studies suggest that between-method comparability has improved since introduction of the International Standards, there are still differences in PSA assays that may lead to clinical misinterpretation if different PSA assays are used when evaluating a single patient (218–220).

ANALYTICAL AND REPORTING CONCERNS

PSA is most frequently used in conjunction with physical examination to screen for prostate cancer. A single positive PSA screen should always be verified by repeating the PSA measurement in a specimen collected separately, before the ordering of confirmatory histopathological tissue examination, e.g., as obtained by biopsy. This protocol may substantially reduce the number of unnecessary biopsies (221). The diagnosis of prostate cancer can be confirmed only by histopathological tissue examination.

Analytical performance should be monitored with QC material containing PSA at concentrations near

clinically relevant decision points. Information on assay characteristics and utility, including the lowest reportable concentration of the assay (often defined as the PSA concentration below which the analytical CV exceeds 20%) and assay CVs at concentrations corresponding to relevant clinical decision points should be available to clinicians through laboratory test information sources.

**NACB PROSTATE CANCER PANEL RECOMMENDATION 9:
ANALYTICAL REQUIREMENTS FOR PSA—QC**

The lowest reportable concentration should be determined by the laboratory and reported to physicians. QC at these concentrations should be in place [LOE, not applicable; SOR, A].

BIOLOGICAL VARIABILITY

To interpret PSA data from any individual or serially collected specimens, PSA variability in the blood should also be taken into account (207, 222). The EGTM recently reviewed publications concerning the variability of PSA and reported that a fair estimate of the biological variation of PSA is 20% in men older than 50 years within the PSA concentration range of 0.1–20 $\mu\text{g/L}$ (223). In healthy men with PSA concentrations $<2 \mu\text{g/L}$, biological variation was $<14\%$, whereas a change of 30% between successive PSA measurements was suggested to be clinically significant (224). In monitoring men with prostate cancer, a critical difference of 50%–60% has been suggested (225). Taking into account that intraindividual biological variation may range up to 20% and that analytical variation for PSA assays is 5%, it has been suggested that the baseline PSA level has to change by 50% to be significant at $P < 0.05$ (223).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 10:
POSTANALYTICAL REQUIREMENTS FOR PSA—
INTRAINDIVIDUAL BIOLOGICAL VARIATION**

The contribution of within-individual biological variation must be taken into account when interpreting clinical results [LOE, not applicable; SOR, A].

It is prudent to include with the PSA result a reminder that a single screening blood test result should not be used as the sole evidence of the presence or absence of malignant disease. The laboratory report should include the manufacturer of the PSA assay used, draw attention to any relevant clinical decision limits, and where necessary warn that the results cannot be used interchangeably with those generated by other as-

says unless the interchange of assay values has previously been validated (212, 220).

**NACB PROSTATE CANCER PANEL RECOMMENDATION 11:
POSTANALYTICAL REQUIREMENTS FOR PSA—INFORMATION
TO BE INCLUDED IN CLINICAL REPORTS**

Clinical reports should include the name of the assay, relevant clinical decision limits, and a reminder that a single screening blood test result should not be used as the sole evidence of the presence or absence of malignant disease [LOE, not applicable; SOR, A].

FUTURE DEVELOPMENTS

Future developments in the use of tumor markers for prostate cancer include the use of experimental assays to measure circulating tumor cells in blood to detect and assess progression of (micro) metastatic stages of prostate cancer

Assays detecting circulating tumor cells (CTCs) in the peripheral blood have been developed and cleared for clinical use by the FDA to provide prognostic information in women with node-positive breast cancer (226). The current ability to detect and profile (micro) metastatic prostate cancer is limited, however. Multiple techniques have been developed and tested to isolate and characterize CTCs. Reverse transcription PCR assays are sensitive and highly specific when the expression of the target gene is limited to the malignant tumor cells. Flow cytometry can be used to detect and verify the identity of the cells as CTCs, but does not allow assessments of morphology and does not allow detection of molecular changes at a subcellular level. Immobilization (e.g., to magnetic beads) of antibodies to the epithelial cell adhesion molecule allows enrichment and inspection by microscopy of circulating epithelial derived tumor cells from peripheral blood. A semiautomated system was recently developed, which uses epithelial cell adhesion molecule–antibody-based immunomagnetic capture and staining methods (227). Factors predictive of detection of CTCs in prostate cancer have been reported, and for patients with metastatic prostate cancer, the detection of >5 CTCs per 7.5 mL of blood predicts shorter progression-free survival and shorter overall survival, with CTC counts found to be more predictive of outcome than standard clinical parameters (228). For prostate cancer, preliminary analysis of the correlation of CTC counts with mRNAs for prostate specific antigen or prostate specific membrane antigen and available clinical predictors (229) are encouraging but are not yet sufficiently evaluated or validated to warrant recommendations for any use in routine clinical practice.

**NACB PROSTATE CANCER PANEL RECOMMENDATION 12:
MEASUREMENT OF CIRCULATING PROSTATE CANCER CELLS
IN PERIPHERAL BLOOD**

Although initial results are encouraging, these techniques are not yet sufficiently validated to warrant recommending their application in routine clinical practice [LOE, IV; SOR, C].

KEY POINTS: TUMOR MARKERS IN PROSTATE CANCER

Measurements of serum PSA markers clearly have an important role in both diagnosis and management of patients with prostate cancer. Further improvement in understanding of the natural history of the disease should enable better use of these markers in the future.

Tumor Markers in Colorectal Cancer^{31,32}

BACKGROUND

Colorectal cancer (CRC) is the third most common cancer worldwide, with an estimated one million new cases and half a million deaths each year (230). In the US, CRC is also the third most common malignant disease, with an estimated 154 000 new cases diagnosed in 2007 (118). Most CRC are detected in the rectum (38%), followed by sigmoid (29%), cecum (15%), and transverse colon and flexures (10%). Only approximately 5% are found in the ascending colon and 3% in the descending colon (231).

Symptoms of colon cancer may include intermittent abdominal pain, nausea, vomiting, or bleeding. A palpable mass may be found in patients with right-sided colon cancer. Rectal and rectosigmoid cancer are more likely than colonic cancer to be symptomatic before diagnosis because these patients frequently have rectal bleeding. It is important to point out that early colon cancers are rarely symptomatic and that the above-mentioned symptoms are nonspecific.

Disease stage at initial diagnosis is the most widely used prognostic indicator for patients with CRC. Although the original Dukes staging system has been modified several times, the extent of cancer invasion through the bowel wall and extent of regional lymph node invasion is still the mainstay of staging systems. In practice, the most widely used staging system is the TNM system of the International Union against Cancer (232) and the American Joint Committee on Cancer (233) system. In the TNM system, “T” refers to the

local extent of the untreated primary tumor at the time of initial diagnosis. The designation “N” refers to the status of the regional lymph nodes and “M” refers to the presence of distant metastasis at initial presentation (234).

Although surgery is the first-line treatment for most patients with CRC, some patients with rectal cancer may receive radiation and/or chemotherapy before surgery. In 1990, an NIH Consensus Conference recommended that stage III colon cancer patients should be treated with adjuvant chemotherapy (235). A subsequent pooled analysis of patients with stage III CRC confirmed that adjuvant chemotherapy increased both the probability of remaining free of tumor recurrence after 5 years and the probability of surviving for 5 years (236).

The value of adjuvant chemotherapy following resection of stage II (Dukes B) colon cancer is unclear, however. In 2004, an American Society of Clinical Oncology (ASCO) Expert Panel recommended that adjuvant chemotherapy should not, in general, be given to patients with stage II colon cancer (237). However, the panel also stated that “there are populations of patients with stage II disease that could be considered for adjuvant therapy, including patients with inadequately sampled nodes, T₄ lesions, perforation, or poorly differentiated histology” (237).

The 1990 NIH Consensus Conference recommended combined adjuvant chemotherapy and high-dose external-beam radiotherapy for patients with stage II or III rectal cancer (235). Although radiation therapy does not appear to affect overall survival, it decreases local recurrence, which is a cause of considerable morbidity in patients with rectal cancer.

Despite potentially curative surgery, 40%–50% of patients with CRC develop recurrent or metastatic disease (238). In an attempt to detect these relapses when they are resectable, most patients with either stage II or stage III disease currently undergo follow-up or surveillance. Surveillance strategies may include one or more of the following: clinical examination, radiology (e.g., chest x-ray, ultrasound, computed tomography, and magnetic resonance imaging), endoscopy, clinical chemistry testing, and the use of tumor markers.

CRC was one of the first cancers in which a tumor marker, i.e., carcinoembryonic antigen (CEA), was used to aid management. We present NACB guidelines on the use of CEA as well as other markers in the detection and management of patients with CRC. In doing so, we also summarize the guidelines from other expert panels on the use of tumor markers in CRC.

To prepare these guidelines, the literature relevant to the use of tumor markers in CRC was reviewed. Particular attention was given to reviews, including systematic reviews, prospective randomized trials that in-

³¹ NACB Colorectal Cancer Sub-Committee members: Nils Brunner, Chair; Michael J. Duffy; Caj Haglund; Mads Holten-Anderson; and Hans J. Nielsen.

³² All comments received about the NACB Recommendations for Colorectal Cancer are included in the online Data Supplement. Professor Robert Bast, Professor Duncan Jodrell, and Professor Callum Fraser were invited expert reviewers.

Table 9. Currently available markers for CRC.

Cancer marker	Proposed use/uses	Phase of development	LOE ^a	References
Blood-based markers				
CEA	Determining prognosis	Preoperative levels may provide prognostic information but this is rarely used for clinical purposes	III	(239–241)
	Surveillance following curative resection	In clinical use, usually in combination with radiology and clinical history	I	(251–255)
	Monitoring therapy in advanced disease	In clinical use, usually in combination with radiology and clinical history	III	(239–241)
CA19.9	Determining prognosis	Undergoing evaluation	III	(264–269)
	Surveillance following curative resection and monitoring therapy in advanced disease	Undergoing evaluation	IV	(262, 263)
CA 242	Determining prognosis	Undergoing evaluation	III	(270, 271)
TIMP-1	Determining prognosis/Screening high risk populations	Undergoing evaluation	III	(274, 275)
Tissue-based markers				
Thymidylate synthase	Determining prognosis	Undergoing evaluation, a meta-analysis suggested that high levels of thymidylate synthase predicted poor outcome (279). Assay not standardized	I	(276–279, 564)
	Predicting response to chemotherapy (5-FU ^b) in advanced disease	Undergoing evaluation. High levels may predict lack of response to 5-FU in advanced disease. Some studies suggest that thymidylate synthase should be determined on metastatic site to be treated	III	(276–280, 564)
MSI	Determining prognosis	Undergoing evaluation, a pooled analysis showed that MSI-tumors were associated with a 15% better prognosis compared with MS-stable tumors (285). Overall, data conflicting	I	(282–284, 565)
	Predicting response to chemotherapy	Results conflicting, undergoing further evaluation	III	(284, 285, 565, 566)
Deleted in colon cancer/18q phenotype	Determining prognosis	Undergoing evaluation, prognostic value validated in a meta-analysis. Assay not standardized	I	(286–288)
uPA/PAI-1	Determining prognosis	Undergoing evaluation	III	(289–291)
Ras	Determining prognosis	A pooled analysis showed that a mutant ras gene was weakly prognostic in Dukes C but not in Dukes B disease. Unlikely to be used for clinical purposes	I	(292)
	Predicting benefit from therapy	May be of value in predicting benefit from the anti-epidermal growth-factor receptor antibodies, cetuximab and panitumumab	III	(294–297)
P53	Determining prognosis	A meta-analysis showed that abnormal p53 was weakly associated with poor outcome. Unlikely to be used for clinical purposes	I	(293)

Continued on page e37

Table 9. Currently available markers for CRC. (Continued from page e36)

Cancer marker	Proposed use/uses	Phase of development	LOE ^a	References
Fecal markers				
FOBT	Screening asymptomatic populations	Shown in randomized trials that screening with FOBT reduced mortality from CRC. Used for ad hoc CRC screening. Feasibility screening trials underway in a number of countries. Lacks sensitivity for early CRC and advanced adenomas and gives rise to many false-positive results	I	(300, 302–306)
DNA Panels	Screening asymptomatic populations	A large study on asymptomatic subjects showed that a DNA panel was more sensitive than FOBT for detecting both advanced adenomas and invasive CRC (79).	III/IV for most panels. I for a specific panel (317)	(313–317)
Genetic Markers				
APC gene	For identifying subjects at high risk of developing FAP	In clinical use in specialized centers	Expert opinion	(322, 323, 326, 567, 568)
MSI	Prescreen for HNPCC	In clinical use in specialized centers	III	(322, 323, 567–569)
MMR genes, e.g., MLH1/MSH2/MSH6/PMS2	For identifying subjects at high risk of developing HNPCC	In clinical use in specialized centers	III/IV	(322, 323, 326, 567–569)

^a LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.
^b 5-FU, 5-fluorouracil; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mutations in mismatch repair.

cluded the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence based.

CURRENTLY AVAILABLE MARKERS FOR CRC

The most widely investigated tumor markers for CRC and the phase of development of each marker and the LOE for its clinical use are listed in Table 9.

TUMOR MARKERS IN CRC: NACB RECOMMENDATIONS

Table 10 presents a summary of recommendations from representative guidelines published on the use of tumor markers in CRC. This table also summarizes the NACB guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion of the most widely investigated markers listed in Table 10.

CARCINOEMBRYONIC ANTIGEN

CEA in screening. Lack of sensitivity and specificity when combined with the low prevalence of CRC in asymptomatic populations preclude the use of CEA in screening for CRC (239–241). In agreement with ASCO (242–244) and EGTM recommendations (245, 246), the NACB Panel states that CEA cannot be used in screening healthy individuals for early CRC.

**NACB CRC PANEL RECOMMENDATION 1:
SERUM CEA IN SCREENING HEALTHY INDIVIDUALS**
CEA cannot be used in screening of healthy subjects for early CRC [LOE, IV/V; SOR, A].

CEA in determining prognosis. As mentioned above, disease stage at initial diagnosis is universally used to determine prognosis in patients with CRC. Several studies, however, have demonstrated that preoperative concentrations of CEA can also provide prognostic information, which in some situations was found to be independent of stage (239–241, 247–249). The NACB Panel therefore states that preoperative concentrations of CEA might be used in combination with other factors in planning surgical treatment. Preoperative CEA concentrations, however, should not be used at present to select patients for adjuvant therapy. These guidelines are broadly in agreement with those previously published by ASCO and EGTM (242, 244–246).

A College of American Pathologists Expert Panel recently ranked preoperative serum CEA together with TNM stage, regional lymph node metastasis, blood or lymphatic vessel invasion, and residual tumor following surgery with curative intent as a category I prognostic marker for CRC (250). According to the College of American Pathologists Panel, Category I prognostic

factors are those “definitely proven to be of prognostic importance based on evidence from multiple statistically robust published trials and generally used in patient management”.

NACB CRC PANEL RECOMMENDATION 2: SERUM CEA IN PROGNOSIS AND PREDICTION

Preoperative CEA concentrations might be used in combination with other factors in planning surgical treatment. Patients with increased concentrations of CEA (e.g., $>5 \mu\text{g/L}$) should be evaluated for the presence of distant metastases [LOE, III; SOR, C]. Preoperative CEA concentrations should not be used at present to select patients for adjuvant chemotherapy [LOE, III; SOR, C].

CEA in postoperative surveillance. The main aims of surveillance following curative resection of CRC are to provide reassurance, address possible complications due to therapy, and identify resectable recurrences or metastases. Six separate metaanalyses have compared outcome in patients with intensive follow-up vs those with minimal or no follow-up (251–256). All concluded that the use of an intensive follow-up regime resulted in a modest but statistically significant improved outcome when compared with regimes with minimal follow-up. In one of these metaanalyses, it was shown that only the studies including CEA demonstrated a significant impact on survival (254).

The most recent ASCO guidelines state that CEA should be measured every 3 months in patients with stage II or III CRC for at least 3 years after diagnosis, if the patient is a candidate for surgery or systemic therapy of metastatic disease (244, 257). The NACB Panel supports this recommendation.

Although serial measurements of CEA are widely used in surveillance, no agreement exists as to the magnitude of concentration change that constitutes a clinically significant increase in CEA during serial monitoring. According to the EGTM Panel, a significant increase in CEA occurs if the elevation is at least 30% over that of the previous value. This increase, however, must be confirmed by a second sample taken within 1 month. If this latter sample is also increased, the patient should undergo further investigations (246). This 30% increase, however, has not been clinically validated. Furthermore, it should not be regarded as exclusive. For example, small increases in CEA (e.g., 15%–20%, maintained over at least 3 successive assays) may also prompt intervention (246). It should also be remembered that low CEA concentrations do not necessarily exclude progression, and in patients with clinical

Table 10. Recommendations for use of markers in colorectal cancer by different expert groups.

Marker	Application	ASCO (242, 244, 257, 324, 325) ^a	EGTM (245, 246, 570)	NACB 2002 (15)	ESMO ^b (571–574)	NCCN (575)	ACS (311)	USPSTF (310)	NACB 2008	SOR ^c
CEA	Screening	No (257)	No	No	None published	None published	None published	None published	No	A
	Determining prognosis	Yes, if it could assist in staging or surgical treatment planning (257)	Yes	None published	Yes	Yes, as part of a complete staging work-up	None published	None published	Yes, may be combined with other factors, if this would aid in the planning of surgical treatment	C
	Postoperative surveillance	Yes, if patient is a candidate for surgery or systemic therapy (257)	Yes, for the early detection of liver metastasis	Yes, if resection of liver metastasis would be clinically indicated	Yes	Yes, if the patient is a candidate for aggressive surgical resection, should recurrence be detected	None published	None published	Yes, if patients is a suitable candidate for undergoing liver resection or receiving systemic chemotherapy	A
	Monitoring advanced disease	Yes (257)	Yes	Yes, especially if metastasis difficult to measure by other means	NR	NR	None published	None published	Yes, especially for disease that cannot be evaluated by other modalities	B
APC gene	Screening for FAP	See ASCO general guidelines for genetic testing for cancer susceptibility (324, 325)	None published	None published	Yes	Yes	None published	None published	Yes	A
MSI	Initial screening test for HNPCC	None published	None published	None published	None published	Yes	None published	None published	Yes	A
MMR genes, e.g. MLH1, MSH2, MSH6, PMS2	Screening for HNPCC for genetic testing for cancer susceptibility (324, 325)	See general guidelines for genetic testing for cancer susceptibility (324, 325)	None published	None published	Yes	Yes	None published	None published	Yes	A
FOBT	Screening asymptomatic subjects	None published	Yes, for subjects ≥ 50 years old	None published	None published	None published	Yes, for subjects ≥ 50 years old	Yes, for subjects ≥ 50 years old	Yes, for subjects ≥ 50 years old	A

^a Ref (325) was a joint study published by ASCO and the Society of Surgical Oncology
^b ESMO, European Society of Medical Oncology; AGA, American Gastroenterology Society; ACS, American Cancer Society; NCCN, National Comprehensive Network; USPSTF, US Preventive Services Task Force; NR, no recommendation published; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mutations in mismatch repair.
^c SOR (520). A, high (further research is very unlikely to change the panel's confidence in the estimate of effect); B, moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); C, low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate); D, very low (any estimate of effect is very uncertain).

symptoms of disease recurrence, additional tests such as computed tomographic scan, x-rays, and colonoscopy are required, irrespective of the CEA concentration (246).

**NACB CRC PANEL RECOMMENDATION 3:
SERUM CEA IN POSTOPERATIVE SURVEILLANCE**

CEA should be measured every 3 months in patients with stage II or III CRC for at least 3 years after diagnosis if the patient is a candidate for surgery or systemic therapy of metastatic disease [LOE, I; SOR, A].

CEA in monitoring therapy in advanced disease. The prognosis for patients with advanced CRC has greatly improved in recent years owing to the introduction of new cytotoxic agents such as irinotecan and oxaliplatin and monoclonal antibodies such as bevacuzimab (Avastin®), panitumumab (Vectibix®), and cetuximab (Erlotinib®), as has recently been reviewed (258, 259). Indeed, the median survival for patients with metastatic CRC has almost doubled in the past 10 years as a result of these new treatments (258–260). However, because these treatments are potentially toxic as well as expensive, it is important to establish as quickly as possible that they are effective in halting tumor progression.

According to the 2006 ASCO guidelines, CEA is the marker of choice for monitoring metastatic CRC during systemic therapy (244). CEA should be measured at the start of treatment for metastatic disease and every 1–3 months during active treatment. Persistently increasing concentrations suggest progressive disease even in the absence of corroborating radiographs (242, 243). In 2003, the EGTM Panel recommended that serial CEA concentrations should be measured every 2–3 months while patients are receiving systemic therapy (246). Both the ASCO and EGTM guidelines stated that caution should be used when interpreting increasing CEA concentrations during the early phase of systemic treatment (16, 18). This is because certain treatments (e.g., 5-fluorouracil and levamisole; oxaliplatin) can cause transient elevations in CEA levels in the absence of disease progression (246).

For monitoring patients with advanced CRC undergoing systemic therapy, the NACB Panel recommends that regular CEA determinations should be carried out. In agreement with the ASCO Panel (242, 243), a confirmed CEA increase (e.g., >30%) may be regarded as evidence of progressive disease. Of course, it should be established that the increases are not false-positive elevations due to either chemotherapy-mediated release of marker or the development of a benign disease that produces CEA.

**NACB CRC PANEL RECOMMENDATION 4:
SERUM CEA IN MONITORING PATIENTS WITH
ADVANCED DISEASE**

In patients with advanced CRC undergoing systemic therapy, regular CEA determinations should be carried out. A confirmed CEA increase (e.g., >30%) suggests progressive disease provided the possibility of false-positive elevations can be excluded [LOE, III; SOR, B].

OTHER SERUM MARKERS

CA 19–9. The CA 19–9 assay detects a mucin containing the sialated Lewis-a pentasaccharide epitope, fucopentaose II [for review, see ref (261)]. CA 19–9 is a less sensitive marker than CEA for CRC (262, 263). Preliminary findings suggest that like CEA, preoperative concentrations of CA 19–9 are also prognostic in patients with CRC (264–268). Based on available data, routine measurement of CA 19–9 cannot be recommended for patients with CRC.

CA 242. The CA 242 assay also detects a mucinlike molecule. Although less sensitive than CEA for CRC, assay of CA 242 may complement CEA in the surveillance of patients with CRC (263, 269). Furthermore, a number of preliminary reports suggest that preoperative concentrations of CA 242 are prognostic in CRC (270, 271). Routine determinations of CA 242 should not be used at present in patients with CRC.

TISSUE INHIBITOR OF METALLOPROTEINASES TYPE 1

Tissue inhibitor of metalloproteinases type 1 (TIMP-1) is a 25-kDa glycoprotein with multiple activities including inhibition of matrix metalloproteinases, promotion of cell proliferation, and inhibition of apoptosis. With use of a research ELISA that detects total TIMP-1 (i.e., the noncomplex form as well as TIMP-1 complexed to matrix metalloproteinases), plasma concentrations of the inhibitor were found to be significantly higher in patients with CRC than in healthy controls or patients with inflammatory bowel diseases, adenomas, or breast cancer (272, 273). For patients with Dukes A and B colon cancers, TIMP-1 appeared to be more sensitive than CEA for the detection of cancer, i.e., 58% vs 40% at 95% specificity and 56% vs 30% at 98% specificity. For patients with early rectal cancer, TIMP-1 and CEA had similar sensitivity (272). Other studies have shown that preoperative plasma TIMP-1 concentration is an independent prognostic factor in patients with CRC, i.e., independent of Dukes stage and tumor location (274, 275). Of particular note was the finding that stage II patients with low plasma TIMP-1 concentrations (dichotomized at the 70% per-

centile) exhibited a survival pattern similar to an age and sex-matched background population.

Although these preliminary findings with TIMP-1 are promising, the marker cannot be recommended at present either for detecting early CRC or for evaluating prognosis in patients with this malignancy.

NACB CRC PANEL RECOMMENDATION 5:

CA19.9, CA 242 AND TIMP-1 IN CRC

Routine measurement of CA19.9, CA 242, or TIMP-1 is not recommended [LOE, III/IV; SOR, B/C].

TISSUE MARKERS

Several tumor tissue markers have been evaluated for potential prognostic and predictive value in patients with CRC. These include thymidylate synthase (276–280), MSI (281–285), deleted in colon cancer (286–288), urokinase plasminogen activator (uPA)/plasminogen activator inhibitor 1 (PAI-1) (289–291), mutant ras (292), and mutant/overexpression of p53 (293). Based on available evidence, none of these markers can at present be recommended for routinely determining prognosis or for therapy prediction. Emerging evidence however, suggests that the presence of wild type k-ras is associated with benefit from the anti-epidermal growth-factor receptor (EGFR) antibodies cetuximab and panitumumab (294–297).

NACB CRC PANEL RECOMMENDATION 6:

TISSUE MARKERS IN CRC

The use of thymidylate synthase, MSI, deleted in colon cancer, uPA, PAI-1, or p53 for determining prognosis or predicting response to therapy is not recommended [LOE, III; SOR, B]. Determination of the mutation status of k-ras may in the future be used for predicting benefit from specific anti-EGFR antibodies.

FECAL MARKERS

The most widely used fecal marker involves testing for occult blood, i.e., the fecal occult blood test (FOBT). Two of the most widely described FOBTs are the guaiac test and the fecal immunochemical test (FIT) (298–301). The guaiac test measures the pseudoperoxidase activity of heme in hemoglobin, and the immunochemical test detects human globin. Because peroxidase activity is also present in certain fruits and vegetables, intake of these foods may give rise to false-positive results in the guaiac test. Certain medicines such as nonsteroidal antiinflammatory drugs can also interfere with this test. Despite these limitations, a number of

large randomized trials have shown that screening with the guaiac test reduced mortality from CRC (302–306).

The efficacy of the FIT in reducing either the incidence or mortality from CRC has not yet been investigated in large population-based studies. However, based on available evidence, it should be at least as accurate if not more accurate than guaiac-based tests in screening for CRC (298, 301, 307). The advantages of the immunochemical test over the guaiac tests include the following (for review, see refs (298, 299, 307):

- FITs have better sensitivity for human blood.
- FITs are not affected by diet or medications.
- Some FITs can be automated.
- Evidence suggests that the use of FITs increases patient participation in screening for CRC.
- FITs can be quantitated, enabling adjustment of sensitivity, specificity and positivity rates.
- Because digested blood from the upper gastrointestinal tract is not usually detected by FITs, the latter are better for detecting bleeding from the lower gastrointestinal tract.

In agreement with other expert panels (308–310), the NACB Panel recommends that all individuals 50 years or older should undergo screening for CRC. Multiple screening procedures for CRC exist, however (306–308), and to date no one procedure has been shown to be significantly superior to the others. The option chosen may therefore depend on availability, personal preference, and risk of developing CRC (311).

According to the National Comprehensive Cancer Network (NCCN), FOBT should be performed on 3 successive stool specimens that are obtained while the patient adheres to a prescribed diet (308). This organization specifically recommends the Hemoccult SENSAs as the testing method. Both the NCCN and the American Cancer Society recommend against use of FOBT of a specimen obtained during a digital rectal examinations (308, 311).

Although screening has been shown to result in reduced mortality from CRC (302–305, 312), it may be associated with certain harmful effects. These include the psychosocial consequence of false-positive results, potential complications of colonoscopy, a false negative result, or the possibility of over-diagnosis (312). Overdiagnosis could give rise to unnecessary investigations or treatment.

Because of the lack of sensitivity and specificity of FOBT for adenomas and early CRC, a considerable amount of research in recent years has focused on other fecal markers, especially on the genes that undergo mutation during CRC carcinogenesis. Among the most widely investigated DNA markers are mutant ras, mutant p53, mutant APC, specific methylated genes, MSI, and long DNA (231, 313–316). Almost all of

the studies published to date on fecal DNA markers contained small numbers of patients. Following an overview of the literature, Allison and Lawson (298) found that the sensitivities of the different DNA panels for invasive CRC varied from 52%–98% (mean, 64%), and the specificity varied from 93%–97% (mean, 95%).

Although most of the studies that evaluated DNA markers for the detection of CRC included only small numbers of patients, a specific panel was recently investigated as a screening test for CRC in a large asymptomatic population (317). Of the 31 invasive CRCs detected, the DNA panel diagnosed 16, whereas FOBT detected only 4 (51.6% vs 12.9%, $P = 0.003$). Of the 71 invasive cancers and adenomas with high-grade dysplasia, the DNA panel diagnosed 29, while FOBT detected only 10 ($P < 0.001$). Although the DNA panel displayed a higher sensitivity than FOBT, clearly neither test detected the majority of advanced adenomas or carcinomas (317). However, because the DNA-based test was superior to FOBT, it might be expected to be at least as good as the latter in reducing mortality from CRC. However, it should be pointed out that compared to FOBTs, measurement of fecal DNA markers is more expensive and technically demanding. Furthermore, it is not clear which combination of DNA markers provides the optimum balance of sensitivity and specificity (231).

One of the main arguments against the use of a DNA panel at present, especially when applied to large populations, is the relative cost compared to FOBT (318, 319). In 2004, Song et al. (318), using a modeling approach, compared the cost-effectiveness of fecal DNA to that of standard CRC screening methods. The main conclusions were as follows:

- Compared with no screening, all screening strategies increased life expectancy at what was regarded as reasonable cost.
- Compared with no screening, the use of fecal DNA testing gained 4560 life-years per 100 000 persons at an incremental cost of \$47 700/life-year gained.
- The use of colonoscopy and FOBT/flexible sigmoidoscopy were more effective strategies, gaining an incremental 6190 and 6270 life-years per 100 000 persons compared to no screening, at incremental costs per life-year gained of \$17 010 and \$17 000.
- All the conventional approaches gained more life-years at lower cost than fecal DNA testing.

Despite their relatively high costs, the technically demanding nature of the assays, and the fact that these tests have not been validated in a prospective randomized trial, recent joint guidelines from the American Cancer Society, the US Multi-Society Task Force, and the American College of Radiology state that there is

now sufficient data to include fecal DNA “as an acceptable option for CRC screening” (320, 321).

NACB CRC PANEL RECOMMENDATION 7:

USE OF FECAL MARKERS IN SCREENING FOR CRC

The NACB recommends that all individuals 50 years or older should undergo screening for CRC. Because the most effective screening test is unknown, the method chosen is likely to depend on risk of CRC, local availability, and personal preference. Although FOBT is the best-validated stool-based method for screening for CRC [LOE, I; SOR, A], fecal DNA testing may also be an option. Potential harmful consequences of screening include complications due to colonoscopy and treatment, the possibility of overdiagnosis leading to unnecessary investigations, and false-negative and false-positive results.

GENETIC TESTS

For genetic testing for CRC susceptibility, i.e., familial adenomatous polyposis coli and hereditary nonpolyposis CRC, the NACB Panel supports previously published guidelines (308, 322–326).

NACB CRC PANEL RECOMMENDATION 8:

GENETIC TESTING FOR CRC

Screening for genetic susceptibility to CRC should commence with a detailed family history. Before undergoing testing, individuals should receive genetic counseling. For persons with suspected familial adenomatous polyposis, genetic testing can be used both to confirm diagnosis in a suspected proband and to assess risk in presymptomatic family members. Provided the mutation responsible for familial adenomatous polyposis within a family is known, testing for adenomatous polyposis coli (*APC*) gene mutations can be considered for at-risk family members. [LOE, Expert opinion; SOR, A].

MSI testing and/or immunohistochemistry for specific mismatch repair enzymes can be used as a prescreen for hereditary nonpolyposis CRC. If an individual is found to possess high MSI, genetic testing for mutations in *MLH1*, *MLH2*, *MSH6*, or *PMS2* genes should be carried out [LOE, III/IV; SOR, A].³³

³³ *MLH1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*); *MSH2*, mutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*); *MSH6*, mutS homolog 6 (*E. coli*); *PMS2*, postmeiotic segregation increased 2 (*S. cerevisiae*).

KEY POINTS: TUMOR MARKERS IN CRC

Although many different markers have been evaluated for CRC, only a small number can be recommended for clinical use. These include CEA in the postoperative surveillance of patients who may be suitable candidates for either surgical resection or systemic chemotherapy, FOBT in screening for early CRC in persons 50 years or older, MSI as a surrogate marker for identifying persons who should undergo genetic testing for *MLH1/MSH2/MSH6/PMS2* to identify hereditary nonpolyposis CRC, and *APC* to identify familial adenomatous polyposis. A promising new plasma marker is TIMP-1. As mentioned above, preliminary findings suggest that this marker may be more sensitive than CEA in detecting early CRC as well as being an independent prognostic factor for CRC. These findings now must be confirmed in large prospective studies. One of the most promising fecal CRC screening tests is a fecal DNA panel (317). This test should be simplified, made available at reduced costs, and subjected to further investigations.

Tumor Markers in Breast Cancer^{34,35}**BACKGROUND**

Breast cancer is by far the most common cancer affecting women worldwide, with approximately one million new cases diagnosed each year (327). In 2007, an estimated 180 000 women were diagnosed with breast cancer in the US and approximately 41 000 died from the disease (118). Currently, there are more than 2 million women in the US who are living with a history of breast cancer (328). Although the worldwide incidence of the disease appears to be increasing, mortality rates are now declining in a number of Western countries including the US and the United Kingdom (329).

The main presenting features in women with symptomatic breast cancer include a lump in the breast, nipple change, or discharge and skin contour changes. Definitive diagnosis requires biopsy and histopathology. Currently available blood-based biomarkers are of no value in the early diagnosis of breast cancer.

The primary treatment for localized breast cancer is either breast-conserving surgery and radiation or mastectomy. Following primary treatment, most women with invasive breast cancer receive systemic adjuvant therapy such as chemotherapy, hormone

therapy, or a combination of chemotherapy and hormone therapy. Both adjuvant chemotherapy and hormone therapy have been shown to reduce systemic recurrence and mortality from breast cancer (330). For example, a meta-analysis of approximately 145 000 women participating in 194 randomized trials of adjuvant systemic therapy concluded that anthracycline-based polychemotherapy reduced the annual breast cancer death rate by about 38% for women younger than 50 years at diagnosis and by about 20% for those age 50–69 years at diagnosis (330). For estrogen receptor (ER)-positive patients, 5 years of adjuvant tamoxifen reduced annual breast cancer death rates by 31% (330). Patients with ER-negative tumors, however, did not benefit from adjuvant tamoxifen (331).

Because not all patients with breast cancer may need adjuvant treatment [e.g., approximately, 70% of lymph node-negative patients are cured of their disease by surgery and radiotherapy (332)] and not all patients benefit from this treatment, rational management requires the availability of reliable prognostic and predictive markers. Recommendations regarding the use of currently available prognostic and predictive markers for breast cancer are discussed below.

Subsequent to primary therapy, patients with a diagnosis of breast cancer are usually followed up at regular intervals. Historically, surveillance has included clinical history, physical examination, mammography, chest x-ray, biochemical testing, and the use of tumor markers. This practice is based on the assumption that the early detection of recurrent disease leads to a better outcome. However, at present, the clinical benefit of close surveillance is unclear (333).

Although adjuvant therapy improves patient outcome, 25%–30% of women with lymph node-negative and at least 50%–60% of those with node-positive disease develop recurrent or metastatic disease (334). Therapy options for metastatic breast cancer include chemotherapy (e.g., anthracycline or taxane-based), hormone therapy, or targeted therapies such as Trastuzumab (Herceptin®), Lapatinib, or Bevacizumab, alone or combined with chemotherapy (334, 335). Currently, metastatic breast cancer is regarded as incurable and thus the goal of treatment is generally palliative. In this context, the use of serial levels of serum tumor markers is potentially useful in deciding whether to persist in using a particular type of therapy, terminate its use, or switch to an alternative therapy.

Based on the above, it is clear that optimal management of patients with breast cancer requires the use of a number of tumor markers. The aim of this article is to present new NACB guidelines on the use of both tissue- and serum-based tumor markers in breast cancer. A summary of guidelines published by other expert panels on this topic is also provided.

³⁴ NACB Breast Cancer Sub-Committee Members: Michael J. Duffy, Chair; Francisco J. Esteva; Nadia Harbeck; Daniel F. Hayes; and Rafael Molina.

³⁵ All comments received about the NACB Recommendations for Breast Cancer are included in the online Data Supplement. Professor Dorte Nielsen, Professor John Smyth, and Professor M. Tuxen were invited expert reviewers.

To prepare these guidelines, the literature relevant to the use of tumor markers in breast cancer was reviewed. Particular attention was given to reviews, including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence-based.

CURRENTLY AVAILABLE MARKERS FOR BREAST CANCER

Table 11 lists the mostly widely investigated tissue-based and serum-based tumor markers for breast cancer. Also listed, is the phase of development of each marker as well as the LOE for its clinical use.

TUMOR MARKERS IN BREAST CANCER: NACB RECOMMENDATIONS

Table 12 presents a summary of recommendations from various expert panels on the use of tumor markers in breast cancer. This table also summarizes the NACB guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion on the most clinically useful markers listed in Table 12.

ER and progesterone receptor. Routine assay of ERs (i.e., ER- α) and progesterone receptors (PR) in all newly diagnosed breast cancers has been recommended by Expert Panels of ASCO, EGTM, the European Society of Medical Oncology, and the St Gallen Conference Consensus Panel (Table 12). The NACB Panel agrees with these recommendations. The primary purpose of determining ER and PR is to select for likely response to endocrine therapy in patients with either early or advanced breast cancer. Additionally, in combination with other factors, ER and PR may also be used for prognostic purposes. However, as predictors of patient outcome, hormone receptors are relatively weak factors and are of little clinical value in lymph node-negative patients. Hormone receptors should therefore not be used alone for determining outcome in breast cancer. However, in combination with established prognostic factors, hormone receptors may be used to predict risk of recurrence. Determination of ER- β has no clinical application at present.

Recommended assays for ER and PR. ER (i.e., ER- α) and PR can be measured by ligand-binding assay, ELISA, or immunohistochemistry. The advantages and disadvantages of these different assays are summarized in Table 13. It is important to note that most of the clinical data relating to both ER and PR were derived from biochemical (ligand-binding and ELISA) assays. Some recent investigations, however, have shown that the immunohistochemical determination of ER provides

clinical information at least as powerful as that obtained with the biochemical assays (336–341). Indeed, one report stated that the use of immunohistochemistry to determine ER was superior to that of biochemical assays for predicting response to therapy (336). Compared to ER, fewer data are available on the clinical value of PR as determined by immunohistochemistry (341–343). As with ER, the predictive power of PR as determined by immunohistochemistry appears to be superior to that obtained using ligand-binding assays (343).

Because of its ease of use and application to a wider range of tumors (e.g., small as well as large tumors and paraffin-embedded as well as frozen tissue), the NACB Panel recommends the use of immunohistochemistry for the determination of both ER and PR.

The following points should be borne in mind when determining ER and PR by immunohistochemistry:

- Immunohistochemical assays used should have been shown to give values that correlate with biochemical assays and should be validated for both predictive and prognostic purposes. Validated antibodies include 6F11 MAb (Novocastra) or antibody ID5 (Dako) for ER and antibody 1A6 (Novocastra), PR88 (Biogenex, Menarini Diagnostics) or monoclonal antibody 1294 (Dako) for PR (336, 337, 343–345).
- Internal controls should be included in each examination. A tissue control with receptor-positive cancer cells and adjacent benign epithelium has been previously recommended (345).
- Participation in an external quality assessment scheme is essential (344, 345).
- Scoring of stain may be based either on percentage of cells stained or on a combination of percentage of cells stained plus intensity of stain. A semiquantitative score should be reported rather than a negative or positive value (344, 345). It is important to state that patients with low ER levels (e.g., staining in 1%–10% of the cells) have been reported to respond to endocrine therapy (336).
- Only nuclear staining should be evaluated.
- The report should mention source of primary antibody as well as type of tissue used (e.g., paraffin-embedded or frozen) (345).

NACB BREAST CANCER PANEL RECOMMENDATION 1:

ER AND PR AS PREDICTIVE AND PROGNOSTIC MARKERS

ER and PR should be measured in all patients with breast cancer. The primary purpose of measuring these receptors is to identify patients with breast cancer that can be treated with hormone therapy [LOE, I; SOR, A].

Table 11. Useful and potentially useful markers for breast cancer.

Cancer marker	Proposed use/uses	Phase of development	LOE ^a	Reference
Tissue-based markers				
ER	For predicting response to hormone therapy in both early and advanced breast cancer	In clinical use	I	(330, 331, 576)
	In combination with other factors for assessing prognosis in breast cancer. ER alone is a relatively weak prognostic factor	In clinical use	III	(576, 577)
PR	Usually combined with ER for predicting response to hormone therapy	In clinical use	I/II	(578, 579)
HER-2	Determining prognosis, most useful in node-positive patients. Conflicting data in node-negative patients	In clinical use in some centers	II-III	(580)
	For selecting patients with either early or metastatic breast cancer for treatment with Trastuzumab (Herceptin)	In clinical use	I	(581–583)
	For predicting resistance to tamoxifen therapy in breast cancer, may be predictive of relative resistance to tamoxifen in patients with early breast cancer	Results conflicting, undergoing further evaluation	III	(348, 349)
	For predicting resistance to CMF ^b in early breast cancer, may be predictive of relative resistance to CMF in patients with early breast cancer	Results conflicting, undergoing further evaluation	III	(348, 349)
	For selecting response to anthracycline-based therapy in early breast cancer, HER-2 may be associated with an enhanced response to anthracycline-based therapy ^c	Undergoing further evaluation	II/III	(348, 349, 351, 352)
uPA	For determining prognosis in breast, cancer, including the subgroup with axillary node-negative disease	Prognostic value validated in both a prospective randomized trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany	I	(361–363)
	For predicting resistance to hormone therapy in advanced breast cancer	Undergoing evaluation	III-IV	(584, 585)
	For predicting enhanced response to chemotherapy in early breast cancer	Undergoing evaluation	III	(364, 365, 586)
PAI-1	Usually assayed in combination with uPA, i.e. for determining prognosis in breast cancer including the subgroup with node-negative disease. Provides prognostic information additional to that of uPA	Prognostic value validated in both a prospective randomized trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany	I	(361–363)
	In combination with uPA may be of value for predicting enhanced response to adjuvant chemotherapy and resistance to hormone therapy in advanced disease	Undergoing further evaluation	III	(364, 365, 584–586)
Cathepsin D	For determining prognosis in breast cancer	Results conflicting. However, using a specific ELISA, most reports show a prognostic value. Prognostic value in node-negative breast cancer validated by meta-analysis. Not in clinical use	I (only in node-negative disease)	(587–589)
p53	For evaluating prognosis in breast cancer	Results conflicting when p53 protein is determined by IHC. Specific mutations in the p53 gene however, correlate with adverse outcome. Undergoing further evaluation	III (with IHC), I (with mutation testing)	(590, 591)
	For predicting response to chemotherapy or hormone therapy in breast cancer	Results conflicting. Undergoing further evaluation	III	(591, 592)

Continued on page e46

Table 11. Useful and potentially useful markers for breast cancer. (Continued from page e45)

Cancer marker	Proposed use/uses	Phase of development	LOE ^a	Reference
DNA ploidy/S-phase	For assessing prognosis in breast cancer	Results conflicting. Undergoing further evaluation	III	(593, 594)
Gene expression microarray	For assessing prognosis ^d	Undergoing evaluation. For one of these profiles (387–390), a prospective multicenter validation study is planned	III	(385–389)
Oncotype DX TM (a multiplex reverse-transcription PCR assay)	For predicting recurrence in lymph node-negative, ER-positive patients receiving adjuvant tamoxifen. May also predict benefit from adjuvant chemotherapy in node-negative, ER-positive patients	Validated in prospectively designed studies, assay can be carried out on paraffin-embedded tissue. In clinical use. A prospective multicenter validation of the chemopredictive utility is underway	I-II (for patients receiving adjuvant tamoxifen)	(391–395)
Serum-based markers				
CA 15-3	Postoperative surveillance in patients with no evidence of disease	In clinical use, but value of changing therapy for patients with rising levels not validated in a high-level evidence study	III	(381, 595)
	Monitoring therapy in advanced disease	In clinical use, but value not validated in a high-level evidence study	III	(381, 595)
	Assessing prognosis. High preoperative levels (e.g. > 30 U/L) predict adverse outcome	Not in clinical use	III	(596–599)
BR 27.29	Provides similar information to CA 15-3 but not as widely investigated as CA 15-3	In clinical use, but value not validated in a high-level evidence study	III	(600, 601)
CEA	Post-operative surveillance in patients with no evidence of disease. Overall, appears to be less sensitive than CA 15-3/BR 27.29	In clinical use, but value not validated in a high-level evidence study	III	(377, 602–604)
	Monitoring therapy in advanced disease, especially if CA 15-3/BR 27.29 is not elevated	In clinical use, but value not validated in a high-level evidence study	III	(377, 602–604)
	Assessing prognosis. High preoperative levels predict adverse outcome	Not in clinical use	III	(596, 598, 604)
TPA	Postoperative surveillance in patients with no evidence of disease	In clinical use in some countries, but value not validated in a high level evidence study	III	(377, 603)
	Monitoring therapy in advanced disease. May be useful if CA 15-3, BR 27.29 or CEA are not elevated	In clinical uses in certain countries, but value not validated by a high level evidence study		(595, 603)
TPS	As for TPA	As for TPA	III	(605, 606)
HER-2 (shed form)	Determining prognosis; predicting response to hormone therapy, chemotherapy and Trastuzumab; post-operative surveillance and monitoring therapy in advanced disease. Less sensitive than either CA 15-3 or CEA but may be useful in monitoring if CA 15-3, BR 27.29 or CEA are not elevated. Preliminary results suggest that serum HER-2 may be of value in monitoring Trastuzumab therapy in patients with advanced breast cancer	Undergoing evaluation	III-IV	(353, 607)
Proteomics	Detecting early disease and monitoring	Undergoing evaluation, results to date conflicting	IV/V	(608, 609)

Continued on page e47

Table 11. Useful and potentially useful markers for breast cancer. (Continued from page e46)

Cancer marker	Proposed use/uses	Phase of development	LOE ^a	Reference
Tumor cells (detected by other than hematoxylin and eosin staining)				
Tumor cells in bone marrow	For assessing prognosis	Prognostic value validated in a pooled analysis. Not in widespread clinical use. Not clear if of value in otherwise favorable prognostic patients	I	(610–612)
Tumor cells in axillary nodes	Prognosis/staging	Most studies conclude that the detection of tumor cells in axillary nodes predicts adverse prognosis but prognostic impact appears relatively weak. Undergoing further evaluation	II–III	(613, 614)
Tumor cells in sentinel lymph nodes	Prognosis/staging ^e	Undergoing evaluation. Two prospective trials are currently in progress	IV/V	(615, 616)
Tumor cells in circulation	For assessing prognosis and monitoring therapy in advanced disease	Undergoing evaluation. Available but not widely used in clinical practice. Prospective randomized trial underway	III	(226, 617, 618)
Genetic markers				
<i>BRCA1</i>	For identifying individuals who are at high risk of developing breast or ovarian cancer in high risk families	In clinical use in specialized centers	Expert opinion	(324, 347, 382–384)
<i>BRCA2</i>	As for <i>BRCA1</i>	In clinical use in specialized centers	Expert opinion	(324, 347, 382–384)

^a LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.

^b CMF, cyclophosphamide, methotrexate, 5-fluorouracil; TPS, tissue polypeptide specific-antigen.

^c This effect may be due to amplification of the topoisomerase IIa gene (619, 620).

^d Recently, a specific gene profile (MammaPrint[®], Agendia, the Netherlands) was cleared by the FDA for determining prognosis in breast cancer patients <61 years of age with stage I or stage II breast cancer, with tumors 5 cm or less in size and lymph node–negative disease.

^e A molecular test, GeneSearch™ Breast Lymph Node (BLN) Assay (Veridex, Raritan, New Jersey), was recently cleared by the FDA for the detection of metastases in axillary sentinel lymph nodes.

In combination with established prognostic factors, i.e., tumor stage, tumor grade, and number of lymph node metastases, ER and PR may also be used for determining short-term prognosis in patients with newly diagnosed breast cancer [LOE, III; SOR, B].

HER-2 gene. In agreement with the ASCO (243), joint ASCO/College of American Pathologists (346), and NCCN Panels (347), the NACB Panel also recommends determination of the gene *HER-2*³⁶ on all newly diagnosed patients with invasive breast cancers (Table 12). At present, the primary purpose for determining *HER-2* is to select patients who may be treated with Trastuzumab in either early or advanced breast cancer. In combination with other factors, *HER-2* may also be

used to determine prognosis. Insufficient data are currently available to recommend *HER-2* for predicting response either to adjuvant endocrine therapy or to cyclophosphamide, methotrexate and 5-fluorouracil–based adjuvant chemotherapy (243, 348–351). *HER-2*, however, may be used to predict the superiority of anthracycline-based adjuvant chemotherapy over methotrexate and 5-fluorouracil–based adjuvant chemotherapy (243, 348–350, 352). Insufficient data are presently available to recommend routine use of serum *HER-2* testing. Preliminary findings, however, suggest that serum *HER-2* may be of value in monitoring patients with advanced breast cancer undergoing treatment with Trastuzumab (353).

Recommended assays for HER-2. Two main types of assay are used to detect *HER-2* in breast tumors, i.e., immunohistochemical analysis (IHC) and FISH (354–360). The advantages and disadvantages of these methods are summarized in Table 14 (354–360).

³⁶ *HER-2* and *NEU* are aliases for *ERBB2* [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)].

Table 12. Recommendations for use of markers in breast cancer by different expert groups.

Marker(s)	Application	ASCO (242, 243, 375)	EGTM (371)	Joint EGTM/NACB (15)	ESMO ^a (372, 373)	St Gallen Conference (350, 374)	NCCN (621)	NACB 2008	SOR ^b
ER + PR	For predicting response to hormone therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	A (for ER) B (for PR)
	For prognosis	Should not be used alone in determining prognosis	Yes, in combination with other factors	None published	None published	Yes	Yes	Yes, in combination with existing factors	B
HER-2	For predicting response to trastuzumab in early and advanced disease	Yes	Yes	None published	Yes	Yes	Yes	Yes	A
	For prognosis	No	Yes, in combination with other factors	None published	None published	Yes	Yes	Yes, in combination with other factors	B
For predicting response to hormone therapy		No	No	None published	None published	None published	None published	No	C
	For predicting response to adjuvant CMF	No	No	None published	None published	None published	None published	No	C
For predicting response to adjuvant anthracycline-based therapy		Yes	Yes	None published	None published	None published	Yes, may be used for predicting superiority of anthracycline-based over non-anthracycline-based adjuvant therapy	Yes, as per NCCN	B
	For determining prognosis	Yes, may be of value for determining prognosis in newly diagnosed node-negative patients	Yes	None published	None published	None published	None published	Yes	A (if ELISA used for assay)
OncoType DX test	For determining prognosis	Yes, for predicting risk of recurrence in patients treated with adjuvant tamoxifen	No	None published	None published	No	May be an option in specific subgroups for estimating probability of recurrence and benefit from chemotherapy (622)	Yes, for patients treated with adjuvant tamoxifen	A

Continued on page 49

Table 12. Recommendations for use of markers in breast cancer by different expert groups. (Continued from page e48)

Marker(s)	Application	ASCO (242, 243, 375)	EGTM (371)	Joint EGTM/NACB (15)	ESMO ^a (372, 373)	St Gallen Conference (350, 374)	NCCN (621)	NACB 2008	SOR ^b
CA 15-3/ BR27.29	Surveillance following surgery	No	Yes	Yes	No	No ^c	No	May provide lead-time for early detection of metastasis but clinical value of lead-time unclear	C
CA 15-3/ BR27.29	Monitoring therapy in advanced disease	Yes, in selected cases, e.g., in absence of measurable disease	Yes	Yes	Yes, in non easily measurable disease	None published	None published	Yes, especially in patients with non-evaluable disease	C
CEA	Surveillance following surgery	No	Yes	None published	No	No ^c	None published	No	C
	Monitoring therapy in advanced disease	Yes, in selected cases, e.g., in absence of measurable disease	Yes	None published	No	None published	None published	Yes, as per ASCO and EUSOMA	C
BRCA1/ BRCA2	For identifying women at high risk of developing breast cancer	See ref. (324) for general guidelines on genetic testing for cancer susceptibility	None published	None published	None published	Yes	Yes	NACB supports documents of CGSC, ASCO, USPSTF and St Gallen Consensus Group (324, 350, 382–384)	B

^a ESMO, European Society of Clinical Oncology; NR, no recommendation published; CGSC, Cancer Genetics Studies Consortium; USPSTF, US Preventive Services Task Force.

^b SOR (520), A, high (further research is very unlikely to change the panel's confidence in the estimate of effect); B, moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); C, low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate); D, very low (any estimate of effect is very uncertain).

^c Recommendations state tumor markers without referring to specific markers.

Table 13. Advantages and disadvantages of different assays for hormone receptors

Ligand-binding assay	ELISA	Immunohistochemistry
Advantages		
<ul style="list-style-type: none"> Quantitative Can determine functionality of receptor with respect to hormone binding Can determine K_m of receptor for ligand 	<ul style="list-style-type: none"> Quantitative No radioactivity required Simpler than ligand binding 	<ul style="list-style-type: none"> Simple and relatively inexpensive Can assess tissue architecture, distinguishing invasive, in situ and normal breast tissue Can use small amounts of tissue including fine needle aspirates and core needle biopsies Normal breast epithelial cells in adjacent tissue provide an internal positive control, at least for ER
Disadvantages		
<ul style="list-style-type: none"> Time-consuming Cumbersome Expensive Requires large amount of tumor tissue Requires frozen tissue (must be rapidly frozen in liquid nitrogen and maintained at low temperature) Requires radioactivity May yield false-negative ER values^a 	<ul style="list-style-type: none"> Requires large amount of frozen tissue Relatively time-consuming 	<ul style="list-style-type: none"> Semiquantitative Interpretation subjective Difficult to standardize Different antibodies can give different results
<p>^a In tumors removed from patients receiving tamoxifen, in which endogenous levels of steroid ligand are high, or when insufficient breast cancer is present in the tissue mass.</p>		

Following a systematic review of the literature, a joint ASCO/College of American Pathologists panel recently published comprehensive guidelines for HER-2 testing in patients with invasive breast cancer (346). Some of the key conclusions are as follows:

- As presently performed, approximately 20% of HER-2 testing may be inaccurate.
- When properly validated assays are used, existing data does not clearly show a superiority for either IHC or FISH for predicting response to Trastuzumab.
- HER-2 should be measured on the invasive component of the breast cancer.
- Laboratories performing HER-2 assays should show at least 95% concordance with another validated test.
- Validation of assays or modifications, the use of standard operating procedures, and compliance with new testing criteria should be monitored by using stringent laboratory accreditation standards, proficiency testing, and competency (346).

The ASCO/College of American Pathologists panel recommended the following algorithm for defining HER-2 status:

- HER-2 positivity was defined as IHC staining of 3+ (uniform and intense membrane staining of >30%

of invasive cancer cells), a FISH value >6 HER-2 gene copies per nucleus or a FISH ratio (HER-2:CEP 17) of >2.2 (CEP, centromeric probe for chromosome 17).

- HER-2 negativity was defined as an IHC score of 0 or 1+, a FISH value of <4 HER-2 gene copies per nucleus or a FISH ratio of <1.8.
- HER-2 IHC was regarded to be equivocal with a score of 2+, i.e., complete membrane staining that is either nonuniform or weak in intensity but with clear circumferential distribution in at least 10% of cells. The equivocal range for FISH was an HER-2:CEP 17 ratio from 1.8–2.2 or an average gene copy number from 4.0–6.0 for those assays without an internal probe. For samples with equivocal IHC scores, FISH should be performed. For samples with equivocal FISH results, the test should be either repeated or additional cells counted.

The NACB Panel supports the above recommendations.

Currently, the FDA has approved a number of assays for detecting HER-2 in breast cancer. Two of these assays are based on immunohistochemistry (Dako and Ventana Medical Systems) and 2 on FISH (Ventana

Table 14. Advantages and disadvantages of different assays for HER-2 IHC^a

Immunohistochemistry	FISH
Advantages	
<ul style="list-style-type: none"> • Low cost • Simple • Widely available 	<ul style="list-style-type: none"> • Relatively more objective scoring system and easier to standardize • Provides a more robust signal than immunohistochemistry
Disadvantages	
<ul style="list-style-type: none"> • Evaluation is subjective and thus difficult to standardize • Loss of sensitivity due to antigenic alteration due to fixation • Wide variability in sensitivity of different antibodies and different results from the same antibody, depending on staining procedure • Borderline values (e.g. 2+) require additional testing, such as FISH 	<ul style="list-style-type: none"> • Relatively expensive • Less widely available than immunohistochemistry (requires fluorescent microscope) • May sometimes be difficult to identify carcinoma in tissues with ductal carcinoma in situ • Requires longer time for scoring than immunohistochemistry • Unable to preserve slide for storage and review • Cutoff to establish critical level of amplification and clinical outcome uncertain
^a Data summarized from refs (354–360).	

Medical Systems and Vysis Inc). Both IHC assays were originally approved for identifying women with advanced breast cancer for therapy with Trastuzumab. The FISH-based tests were originally cleared for the selection of women with node-negative disease at high risk for progression and for response to doxorubicin-based therapy. More recently, these tests have also been approved for selecting women with metastatic breast cancer for treatment with Trastuzumab. In 2008, the FDA gave premarket clearance for a new chromogenic in situ hybridization (CISH) assay (Invitrogen) for identifying patients eligible for Trastuzumab. A serum-based-HER-2 test has been cleared by the FDA for follow-up and monitoring patients with advanced breast cancer (Siemens Healthcare Diagnostics).

**NACB BREAST CANCER PANEL RECOMMENDATION 2:
HER-2 AS A PREDICTIVE AND PROGNOSTIC MARKER**
HER-2 should be measured in all patients with invasive breast cancer. The primary purpose of measuring HER-2 is to select patients with breast cancer that may be treated with Trastuzumab [LOE, I; SOR, A].
HER-2 may also identify patients that preferentially benefit from anthracycline-based adjuvant chemotherapy [LOE, II/III; SOR, B].

uPA and PAI-1. Results from a pooled analysis comprising more than 8000 patients have shown that both uPA and PAI-1 are strong (relative risk >2) and inde-

pendent (i.e., independent of nodal metastases, tumor size, and hormone receptor status) prognostic factors in breast cancer (361). For axillary node-negative patients, the prognostic impact of these 2 proteins has been validated by use of both a randomized prospective trial (Chemo N₀ study) and a pooled analysis of small-scale retrospective and prospective studies (361, 362). uPA and PAI-1 are thus the first biological factors in breast cancer to have their prognostic value validated by using level 1-evidence studies (363).

The NACB Panel therefore states that testing for uPA and PAI-1 may be carried out to identify lymph node-negative patients who do not need or are unlikely to benefit from adjuvant chemotherapy. Measurement of both proteins should be performed because the information provided by the combination is superior to that from either alone (361, 364). Lymph node-negative patients with low levels of both uPA and PAI-1 have a low risk of disease relapse and thus may be spared from the toxic side effects and costs of adjuvant chemotherapy. Lymph node-negative women with high levels of either uPA or PAI-1 should be treated with adjuvant chemotherapy. Indeed, results from the Chemo N₀ trial (362) as well as data from recent large retrospective studies (364, 365) suggest that patients with high levels of uPA/PAI-1 derive an enhanced benefit from adjuvant chemotherapy.

Recommended assays for uPA and PAI-1. Measurement of both uPA and PAI-1 should be carried out using a

validated ELISA. A number of ELISAs have undergone technical validation (366), and some have also been evaluated in an external quality assessment scheme (367). For determining prognosis in breast cancer, the NACB Panel recommends use of an ELISA that has been both technically and clinically validated (e.g., from American Diagnostic). Extraction of tumor tissue with Triton X-100 is recommended (368). It is important to note that to perform an ELISA for uPA or PAI-1, a representative piece of fresh (i.e., not fixed in formalin) breast tumor (>200–300 mg) must be stored in liquid nitrogen immediately after histological diagnosis.

Recently, a microassay using as little as 100 mg of tumor tissue was described for the measurement of uPA and PAI-1 (369, 370). This assay can also use material from 2 or 3 core biopsies or from 5–10 90- μ m-thick cryosections. Although not yet clinically validated, preliminary data showed that uPA and PAI-1 levels in core biopsies correlated well with corresponding levels in surgically removed tissue. Because IHC determination of uPA/PAI-1 has not yet been clinically validated, this method cannot be recommended, at present, for the routine determination of these proteins in breast cancer.

NACB BREAST CANCER PANEL RECOMMENDATION 3:

uPA AND PAI-1 FOR DETERMINING PROGNOSIS

uPA and PAI-1 may be used to identify lymph node-negative breast cancer patients who do not need or are unlikely to benefit from adjuvant chemotherapy. uPA and PAI-1 should be measured by a validated ELISA using extracts of fresh or freshly frozen tumor [LOE, I; SOR, A].

CA 15-3/BR 27.29. The CA 15-3 and BR 27.29 (also known as CA 27.29) serum assays detect the same antigen, i.e., MUC1 protein, and provide similar clinical information. CA 15-3 has, however, been more widely investigated than BR 27.29. There are conflicting views about the value of CA 15-3 and BR 27.29 in the post-operative surveillance of asymptomatic patients who have undergone curative surgery for breast cancer (15, 242, 243, 371–375). Although increasing CA 15-3 or BR 27.29 levels can preclinically detect distant metastatic disease in approximately 70% of asymptomatic patients, there is no high-level evidence study showing that the early diagnosis of progressive disease followed by initiation of therapy positively impacts either patient survival or quality of life. Furthermore, there is no universally accepted or clinically validated definition of a clinically significant tumor marker increase. A con-

firmed increase of at least 25% however, is widely interpreted to signify a clinically significant increase.

Based on current evidence, the NACB Panel recommends against routine CA 15-3 (or BR 27.29) testing in asymptomatic patients following diagnosis of operable breast cancer. The Panel, however, would like to note that there are a number of small studies suggesting that the early initiation of therapy based on increasing serum markers levels can lead to an enhanced outcome (376–378). Although these studies do not provide high-level evidence that early treatment based on rising tumor marker levels positively impacts on patient outcome, some doctors as well as some patients may wish to have serial levels of CA 15-3 (or BR 27.29) determined following primary surgery. The ultimate decision about whether or not to use CA 15-3 (BR 27.29) in this situation must be made by the doctor in consultation with the patient.

According to both ASCO and NCCN, CA 15-3 (or BR 27.29) should not be used alone for monitoring therapy in advanced disease (242, 243, 347, 375). The EGTM Panel recommends that in patients with metastatic disease, markers should be determined before each course of chemotherapy and at least every 3 months for patients receiving hormone therapy (371).

The NACB Panel states that CA 15-3 or BR 27.29 in combination with imaging and clinical examination may be used to monitor chemotherapy in patients with advanced breast cancer. These markers may be particularly helpful in patients with nonevaluable disease. In such patients, 2 successive increases (e.g., each >30%) are likely to indicate progressive disease and may result in cessation of therapy, change in therapy, or entry of the patient into clinical trials evaluating new anticancer treatments. However, as with markers during postoperative surveillance, there is no universally accepted or clinically validated definition of a clinically significant increase in marker concentration during therapy of advanced disease.

It is important to bear in mind that following the initiation of chemotherapy, a transient increase in serum marker levels may occur (379, 380). Such transient increases or spikes usually subside within 6–12 weeks after starting chemotherapy. Increases in marker levels unrelated to tumor progression might also occur as a result of certain benign diseases (381). These increases may be transient or progressive depending on whether the benign disease is short-lived or continues to deteriorate.

Recommended assays for CA 15-3/BR 27.29. The FDA has cleared a number of commercially available CA 15-3 and BR 27.29 assays.

**NACB BREAST CANCER PANEL RECOMMENDATION 4:
CA 15-3 AND BR 27.29 IN POSTOPERATIVE SURVEILLANCE
AND MONITORING THERAPY IN ADVANCED DISEASE**

CA 15-3 and BR 27.29 should not be routinely used for the early detection of recurrences/metastases in asymptomatic patients with diagnosed breast cancer. However, because some patients, as well as some doctors, may wish to have these measurements, the ultimate decision on whether or not to use CA 15-3 or BR 27.29 must be made by the doctor in consultation with the patient [LOE, III; SOR, B].

In combination with radiology and clinical examination, CA 15-3 or BR 27.29 may be used to monitor chemotherapy in patients with advanced breast cancer. For patients with nonevaluable disease, sustained increases in marker concentrations suggest progressive disease [LOE, III; SOR, B].

Carcinoembryonic antigen. As for CA 15-3 and BR 27.29, the NACB Panel does not recommend routine use of CEA in the surveillance of patients with diagnosed breast cancer. For monitoring patients with advanced disease, CEA should not be used alone. For monitoring patients with nonevaluable disease, CEA may occasionally be informative when CA 15-3/BR 27.29 is not. As a marker for breast cancer, CEA is generally less sensitive than CA 15-3/BR 27.29, but on occasion, CEA can be informative when levels of MUC-1-related markers remain below the cutoff point.

Recommended assay for CEA. The FDA has cleared a number of commercially available CEA assays.

**NACB BREAST CANCER PANEL RECOMMENDATION 5:
CEA IN POSTOPERATIVE SURVEILLANCE AND MONITORING
OF THERAPY IN ADVANCED DISEASE**

CEA should not be routinely used for the early detection of recurrences/metastases in patients with diagnosed breast cancer. However, because some patients as well as some doctors may wish to have these measurements, the ultimate decision on whether or not to use CEA must be made by the doctor in consultation with the patient [LOE, III; SOR, B].

In conjunction with radiology and clinical examination, CEA may be used to monitor chemotherapy in patients with advanced breast cancer. In patients with nonevaluable disease, sustained increases in CEA concentrations suggest progressive disease [LOE, III; SOR, B].

BRCA1 and BRCA2 genes. According to the task force of the Cancer Genetics Studies Consortium, early breast and ovarian cancer screening are recommended for individuals with breast cancer 1, early onset (*BRCA1*) mutations and early breast cancer screening for those with breast cancer 2, early onset (*BRCA2*) mutations (382). No recommendation, however, was made for or against prophylactic surgery (e.g., mastectomy or oophorectomy). The guidelines further stated that “these surgeries are an option for mutation carriers, but evidence of benefit is lacking, and case reports have documented the occurrence of cancer following prophylactic surgery. It is recommended that individuals considering genetic testing be counselled regarding the unknown efficacy of measures to reduce risk and that care for individuals with cancer-predisposing mutations be provided whenever possible within the context of research protocols designed to evaluate clinical outcome” (382). It is important to point out that these guidelines were based on expert opinion only.

In 2003, an ASCO Panel published a detailed policy statement regarding genetic testing for cancer susceptibility (324). This statement included recommendations in the following areas: indications for genetic testing, regulation of testing, insurance reimbursement, protection from discrimination, confidentiality issues associated with genetic testing, and continuing educational challenges and special research issues surrounding genetic testing of human tissues.

According to the 2005 Consensus Panel of the 8th St Gallen Conference, treatment decisions for women with mutations in *BRCA1* or *BRCA2* genes “need to include consideration of bilateral mastectomy with plastic surgical reconstruction, prophylactic oophorectomy, chemoprevention and intensified surveillance” (350).

The NACB Panel supports the statements published by the Cancer Genetics Studies Consortium, ASCO, US Preventive Services Task Force, and the St Gallen Consensus Panel (324, 350, 382–384).

**NACB BREAST CANCER PANEL RECOMMENDATION 6:
BRCA1 AND BRCA2 MUTATION TESTING FOR IDENTIFYING
WOMEN AT HIGH RISK OF DEVELOPING BREAST CANCER**

BRCA1 and BRCA2 mutation testing may be used for identifying women who are at high risk of developing breast or ovarian cancer in high-risk families. For those with such mutations, screening should begin at 25–30 years of age. Insufficient data exist, however, to recommend a specific surveillance/screening strategy for young women at high risk. Appropriate counselling should be given to any individual considering BRCA1/2 testing [LOE, expert opinion; SOR, B].

MULTIGENE GENE SIGNATURES

Gene expression profiling. Gene expression profiling uses microarray technology to measure the simultaneous expression of thousands of genes. At least 8 gene signatures have been described for predicting outcome in patients with breast cancer [for review, see ref (385)]. Although these signatures contain few genes that overlap, most give similar prognostic information (386).

In one of the first clinical microarray studies, van't Veer et al. (387) described a 70-gene signature that correctly predicted the later appearance of distant metastasis in 65 of 78 patients with newly diagnosed lymph node–negative breast cancer patients younger than 55 years who had not received systemic treatment. Application of this signature to an independent set of 19 breast cancers resulted in only 2 incorrect classifications. This 70-gene signature was subsequently both internally (388) and externally validated (389). In both the internal and external validations studies, the prognostic impact of the gene signature was independent of the conventional prognostic factors for breast cancer.

Currently this 70-gene signature is undergoing prospective validation as part of the Microarray for Node-Negative Disease Avoids Chemotherapy (MINDACT) trial (390). The primary objective of this trial is to establish if lymph node–negative breast cancer patients with low risk of recurrence based on the above gene signature but at high risk of recurrence based on clinicopathological factors can be safely spared adjuvant chemotherapy without affecting distant metastasis-free survival.

**NACB BREAST CANCER PANEL RECOMMENDATION 7:
GENE EXPRESSION PROFILING, AS DETERMINED BY
MICROARRAY, FOR PREDICTING OUTCOME**

None of the microarray-based gene signatures currently available should be routinely used for predicting patient outcome [LOE, III; SOR, B].

Oncotype DX™ test. Oncotype DX™ is a multigene assay that quantifies the likelihood of breast cancer recurrence in women with newly diagnosed, early-stage breast cancer (for review, see (391)). Rather than using microarray technology, this test uses reverse-transcription PCR to measure the expression of 21 genes (16 cancer-associated and 5 control genes). Based on the expression of these genes, a recurrence score (RS) was calculated that predicted low, intermediate, and high risk of distant metastasis for ER-positive patients treated with adjuvant tamoxifen (392). The RS was prospectively validated in an independent population of lymph node–negative ER-positive patients treated with adjuvant tamoxifen, as part of the National Surgi-

cal Adjuvant Breast and Bowel Project trial B14 (392). In this validation study, the RS was an independent predictor of patient outcome. The independent prognostic impact of the RS was later confirmed in a population-based case-control study (393). Although a low RS predicted good outcome in patients treated with adjuvant tamoxifen, a high RS was found to be associated with favorable outcome in patients treated with either neoadjuvant or adjuvant chemotherapy (394, 395). A particular advantage of this test is that it may be carried out on formal-fixed paraffin embedded tissue.

Currently, the RS is undergoing prospective validation as part of the Trial Assigning Individualized Options for Treatment (TAILORx) trial (396). In this trial, patients with intermediate RS are being randomized to receive hormonal therapy alone or hormone therapy plus chemotherapy. The aim is to establish if adjuvant chemotherapy improves survival in the group of patients with the intermediate score. Also, in this trial, patients with low RS after tamoxifen therapy will receive endocrine treatment, whereas those with high RS will be given chemotherapy and hormone therapy.

**NACB BREAST CANCER PANEL RECOMMENDATION 8:
ONCOTYPE DX TEST FOR PREDICTING OUTCOME**

The Oncotype DX test may be used for predicting recurrence in lymph node–negative, ER-positive patients receiving adjuvant tamoxifen. Patients predicted to have a good outcome may be able to avoid having to undergo treatment with adjuvant chemotherapy [LOE, I/II; SOR, A].

The Oncotype DX test may also be used to predict benefit from adjuvant chemotherapy (cyclophosphamide-methotrexate-5-fluorouracil or methotrexate-5-fluorouracil) in node-negative, ER-positive patients, i.e., patients with a high recurrence score appear to derive greater benefit from chemotherapy than those with low scores [LOE, III; SOR, B].

KEY POINTS: TUMOR MARKERS IN BREAST CANCER

The best-validated markers in breast cancer are all tissue based and include ER, PR, HER-2, uPA, and PAI-1. Assay of ER, PR, and HER-2 is now mandatory for all newly diagnosed breast cancer patients. The measurement of uPA and PAI-1, although technically and clinically validated (361–363, 366, 367), is not presently in widespread clinical use, mainly due to the requirement of a minimum amount of fresh or freshly frozen tissue. Assay of these proteins however, may be used to aid in the selection of lymph node–negative breast cancer patients who do not need adjuvant chemotherapy. Similarly, the Oncotype DX test may be used for predicting recurrence in lymph node–negative, ER-positive pa-

tients receiving adjuvant tamoxifen. Although widely used in postoperative surveillance and monitoring therapy in advanced disease, the clinical value of CA 15-3 and other serum markers has not yet been validated by a level I evidence study.

Tumor Markers in Ovarian Cancer^{37,38}

BACKGROUND

In the US, ovarian cancer is among the top 4 most lethal malignant diseases in women, who have a lifetime probability of developing the disease of 1 in 59 (397). Worldwide, the incidence of ovarian cancer was estimated as 204 499 cases per year with a corresponding 124 860 deaths (398).

The overall mortality of ovarian cancer is still high despite new chemotherapeutic agents, which have significantly improved the 5-year survival rate (118). The main reason for high mortality is lack of success in diagnosing ovarian cancer at an early stage, because the great majority of patients with advanced stage ovarian carcinoma die of the disease. In contrast, if ovarian cancer is detected early, 90% of those with well-differentiated disease confined to the ovary survive. Furthermore, biomarkers that can reliably predict clinical behavior and response to treatment are generally lacking. The search for tumor markers for the early detection and outcome prediction of ovarian carcinoma is therefore of profound importance and is a subject of critical importance in the study of ovarian cancer.

Although ovarian cancer is often considered to be a single disease, it is composed of several related but distinct tumor categories including surface epithelial tumors, sex-cord stromal tumors, and germ cell tumors (399). Within each category are several histological subtypes. Of these, epithelial tumors (carcinomas) are the most common and are divided, according to the International Federation of Gynecology and Obstetrics (FIGO) and WHO classifications, into 5 histologic types: serous, mucinous, endometrioid, clear cell, and transitional (400). The different types of ovarian cancers are not only histologically distinct but are characterized by different clinical behavior, tumorigenesis, and pattern of gene expression. Based on prevalence and mortality, serous ovarian carcinoma is the most important and represents the majority of all primary ovarian carcinomas (401). Therefore, unless otherwise specified, serous carcinoma is what is generally thought of as "ovarian cancer."

The search for more effective biomarkers depends on a better understanding of the pathogenesis of ovarian cancer, i.e., the molecular events in its development. Based on a review of recent clinicopathological and molecular studies, a model for the development of ovarian carcinomas has been proposed (402). In this model, surface epithelial tumors are divided into 2 broad categories designated type I and type II tumors, which correspond to 2 main pathways of tumorigenesis.

Type I tumors tend to be low-grade neoplasms that arise in a stepwise fashion from borderline tumors, whereas type II tumors are high-grade neoplasms for which morphologically recognizable precursor lesions have not been identified, so-called *de novo* development. Because serous tumors are the most common surface epithelial tumors, low-grade serous carcinoma is the prototypic type I tumor, and high-grade serous carcinoma is the prototypic type II tumor.

In addition to low-grade serous carcinomas, type I tumors are composed of mucinous carcinomas, endometrioid carcinomas, malignant Brenner tumors, and clear cell carcinomas. Type I tumors are associated with distinct molecular changes that are rarely found in type II tumors, such as *BRAF*³⁹ and *KRAS* mutations for serous tumors, *KRAS* mutations for mucinous tumors, and β -catenin and *PTEN* mutations and MSI for endometrioid tumors.

Type II tumors include high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcoma), and undifferentiated carcinoma. There are very limited data on the molecular alterations associated with type II tumors, except frequent p53 mutations in high-grade serous carcinomas and malignant mixed mesodermal tumors (carcinosarcomas). This model of carcinogenesis provides a molecular platform for the discovery of new ovarian cancer markers.

To prepare these guidelines, the literature relevant to the use of tumor markers in ovarian cancer was reviewed. Particular attention was given to reviews including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence-based.

CURRENTLY AVAILABLE MARKERS FOR OVARIAN CANCER

The most widely studied ovarian cancer body fluid- and tissue-based tumor markers for ovarian cancer are listed in Table 15, which also summarizes the phase of

³⁷ NACB Ovarian Cancer Sub-Committee Members: Daniel W. Chan, Chair; Robert C. Bast, Jr.; le-Ming Shih; Lori J. Sokoll; and György Sölétormos.

³⁸ All comments received about the NACB Recommendations for Ovarian Cancer are included in the online Data Supplement. Professor Gordon Rustin, Professor Bengt Tholander, and Professor M. Tuxen were invited expert reviewers.

³⁹ *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *PTEN*, phosphatase and tensin homolog.

Table 15. Currently available serum markers for ovarian cancer.

Cancer marker	Proposed uses	Phase of development	LOE ^a	References
CA125 ^b	Differential diagnosis of pelvic masses	Accepted clinical use	III	(407, 411)
	Monitoring treatment with chemotherapy	Accepted clinical use	I, II	(407, 408, 411, 428, 623–627)
Her-2/neu	Tissue marker for prognosis prediction and treatment outcome	Evaluation	IV	(628)
Akt-2	Tissue marker for prognosis prediction	Research/discovery	V	(500)
Inhibin	Detection	Evaluation	IV	(506–508)
HLA-G	Differential diagnosis	Research/discovery	V	(629)
TATI	Tumor monitoring	Research/discovery	IV, V	(480)
CASA	Tumor monitoring, prognosis prediction	Research/discovery	IV	(473, 482–484, 630)
TPA	Tumor monitoring	Research/discovery	IV	(472, 473)
CEA	Tumor monitoring	Research/discovery	IV	(473)
LPA	Detection	Evaluation	IV, V	(474, 631)
PAI-1	Prognosis prediction	Research/discovery	V	(485, 486, 632)
Interleukin-6	Prognosis prediction	Research/discovery	IV	(487–489)
Kallikreins 5, 6, 7, 8, 9, 10, 11, 13, 14, 15	Differential diagnosis, tumor monitoring, prognosis prediction	Research/discovery	IV, V	(445–465)
hCGβcf	Prognosis prediction	Evaluation	III, IV	(491, 492)
Prostasin	Differential diagnosis	Research/discovery	IV	(470)
Osteopontin	Tumor monitoring	Research/discovery	III, IV	(468, 469, 633, 634)
HE4 ^c	Differential diagnosis of pelvic masses, monitoring therapy	In clinical use in some centers	III, IV	(635–637)
Mitogen-activated protein kinase	Tissue marker for prognosis prediction	Research/discovery	V	(504, 505)
Insulin-like growth factor binding protein-2 (IGFBP-2)	Prognosis prediction	Research/discovery	IV	(638)
RSF-1	Prognosis prediction	Research/discovery	V	(512, 513)
NAC-1	Prognosis prediction	Research/discovery	V	(516, 518)

^a LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.

^b Refer to Table 16 for additional information.

^c Note added at proofs stage: HE4 was recently cleared by the FDA as an aid for monitoring patients with ovarian cancer.

development of each marker and LOE for its clinical use. The LOE grading system is based on a previous report describing the framework to evaluate clinical utility of tumor markers (120). The following discussion will focus mainly on CA125, which is the most widely investigated marker in ovarian cancer.

TUMOR MARKERS IN OVARIAN CANCER:

NACB RECOMMENDATIONS

Several organizations, including the EGTM (403, 404), the American College of Physicians (405), the European Society for Medical Oncology (406) and the NCCN (407) have developed guidelines for the use of

CA125 as a tumor marker for ovarian cancer. In addition, an NIH Consensus Conference on screening, prevention, diagnosis, and treatment of ovarian cancer was held in 1994 (408). Recommendations from these groups are summarized in Table 16. Table 16 also includes previous recommendations from the NACB as well as current recommendations based on the information below and other established guidelines.

CA125

In 1981, Bast et al. identified the CA125 antigen with the development of the OC 125 murine monoclonal antibody against cell line OVCA 433, which was de-

Table 16. Recommendations for use of CA125 as a tumor marker in ovarian cancer by different expert groups.

Use	American College of Physicians (405)	EGTM 2005 (404)	ESMO ^a (406)	NACB and EGTM 2002 (15)	NCCN (639)	NIH Panel (408)	NACB 2008	
							Recommendation	LOE ^b SOR ^c
Screening—no family history or other risk factors	No	No	None published	No	None published	No	No	III B
Early detection in hereditary syndromes, with transvaginal ultrasound	No	Yes	None published	Yes	None published	Yes	Yes	III B
Differential diagnosis—suspicious pelvic mass	None published	Yes (Postmenopausal women only)	None published	Yes (Postmenopausal women only)	Yes	Yes (Postmenopausal women)	Yes (Postmenopausal women)	III/IV A
Monitoring therapy	None published	Yes	Yes	Yes	Yes	None published	Yes	I/II A
Detection of recurrence	None published	Yes, in certain situations	Yes	Yes	Yes	Yes	Yes	III B
Prognosis	None published	No	Yes	Yes	None published	Yes	Yes	III A/B

^a ESMO, European Society of Clinical Oncology.

^b LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV, evidence from small retrospective studies; level V, evidence from small pilot studies.

^c SOR (520), A, high (further research is very unlikely to change the panel's confidence in the estimate of effect); B, moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); C, low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate); D, very low (any estimate of effect is very uncertain).

rived from a patient with ovarian serous carcinoma (409). The CA125 molecule has since been cloned by use of a partial cDNA sequence originating from the peptide core of the molecule identified (410). This new mucin molecule has been designated CA125/MUC16 [mucin 16, cell surface associated (*MUC16*) gene] and consists of a 156–amino-acid tandem-repeat region in the *N*-terminus and a possible transmembrane region and tyrosine phosphorylation site in the *C*-terminus.

The first immunoassay for CA125, commercialized in 1983, used the OC 125 antibody for both capture and detection (411, 412). A second-generation assay (CA125 II) was subsequently developed, incorporating M11 and OC 125 antibodies, which have distinct nonoverlapping epitopes. Assays for CA125 have since been adapted to automated platforms, and although the majority of manufacturers quote a similar reference interval, concentrations of CA125 may vary among manufacturers owing to differences in calibration, assay design, and reagent specificities. The lack of an International Standard for CA125 hampers progress in improving between-method comparability, and the clinical and laboratory communities should work toward producing and adopting such a standard. For the present, values from different methods are not interchangeable, and patients who are serially monitored should be rebaselined if there is a change in methodology (413). Manufacturers should specify the standard preparation against which their method is calibrated, and laboratories should indicate the CA125 method used on their clinical reports.

The cutoff of 35 kU/L for the CA125 and CA125II assays was determined from the distribution of values in healthy individuals so as to include 99% of normals (414). Values tend to decline with menopause and aging (415). It has recently been reported that CA125II concentrations vary 20%–50% by race in postmenopausal women, with concentrations in African and Asian women lower than in white women (415). Menstrual cycle variations can also be found (412). Increased values may be found in 1%–2% of normal healthy individuals, 5% of those with benign diseases, and 28% of those with nongynecologic cancers (15, 411, 412).

It is recommended that analysis be performed shortly after prompt centrifugation of the specimen and separation of serum from the clot, and that specimens be stored at either 4 °C (1–5 days) or –20 °C (2 weeks–3 months) in the short term, or –70 °C in the long term to ensure stability (15). Plasma is an acceptable specimen type for some assays, where indicated by the manufacturer. As in other immunoassays, assay interferences may be observed if heterophilic antibodies are present in the serum, particularly following therapeutic or diagnostic use of monoclonal antibodies.

**NACB OVARIAN CANCER PANEL RECOMMENDATION 1:
HANDLING OF SPECIMENS FOR CA125 DETERMINATION**

Analysis should be performed shortly after prompt centrifugation of the specimen and separation of serum from the clot, and specimens stored at either 4 °C (1–5 days) or –20 °C (2 weeks–3 months) in the short term or –70 °C in the long term [LOE, not applicable; SOR, A].

The recommendations of the current NACB panel and other groups with respect to the potential clinical utility for CA125 are summarized in Table 16 and are described below.

SCREENING/EARLY DETECTION

In women with epithelial ovarian cancer, 80% have CA125 levels >35 kU/L, with elevations of 50%–60% in clinically detected stage I disease, 90% in stage II, and >90% in stages III and IV (412, 416). Concentrations correlate with tumor burden and stage. Owing to the lack of sensitivity and specificity for a single determination of the marker, CA125 is not recommended for use in screening asymptomatic women by the NACB Panel or by other authoritative organizations (15, 403, 405–408). An NIH Consensus Development Panel has concluded that evidence is not yet available to indicate that either CA125 or transvaginal ultrasonography effectively reduce mortality from ovarian cancer (408). However, the same panel did recommend annual CA125 determinations, in addition to pelvic and ultrasound examinations, in women with a history of hereditary ovarian cancer who have an estimated lifetime risk of 40%, because early intervention may be beneficial.

A number of approaches have been proposed to improve the specificity of CA125 for early detection, because very high specificity (99.7%) is needed to achieve an acceptable positive predictive value of 10% with a prevalence of disease of 40 per 100 000 in women older than 50 years (417). Strategies have included sequential or 2-stage strategies combining CA125 with ultrasound, longitudinal measurements of CA125, and measurement of CA125 in combination with other markers such as OVX1, macrophage colony-stimulating factor, or other new biomarkers discovered using proteomic profiling approaches (411, 417–419). To evaluate the potential role for CA125 in screening for ovarian cancer in asymptomatic populations, 2 major prospective randomized trials are currently in progress in the US (420) and the United Kingdom (421). In total 200 000 women will be randomized to either screening with ultrasound, screening with CA125 plus ultrasound, or no screening. The studies are adequately powered to detect a significant improvement in sur-

vival among women screened with serial CA125 measurements and transvaginal sonography.

NACB OVARIAN CANCER PANEL RECOMMENDATION 2:

CA125 IN SCREENING

CA125 is not recommended for screening asymptomatic women [LOE, III; SOR, B].

CA125 is recommended, together with transvaginal ultrasound, for early detection of ovarian cancer in women with hereditary syndromes because early intervention may be beneficial in these women [LOE, III; SOR B].

DISCRIMINATION OF PELVIC MASSES

In contrast to its use in early detection, CA125 is more widely accepted as an adjunct in distinguishing benign from malignant disease in women, particularly in postmenopausal women presenting with ovarian masses (407, 408, 422), facilitating triage for operations by optimally qualified surgeons. Benign conditions resulting in increased CA125 levels may be a confounding factor in premenopausal women. In the United Kingdom CA125 measurement is an integral part of the RMI (risk of malignancy index), which forms the basis of patient pathway guidelines for the management of pelvic masses and/or adnexal cysts (423). The RMI is calculated as a product of CA125 concentration multiplied by menopausal status (1 for premenopausal and 3 for postmenopausal) multiplied by ultrasound score (0, 1, or 3 depending on ultrasound features). A cutoff of 200 or 250 is frequently used, with patients with scores above this referred to specialist gynecology–oncology teams. Sensitivities of 71%–78% and specificities of 75%–94% have been reported in other studies (414). Increased concentrations of CA125 >95 kU/L in postmenopausal women can discriminate malignant from benign pelvic masses with a positive predictive value of 95% (411). Therefore, based on current evidence, CA125 is recommended as an adjunct in distinguishing benign from malignant pelvic masses, particularly in postmenopausal women. When there is a suspicion of germ cell tumor, particularly in women younger than 40 years or in older women where scan features suggest a germ cell tumor, AFP and hCG are also important markers for triage, as for testicular germ cell tumors.

NACB OVARIAN CANCER PANEL RECOMMENDATION 3:

CA125 IN DISCRIMINATION OF PELVIC MASSES

CA125 is recommended as an adjunct in distinguishing benign from malignant suspicious pelvic masses, particularly in postmenopausal women [LOE, III/IV; SOR, A].

MONITORING TREATMENT

Serial measurement of CA125 may also play a role in monitoring response to chemotherapy. Declining CA125 concentrations appear to correlate with treatment response even when disease is not detectable by either palpation or imaging. In a meta-analysis, serial CA125 concentrations in 89% of 531 patients correlated with clinical outcome of disease (424–426). There is general consensus among current guidelines in recommending that CA125 be used to monitor therapeutic response, but there is no consensus as to how best to define a CA125-based response (404, 427, 428). A response has been defined as a reduction of 50% or more in pretreatment CA125 level that is maintained for at least 28 days (428–431). The pretreatment sample must be at least twice the upper limit of the reference range, which means that patients with pretreatment concentrations between the upper limit and twice the upper limit are nonassessable by this criterion. The first sample is recommended within 2 weeks before treatment, with subsequent samples at 2–4 weeks during treatment and at intervals of 2–3 weeks during follow-up. The same assay method is required throughout, and patients who received immunotherapy (mouse antibodies) cannot be evaluated.

In addition to monitoring initial chemotherapeutic regimens, CA125 measurements may be useful in monitoring salvage therapy, because a doubling of values is associated with disease progression and treatment failure in more than 90% of cases (411). However, disease progression may also occur without an increase in CA125, and therefore the presence of tumor should also be assessed by physical examination and imaging (15). Tuxen et al. (427) suggested that interpretation of changes in serial CA125 levels should be based on a statistical estimation that takes account both the analytical variation of the method used and of the normal background intraindividual biological variation of the marker (432, 433). The theoretical background for this statistical procedure has recently been reviewed in detail (434). Serial measurement of CA125 to aid in monitoring response to therapy is a second FDA-indicated use for the marker. Trials currently in progress, including the UK Medical Research Council OV05 trial, have been designed to evaluate the benefit of early chemotherapy for recurrent ovarian cancer, based on a raised CA125 level alone, vs chemotherapy based on conventional clinical indicators (435). Pending results of these trials, practice is likely to vary.

NACB OVARIAN CANCER PANEL RECOMMENDATION 4:

CA125 IN MONITORING TREATMENT

CA125 measurements may be used to monitor response to chemotherapeutic response. The first

sample should be taken within 2 weeks before treatment with subsequent samples at 2–4 weeks during treatment and at intervals of 2–3 weeks during follow-up. The same assay method should be used throughout, and patients who received therapy with anti-CA125 antibodies cannot be evaluated [LOE, I/II; SOR, A].

CA125 MEASUREMENT POSTOPERATIVELY: SECOND-LOOK OPERATION

Early studies on CA125 indicated that it was useful postoperatively in predicting the likelihood that tumor would be found at a second-look operation, and CA125 assays were initially cleared by the FDA for this indication (412, 424). Elevations of CA125 > 35 kU/L after debulking surgery and chemotherapy indicate that residual disease is likely (>95% accuracy) and that chemotherapy will be required (436). Second-look laparotomy is now considered to be controversial and suggested only for patients enrolled in clinical trials or in situations in which surgical findings would alter clinical management. Monitoring with CA125 testing in women with increased preoperative CA125 concentrations, along with a routine history and physical and rectovaginal pelvic examination, has been advocated instead of surgery for asymptomatic women after primary therapy (408).

CA125 MEASUREMENT POSTOPERATIVELY: DETECTION OF RECURRENCE

Increased, rising, or doubling CA125 concentrations predict relapse. However, it should be noted that postoperative CA125 levels below the cutoff concentration do not necessarily exclude disease presence.

The Gynecologic Cancer Intergroup (GCIg) is an organization consisting of representatives from thirteen international groups performing clinical trials in gynecologic cancer (437). The GCIg has defined criteria progression using serial CA125 measurements (431) as:

- CA125 concentrations \geq twice the upper limit of normal on 2 occasions in patients with increased CA125 levels pretreatment that normalize, or patients with CA125 in the reference range *or*
- CA125 concentrations \geq the nadir value on 2 occasions in patients with increased CA125 levels pretreatment that do not normalize.

The 2 measurements must be at least 1 week apart (431).

Although monitoring intervals are as yet undefined, current practice suggests following patients every 2 to 4 months for 2 years and then less frequently (407). Ele-

vations in CA125 can precede clinical or radiological evidence of recurrence, with a median time of 2 to 6 months, although there is no evidence to date that initiating salvage chemotherapy before clinical recurrence improves survival (436). Early detection of recurrent disease, however, permits the timely evaluation of the multiple drugs available for salvage therapy. Because only a fraction of patients will respond to any single drug and reliable predictive tests are not yet available, chemotherapeutic agents are generally used individually and sequentially to identify those drugs that are active against a particular patient's cancer. Given the modest difference between time to recurrence and overall survival, early detection of recurrence provides time in which to identify effective palliative therapy. Therefore, measurement of CA125 at follow-up visits is recommended if values were initially increased. Low preoperative concentrations do not exclude the possibility that CA125 concentrations may increase above the cutoff before clinical relapse, and progressive increases in CA125 within the reference interval may be predictive of recurrence (438).

NACB OVARIAN CANCER PANEL RECOMMENDATION 5: CA125 IN MONITORING PATIENTS AFTER THERAPY

Measurement of CA125 at follow-up visits is recommended if values were initially increased. Although monitoring intervals are as yet undefined, current practice suggests following patients every 2 to 4 months for 2 years and then less frequently [LOE, III; SOR, B].

PROGNOSIS

CA125 is recommended during primary therapy as a potential prognostic marker because CA125 concentrations, both preoperative and postoperative, may be of prognostic significance (439–442). After primary surgery and chemotherapy, declines in CA125 concentrations during chemotherapy have generally been observed to be independent prognostic factors, and in some studies the most important indicator. Persistent elevations indicate a poor prognosis. In patients who had a preoperative CA125 concentration >65 kU/L, the 5-year survival rates were significantly lower and conferred a 6.37-fold risk of death compared to patients who had values <65 kU/L (412, 426). In addition to the measured level, the half-life of the CA125 marker indicates prognosis after chemotherapy. A half-life of <20 days was associated with significantly improved survival (28 months vs 19 months) compared to >20 days (411, 443). Improved survival also correlates with normalization of CA125 after 3 cycles of combination chemotherapy. These findings have been supported by a recent study suggesting that CA125

half-life and CA125 nadir during induction chemotherapy are independent predictors of epithelial ovarian cancer outcome (444).

NACB OVARIAN CANCER PANEL RECOMMENDATION 6:

CA125 IN PROGNOSIS

CA125 measurement during primary therapy is recommended because CA125 concentrations, both preoperative and postoperative, may be of prognostic significance. Persistent elevations indicate poor prognosis [LOE, III; SOR, A/B].

OTHER MARKERS FOR OVARIAN CANCER

Several other potential tumor-associated markers have been reported in body fluids and tissue specimens from ovarian cancer patients. Although these experimental markers could represent promising new biomarkers for future ovarian cancer screening, diagnosis, and monitoring, it is uncertain whether they will become viable clinical tools, i.e., their clinical usefulness needs to be validated by assessing their sensitivity and specificity in larger groups of patients with stage I disease.

The Kallikrein family. Kallikreins are a subgroup of the serine protease enzyme family that play an important role in the progression and metastasis of human cancers (445). Kallikreins 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, and 15 in ovarian cancer have been shown to have value in detection, diagnosis, prognosis prediction, and monitoring of ovarian cancer (446–463). Kallikrein 4, for example, is expressed in the majority of serous carcinomas but rarely in normal ovarian surface epithelium (449, 450). Kallikrein 4 expression is associated with higher clinical stage and tumor grade in ovarian cancer: a univariate survival analysis revealed that patients with ovarian tumors positive for kallikrein 4 expression had an increased risk for relapse and death (450). Similarly, kallikrein 5 has been suggested to be a useful independent prognostic indicator in patients with stage I and II disease (451). Assessment of kallikrein 5 expression could help oncologists determine those patients at higher risk of relapse. Kallikrein 7 expression in ovarian cancer tissue is associated with poorer prognosis of ovarian cancer patients, especially those with lower grade disease and those who have been optimally debulked (464). In contrast, kallikrein 8 (neuropsin or ovasin) (452), kallikrein 9 (465), and kallikrein 11 (462) are favorable prognostic markers in ovarian cancer. Patients with higher kallikrein 8 expression in their tumors have lower-grade disease, lower residual tumor, longer survival, and low rate of recurrence. In a multivariate analysis, higher kallikrein 8 expression was significantly associated with longer disease-free survival. As well as their roles as tissue markers, kal-

likrein 6, 10, and 11 can be detected in serum, and are potential serological markers of the disease (446, 448, 466). A recent comprehensive and parallel analysis of different secreted kallikreins in ovarian cancer has demonstrated that kallikreins 6, 7, 8, and 10 are the 4 most specific secreted kallikreins in ovarian cancer effusions (467). These kallikreins may have clinical applications in the differential diagnosis of ovarian carcinoma from benign controls and other cancer types.

Osteopontin. Osteopontin was first identified by a cDNA microarray approach used to identify upregulated genes in ovarian cancer cells, and osteopontin has been found to be a potential diagnostic biomarker for ovarian cancer (468). In the original report, osteopontin expression was higher in invasive ovarian cancer than in borderline ovarian tumors, benign ovarian tumors, and normal ovarian surface epithelium (468). Plasma levels of osteopontin were significantly higher in patients with epithelial ovarian cancer compared to healthy controls, patients with benign ovarian disease, and patients with other gynecologic cancers. In a more recent report (469), osteopontin has been shown to be less sensitive than CA125 in predicting clinical response to therapy. However, osteopontin increased earlier than CA125 in 90% of the study patients who developed recurrent disease, indicating that osteopontin may be a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer.

Prostasin. Using gene expression profiling by cDNA microarrays, Mok et al. have identified an overexpressed gene called prostasin that produces a secretory product (470). Prostasin [alias for PRSS8 (protease, serine, 8)] was originally isolated from human seminal fluid and its highest levels are found in the prostate gland (471). Prostasin was detected more strongly in ovarian carcinoma than in normal ovarian tissue. The mean level of serum prostasin was 13.7 $\mu\text{g/mL}$ in patients with ovarian cancer and 7.5 $\mu\text{g/mL}$ in control subjects. In a series of patients with nonmucinous ovarian carcinoma, the combination of prostasin and CA125 gave a sensitivity of 92% and a specificity of 94% for detecting ovarian cancer. Although the above finding is promising, prostasin should be investigated further as a screening or tumor marker, both alone and in combination with CA125.

Tissue polypeptide antigen. Tissue polypeptide antigen (TPA) is a single-chain polypeptide that may be made up of proteolytic fragments of the cytokeratins (472). Production of TPA may be associated with rapid cell turnover, and increased TPA levels in serum have been reported in patients suffering from cancers and other disease (473). In ovarian cancers of serous and mucinous type, TPA levels correlate with FIGO stage; 33%–

50% of patients with stage I–II disease, and 88%–96% of patients with stage III–IV disease presented with increased serum TPA. Serial TPA measurements correlated with the clinical course of ovarian cancer in 42%–79% of the matched events. These findings suggest that TPA may be a potential marker for following ovarian cancer in patients.

Lysophosphatidic acid. Lysophosphatidic acid (LPA) was first identified in ascites of ovarian cancer patients and has since been demonstrated to play a biological role in ovarian cancer cell growth (474–477). In a preliminary study in a small number of patients (474), plasma LPA concentrations were increased in 90% of patients with stage I disease and 100% of patients with advanced and recurrent disease compared to controls without apparent diseases, although 80% of women with other gynecologic cancers also had increased levels. CA125 concentrations appeared to complement LPA levels.

Tumor-associated trypsin inhibitor. Tumor-associated trypsin inhibitor (TATI) was first identified from the urine of patients with ovarian cancer (478). The amino acid sequence and biochemical properties of TATI are identical to those of pancreatic secretory trypsin inhibitor (479). Increased serum and urinary concentrations of TATI are frequently observed in postoperative patients, in severe inflammatory diseases, and in various types of cancer, especially gynecological and pancreatic cancer (473). Increased concentrations of TATI can be observed in ovarian cancers, especially the mucinous types. The increased serum levels of TATI appear to correlate with higher stages of disease. In one report, the sensitivity was only 8% in patients with stage I–II and 62% of patients with stage III–IV disease (480). Several reports suggest that TATI is not a good marker for monitoring disease during therapy, because TATI has a lower sensitivity for residual tumor than CA125, and <50% of the matched clinical events are observed to correlate serum levels of TATI.

Carcinoembryonic antigen. CEA is an oncofetal antigen (473), and increased serum levels of CEA are frequently found in a variety of benign diseases and cancers, including ovarian carcinoma. The frequency of increased concentration in ovarian carcinoma varies with the histological type and disease stage, generally being higher in patients with mucinous ovarian cancers and with metastatic disease. The sensitivity of CEA as a marker to detect ovarian cancer is approximately 25%, and the positive predictive value of an increased CEA concentration is only 14% (473). Although CEA is not a marker for early diagnosis owing to its low sensitivity, CEA can be useful in determining treatment response in ovarian cancer patients.

Cancer-associated serum antigen. Cancer-associated serum antigen (CASA) was initially defined by a monoclonal antibody that bound to an epitope on the polymorphic epithelial mucin (481). Increased CASA levels in serum were found in individuals in the later stage of pregnancy, in the elderly, in smokers, and in patients with cancers. CASA is expressed in all histological types of ovarian cancer and appears to have a sensitivity of 46%–73% in patients with ovarian cancer (473). Only a few studies have indicated that CASA is a potentially useful marker in monitoring ovarian cancer. Ward et al. reported that inclusion of CASA in a diagnostic tumor panel might improve the detection of residual disease by increasing the sensitivity from 33% to 62% and the negative predictive value from 66% to 78% (482, 483). One study has demonstrated that CASA can detect more cases with small volume disease than CA125, and that 50% of patients with microscopic disease are detected by CASA alone (473). Another study has shown that the prognostic value of postoperative serum CASA level is superior to CA125 and other parameters including residual disease, histological type, tumor grade, and the cisplatin-based chemotherapy (484).

PAI-1 and PAI-2. Fibrinolytic markers include PAI-1 and PAI-2, for which diagnostic and prognostic values have recently been reported in ovarian cancer (485). In this pilot study, PAI-1 appeared to be a poor prognostic factor (486), because plasma levels of PAI-1 are significantly higher in patients with ovarian cancer, and their levels correlate with the diseases at higher clinical stages. Whether PAI-1 can be used clinically for screening and/or monitoring ovarian cancer awaits further studies, including correlation with clinical treatment events and comparison with CA125. In contrast, expression of PAI-2 in tumors has been shown to be a favorable prognostic factor in ovarian cancer patients (485).

Interleukin-6. High levels of interleukin-6 have been detected in the serum and ascites of ovarian cancer patients (487). Interleukin-6 correlates with tumor burden, clinical disease status, and survival time of patients with ovarian cancer, implying that this marker may be useful in diagnosis. Based on a multivariate analysis, investigators have found serum levels of interleukin-6 to be of prognostic value, but less sensitive than CA125 (488, 489).

Human chorionic gonadotropin. hCG normally is produced by the trophoblast, and clinically has been used as a serum or urine marker for pregnancy and gestational trophoblastic disease (490). Ectopic hCG production, however, has been detected in a variety of human cancers. Recent studies have demonstrated that

the immunoreactivity of total hCG in serum and urine provides a strong independent prognostic factor in ovarian carcinoma, and its prognostic value is similar to that of grade and stage (491, 492). When serum hCG is normal, the 5-year survival rate can be as high as 80%, but it is only 22% when hCG is increased (491). In patients with stage III or IV and minimal residual disease, the 5-year survival is 75% if hCG is not detectable compared to 0% if hCG is increased. Similarly, hCG β -core fragment (hCG β cf) can be detected in urine in 84% of ovarian cancer patients (492). The incidence of positive urinary hCG β cf correlates with disease progression, with elevations observed in a higher proportion of patients in advanced clinical stages. Although the availability of this marker before surgery could facilitate selection of treatment modalities, the clinical application of hCG and hCG β for screening and diagnosis is limited. Since several different types of tumors can produce hCG \pm hCG β , and only a small proportion of ovarian tumors express these, detection of serum hCG \pm hCG β or urinary hCG β cf will not provide a specific or sensitive tool for screening or diagnosis in ovarian cancer.

HER-2/neu gene. The *c-erbB-2* oncogene expresses a transmembrane protein, p185, with intrinsic tyrosine kinase activity, also known as HER2/neu. Amplification of Her2/neu has been found in several human cancers, including ovarian carcinoma. In ovarian cancer, 9% to 38% of patients have increased levels of p105, the shed extracellular domain of the HER-2/neu protein (493–495). According to one report, measurement of Her2/neu alone or in combination with CA125 is not useful for differentiating benign from malignant ovarian tumors (495). However, elevation of p105 in serum or the overexpression immunohistochemically of Her2/neu in tumors has correlated with an aggressive tumor type, advanced clinical stages, and poor clinical outcome (496). Screening for increased p105 levels might therefore make it possible to identify a subset of high-risk patients (494). Furthermore, the test could be potentially useful for detecting recurrent disease.

AKT2 gene. The *v-akt* murine thymoma viral oncogene homolog 2 (*AKT2*) gene is one of the human homologs of *v-akt*, the transduced oncogene of the AKT8 virus, which experimentally induces lymphomas in mice. *AKT2*, which codes for a serine-threonine protein kinase, is activated by growth factors and other oncogenes such as *v-Ha-ras* and *v-src* through phosphatidylinositol 3-kinase in human ovarian cancer cells (497, 498). Studies have shown that the *AKT2* gene is amplified and overexpressed in approximately 12–36% of ovarian carcinomas (499–501). In contrast, *AKT2*

alteration was not detected in 24 benign or borderline tumors.

Ovarian cancer patients with *AKT2* alterations appear to have a poor prognosis. Amplification of *AKT2* is more frequently found in histologically high-grade tumors or tumors at advanced stages (III or IV), suggesting that *AKT2* gene overexpression, like *c-erbB-2*, may be associated with tumor aggressiveness (500).

Mitogen-activated protein kinase. Activation of mitogen-activated protein kinase occurs in response to various growth stimulating signals and as a result of activating mutations of the upstream regulators, *KRAS* and *BRAF*, which can be found in many types of human cancer. Activation of mitogen-activated protein kinase activates downstream cellular targets (502, 503) including a variety of cellular and nuclear proteins. Two studies have reported that expression of active mitogen-activated protein kinase in ovarian cancer tissue or ascites cells correlates with better prognosis in the advanced stage ovarian cancer (504, 505).

Inhibin. Inhibin is a glycoprotein and member of the transforming growth factor β family. Inhibins A and B are heterodimers consisting of identical α -subunits and either βA or βB subunits linked with disulfide bonds (506–508). Inhibin is primarily produced by the gonads and functions as a regulator of follicle-stimulating hormone secretion. Inhibin is associated with granulosa cell tumors and mucinous carcinomas as opposed to CA125, which is associated with serous, endometrioid, and undifferentiated tumors. In addition the α subunit may function as an ovarian tumor suppressor. Using a total inhibin ELISA in combination with CA125 has been shown to detect the majority of ovarian cancer types with 95% sensitivity and specificity (507).

RSF-1 gene. The clinical significance of the remodeling and spacing factor 1 (*RSF-1*) gene in ovarian cancer was first demonstrated by analyzing a new amplified chromosomal region, 11q13.5, in the ovarian cancer genome by use of digital karyotyping. The *RSF-1* gene belongs to the SWI/SNF chromatin remodelling gene family and Rsf-1 protein partners with hSNF2h to form the chromatin remodelling complex, RSF (remodelling and spacing factor) (509). It has been shown that Rsf-1 participates in chromatin remodelling (509) and transcriptional regulation (510, 511). Previous studies have demonstrated that *RSF-1* amplification and overexpression are associated with the most aggressive type of ovarian cancer, and patients with *RSF-1* gene amplification in their carcinomas had a significantly shorter overall survival (512–514). Further multiinstitutional studies are required to validate the clinical significance of *RSF-1* gene amplification for future clinical practice.

NAC-1 gene.⁴⁰ The genes within the BTB/POZ family participate in several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance (515). The roles of BTB/POZ proteins in human cancer have been recently revealed as several of BTB/POZ proteins such as BCL-6 are involved in cancer development. Based on analyzing gene expression levels in all 130 deduced human BTB/POZ genes using the serial analysis of gene expression data, Nakayama et al. have recently identified NAC-1 as a carcinoma-associated BTB/POZ gene (516). NAC-1 is a transcription repressor and is involved in self-renewal and maintaining pluripotency of embryonic stem cells (517). In ovarian carcinomas, NAC-1 is significantly over expressed in high-grade carcinoma but not in borderline tumors or benign cystadenomas. The levels of NAC-1 expression correlate with tumor recurrence in ovarian serous carcinomas and intense NAC-1 immunoreactivity in primary ovarian tumors predicts early recurrence (516, 518). As the NAC-1 specific antibody is available to evaluate NAC-1 protein levels in archival paraffin sections, the marker alone or in combination with other biomarkers may hold promise for prognosis and prediction in ovarian carcinoma patients.

**NACB OVARIAN CANCER PANEL RECOMMENDATION 7:
TUMOR MARKERS OTHER THAN CA125**

CA125 is the only marker that can be recommended for use in serous ovarian malignancies. New ovarian cancer markers offer promise; however, their contribution to the current standard of care is unknown and further investigations in properly designed clinical trials are needed [LOE, not applicable; SOR, B].

KEY POINTS: TUMOR MARKERS IN OVARIAN CANCER

The NACB Panel recommends CA125 as the only marker for clinical use in ovarian cancer for the following indications: early detection in combination with transvaginal ultrasound in hereditary syndromes, differential diagnosis in suspicious pelvic mass, detection of recurrence, monitoring of therapy, and prognosis. The NACB Panel does not recommend CA125 for screening of ovarian cancer in asymptomatic women.

All other markers are either in the evaluation phase or in the research/discovery phase, therefore the NACB Panel does not recommend these biomarkers for clinical use in ovarian cancer.

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⁴⁰ NAC-1, alias for NACC1 (nucleus accumbens associated 1, BEN and BTB (POZ) domain containing).

References

- Field M, Lohr K, editors. Clinical practice guidelines: directions for a new program. Washington (DC): National Academy Press, 1990; 168 p.
- Diamandis EP, Hoffman BR, Sturgeon CM. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for the Use of Tumor Markers. *Clin Chem* 2008;54:1935–1939.
- Sturgeon CM, Hoffman BR, Chan DW, Ch'ng S-L, Hammond E, Hayes DF, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Clinical Practice: Quality Requirements. *Clin Chem* 2008;54:e1–e10 [Epub 2008 Jul 7].
- Bosl GJ, Motzer RJ. Testicular germ-cell cancer. *N Engl J Med* 1997;337:242–53.
- Mead GM, Stenning SP. Prognostic factors in metastatic non-seminomatous germ cell tumours: the Medical Research Council studies. *Eur Urol* 1993;23:196–200.
- Looijenga LH, Gillis AJ, van Gurp RJ, Verkerk AJ, Oosterhuis JW. X inactivation in human testicular tumors: XIST expression and androgen receptor methylation status. *Am J Pathol* 1997;151:581–90.
- Looijenga LH, Oosterhuis JW. Pathobiology of testicular germ cell tumors: views and news. *Anal Quant Cytol Histol* 2002;24:263–79.
- Woodward PJ, Heidenreich A, Looijenga LHJ, Oosterhuis JW, McLeod DG, Moller H, et al. Testicular germ cell tumors. In: Eble JN, Sauter G, Epstein JI, Sesterhann IA, eds. World Health Organization classification of tumours: pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC Press; 2004. p 217–278.
- Oosterhuis JW, Looijenga LH, van Echten J, de Jong B. Chromosomal constitution and developmental potential of human germ cell tumors and teratomas. *Cancer Genet Cytogenet* 1997;95:96–102.
- Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003;170:5–11.
- McGlynn KA, Devesa SS, Sigurdson AJ, Brown LM, Tsao L, Tarone RE. Trends in the incidence of testicular germ cell tumors in the United States. *Cancer* 2003;97:63–70.
- Oliver RT, Mason MD, Mead GM, von der Maase H, Rustin GJ, Joffe JK, et al. Radiotherapy versus single-dose carboplatin in adjuvant treatment of stage I seminoma: a randomised trial. *Lancet* 2005;366:293–300.
- Tumour markers in germ cell cancer: EGTM recommendations. *Anticancer Res* 1999;19:2795–8.
- Laguna MP, Pizzocaro G, Klepp O, Algaba F, Kisbenedek L, Leiva O. EAU guidelines on testicular cancer. *Eur Urol* 2001;40:102–10.
- Fleisher M, Dnistrian A, Sturgeon C, Lamerz R, Witliff J. Practice guidelines and recommendations for use of tumor markers in the clinic. *Tumor Markers: physiology, pathobiology, technology and clinical applications*. Washington (DC): AACCC Press, 2002;33–63.
- Schmoll HJ, Souchon R, Kregge S, Albers P, Beyer J, Kollmannsberger C, et al. European consensus on diagnosis and treatment of germ cell cancer: a report of the European Germ Cell Cancer Consensus Group (EGCCCG). *Ann Oncol* 2004;15:1377–99.
- Testicular seminoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2007;18 Suppl 2:ii40–1.
- NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Testicular cancer. Version 1. 2007. http://www.nccn.org/professionals/physician_gls/PDF/testicular.pdf (Accessed June 2007).
- Mixed or non-seminomatous germ-cell tumors: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2007;18 Suppl 2:ii42–3.
- Albers P, Albrecht W, Algaba F, Bokemeyer C, Cohn-Cedermark G, Horwich A, et al. Guidelines on testicular cancer. *Eur Urol* 2005;48:885–94.
- Huddart R, Kataja V. Testicular seminoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2008;19 Suppl 2:ii49–51.
- Mostofi FK, Sesterhenn IA, Davis CJ Jr. Developments in histopathology of testicular germ cell tumors. *Semin Urol* 1988;6:171–88.
- Pugh R. Combined tumours: pathology of testis. Oxford: Blackwell; 1976;245–58.
- Mostofi FK, Sesterhenn IA, Davis CJ Jr. Immunopathology of germ cell tumors of the testis. *Semin Diagn Pathol* 1987;4:320–41.
- Skakkebaek NE. Possible carcinoma-in-situ of the testis. *Lancet* 1972;2:516–7.
- Gondos B, Hobel CJ. Ultrastructure of germ cell development in the human fetal testis. *Z Zellforsch Mikrosk Anat* 1971;119:1–20.
- Oosterhuis JW, Kersemaekers AM, Jacobsen GK, Timmer A, Steyerberg EW, Molier M, et al. Morphology of testicular parenchyma adjacent to germ cell tumours: an interim report. *APMIS* 2003;111:32–40.
- Rajpert-De ME, Skakkebaek NE. Expression of the c-kit protein product in carcinoma-in-situ and invasive testicular germ cell tumours. *Int J Androl* 1994;17:85–92.
- van Gurp RJ, Oosterhuis JW, Kalscheuer V, Mariman EC, Looijenga LH. Biallelic expression of the H19 and IGF2 genes in human testicular germ cell tumors. *J Natl Cancer Inst* 1994;86:1070–5.
- de Gouveia Brazao CA, Pierik FH, Oosterhuis JW, Dohle GR, Looijenga LH, Weber RF. Bilateral testicular microlithiasis predicts the presence of the precursor of testicular germ cell tumors in subfertile men. *J Urol* 2004;171:158–60.
- Dieckmann KP, Classen J, Loy V. Diagnosis and management of testicular intraepithelial neoplasia (carcinoma in situ): surgical aspects. *APMIS* 2003;111:64–8.
- Motzer RJ, Rodriguez E, Reuter VE, Samaniego F, Dmitrovsky E, Bajorin DF, et al. Genetic analysis as an aid in diagnosis for patients with midline carcinomas of uncertain histologies. *J Natl Cancer Inst* 1991;83:341–6.
- Rao PH, Houldsworth J, Palanisamy N, Murty VV, Reuter VE, Motzer RJ, et al. Chromosomal amplification is associated with cisplatin resistance of human male germ cell tumors. *Cancer Res* 1998;58:4260–3.
- Roelofs H, Mostert MC, Pompe K, Zafarana G, van Oorschot M, van Gurp RJ, et al. Restricted 12p amplification and RAS mutation in human germ cell tumors of the adult testis. *Am J Pathol* 2000;157:1155–66.
- Zafarana G, Gillis AJ, van Gurp RJ, Olsson PG, Elstrodt F, Stoop H, et al. Coamplification of DAD-R, SOX5, and EK1 in human testicular seminomas, with specific overexpression of DAD-R, correlates with reduced levels of apoptosis and earlier clinical manifestation. *Cancer Res* 2002;62:1822–31.
- Spierings DC, de Vries EG, Vellenga E, de Jong S. The attractive Achilles heel of germ cell tumours: an inherent sensitivity to apoptosis-inducing stimuli. *J Pathol* 2003;200:137–48.
- Masters JR, Koberle B. Curing metastatic cancer: lessons from testicular germ-cell tumours. *Nat Rev Cancer* 2003;3:517–25.
- Mayer F, Honecker F, Looijenga LH, Bokemeyer C. Towards an understanding of the biological basis of response to cisplatin-based chemotherapy in germ-cell tumors. *Ann Oncol* 2003;14:825–32.
- Mayer F, Gillis AJ, Dinjens W, Oosterhuis JW, Bokemeyer C, Looijenga LH. Microsatellite instability of germ cell tumors is associated with resistance to systemic treatment. *Cancer Res* 2002;62:2758–60.
- Velasco A, Riquelme E, Schultz M, Wistuba II, Villarroel L, Koh MS, Leach FS. Microsatellite instability and loss of heterozygosity have distinct prognostic value for testicular germ cell tumor recurrence. *Cancer Biol Ther* 2004;3:1152–8; discussion 1159–61.
- Velasco A, Riquelme E, Schultz M, Wistuba II, Villarroel L, Pizarro J, et al. Mismatch repair gene expression and genetic instability in testicular germ cell tumor. *Cancer Biol Ther* 2004;3:977–82.
- Mueller T, Voigt W, Simon H, Fruehauf A, Bulankin A, Grothey A, Schmoll HJ. Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. *Cancer Res* 2003;63:513–21.
- van Echten J, Oosterhuis JW, Looijenga LH, van de Pol M, Wiersema J, te Meersema GJ, et al. No recurrent structural abnormalities apart from i(12p) in primary germ cell tumors of the adult testis. *Genes Chromosomes Cancer* 1995;14:133–44.
- Kawakami T, Okamoto K, Ogawa O, Okada Y. XIST unmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer. *Lancet* 2004;363:40–2.
- Strohmeier T, Reissmann P, Cordon-Cardo C, Hartmann M, Ackermann R, Slamon D. Correlation between retinoblastoma gene expression and differentiation in human testicular tumors. *Proc Natl Acad Sci U S A* 1991;88:6662–6.
- Fan S, Chang JK, Smith ML, Duba D, Fornace AJ Jr, O'Connor PM. Cells lacking CIP1/WAF1 genes exhibit preferential sensitivity to cisplatin and nitrogen mustard. *Oncogene* 1997;14:2127–36.
- Bartkova J, Thullberg M, Rajpert-De Meyts E, Skakkebaek NE, Bartek J. Cell cycle regulators in testicular cancer: loss of p18INK4C marks pro-

- gression from carcinoma in situ to invasive germ cell tumours. *Int J Cancer* 2000;85:370–5.
48. Mayer F, Stoop H, Scheffer GL, Scheper R, Oosterhuis JW, Looijenga LH, Bokemeyer C. Molecular determinants of treatment response in human germ cell tumors. *Clin Cancer Res* 2003;9:767–73.
 49. Steyerberg EW, Keizer HJ, Habbema JD. Prediction models for the histology of residual masses after chemotherapy for metastatic testicular cancer. ReHIT Study Group. *Int J Cancer* 1999;83:856–9.
 50. Suurmeijer AJ, Oosterhuis JW, Sleijfer DT, Koops HS, Fleuren GJ. Non-seminomatous germ cell tumors of the testis: morphology of retroperitoneal lymph node metastases after chemotherapy. *Eur J Cancer Clin Oncol* 1984;20:727–34.
 51. Rajpert-De Meyts E, Bartkova J, Samson M, Høei-Hansen CE, Frydelund-Larsen L, Bartek J, Skakkebaek NE. The emerging phenotype of the testicular carcinoma in situ germ cell. *APMIS* 2003;111:267–78; discussion 278–9.
 52. Bartkova J, Thullberg M, Rajpert-De Meyts E, Skakkebaek NE, Bartek J. Lack of p19INK4d in human testicular germ-cell tumours contrasts with high expression during normal spermatogenesis. *Oncogene* 2000;19:4146–50.
 53. Bartkova J, Rajpert-De Meyts E, Skakkebaek NE, Lukas J, Bartek J. Deregulation of the G1/S-phase control in human testicular germ cell tumours. *Apms* 2003;111:252–65; discussion 265–6.
 54. Freedman LS, Parkinson MC, Jones WG, Oliver RT, Peckham MJ, Read G, et al. Histopathology in the prediction of relapse of patients with stage I testicular teratoma treated by orchidectomy alone. *Lancet* 1987;2:294–8.
 55. Vergouwe Y, Steyerberg EW, Eijkemans MJ, Albers P, Habbema JD. Predictors of occult metastasis in clinical stage I nonseminoma: a systematic review. *J Clin Oncol* 2003;21:4092–9.
 56. Albers P, Siener R, Kliesch S, Weissbach L, Krege S, Sparwasser C, et al. Risk factors for relapse in clinical stage I nonseminomatous testicular germ cell tumors: results of the German Testicular Cancer Study Group Trial. *J Clin Oncol* 2003;21:1505–12.
 57. Mazumdar M, Bacik J, Tickoo SK, Dobrzynski D, Donadio A, Bajorin D, et al. Cluster analysis of p53 and Ki67 expression, apoptosis, alpha-fetoprotein, and human chorionic gonadotrophin indicates a favorable prognostic subgroup within the embryonal carcinoma germ cell tumor. *J Clin Oncol* 2003;21:2679–88.
 58. Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, van Roozendaal KE, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res* 2003;63:2244–50.
 59. Cheng L, Thomas A, Roth LM, Zheng W, Michael H, Karim FW. OCT4: a novel biomarker for dysgerminoma of the ovary. *Am J Surg Pathol* 2004;28:1341–6.
 60. Jones TD, Ulbright TM, Eble JN, Baldrige LA, Cheng L. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. *Am J Surg Pathol* 2004;28:935–40.
 61. Rajpert-De ME, Hanstein R, Jorgensen N, Graem N, Vogt PH, Skakkebaek NE. Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. *Hum Reprod* 2004;19:1338–44.
 62. Scottish Intercollegiate Guidelines Network (SIGN). Management of adult testicular germ cell tumours. <http://www.sign.ac.uk/> (Accessed October 2007). (SIGN 28; 1998 Sep)
 63. Germa-Lluch JR, Garcia del Muro X, Maroto P, Paz-Ares L, Arranz JA, Guma J, et al. Clinical pattern and therapeutic results achieved in 1490 patients with germ-cell tumours of the testis: the experience of the Spanish Germ-Cell Cancer Group (GG). *Eur Urol* 2002;42:553–62; discussion 562–3.
 64. Stenman UH, Alftan H, Diamandis EP, Fritsche HA, Lilja H, Chan DW, Schwartz MK. Markers for testicular cancer. In: Diamandis EP, Fritsche HA, Chan DW, Schwartz MK, editors. *Tumor markers physiology, pathobiology, technology, and clinical applications*. Washington (DC): AAC Press, 2002:351–9.
 65. Bosl GJ, Lange PH, Fraley EE, Goldman A, Nohomovitz LE, Rosai J, et al. Human chorionic gonadotropin and alphafetoprotein in the staging of nonseminomatous testicular cancer. *Cancer* 1981;47:328–32.
 66. International Germ Cell Consensus Classification: a prognostic factor-based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J Clin Oncol* 1997;15:594–603.
 67. Vogelzang NJ. Prognostic factors in metastatic testicular cancer. *Int J Androl* 1987;10:225–37.
 68. Davis BE, Herr HW, Fair WR, Bosl GJ. The management of patients with nonseminomatous germ cell tumors of the testis with serologic disease only after orchidectomy. *J Urol* 1994;152:111–3; discussion 114.
 69. Rabbani F, Sheinfeld J, Farivar-Mohseni H, Leon A, Rentzepis MJ, Reuter VE, et al. Low-volume nodal metastases detected at retroperitoneal lymphadenectomy for testicular cancer: pattern and prognostic factors for relapse. *J Clin Oncol* 2001;19:2020–5.
 70. Toner GC, Geller NL, Tan C, Nisselbaum J, Bosl GJ. Serum tumor marker half-life during chemotherapy allows early prediction of complete response and survival in nonseminomatous germ cell tumors. *Cancer Res* 1990;50:5904–10.
 71. Coogan CL, Foster RS, Rowland RG, Bihle R, Smith ER Jr, Einhorn LH, et al. Postchemotherapy retroperitoneal lymph node dissection is effective therapy in selected patients with elevated tumor markers after primary chemotherapy alone. *Urology* 1997;50:957–62.
 72. Vogelzang NJ, Lange PH, Goldman A, Vessela RH, Fraley EE, Kennedy BJ. Acute changes of alpha-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors. *Cancer Res* 1982;42:4855–61.
 73. Kohn J. The dynamics of serum alpha-fetoprotein in the course of testicular teratoma. *Scand J Immunol* 1978;8(Suppl 8):103.
 74. Lange PH, Vogelzang NJ, Goldman A, Kennedy BJ, Fraley EE. Marker half-life analysis as a prognostic tool in testicular cancer. *J Urol* 1982;128:708–11.
 75. Mazumdar M, Bajorin DF, Bacik J, Higgins G, Motzer RJ, Bosl GJ. Predicting outcome to chemotherapy in patients with germ cell tumors: the value of the rate of decline of human chorionic gonadotrophin and alpha-fetoprotein during therapy. *J Clin Oncol* 2001;19:2534–41.
 76. Seckl MJ, Rustin GJ, Bagshawe KD. Frequency of serum tumour marker monitoring in patients with non-seminomatous germ cell tumours. *Br J Cancer* 1990;61:916–8.
 77. Abelev GI. Alpha-fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. *Transplant Rev* 1974;20:3–37.
 78. Gitlin D, Boesman M. Serum alpha-fetoprotein, albumin, and gamma-G-globulin in the human conceptus. *J Clin Invest* 1966;45:1826–38.
 79. Brewer JA, Tank ES. Yolk sac tumors and alpha-fetoprotein in first year of life. *Urology* 1993;42:79–80.
 80. Blohm ME, Vesterling-Horner D, Calaminus G, Gobel U. Alpha 1-fetoprotein (AFP) reference values in infants up to 2 years of age. *Pediatr Hematol Oncol* 1998;15:135–42.
 81. Carroll WL, Kempson RL, Govan DE, Freiha FS, Shochat SJ, Link MP. Conservative management of testicular endodermal sinus tumor in childhood. *J Urol* 1985;133:1011–4.
 82. Huddart SN, Mann JR, Gonnall P, Pearson D, Barrett A, Raafat F, et al. The UK Children's Cancer Study Group: testicular malignant germ cell tumours 1979–1988. *J Pediatr Surg* 1990;25:406–10.
 83. Christiansen M, Hogdall CK, Andersen JR, Norgaard-Pedersen B. Alpha-fetoprotein in plasma and serum of healthy adults: preanalytical, analytical and biological sources of variation and construction of age-dependent reference intervals. *Scand J Clin Lab Invest* 2001;61:205–15.
 84. Beck SD, Patel MI, Sheinfeld J. Tumor marker levels in post-chemotherapy cystic masses: clinical implications for patients with germ cell tumors. *J Urol* 2004;171:168–71.
 85. Germa JR, Llanos M, Tabernero JM, Mora J. False elevations of alpha-fetoprotein associated with liver dysfunction in germ cell tumors. *Cancer* 1993;72:2491–4.
 86. Morris MJ, Bosl GJ. Recognizing abnormal marker results that do not reflect disease in patients with germ cell tumors. *J Urol* 2000;163:796–801.
 87. Ruoslahti E, Adamson E. Alpha-fetoproteins produced by the yolk sac and the liver are glycosylated differently. *Biochem Biophys Res Commun* 1978;85:1622–30.
 88. de Takats PG, Jones SR, Penn R, Cullen MH. Alpha-fetoprotein heterogeneity: what is its value in managing patients with germ cell tumors? *Clin Oncol (R Coll Radiol)* 1996;8:323–6.
 89. Perlin E, Engeler JE, Jr., Edson M, Karp D, McIntire KR, Waldmann TA. The value of serial measurement of both human chorionic gonadotropin and alpha-fetoprotein for monitoring germinal cell tumors. *Cancer* 1976;37:215–9.
 90. Butler SA, Ikram MS, Mathieu S, Iles RK. The increase in bladder carcinoma cell population induced by the free beta subunit of human chorionic gonadotrophin is a result of an anti-apoptosis effect and not cell proliferation. *Br J Cancer* 2000;82:1553–6.
 91. Birken S, Yershova O, Myers RV, Bernard MP, Moyle W. Analysis of human choriogonadotropin core 2 o-glycan isoforms. *Mol Cell Endocrinol* 2003;204:21–30.

92. Vaitukaitis JL. Human chorionic gonadotropin as a tumor marker. *Ann Clin Lab Sci* 1974;4:276–80.
93. Birken S, Berger P, Bidart JM, Weber M, Bristow A, Norman R, et al. Preparation and characterization of new WHO reference reagents for human chorionic gonadotropin and metabolites. *Clin Chem* 2003;49:144–54.
94. Berger P, Sturgeon C, Bidart JM, Paus E, Gerth R, Niang M, et al. The ISOBM TD-7 Workshop on hCG and related molecules. Towards user-oriented standardization of pregnancy and tumor diagnosis: assignment of epitopes to the 3-dimensional structure of diagnostically and commercially relevant monoclonal antibodies directed against human chorionic gonadotropin and derivatives. *Tumour Biol* 2002;23:1–38.
95. Bristow A, Berger P, Bidart JM, Birken S, Norman R, Stenman UH, Sturgeon C. Establishment, value assignment, and characterization of new WHO reference reagents for six molecular forms of human chorionic gonadotropin. *Clin Chem* 2005;51:177–82.
96. Mann K, Saller B, Hoermann R. Clinical use of hCG and hCG beta determinations. *Scand J Clin Lab Invest Suppl* 1993;216:97–104.
97. Stenman UH, Alfthan H, Ranta T, Vartiainen E, Jaikonen J, Seppala M. Serum levels of human chorionic gonadotropin in nonpregnant women and men are modulated by gonadotropin-releasing hormone and sex steroids. *J Clin Endocrinol Metab* 1987;64:730–6.
98. Alfthan H, Haglund C, Dabek J, Stenman UH. Concentrations of human chorionic gonadotropin, its beta-subunit, and the core fragment of the beta-subunit in serum and urine of men and nonpregnant women. *Clin Chem* 1992;38:1981–7.
99. Lempiainen A, Hotakainen K, Blomqvist C, Alfthan H, Stenman UH. Increased human chorionic gonadotropin due to hypogonadism after treatment of a testicular seminoma. *Clin Chem* 2007;53:1560–1.
100. Catalona WJ, Vaitukaitis JL, Fair WR. Falsely positive specific human chorionic gonadotropin assays in patients with testicular tumors: conversion to negative with testosterone administration. *J Urol* 1979;122:126–8.
101. Stenman UH, Alfthan H, Hotakainen K. Human chorionic gonadotropin in cancer. *Clin Biochem* 2004;37:549–61.
102. Cole LA, Rinne KM, Shahabi S, Omrani A. False-positive hCG assay results leading to unnecessary surgery and chemotherapy and needless occurrences of diabetes and coma. *Clin Chem* 1999;45:313–4.
103. Saller B, Clara R, Spottl G, Siddle K, Mann K. Testicular cancer secretes intact human chorionic gonadotropin (hCG) and its free beta-subunit: evidence that hCG (+hCG-beta) assays are the most reliable in diagnosis and follow-up. *Clin Chem* 1990;36:234–9.
104. Summers J, Raggatt P, Pratt J, Williams MV. Experience of discordant beta hCG results by different assays in the management of non-seminomatous germ cell tumours of the testis. *Clin Oncol (R Coll Radiol)* 1999;11:388–92.
105. Li SS, Luedemann M, Sharief FS, Takano T, Deaven LL. Mapping of human lactate dehydrogenase-A, -B, and -C genes and their related sequences: the gene for LDHC is located with that for LDHA on chromosome 11. *Cytogenet Cell Genet* 1988;48:16–8.
106. Looijenga LH, Zafarana G, Grygalewicz B, Summersgill B, bic-Rychter M, Veltman J, et al. Role of gain of 12p in germ cell tumour development. *APMIS* 2003;111:161–71.
107. Rosenberg C, van Gurp RJ, Geelen E, Oosterhuis JW, Looijenga LH. Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas. *Oncogene* 2000;19:5858–62.
108. Summersgill B, Osin P, Lu YJ, Huddart R, Shipley J. Chromosomal imbalances associated with carcinoma in situ and associated testicular germ cell tumours of adolescents and adults. *Br J Cancer* 2001;85:213–20.
109. von Eyben FE. A systematic review of lactate dehydrogenase isoenzyme 1 and germ cell tumors. *Clin Biochem* 2001;34:441–54.
110. Fishman WH. Perspectives on alkaline phosphatase isoenzymes. *Am J Med* 1974;56:617–50.
111. Roelofs H, Manes T, Janszen T, Millan JL, Oosterhuis JW, Looijenga LH. Heterogeneity in alkaline phosphatase isozyme expression in human testicular germ cell tumours: an enzyme-/immunohistochemical and molecular analysis. *J Pathol* 1999;189:236–44.
112. Lange PH, Millan JL, Stigbrand T, Vessella RL, Ruoslahti E, Fishman WH. Placental alkaline phosphatase as a tumor marker for seminoma. *Cancer Res* 1982;42:3244–7.
113. De Broe ME, Pollet DE. Multicenter evaluation of human placental alkaline phosphatase as a possible tumor-associated antigen in serum. *Clin Chem* 1988;34:1995–9.
114. Mosselman S, Looijenga LH, Gillis AJ, van Rooijen MA, Kraft HJ, van Zoelen EJ, Oosterhuis JW. Aberrant platelet-derived growth factor alpha-receptor transcript as a diagnostic marker for early human germ cell tumors of the adult testis. *Proc Natl Acad Sci U S A* 1996;93:2884–8.
115. de Bruijn HW, Sleijfer DT, Schraffordt Koops H, Suurmeijer AJ, Marrink J, Ockhuizen T. Significance of human chorionic gonadotropin, alpha-fetoprotein, and pregnancy-specific beta-1-glycoprotein in the detection of tumor relapse and partial remission in 126 patients with nonseminomatous testicular germ cell tumors. *Cancer* 1985;55:829–35.
116. Kuzmits R, Scherthaner G, Krusch K. Serum neuron-specific enolase: a marker for responses to therapy in seminoma. *Cancer* 1987;60:1017–21.
117. Fossa SD, Klepp O, Paus E. Neuron-specific enolase—a serum tumour marker in seminoma? *Br J Cancer* 1992;65:297–9.
118. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43–66.
119. Sakr WA, Grignon DJ. Prostate cancer: indicators of aggressiveness. *Eur Urol* 1997;32 Suppl 3:15–23.
120. Hayes DF, Bast RC, Desch CE, Fritsche H, Jr., Kemeny NE, Jessup JM, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. *J Natl Cancer Inst* 1996;88:1456–66.
121. National Institute for Health and Clinical Excellence (NICE). Prostate cancer: diagnosis and treatment. <http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11924> (Accessed May 2008).
122. Mäkinen T, Tammela TL, Hakama M, Stenman UH, Rannikko S, Aro J, et al. Tumor characteristics in a population-based prostate cancer screening trial with prostate-specific antigen. *Clin Cancer Res* 2003;9:2435–9.
123. Draisma G, Boer R, Otto SJ, van der Crujns I, Damhuis RA, Schroder FH, de Koning HJ. Lead times and over-detection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 2003;95:868–78.
124. Punglia RS, D'Amico AV, Catalona WJ, Roehl KA, Kuntz KM. Effect of verification bias on screening for prostate cancer by measurement of prostate-specific antigen. *N Engl J Med* 2003;349:335–42.
125. Stamey TA, Caldwell M, McNeal JE, Nolley R, Hemenez M, Downs J. The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? *J Urol* 2004;172:1297–301.
126. Stenman UH, Hakama M, Knekt P, Aromaa A, Teppo L, Leinonen J. Serum concentrations of prostate specific antigen and its complex with alpha 1-antichymotrypsin before diagnosis of prostate cancer. *Lancet* 1994;344:1594–8.
127. Gann PH, Hennekens CH, Stampfer MJ. A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. *Jama* 1995;273:289–94.
128. Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004;350:2239–46.
129. Whittemore AS, Cirillo PM, Feldman D, Cohn BA. Prostate specific antigen levels in young adulthood predict prostate cancer risk: results from a cohort of Black and White Americans. *J Urol* 2005;174:872–6; discussion 876.
130. Lilja H, Ulmert D, Bjork T, Becker C, Serio AM, Nilsson JA, et al. Long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using prostate kallikreins measured at age 44 to 50 years. *J Clin Oncol* 2007;25:431–6.
131. Wirth MP, Frohmuller HG. Prostate-specific antigen and prostate acid phosphatase in the detection of early prostate cancer and the prediction of regional lymph node metastases. *Eur Urol* 1992;22:27–32.
132. Kontturi M. Is acid phosphatase (PAP) still justified in the management of prostatic cancer? *Acta Oncol* 1991;30:169–70.
133. Hugosson J, Aus G, Lilja H, Loddning P, Pihl CG. Results of a randomized, population-based study of biennial screening using serum prostate-specific antigen measurement to detect prostate carcinoma. *Cancer* 2004;100:1397–405.
134. Oesterling JE, Jacobsen SJ, Klee GG, Pettersson K, Piironen T, Abrahamson PA, et al. Free, complexed and total serum prostate specific antigen: the establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* 1995;154:1090–5.
135. Aus G, Damber JE, Khatami A, Lilja H, Stranne J,

- Hugosson J. Individualized screening interval for prostate cancer based on prostate-specific antigen level: results of a prospective, randomized, population-based study. *Arch Intern Med* 2005; 165:1857–61.
136. Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, et al. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. *J Urol* 1994;151:1283–90.
137. Crawford ED, Leewangsong S, Goktas S, Holthaus K, Baier M, Macbeth F, Titshall V. Diagnosis and treatment of prostate cancer: summary of NICE guidance. *BMJ* 2008;336:610–2.
140. Wilt TJ. Commentary: controversies in NICE guidance on prostate cancer. *BMJ* 2008;336: 612–4.
141. Myrtle JF, Klimley PG, Ivor IP, Bruni JF. Clinical utility of prostate specific antigen (PSA) in the management of prostate cancer. *San Diego: Hybritech*; 1986. p. 1–5.
142. Catalona WJ, Smith DS, Ornstein DK. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination: enhancement of specificity with free PSA measurements. *JAMA* 1997; 277:1452–5.
143. Lodding P, Aus G, Bergdahl S, Frosing R, Lilja H, Pihl CG, Hugosson J. Characteristics of screening detected prostate cancer in men 50 to 66 years old with 3 to 4 ng/mL. Prostate specific antigen. *J Urol* 1998;159:899–903.
144. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 2006;98: 529–34.
145. Gann PH. Interpreting recent trends in prostate cancer incidence and mortality. *Epidemiology* 1997;8:117–20.
146. Oesterling JE, Jacobsen SJ, Chute CG, Guess HA, Girman CJ, Panser LA, Lieber MM. Serum prostate-specific antigen in a community-based population of healthy men: establishment of age-specific reference ranges. *JAMA* 1993;270: 860–4.
147. Catalona WJ, Smith DS. Comparison of different serum prostate specific antigen measures for early prostate cancer detection. *Cancer* 1994; 74:1516–8.
148. Tumour markers in prostate cancer: EGTM recommendations. European Group on Tumour Markers. *Anticancer Res* 1999;19:2799–801.
149. Partin AW, Brawer MK, Bartsch G, Horninger W, Taneja SS, Lepor H, et al. Complexed prostate specific antigen improves specificity for prostate cancer detection: results of a prospective multicenter clinical trial. *J Urol* 2003;170:1787–91.
150. Pettersson K, Piironen T, Seppala M, Liukkonen L, Christensson A, Matikainen MT, et al. Free and complexed prostate-specific antigen (PSA) in vitro stability, epitope map, and development of immunofluorometric assays for specific and sensitive detection of free PSA and PSA-alpha 1-antichymotrypsin complex. *Clin Chem* 1995; 41:1480–8.
151. Babaian RJ, Fritsche HA, Evans RB. Prostate-specific antigen and prostate gland volume: correlation and clinical application. *J Clin Lab Anal* 1990;4:135–7.
152. Veneziano S, Pavlica P, Querze R, Nanni G, Lalanne MG, Vecchi F. Correlation between prostate-specific antigen and prostate volume, evaluated by transrectal ultrasonography: usefulness in diagnosis of prostate cancer. *Eur Urol* 1990;18:112–6.
153. Benson MC, Whang IS, Pantuck A, Ring K, Kaplan SA, Olsson CA, Cooner WH. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. *J Urol* 1992;147:815–6.
154. Carter HB, Pearson JD, Metter EJ, Brant LJ, Chan DW, Andres R, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA* 1992;267: 2215–20.
155. Schmid HP, McNeal JE, Stamey TA. Observations on the doubling time of prostate cancer: the use of serial prostate-specific antigen in patients with untreated disease as a measure of increasing cancer volume. *Cancer* 1993;71:2031–40.
156. Semjonow A, Schmid HP. The rise and fall of PSA: clinical implications of prostate specific antigen kinetics. *Urol Res* 2002;30:85–8.
157. Lilja H, Christensson A, Dahlen U, Matikainen MT, Nilsson O, Pettersson K, Lovgren T. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem* 1991;37:1618–25.
158. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res* 1991;51: 222–6.
159. Catalona WJ, Partin AW, Finlay JA, Chan DW, Rittenhouse HG, Wolfert RL, Woodrum DL. Use of percentage of free prostate-specific antigen to identify men at high risk of prostate cancer when PSA levels are 2.51 to 4 ng/mL and digital rectal examination is not suspicious for prostate cancer: an alternative model. *Urology* 1999;54: 220–4.
160. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA* 1998;279:1542–7.
161. Raaijmakers R, Blijenberg BG, Finlay JA, Rittenhouse HG, Wildhagen MF, Roobol MJ, Schroder FH. Prostate cancer detection in the prostate specific antigen range of 2.0 to 3.9 ng/ml: value of percent free prostate specific antigen on tumor detection and tumor aggressiveness. *J Urol* 2004;171:2245–9.
162. Stephan C, Lein M, Jung K, Schnorr D, Loening SA. The influence of prostate volume on the ratio of free to total prostate specific antigen in serum of patients with prostate carcinoma and benign prostate hyperplasia. *Cancer* 1997;79: 104–9.
163. Roddam AW, Duffy MJ, Hamdy FC, Ward AM, Patrick J, Price CP, et al. Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2–10 ng/ml: systematic review and meta-analysis. *Eur Urol* 2005;48:386–98; discussion 398–9.
164. Lee R, Localio AR, Armstrong K, Malkowicz SB, Schwartz JS. A meta-analysis of the performance characteristics of the free prostate-specific antigen test. *Urology* 2006;67:762–8.
165. Oberpenning F, Weining C, Brandt B, De AG, Heinecke A, Hamm M, et al. Combining free and total prostate specific antigen assays from different manufacturers: the pitfalls. *Eur Urol* 2002;42:577–82.
166. Lilja H, Haese A, Bjork T, Friedrich MG, Piironen T, Pettersson K, et al. Significance and metabolism of complexed and noncomplexed prostate specific antigen forms, and human glandular kallikrein 2 in clinically localized prostate cancer before and after radical prostatectomy. *J Urol* 1999;162:2029–34.
167. Allard WJ, Zhou Z, Yeung KK. Novel immunoassay for the measurement of complexed prostate-specific antigen in serum. *Clin Chem* 1998; 44:1216–23.
168. Finne P, Zhang WM, Auvinen A, Leinonen J, Maattanen L, Rannikko S, et al. Use of the complex between prostate specific antigen and alpha 1-protease inhibitor for screening prostate cancer. *J Urol* 2000;164:1956–60.
169. Lein M, Jung K, Hammerer P, Graefen M, Semjonow A, Stieber P, et al. A multicenter clinical trial on the use of alpha1-antichymotrypsin-prostate-specific antigen in prostate cancer diagnosis. *Prostate* 2001;47:77–84.
170. Lein M, Kwiatkowski M, Semjonow A, Luboldt HJ, Hammerer P, Stephan C, et al. A multicenter clinical trial on the use of complexed prostate specific antigen in low prostate specific antigen concentrations. *J Urol* 2003;170:1175–9.
171. Okihara K, Fritsche HA, Ayala A, Johnston DA, Allard WJ, Babaian RJ. Can complexed prostate specific antigen and prostatic volume enhance prostate cancer detection in men with total prostate specific antigen between 2.5 and 4.0 ng/mL. *J Urol* 2001;165:1930–6.
172. Keller T, Butz H, Lein M, Kwiatkowski M, Semjonow A, Luboldt HJ, et al. Discordance analysis characteristics as a new method to compare the diagnostic accuracy of tests: example of complexed versus total prostate-specific antigen. *Clin Chem* 2005;51:532–9.
173. Moul JW, Sesterhenn IA, Connelly RR, Douglas T, Srivastava S, Mostofi FK, McLeod DG. Prostate-specific antigen values at the time of prostate cancer diagnosis in African-American men. *JAMA* 1995;274:1277–81.
174. National Health Service (NHS). NHS cancer screening programmes: prostate cancer risk management. <http://www.cancerscreening.nhs.uk/prostate/index.html> (Accessed October 2007).
175. Evans R, Edwards A, Brett J, Bradburn M, Watson E, Austoker J, Elwyn G. Reduction in uptake of PSA tests following decision aids: systematic review of current aids and their evaluations. *Patient Educ Couns* 2005;58:13–26.

176. Sturgeon C. Practice guidelines for tumor marker use in the clinic. *Clin Chem* 2002;48:1151–9.
177. Zorob R, Anderson R, Cefalu C, Sidani M. Cancer screening guidelines. *Am Fam Physician* 2001;63:1101–12.
178. Harris R, Lohr KN. Screening for prostate cancer: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002;137:917–29.
179. Bangma CH, Roemeling S, Schroder FH. Overdiagnosis and overtreatment of early detected prostate cancer. *World J Urol* 2007;25:3–9.
180. Schroder FH, Denis LJ, Roobol M, Nelen V, Auvinen A, Tammela T, et al. The story of the European Randomized Study of Screening for Prostate Cancer. *BJU Int* 2003;92 Suppl 2:1–13.
181. de Koning HJ, Liem MK, Baan CA, Boer R, Schroder FH, Alexander FE. Prostate cancer mortality reduction by screening: power and time frame with complete enrollment in the European Randomised Screening for Prostate Cancer (ERSPC) trial. *Int J Cancer* 2002;98:268–73.
182. Andriole GL, Levin DL, Crawford ED, Gelmann EP, Pinsky PF, Chia D, et al. Prostate Cancer Screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: findings from the initial screening round of a randomized trial. *J Natl Cancer Inst* 2005;97:433–8.
183. Bartsch G, Horninger W, Klocker H, Reissigl A, Oberaigner W, Schonitzer D, et al. Prostate cancer mortality after introduction of prostate-specific antigen mass screening in the Federal State of Tyrol, Austria. *Urology* 2001;58:417–24.
184. Oberaigner W, Horninger W, Klocker H, Schonitzer D, Stuhlinger W, Bartsch G. Reduction of prostate cancer mortality in Tyrol, Austria, after introduction of prostate-specific antigen testing. *Am J Epidemiol* 2006;164:376–84.
185. Xia Z, Jacobsen SJ, Bergstralh EJ, Chute CG, Katusic SK, Lieber MM. Secular changes in radical prostatectomy utilization rates in Olmsted County, Minnesota 1980 to 1995. *J Urol* 1998;159:904–8.
186. Collin SM, Martin RM, Metcalfe C, Gunnell D, Albertsen PC, Neal D, et al. Prostate-cancer mortality in the USA and UK in 1975–2004: an ecological study. *Lancet Oncol* 2008;9:445–52.
187. Hugosson J, Aus G, Becker C, Carlsson S, Eriksson H, Lilja H, et al. Would prostate cancer detected by screening with prostate-specific antigen develop into clinical cancer if left undiagnosed? A comparison of two population-based studies in Sweden. *BJU Int* 2000;85:1078–84.
188. Ilic D, O'Connor D, Green S, Wilt T. Screening for prostate cancer: a Cochrane systematic review. *Cancer Causes Control* 2006;18:279–285.
189. Hammerer P, Graefen M, Henke RP, Haese A, Palisaar J, Huland E, Huland H. Analysis of molecular isoforms of PSA and their ratios in men with PSA-relapse after radical prostatectomy. *Anticancer Res* 2000;20:5253–5.
190. Hsu CY, Joniau S, Oyen R, Roskams T, Van Poppel H. Outcome of surgery for clinical unilateral T3a prostate cancer: a single-institution experience. *Eur Urol* 2007;51:121–8; discussion 128–9.
191. Narayan P, Gajendran V, Taylor SP, Tewari A, Presti JC Jr, Leidich R, et al. The role of transrectal ultrasound-guided biopsy-based staging, preoperative serum prostate-specific antigen, and biopsy Gleason score in prediction of final pathologic diagnosis in prostate cancer. *Urology* 1995;46:205–12.
192. Kattan MW, Stapleton AM, Wheeler TM, Scardino PT. Evaluation of a nomogram used to predict the pathologic stage of clinically localized prostate carcinoma. *Cancer* 1997;79:528–37.
193. Partin AW, Kattan MW, Subong EN, Walsh PC, Wojno KJ, Oesterling JE, et al. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer: a multi-institutional update. *JAMA* 1997;277:1445–51.
194. Ohori M, Kattan MW, Koh H, Maru N, Slawin KM, Shariat S, et al. Predicting the presence and side of extracapsular extension: a nomogram for staging prostate cancer. *J Urol* 2004;171:1844–9.
195. D'Amico AV, Chen MH, Roehl KA, Catalona WJ. Preoperative PSA velocity and the risk of death from prostate cancer after radical prostatectomy. *N Engl J Med* 2004;351:125–35.
196. D'Amico AV, Manola J, Loffredo M, Renshaw AA, DellaCrocce A, Kantoff PW. 6-month androgen suppression plus radiation therapy vs radiation therapy alone for patients with clinically localized prostate cancer: a randomized controlled trial. *Jama* 2004;292:821–7.
197. Carter HB, Ferrucci L, Kettermann A, Landis P, Wright EJ, Epstein JI, et al. Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. *J Natl Cancer Inst* 2006;98:1521–7.
198. Pontes JE, Jabalamei P, Montie J, Foemmel R, Howard PD, Boyett J. Prognostic implications of disappearance rate of biologic markers following radical prostatectomy. *Urology* 1990;36:415–9.
199. Semjonow A, Hamm M, Rathert P. Half-life of prostate-specific antigen after radical prostatectomy: the decisive predictor of curative treatment? *Eur Urol* 1992;21:200–5.
200. Amling CL, Bergstralh EJ, Blute ML, Slezak JM, Zincke H. Defining prostate specific antigen progression after radical prostatectomy: what is the most appropriate cut point? *J Urol* 2001;165:1146–51.
201. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *JAMA* 1999;281:1591–7.
202. Semjonow A, De Angelis G. "Ultrasensitive" prostate specific antigen (PSA) assays: how low do we want to go? *J Lab Med* 2003;27:16–9.
203. Stephenson AJ, Scardino PT, Bianco FJ, Jr., Eastham JA. Salvage therapy for locally recurrent prostate cancer after external beam radiotherapy. *Curr Treat Options Oncol* 2004;5:357–65.
204. Coquard R, Bachaud J. Report of the 38th meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO). *Cancer Radiother* 1997;1:88–93.
205. Kattan MW, Eastham JA, Stapleton AM, Wheeler TM, Scardino PT. A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *J Natl Cancer Inst* 1998;90:766–71.
206. Kattan MW, Wheeler TM, Scardino PT. Postoperative nomogram for disease recurrence after radical prostatectomy for prostate cancer. *J Clin Oncol* 1999;17:1499–507.
207. Price CP, Allard J, Davies G, Dawnay A, Duffy MJ, France M, et al. Pre-and post-analytical factors that may influence use of serum prostate specific antigen and its isoforms in a screening programme for prostate cancer. *Ann Clin Biochem* 2001;38:188–216.
208. Oberpenning F, Schmid HP, Fuchs-Surdel W, Hertle L, Semjonow A. The impact of intraoperative manipulation of the prostate on total and free prostate-specific antigen. *Int J Biol Markers* 2002;17:154–60.
209. Bjork T, Ljungberg B, Piironen T, Abrahamsson PA, Pettersson K, Cockett AT, Lilja H. Rapid exponential elimination of free prostate-specific antigen contrasts the slow, capacity-limited elimination of PSA complexed to alpha 1-antichymotrypsin from serum. *Urology* 1998;51:57–62.
210. Piironen T, Pettersson K, Suonpaa M, Stenman UH, Oesterling JE, Lovgren T, Lilja H. In vitro stability of free prostate-specific antigen (PSA) and prostate-specific antigen (PSA) complexed to alpha 1-antichymotrypsin in blood samples. *Urology* 1996;48:81–87.
211. Woodrum D, French C, Shamel LB. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions. *Urology* 1996;48:33–39.
212. Sturgeon CM, Ellis AR. Improving the comparability of immunoassays for prostate-specific antigen (PSA): progress and problems. *Clin Chim Acta* 2007;381:85–92.
213. Graves HC, Kamarei M, Stamey TA. Identity of prostate specific antigen and the semen protein P30 purified by a rapid chromatography technique. *J Urol* 1990;144:1510–5.
214. Vessella RL, Notebook J, Lange PH. Evaluation of the Abbott IMx automated immunoassay of prostate-specific antigen. *Clin Chem* 1992;38:2044–54.
215. Stamey TA, Teplow DB, Graves HC. Identity of PSA purified from seminal fluid by different methods: comparison by amino acid analysis and assigned extinction coefficients. *Prostate* 1995;27:198–203.
216. Rafferty B, Rigby P, Rose M, Stamey T, Gaines DR. Reference reagents for prostate-specific antigen (PSA): establishment of the first international standards for free PSA and PSA (90:10). *Clin Chem* 2000;46:1310–7.
217. Chan DW, Sokoll LJ. WHO first international standards for prostate-specific antigen: the beginning of the end for assay discrepancies? *Clin Chem* 2000;46:1291–2.
218. Roddam AW, Rimmer J, Nickerson C, Ward AM. Prostate-specific antigen: bias and molarity of commercial assays for PSA in use in England. *Ann Clin Biochem* 2006;43:35–48.
219. Kort SA, Martens F, Vanpoucke H, van Duijnhoven HL, Blankenstein MA. Comparison of 6 automated assays for total and free prostate-specific antigen with special reference to their reactivity toward the WHO 96/670 reference preparation. *Clin Chem* 2006;52:1568–74.
220. Stephan C, Klaas M, Muller C, Schnorr D, Loening SA, Jung K. Interchangeability of measurements of total and free prostate-specific antigen in serum with 5 frequently used assay combinations: an update. *Clin Chem* 2006;52:59–64.
221. Singh R, Cahill D, Popert R, O'Brien TS. Repeat-

- ing the measurement of prostate-specific antigen in symptomatic men can avoid unnecessary prostatic biopsy. *BJU Int* 2003;92:932–5.
222. Eastham JA, Riedel E, Scardino PT, Shike M, Fleisher M, Schatzkin A, et al. Variation of serum prostate-specific antigen levels: an evaluation of year-to-year fluctuations. *JAMA* 2003; 289:2695–700.
 223. Soletormos G, Semjonow A, Sibley PE, Lamerz R, Petersen PH, Albrecht W, et al. Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice. *Clin Chem* 2005;51:1342–51.
 224. Bruun L, Becker C, Hugosson J, Lilja H, Christensson A. Assessment of intra-individual variation in prostate-specific antigen levels in a biennial randomized prostate cancer screening program in Sweden. *Prostate* 2005;65:216–21.
 225. Bunting PS, DeBoer G, Choo R, Danjoux C, Klotz L, Fleshner N. Intraindividual variation of PSA, free PSA and complexed PSA in a cohort of patients with prostate cancer managed with watchful observation. *Clin Biochem* 2002;35: 471–5.
 226. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004;351: 781–91.
 227. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004; 10:6897–904.
 228. Moreno JG, Miller MC, Gross S, Allard WJ, Gommella LG, Terstappen LW. Circulating tumor cells predict survival in patients with metastatic prostate cancer. *Urology* 2005;65:713–8.
 229. Rissanen M, Helo P, Vaananen RM, Wahlroos V, Lilja H, Nurmi M, et al. Novel homogenous time-resolved fluorometric RT-PCR assays for quantification of PSA and hK2 mRNAs in blood. *Clin Biochem* 2007;40:111–8.
 230. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55:74–108.
 231. Davies RJ, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. *Nat Rev Cancer* 2005;5:199–209.
 232. Sobin LH, Wittekind C, eds. *TNM: classification of malignant tumors*. 6th. ed. New York: Wiley-Liss; 2002. 239 p.
 233. Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M, eds. *AJCC cancer staging manual*. New York: Springer-Verlag; 2002. 421 p.
 234. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. *Cancer* 2000;88: 1739–57.
 235. NIH Consensus Conference. Adjuvant therapy for patients with colon and rectal cancer. *JAMA* 1990;264:1444–50.
 236. Gill S, Loprinzi CL, Sargent DJ, Thome SD, Alberts SR, Haller DG, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004;22:1797–806.
 237. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol* 2004;22:3408–19.
 238. Kievit J. Follow-up of patients with colorectal cancer: numbers needed to test and treat. *Eur J Cancer* 2002;38:986–99.
 239. Fletcher RH. Carcinoembryonic antigen. *Ann Intern Med* 1986;104:66–73.
 240. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? *Clin Chem* 2001;47:624–30.
 241. Goldstein MJ, Mitchell EP. Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. *Cancer Invest* 2005;23: 338–51.
 242. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. *J Clin Oncol* 1996; 14:2843–77.
 243. Bast RC, Jr., Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2001;19:1865–78.
 244. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006;24:5313–27.
 245. Klapdor R, Aronsson AC, Duffy MJ, Hansson LO, Khalifa R, Lamerz R, et al. Tumor markers in gastrointestinal cancers: EGTM recommendations. *Anticancer Res* 1999;19:2811–15.
 246. Duffy MJ, van Dalen A, Haglund C, Hansson L, Klapdor R, Lamerz R, et al. Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines. *Eur J Cancer* 2003;39:718–27.
 247. Grem J. The prognostic importance of tumor markers in adenocarcinomas of the gastrointestinal tract. *Curr Opin Oncol* 1997;9:380–7.
 248. Watine J, Miedouge M, Friedberg B. Carcinoembryonic antigen as an independent prognostic factor of recurrence and survival in patients resected for colorectal liver metastases: a systematic review. *Dis Colon Rectum* 2001;44: 1791–9.
 249. Watine J, Friedberg B. Laboratory variables and stratification of metastatic colorectal cancer patients: recommendations for therapeutic trials and for clinical practice guidelines. *Clin Chim Acta* 2004;345:1–15.
 250. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;124:979–94.
 251. Bruinvels DJ, Stiggelbout AM, Kievit J, van Houwelingen HC, Habbema JD, van de Velde CH. Follow-up of patients with colorectal cancer: a meta-analysis. *Ann Surg* 1994;219:174–82.
 252. Rosen M, Chan L, Beart RW, Jr., Vukasin P, Anthon G. Follow-up of colorectal cancer: a meta-analysis. *Dis Colon Rectum* 1998;41:1116–26.
 253. Renehan AG, Egger M, Saunders MP, O'Dwyer ST. Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trials. *BMJ* 2002;324:813–20.
 254. Figueredo A, Rumble RB, Maroun J, Earle CC, Cummings B, McLeod R, et al. Follow-up of patients with curatively resected colorectal cancer: a practice guideline. *BMC Cancer* 2003; 3:26–38.
 255. Jeffery M, Hickey BE, Hider PN. Follow-up strategies for patients treated for nonmetastatic colorectal cancer. *Cochrane Database Syst Rev* 2007;(1):CD002200. DOI: 10.1002/14651858.CD002200.pub2. Update of Cochrane Database Syst Rev 2002;(1):CD002200.
 256. Tjandra JJ, Chan MK. Follow-up after curative resection of colorectal cancer: a meta-analysis. *Dis Colon Rectum* 2007;50:1783–99.
 257. Desch CE, Benson AB 3rd, Somerfield MR, Flynn PJ, Krause C, Loprinzi CL, et al. Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. *J Clin Oncol* 2005;23:8512–9.
 258. Thirion P, Michiels S, Pignon JP, Buyse M, Braud AC, Carlson RW, et al. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. *J Clin Oncol* 2004;22:3766–75.
 259. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. *N Engl J Med* 2005;352: 476–87.
 260. Gollinopoulos V, Salanti G, Pavlidis N, Ioannidis JP. Survival and disease-progression benefits with treatment regimens for advanced colorectal cancer: a meta-analysis. *Lancet Oncol* 2007; 8:898–911.
 261. Duffy MJ. CA 19–9 as a marker for gastrointestinal cancers: a review. *Ann Clin Biochem* 1998;35(Pt 3):364–70.
 262. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Haglund C. CEA, CA 19–9 and CA 72–4 improve the diagnostic accuracy in gastrointestinal cancers. *Anticancer Res* 2002; 22:2311–6.
 263. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Jarvinen H, Haglund C. CEA, CA 242, CA 19–9, CA 72–4 and hCGbeta in the diagnosis of recurrent colorectal cancer. *Tumour Biol* 2004;25:228–34.
 264. Lindmark G, Bergstrom R, Pahlman L, Glimeilius B. The association of preoperative serum tumour markers with Dukes' stage and survival in colorectal cancer. *Br J Cancer* 1995; 71:1090–4.
 265. Nakayama T, Watanabe M, Teramoto T, Kitajima M. CA19–9 as a predictor of recurrence in patients with colorectal cancer. *J Surg Oncol* 1997;66:238–43.
 266. Reiter W, Stieber P, Reuter C, Nagel D, Lauwerner U, Lamerz R. Multivariate analysis of the prognostic value of CEA and CA 19–9 serum levels in colorectal cancer. *Anticancer Res* 2000; 20:5195–8.
 267. Behbehani AI, Al-Sayer H, Farghaly M, Kanawati N, Mathew A, al-Bader A, van DA. Prognostic significance of CEA and CA 19–9 in colorectal cancer in Kuwait. *Int J Biol Markers* 2000;15: 51–5.
 268. Filella X, Molina R, Pique JM, Garcia-Valdecasas JC, Grau JJ, Novell F, et al. Use of CA 19–9 in the early detection of recurrences in colorectal

- cancer: comparison with CEA. *Tumour Biol* 1994;15:1–6.
269. Nilsson O, Johansson C, Glimelius B, Persson B, Norgaard-Pedersen B, Andren-Sandberg A, Lindholm L. Sensitivity and specificity of CA242 in gastro-intestinal cancer. A comparison with CEA, CA50 and CA 19–9. *Br J Cancer* 1992;65:215–21.
 270. Carpelan-Holmstrom M, Haglund C, Lundin J, Alfthan H, Stenman UH, Roberts PJ. Independent prognostic value of preoperative serum markers CA 242, specific tissue polypeptide antigen and human chorionic gonadotrophin beta, but not of carcinoembryonic antigen or tissue polypeptide antigen in colorectal cancer. *Br J Cancer* 1996;74:925–9.
 271. Carpelan-Holmstrom M, Haglund C, Lundin J, Jarvinen H, Roberts P. Pre-operative serum levels of CA 242 and CEA predict outcome in colorectal cancer. *Eur J Cancer* 1996;32A:1156–61.
 272. Holten-Andersen MN, Christensen IJ, Nielsen HJ, Stephens RW, Jensen V, Nielsen OH, et al. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. *Clin Cancer Res* 2002;8:156–64.
 273. Holten-Andersen MN, Fenger C, Nielsen HJ, Rasmussen AS, Christensen IJ, Brunner N, Kronborg O. Plasma TIMP-1 in patients with colorectal adenomas: a prospective study. *Eur J Cancer* 2004;40:2159–64.
 274. Holten-Andersen MN, Stephens RW, Nielsen HJ, Murphy G, Christensen IJ, Stetler-Stevenson W, Brunner N. High preoperative plasma tissue inhibitor of metalloproteinase-1 levels are associated with short survival of patients with colorectal cancer. *Clin Cancer Res* 2000;6:4292–9.
 275. Holten-Andersen M, Christensen IJ, Nilbert M, Bendahl PO, Nielsen HJ, Brunner N, Fernebro E. Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival. a validation study. *Eur J Cancer* 2004;40:64–72.
 276. Johnston PG, Benson AB, III, Catalano P, Rao MS, O'Dwyer PJ, Allegra CJ. Thymidylate synthase protein expression in primary colorectal cancer: lack of correlation with outcome and response to fluorouracil in metastatic disease sites. *J Clin Oncol* 2003;21:815–9.
 277. Edler D, Glimelius B, Hallstrom M, Jakobsen A, Johnston PG, Magnusson I, et al. Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. *J Clin Oncol* 2002;20:1721–8.
 278. Allegra C. Thymidylate synthase levels: prognostic, predictive, or both? *J Clin Oncol* 2002;20:1711–3.
 279. Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. *J Clin Oncol* 2004;22:529–36.
 280. Aschele C, Lonardi S, Monfardini S. Thymidylate synthase expression as a predictor of clinical response to fluoropyrimidine-based chemotherapy in advanced colorectal cancer. *Cancer Treat Rev* 2002;28:27–47.
 281. Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, Slattery ML. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. *Cancer Epidemiol Biomarkers Prev* 2001;10:917–23.
 282. Kohonen-Corish MR, Daniel JJ, Chan C, Lin BP, Kwun SY, Dent OF, et al. Low microsatellite instability is associated with poor prognosis in stage C colon cancer. *J Clin Oncol* 2005;23:2318–24.
 283. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005;23:609–18.
 284. Elsaleh H, Powell B, McCaul K, Grief F, Grant R, Joseph D, Iacopetta B. P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. *Clin Cancer Res* 2001;7:1343–9.
 285. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;349:247–57.
 286. Shibata D, Reale MA, Lavin P, Silverman M, Fearon ER, Steele G, Jr., et al. The DCC protein and prognosis in colorectal cancer. *N Engl J Med* 1996;335:1727–32.
 287. Aschele C, Debernardis D, Lonardi S, Bandelloni R, Casazza S, Monfardini S, Gallo L. Deleted in colon cancer protein expression in colorectal cancer metastases: a major predictor of survival in patients with unresectable metastatic disease receiving palliative fluorouracil-based chemotherapy. *J Clin Oncol* 2004;22:3758–65.
 288. Popat S, Houlston RS. A systematic review and meta-analysis of the relationship between chromosome 18q genotype, DCC status and colorectal cancer prognosis. *Eur J Cancer* 2005;41:2060–70.
 289. Skelly MM, Troy A, Duffy MJ, Mulcahy HE, Duggan C, Connell TG, et al. Urokinase-type plasminogen activator in colorectal cancer: relationship with clinicopathological features and patient outcome. *Clin Cancer Res* 1997;3:1837–40.
 290. Mulcahy HE, Duffy MJ, Gibbons D, McCarthy P, Parfrey NA, O'Donoghue DP, Sheahan K. Urokinase-type plasminogen activator and outcome in Dukes' B colorectal cancer. *Lancet* 1994;344:583–4.
 291. Yang JL, Seetoo D, Wang Y, Ranson M, Berney CR, Ham JM, et al. Urokinase-type plasminogen activator and its receptor in colorectal cancer: independent prognostic factors of metastasis and cancer-specific survival and potential therapeutic targets. *Int J Cancer* 2000;89:431–9.
 292. Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001;85:692–6.
 293. Munro AJ, Lain S, Lane DP. P53 abnormalities and outcomes in colorectal cancer: a systematic review. *Br J Cancer* 2005;92:434–44.
 294. Lievre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;26:374–9.
 295. De Rook W, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;19:508–15.
 296. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66:3992–5.
 297. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:1626–34.
 298. Allison JE, Lawson M. Screening tests for colorectal cancer: a menu of options remains relevant. *Curr Oncol Rep* 2006;8:492–8.
 299. Imperiale TF, Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Quantitative immunochemical fecal occult blood tests: is it time to go back to the future? *Ann Intern Med* 2007;146:309–11.
 300. Allison JE, Tekawa IS, Ransom LJ, Adrain AL. A comparison of fecal occult-blood tests for colorectal-cancer screening. *N Engl J Med* 1996;334:155–9.
 301. Allison JE, Sakoda LC, Levin TR, Tucker JP, Tekawa IS, Cuff T, et al. Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. *J Natl Cancer Inst* 2007;99:1462–70.
 302. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993;328:1365–71.
 303. Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996;348:1467–71.
 304. Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, et al. The effect of fecal occult-blood screening on the incidence of colorectal cancer. *N Engl J Med* 2000;343:1603–7.
 305. Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, et al. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996;348:1472–7.
 306. Towler BP IL, Glasziou P, Weller D, Kewenter J. Screening for colorectal cancer using the fecal occult blood test Haemoccult. *Cochrane Database Sys* 1998;Rev 2 CD001216.
 307. Allison JE. Colon Cancer Screening Guidelines 2005: the fecal occult blood test option has become a better FIT. *Gastroenterology* 2005;129:745–8.
 308. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Colorectal cancer screening. Version 1. http://www.nccn.org/physician_gls?PDF/colorectal_screening.pdf (Accessed October 2006).
 309. Smith RA, von Eschenbach AC, Wender R, Levin B, Byers T, Rothenberger D, et al. American Cancer Society guidelines for the early detection of cancer: update of early detection guidelines for prostate, colorectal, and endometrial cancers. Also: update 2001–testing for early lung cancer detection. *CA Cancer J Clin* 2001;51:38–75.
 310. US Preventive Services Task Force. Screening for colorectal cancer: recommendation and ratio-

- nale. *Ann Intern Med* 2002;137:129–31.
311. Smith RA, Cokkinides V, Eyre HJ. American Cancer Society guidelines for the early detection of cancer, 2006. *CA Cancer J Clin* 56:11–25, 2006; quiz 49–50.
312. Hewitson P, Glasziou P, Irwig L, Towler B, Watson E. Screening for colorectal cancer using the faecal occult blood test, Hemoccult. *Cochrane Database Syst Rev* 2007;CD001216.
313. Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science (Wash DC)* 1992;256:102–5.
314. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000;119:1219–27.
315. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J Natl Cancer Inst* 2001;93:858–65.
316. Ahlquist DA, Shuber AP. Stool screening for colorectal cancer: evolution from occult blood to molecular markers. *Clin Chim Acta* 2002;315:157–68.
317. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704–14.
318. Song K, Fendrick AM, Ladabaum U. Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis. *Gastroenterology* 2004;126:1270–9.
319. Loganayagam A. Faecal screening of colorectal cancer. *Int J Clin Pract* 2008;62:454–9.
320. Levin B, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570–95.
321. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. Screening and Surveillance for the Early Detection of Colorectal Cancer and Adenomatous Polyps, 2008: a Joint Guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin* 2008;58:130–60.
322. American Gastroenterological Association Medical Position statement: hereditary colorectal cancer and genetic testing. *Gastroenterology* 2001;121:195–7.
323. Giardiello FM, Brensinger JD, Petersen GM. AGA technical review on hereditary colorectal cancer and genetic testing. *Gastroenterology* 2001; 121:198–213.
324. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. *J Clin Oncol* 2003;21:2397–406.
325. Guillem JG, Wood WC, Moley JF, Berchuck A, Karlan BY, Mutch DG, et al. ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. *J Clin Oncol* 2006; 24:4642–60.
326. Lynch HT, Boland CR, Rodriguez-Bigas MA, Amos C, Lynch JF, Lynch PM. Who should be sent for genetic testing in hereditary colorectal cancer syndromes? *J Clin Oncol* 2007;25: 3534–42.
327. Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. *Int J Cancer* 2002;97: 72–81.
328. Hewitt M, Breen N, Devesa S. Cancer prevalence and survivorship issues: analyses of the 1992 National Health Interview Survey. *J Natl Cancer Inst* 1999;91:1480–6.
329. Peto R, Boreham J, Clarke M, Davies C, Beral V. UK and USA breast cancer deaths down 25% in year 2000 at ages 20–69 years. *Lancet* 2000; 355:1822.
330. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;365: 1687–717.
331. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998; 351:1451–67.
332. Morris AD, Morris RD, Wilson JF, White J, Steinberg S, Okunieff P, et al. Breast-conserving therapy vs mastectomy in early-stage breast cancer: a meta-analysis of 10-year survival. *Cancer J Sci Am* 1997;3:6–12.
333. Khatcheressian JL, Wolff AC, Smith TJ, Grunfeld E, Muss HB, Vogel VG, et al. American Society of Clinical Oncology 2006 update of the breast cancer follow-up and management guidelines in the adjuvant setting. *J Clin Oncol* 2006;24: 5091–7.
334. Bernard-Marty C, Cardoso F, Piccart MJ. Facts and controversies in systemic treatment of metastatic breast cancer. *Oncologist* 2004;9: 617–32.
335. Colozza M, de Azambuja E, Cardoso F, Bernard C, Piccart MJ. Breast cancer: achievements in adjuvant systemic therapies in the pre-genomic era. *Oncologist* 2006;11:111–25.
336. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999;17: 1474–81.
337. Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, et al. Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and p52, by immunohistochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study. *Int J Cancer* 2000; 89:111–7.
338. McCarty KS, Jr., Miller LS, Cox EB, Konrath J, McCarty KS, Sr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 1985;109:716–21.
339. Barnes DM, Harris WH, Smith P, Millis RR, Rubens RD. Immunohistochemical determination of oestrogen receptor: comparison of different methods of assessment of staining and correlation with clinical outcome of breast cancer patients. *Br J Cancer* 1996;74:1445–51.
340. Pertschuk LP, Feldman JG, Kim YD, Braithwaite L, Schneider F, Braverman AS, Axiotis C. Estrogen receptor immunocytochemistry in paraffin embedded tissues with ER1D5 predicts breast cancer endocrine response more accurately than H222Sp gamma in frozen sections or cytosol-based ligand-binding assays. *Cancer* 1996;77: 2514–9.
341. Fisher ER, Anderson S, Dean S, Dabbs D, Fisher B, Siderits R, et al. Solving the dilemma of the immunohistochemical and other methods used for scoring estrogen receptor and progesterone receptor in patients with invasive breast carcinoma. *Cancer* 2005;103:164–73.
342. Chebil G, Bendahl PO, Ferno M. Estrogen and progesterone receptor assay in paraffin-embedded breast cancer—reproducibility of assessment. *Acta Oncol* 2003;42:43–7.
343. Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh ID, et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol* 2004;17:1545–54.
344. Leake R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. UK Receptor Group, UK NEQAS, the Scottish Breast Cancer Pathology Group, and the Receptor and Biomarker Study Group of the EORTC. *J Clin Pathol* 2000;53: 634–5.
345. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;124:966–78.
346. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007;25:118–45.
347. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Breast cancer. Version 2, 2007. http://www.nccn.org/patients/patient_gls_english_breast/contents.asp (Accessed April 2007).
348. Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *J Clin Oncol* 2001;19:2334–56.
349. Duffy MJ. Predictive markers in breast and other cancers: a review. *Clin Chem* 2005;51:494–503.
350. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. *J Clin Oncol* 2003;21:3357–65.
351. Gennari A, Sormani MP, Pronzato P, Puntoni M, Colozza M, Pfeffer U, Bruzzi P. HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials. *J Natl Cancer Inst* 2008;100:14–20.
352. Pritchard KI, Shepherd LE, O'Malley FP, Andrulis IL, Tu D, Bramwell VH, Levine MN. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med* 2006;354: 2103–11.

353. Esteva FJ, Cheli CD, Fritsche H, Fornier M, Slamon D, Thiel RP, et al. Clinical utility of serum HER2/neu in monitoring and prediction of progression-free survival in metastatic breast cancer patients treated with trastuzumab-based therapies. *Breast Cancer Res* 2005;7:R436–43.
354. Bartlett J, Mallon E, Cooke T. The clinical evaluation of HER-2 status: which test to use? *J Pathol* 2003;199:411–7.
355. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R, Slamon DJ. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol* 2000;18:3651–64.
356. Press MF, Hung G, Godolphin W, Slamon DJ. Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. *Cancer Res* 1994;54:2771–7.
357. Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. *J Clin Oncol* 2002;20:3095–105.
358. Bartlett JMS, Going JJ, Mallon EA, Watters AD, Reeves JR, Stanton P, et al. Evaluating HER-2 amplification and over expression in breast cancer. *J Pathol* 2001;195:422–8.
359. Birner P, Oberhuber G, Stani J, Reithofer C, Samonigg H, Hausmaninger H, et al. Evaluation of the United States Food and Drug Administration-approved scoring and test system of HER-2 protein expression in breast cancer. *Clin Cancer Res* 2001;7:1669–75.
360. Yaziji H, Goldstein LC, Barry TS, Werling R, Hwang H, Ellis GK, et al. HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA* 2004;291:1972–7.
361. Look MP, van Putten WL, Duffy MJ, Harbeck N, Christensen IJ, Thomssen C, et al. Pooled analysis of prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in 8377 breast cancer patients. *J Natl Cancer Inst* 2002;94:116–28.
362. Janicke F, Pechtl A, Thomssen C, Harbeck N, Meisner C, Untch M, et al. Randomized adjuvant chemotherapy trial in high-risk, lymph node-negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. *J Natl Cancer Inst* 2001;93:913–20.
363. Duffy MJ. Urokinase plasminogen activator and its inhibitor, PAI-1, as prognostic markers in breast cancer: from pilot to level 1 evidence studies. *Clin Chem* 2002;48:1194–7.
364. Harbeck N, Kates RE, Schmitt M. Clinical relevance of invasion factors urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 for individualized therapy decisions in primary breast cancer is greatest when used in combination. *J Clin Oncol* 2002;20:1000–7.
365. Harbeck N, Kates RE, Look MP, Meijer-van Gelder ME, Klijn JG, Kruger A, et al. Enhanced benefit from adjuvant chemotherapy in breast cancer patients classified high-risk according to urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (n = 3 424). *Cancer Res* 2002;62:4617–22.
366. Benraad TJ, Geurts-Moespot J, Grondahl-Hansen J, Schmitt M, Heuvel JJ, de Witte JH, et al. Immunoassays (ELISA) of urokinase-type plasminogen activator (uPA): report of an EORTC/BIOMED-1 workshop. *Eur J Cancer* 1996;32A:1371–81.
367. Sweep CG, Geurts-Moespot J, Grebenschikov N, de Witte JH, Heuvel JJ, Schmitt M, et al. External quality assessment of trans-European multicentre antigen determinations (enzyme-linked immunosorbent assay) of urokinase-type plasminogen activator (uPA) and its type 1 inhibitor (PAI-1) in human breast cancer tissue extracts. *Br J Cancer* 1998;78:1434–41.
368. Janicke F, Pache L, Schmitt M, Ulm K, Thomssen C, Pechtl A, Graeff H. Both the cytosols and detergent extracts of breast cancer tissues are suited to evaluate the prognostic impact of the urokinase-type plasminogen activator and its inhibitor, plasminogen activator inhibitor type 1. *Cancer Res* 1994;54:2527–30.
369. Abraha RS, Thomssen C, Harbeck N, Mueller V, Baack K, Schmitt M, et al. Micromethod for determination of uPA and PAI-1 from preoperative core-needle biopsies in breast cancer. *Breast Cancer Res Treat* 2003;82:S144.
370. Schmitt M, Sturmheit AS, Welk A, Schnelldorfer C, Harbeck N. Procedures for the quantitative protein determination of urokinase and its inhibitor PAI-1 in human breast cancer tissue extracts using ELISA. In: Brooks SA, Harris AL, eds. *Breast cancer research protocols*. Totowa (NJ): Humana Press Inc.; 2006. p 245–265. (Methods in molecular medicine, Vol. 120)
371. Molina R, Barak V, van DA, Duffy MJ, Einarsson R, Gion M, et al. Tumor markers in breast cancer: European Group on Tumor Markers recommendations. *Tumour Biol* 2005;26:281–93.
372. Pestalozzi BC, Luporsi-Gely E, Jost LM, Bergh J. ESMO Minimum Clinical Recommendations for diagnosis, adjuvant treatment and follow-up of primary breast cancer. *Ann Oncol* 2005;16 Suppl 1:i7–9.
373. Kataja VV, Colleoni M, Bergh J. ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of locally recurrent or metastatic breast cancer (MBC). *Ann Oncol* 2005;16 Suppl 1:i10–2.
374. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. *Ann Oncol* 2007;18:1133–44.
375. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. American Society of Clinical Oncology 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer. *J Clin Oncol* 2007;25:5287–312.
376. Jager W. The early detection of disseminated (metastasized) breast cancer by serial tumour marker measurements. *Eur J Cancer Prev* 1993;2 Suppl 3:133–9.
377. Nicolini A, Anselmi L, Michelassi C, Carpi A. Prolonged survival by 'early' salvage treatment of breast cancer patients: a retrospective 6-year study. *Br J Cancer* 1997;76:1106–11.
378. Kovner F, Merimsky O, Hareuveni M, Wigler N, Chaichik S. Treatment of disease-negative but mucin-like carcinoma-associated antigen-positive breast cancer patients with tamoxifen: preliminary results of a prospective controlled randomized trial. *Cancer Chemother Pharmacol* 1994;35:80–3.
379. Hayes DF, Kiang DT, Korzum AH, Tondini C, Wood WC, Kufe DW. CA15-3 and CEA spikes during chemotherapy for metastatic breast cancer. *Proc Am Soc Clin Oncol* 1998;7:38.
380. Yasasever V, Dincer M, Camlica H, Karaloglu D, Dalay N. Utility of CA 15-3 and CEA in monitoring breast cancer patients with bone metastases: special emphasis on "spiking" phenomena. *Clin Biochem* 1997;30:53–6.
381. Duffy MJ. Serum tumor markers in breast cancer: are they of clinical value? *Clin Chem* 2006;52:345–51.
382. Burke W, Daly M, Garber J, Botkin J, Kahn MJ, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. *JAMA* 1997;277:997–1003.
383. Nelson HD, Huffman LH, Fu R, Harris EL. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: systematic evidence review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2005;143:362–79.
384. US Preventive Services Task Force. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: Recommendation statement. *Ann Intern Med* 2005;143:355–61.
385. Sotiriou C, Piccart MJ. Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? *Nat Rev Cancer* 2007;7:545–53.
386. Fan C, Oh DS, Wessels L, Weigelt B, Nuyten DS, Nobel AB, et al. Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med* 2006;355:560–9.
387. van't Veer LJ, Dai H, van dV, M.J., He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature (Lond)* 2002;415:530–6.
388. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;347:1999–2009.
389. Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst* 2006;98:1183–92.
390. Cardoso F, Van't Veer L, Rutgers E, Loi S, Mook S, Piccart-Gebhart MJ. Clinical application of the 70-gene profile: the MINDACT trial. *J Clin Oncol* 2008;26:729–35.
391. Paik S. Development and clinical utility of a 21-gene recurrence score prognostic assay in patients with early breast cancer treated with tamoxifen. *Oncologist* 2007;12:631–5.
392. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multi-gene assay to predict recurrence of tamoxifen-treated node-negative breast cancer. *N Engl J Med* 2005;347:2817–26.
393. Habel LA, Shak S, Jacobs MK, Capra A, Alexander C, Pho M, et al. A population-based study of tumor gene expression and risk of breast

- cancer death among lymph node-negative patients. *Breast Cancer Res* 2006;8:R25.
394. Gianni L, Zambetti M, Clark K, Baker J, Cronin M, Wu J, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol* 2005; 23:7265–77.
 395. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006;24:3726–34.
 396. Sparano JA, Paik S. Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol* 2008;26:721–8.
 397. American Cancer Society (ACS). American Cancer Society statistics for 2006. http://www.cancer.org/docroot/STT/stt_0.asp (Accessed May 2007).
 398. International Agency for Research on Cancer (IARC). Cancer mondial. <http://www-dep.iarc.fr/> (Accessed May 2007).
 399. Young RH, Clement PB, Scully RE, Sternberg SS. The ovary: diagnostic surgical pathology. Vol. 3. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 2307–94.
 400. Scully RE, ed. Histological typing of ovarian tumours. Sobin LH and pathologists in 5 countries, collaborators. 2nd ed. New York: Springer; 1999. 136 p.
 401. Seidman JD, Horkayne-Szakaly I, Haiba M, Boice CR, Kurman RJ, Ronnett BM. The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. *Int J Gynecol Pathol* 2004;23:41–4.
 402. Shih I, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol* 2004; 164:1511–8.
 403. Bonfrer JMG, Duffy MJ, Radtke M, Segurado O, Torre GC, Van Dalen A, et al. Tumour markers in gynaecological cancers: EGTM recommendations. *Anticancer Res* 1999;19:2807–10.
 404. Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJ, Soletoormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. *Int J Gynecol Cancer* 2005;15:679–91.
 405. American College of Physicians. Screening for ovarian cancer: recommendations and rationale. *Ann Intern Med* 1994;121:141–2.
 406. Vasey PA, Herrstedt J, Jelic S. ESMO minimum clinical recommendations for diagnosis, treatment and follow-up of epithelial ovarian carcinoma. *Ann Oncol* 2005;16 Suppl 1:i13–5.
 407. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Ovarian cancer. Version 1.2008. http://www.nccn.org/professionals/physician_gls/PDF/ovarian.pdf. (Accessed November 2008).
 408. NIH consensus conference. Ovarian cancer: screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer. *JAMA* 1995;273:491–7.
 409. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 1981;68:1331–7.
 410. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. *J Biol Chem* 2001;276: 27371–5.
 411. Bast RC, Jr., Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. *Int J Biol Markers* 1998;13:179–87.
 412. Jacobs I, Bast RC, Jr. The CA 125 tumour-associated antigen: a review of the literature. *Hum Reprod* 1989;4:1–12.
 413. Davelaar EM, van Kamp GJ, Verstraeten RA, Kenemans P. Comparison of seven immunoassays for the quantification of CA 125 antigen in serum. *Clin Chem* 1998;44:1417–22.
 414. Shih Ie M, Sokoll L, Chan DW. Ovarian cancer. In: Diamandis EP, Fritsche HA, Lilja H, Chan DW, Schwartz MK, editors. *Tumor markers: physiology, pathobiology, technology and clinical applications*. Washington DC: AACC Press; 2002. p. 239–52.
 415. Pauler DK, Menon U, McIntosh M, Symecko HL, Skates SJ, Jacobs IJ. Factors influencing serum CA125II levels in healthy postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2001;10:489–93.
 416. Munkarah A, Chatterjee M, Tainsky MA. Update on ovarian cancer screening. *Curr Opin Obstet Gynecol* 2007;19:22–6.
 417. Bast RC, Jr., Urban N, Shridhar V, Smith D, Zhang Z, Skates S, et al. Early detection of ovarian cancer: promise and reality. *Cancer Treat Res* 2002;107:61–97.
 418. Skates SJ, Xu FJ, Yu YH, Sjøvall K, Einhorn N, Chang Y, et al. Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers. *Cancer* 1995;76:2004–10.
 419. Zhang Z, Bast RC, Jr., Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004;64: 5882–90.
 420. Simpson NK, Johnson CC, Ogden SL, Gamito E, Trocky N, McGuire C, et al. Recruitment strategies in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: the first six years. *Control Clin Trials* 2000;21:3565–78S.
 421. Menon U, Jacobs I. Screening for ovarian cancer. *Best Pract Res Clin Obstet Gynaecol* 2002; 16:469–82.
 422. Tholander B, Taube A, Lindgren A, Sjöberg O, Stendahl U, Kiviranta A, et al. Pretreatment serum levels of CA-125, carcinoembryonic antigen, tissue polypeptide antigen, and placental alkaline phosphatase, in patients with ovarian carcinoma, borderline tumors, or benign adnexal masses: relevance for differential diagnosis. *Gynecol Oncol* 1990;39:16–25.
 423. Scottish Intercollegiate Guidelines Network (SIGN). Epithelial ovarian cancer: a national clinical guideline. <http://www.sign.ac.uk/guidelines/fulltext/75/index.html> (Accessed November 2008). (SIGN 75; 2003 Oct)
 424. Fritsche HA, Bast RC. CA 125 in ovarian cancer: advances and controversy. *Clin Chem* 1998;44: 1379–80.
 425. Duffy MJ. Clinical uses of tumor markers: a critical review. *Crit Rev Clin Lab Sci* 2001;38: 225–62.
 426. Meyer T, Rustin GJ. Role of tumour markers in monitoring epithelial ovarian cancer. *Br J Cancer* 2000;82:1535–8.
 427. Tuxen M. CA125 in ovarian cancer. *J Tumor Marker Oncol* 2001;16:49–68.
 428. Gronlund B, Hogdall C, Hilden J, Engelholm SA, Hogdall EV, Hansen HH. Should CA-125 response criteria be preferred to response evaluation criteria in solid tumors (RECIST) for prognostication during second-line chemotherapy of ovarian carcinoma? *J Clin Oncol* 2004;22:4051–8.
 429. Rustin GJ, Quinn M, Thigpen T, du BA, Pujade-Lauraine E, Jakobsen A, et al. Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). *J Natl Cancer Inst* 2004;96:487–8.
 430. Rustin GJ. Can we now agree to use the same definition to measure response according to CA-125? *J Clin Oncol* 2004;22:4035–6.
 431. National Cancer Institute. CA125 definitions agreed by GCIG November 2005. <http://ctep.cancer.gov/resources/gcig/respdef.html> (Accessed May 2007).
 432. Tuxen MK, Soletoormos G, Petersen PH, Schioler V, Dombrowsky P. Assessment of biological variation and analytical imprecision of CA 125, CEA, and TPA in relation to monitoring of ovarian cancer. *Gynecol Oncol* 1999;74:12–22.
 433. Tuxen MK, Soletoormos G, Dombrowsky P. Serum tumour marker CA 125 in monitoring of ovarian cancer during first-line chemotherapy. *Br J Cancer* 2001;84:1301–7.
 434. Fraser C. Biological variation: from principles to practice. Washington (DC): AACC Press; 2001. p. 67–90.
 435. MRC Clinical Trials Unit. OV05: Second-line treatment based on CA125 or clinical progression. <http://www.ctu.mrc.ac.uk/studies/OV05.asp> (Accessed November 2008).
 436. Partridge EE, Barnes MN. Epithelial ovarian cancer: prevention, diagnosis, and treatment. *CA Cancer J Clin* 1999;49:297–320.
 437. Vermorken JB, Avall-Lundqvist E, Pfisterer J, Bacon M. The Gynecologic Cancer Intergroup (GCG): history and current status. *Ann Oncol* 2005;16 Suppl 8:viii39–viii42.
 438. Santillan A, Garg R, Zahurak ML, Gardner GJ, Giuntoli RL, 2nd, Armstrong DK, Bristow RE. Risk of epithelial ovarian cancer recurrence in patients with rising serum CA-125 levels within the normal range. *J Clin Oncol* 2005;23: 9338–43.
 439. Cooper BC, Sood AK, Davis CS, Ritchie JM, Sorosky JI, Anderson B, Buller RE. Preoperative CA 125 levels: an independent prognostic factor for epithelial ovarian cancer. *Obstet Gynecol* 2002;100:59–64.
 440. Gadducci A, Cosio S, Fanucchi A, Negri S, Cristofani R, Genazzani AR. The predictive and prognostic value of serum CA 125 half-life during paclitaxel/platinum-based chemotherapy in patients with advanced ovarian carcinoma. *Gynecol Oncol* 2004;93:131–6.
 441. Rustin GJ. The clinical value of tumour markers in the management of ovarian cancer. *Ann Clin Biochem* 1996;33(Pt 4):284–9.
 442. Fayers PM, Rustin G, Wood R, Nelstrop A, Leonard RC, Wilkinson P, et al. The prognostic value of serum CA 125 in patients with advanced ovarian carcinoma: an analysis of 573 patients by the Medical Research Council Working Party on Gynaecological Cancer. *Int J Gynecol Cancer* 1993;3:285–92.

443. Verheijen RH, von Mensdorff-Pouilly S, van Kamp GJ, Kenemans P. CA 125: fundamental and clinical aspects. *Semin Cancer Biol* 1999;9:117–24.
444. Riedinger JM, Wafflart J, Ricolleau G, Eche N, Larbre H, Basuyau JP, et al. CA 125 half-life and CA 125 nadir during induction chemotherapy are independent predictors of epithelial ovarian cancer outcome: results of a French multicentric study. *Ann Oncol* 2006;17:1234–8.
445. Diamandis EP, Yousef GM. Human tissue kallikreins: a family of new cancer biomarkers. *Clin Chem* 2002;48:1198–205.
446. Diamandis EP, Yousef GM, Soosaipillai AR, Bunting P. Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. *Clin Biochem* 2000;33:579–83.
447. Hoffman BR, Katsaros D, Scorilas A, Diamandis P, Fracchioli S, Rigault de la Longrais IA, et al. Immunofluorometric quantitation and histochemical localisation of kallikrein 6 protein in ovarian cancer tissue: a new independent unfavourable prognostic biomarker. *Br J Cancer* 2002;87:763–71.
448. Diamandis EP, Okui A, Mitsui S, Luo LY, Soosaipillai A, Grass L, et al. Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. *Cancer Res* 2002;62:295–300.
449. Dong Y, Kaushal A, Bui L, Chu S, Fuller PJ, Nicklin J, et al. Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. *Clin Cancer Res* 2001;7:2363–71.
450. Obiezu CV, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, et al. Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. *Clin Cancer Res* 2001;7:2380–6.
451. Kim H, Scorilas A, Katsaros D, Yousef GM, Massobrio M, Fracchioli S, et al. Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. *Br J Cancer* 2001;84:643–50.
452. Magklara A, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, et al. The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. *Clin Cancer Res* 2001;7:806–11.
453. Chang A, Yousef GM, Jung K, Rajpert-De ME, Diamandis EP. Identification and molecular characterization of five novel kallikrein gene 13 (KLK13; KLK-L4) splice variants: differential expression in the human testis and testicular cancer. *Anticancer Res* 2001;21:3147–52.
454. Yousef GM, Scorilas A, Katsaros D, Fracchioli S, Iskander L, Borgono C, et al. Prognostic value of the human kallikrein gene 15 expression in ovarian cancer. *J Clin Oncol* 2003;21:3119–26.
455. Borgono CA, Grass L, Soosaipillai A, Yousef GM, Petraki CD, Howarth DH, et al. Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. *Cancer Res* 2003;63:9032–41.
456. Yousef GM, Polymeris ME, Grass L, Soosaipillai A, Chan PC, Scorilas A, et al. Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. *Cancer Res* 2003;63:3958–65.
457. Kishi T, Grass L, Soosaipillai A, Scorilas A, Harbeck N, Schmalfeldt B, et al. Human kallikrein 8, a novel biomarker for ovarian carcinoma. *Cancer Res* 2003;63:2771–4.
458. Diamandis EP, Scorilas A, Fracchioli S, Van GM, De BH, Henrik A, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma. *J Clin Oncol* 2003;21:1035–43.
459. Luo LY, Katsaros D, Scorilas A, Fracchioli S, Bellino R, Van GM, et al. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. *Cancer Res* 2003;63:807–11.
460. Yousef GM, Polymeris ME, Yacoub GM, Scorilas A, Soosaipillai A, Popalis C, et al. Parallel overexpression of seven kallikrein genes in ovarian cancer. *Cancer Res* 2003;63:2223–7.
461. Kurlender L, Yousef GM, Memari N, Robb JD, Michael IP, Borgono C, et al. Differential expression of a human kallikrein 5 (KLK5) splice variant in ovarian and prostate cancer. *Tumour Biol* 2004;25:149–56.
462. Diamandis EP, Borgono CA, Scorilas A, Harbeck N, Dorn J, Schmitt M. Human kallikrein 11: an indicator of favorable prognosis in ovarian cancer patients. *Clin Biochem* 2004;37:823–9.
463. Scorilas A, Borgono CA, Harbeck N, Dorn J, Schmalfeldt B, Schmitt M, Diamandis EP. Human kallikrein 13 protein in ovarian cancer cytosols: a new favorable prognostic marker. *J Clin Oncol* 2004;22:678–85.
464. Kyriakopoulou LG, Yousef GM, Scorilas A, Katsaros D, Massobrio M, Fracchioli S, Diamandis EP. Prognostic value of quantitatively assessed KLK7 expression in ovarian cancer. *Clin Biochem* 2003;36:135–43.
465. Yousef GM, Kyriakopoulou LG, Scorilas A, Fracchioli S, Ghiringhello B, Zarghooni M, et al. Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. *Cancer Res* 2001;61:7811–8.
466. Luo LY, Bunting P, Scorilas A, Diamandis EP. Human kallikrein 10: a novel tumor marker for ovarian carcinoma? *Clin Chim Acta* 2001;306:111–8.
467. Shih le M, Salani R, Fiegl M, Wang TL, Soosaipillai A, Marth C, et al. Ovarian cancer specific kallikrein profile in effusions. *Gynecol Oncol* 2007;105:501–7.
468. Kim JH, Skates SJ, Uede T, Wong KK, Schorge JO, Feltmate CM, et al. Osteopontin as a potential diagnostic biomarker for ovarian cancer. *JAMA* 2002;287:1671–9.
469. Schorge JO, Drake RD, Lee H, Skates SJ, Rajanbabu R, Miller DS, et al. Osteopontin as an adjunct to CA125 in detecting recurrent ovarian cancer. *Clin Cancer Res* 2004;10:3474–8.
470. Mok SC, Chao J, Skates S, Wong K, Yiu GK, Muto MG, et al. Prostatein, a potential serum marker for ovarian cancer: identification through microarray technology. *J Natl Cancer Inst* 2001;93:1458–64.
471. Yu JX, Chao L, Chao J. Prostatein is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. *J Biol Chem* 1994;269:18843–8.
472. Sundstrom BE, Stigbrand TI. Cytokeratins and tissue polypeptide antigen. *Int J Biol Markers* 1994;9:102–8.
473. Tuxen MK, Soletormos G, Dombernowsky P. Tumor markers in the management of patients with ovarian cancer. *Cancer Treat Rev* 1995;21:215–45.
474. Xu Y, Shen Z, Wiper DW, Wu M, Morton RE, Elson P, et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. *JAMA* 1998;280:719–23.
475. Pustilnik TB, Estrella V, Wiener JR, Mao M, Eder A, Watt MA, et al. Lysophosphatidic acid induces urokinase secretion by ovarian cancer cells. *Clin Cancer Res* 1999;5:3704–10.
476. Hu YL, Albanese C, Pestell RG, Jaffe RB. Dual mechanisms for lysophosphatidic acid stimulation of human ovarian carcinoma cells. *J Natl Cancer Inst* 2003;95:733–40.
477. Fang X, Yu S, Bast RC, Liu S, Xu HJ, Hu SX, et al. Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J Biol Chem* 2004;279:9653–61.
478. Stenman UH, Huhtala ML, Koistinen R, Seppala M. Immunochemical demonstration of an ovarian cancer-associated urinary peptide. *Int J Cancer* 1982;30:53–7.
479. Huhtala ML, Pesonen K, Kalkkinen N, Stenman UH. Purification and characterization of a tumor-associated trypsin inhibitor from the urine of a patient with ovarian cancer. *J Biol Chem* 1982;257:13713–6.
480. Gadducci A, Ferdeghini M, Rispoli G, Prontera C, Bianchi R, Fioretti P. Comparison of tumor-associated trypsin inhibitor (TATI) with CA125 as a marker for diagnosis and monitoring of epithelial ovarian cancer. *Scand J Clin Lab Invest Suppl* 1991;207:19–24.
481. Zotter S, Hagemann PC, Lossnitzer A, Moo WJ, Hilgers J. Tissue and tumor distribution of human polymorphic epithelial mucin. *Cancer Rev* 1988;11:55–101.
482. Ward BG, McGuckin MA. Are CASA and CA125 concentrations in peripheral blood sourced from peritoneal fluid in women with pelvic masses? *Cancer* 1994;73:1699–703.
483. Ward BG, McGuckin MA, Ramm L, Forbes KL. Expression of tumour markers CA125, CASA and OSA in minimal/mild endometriosis. *Aust N Z J Obstet Gynaecol* 1991;31:273–5.
484. Ward BG, McGuckin MA, Ramm LE, Cogan M, Sanderson B, Tripcony L, Free KE. The management of ovarian carcinoma is improved by the use of cancer-associated serum antigen and CA 125 assays. *Cancer* 1993;71:430–8.
485. Chambers SK, Ivins CM, Carcangiu ML. Expression of plasminogen activator inhibitor-2 in epithelial ovarian cancer: a favorable prognostic factor related to the actions of CSF-1. *Int J Cancer* 1997;74:571–5.
486. Chambers SK, Ivins CM, Carcangiu ML. Plasminogen activator inhibitor-1 is an independent poor prognostic factor for survival in advanced stage epithelial ovarian cancer patients. *Int J Cancer* 1998;79:449–54.
487. Gastl G, Plante M, Bartlett JMS. Bioactive interleukin-6 levels in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer. *Methods in molecular medicine-ovarian cancer*, Vol. Totowa: Humana Press Inc., 2000;121–3.
488. Foti E, Ferrandina G, Martucci R, Romanini ME, Benedetti PP, Testa U, et al. IL-6, M-CSF and IAP cytokines in ovarian cancer: simultaneous assessment of serum levels. *Oncology* 1999;57:211–5.

489. Scambia G, Testa U, Benedetti PP, Foti E, Martucci R, Gadducci A, et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br J Cancer* 1995;71:354–6.
490. Cole LA. hCG, its free subunits and its metabolites: roles in pregnancy and trophoblastic disease. *J Reprod Med* 1998;43:3–10.
491. Vartiainen J, Lehtovirta P, Finne P, Stenman UH, Alfthan H. Preoperative serum concentration of hCGbeta as a prognostic factor in ovarian cancer. *Int J Cancer* 2001;95:313–6.
492. Nishimura R, Koizumi T, Das H, Takemori M, Hasegawa K, Bartlett JMS. Enzyme immunoassay of urinary b-core fragment of human chorionic gonadotropin as a tumor marker for ovarian cancer: methods in molecular medicine-ovarian cancer. Totowa: Humana Press; 2000. p. 135–41.
493. Meden H, Fattahi-Meibodi A, Marx D, Bartlett JMS. ELISA-based quantification of p105 (c-erbB-2, HER2/neu) in serum of ovarian carcinoma. Methods in molecular medicine - ovarian cancer. Totowa: Humana Press; 2000. p. 125–33.
494. Meden H, Kuhn W. Overexpression of the oncogene c-erbB-2 (HER2/neu) in ovarian cancer: a new prognostic factor. *Eur J Obstet Gynecol Reprod Biol* 1997;71:173–9.
495. Cheung TH, Wong YF, Chung TK, Maimonis P, Chang AM. Clinical use of serum c-erbB-2 in patients with ovarian masses. *Gynecol Obstet Invest* 1999;48:133–7.
496. Hellstrom I, Goodman G, Pullman J, Yang Y, Hellstrom KE. Overexpression of HER-2 in ovarian carcinomas. *Cancer Res* 2001;61:2420–3.
497. Liu AX, Testa JR, Hamilton TC, Jove R, Nicosia SV, Cheng JQ. AKT2, a member of the protein kinase B family, is activated by growth factors, v-Ha-ras, and v-src through phosphatidylinositol 3-kinase in human ovarian epithelial cancer cells. *Cancer Res* 1998;58:2973–7.
498. Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C, et al. PIK3CA is implicated as an oncogene in ovarian cancer. *Nat Genet* 1999; 21:99–102.
499. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, et al. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci U S A* 1992;89:9267–71.
500. Bellacosa A, de FD, Godwin AK, Bell DW, Cheng JQ, Altomare DA, et al. Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer* 1995;64:280–5.
501. Yuan ZQ, Sun M, Feldman RI, Wang G, Ma X, Jiang C, et al. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. *Oncogene* 2000;19:2324–30.
502. Peyssonnaud C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* 2001;93:53–62.
503. Allen LF, Sebolt-Leopold J, Meyer MB. CI-1040 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK). *Semin Oncol* 2003;30: 105–6.
504. Hsu CY, Bristow R, Cha MS, Wang BG, Ho CL, Kurman RJ, et al. Characterization of active mitogen-activated protein kinase in ovarian serous carcinomas. *Clin Cancer Res* 2004;10:6432–6.
505. Givant-Horwitz V, Davidson B, Lazarovici P, Schaefer E, Nesland JM, Trope CG, Reich R. Mitogen-activated protein kinases (MAPK) as predictors of clinical outcome in serous ovarian carcinoma in effusions. *Gynecol Oncol* 2003;91: 160–72.
506. Robertson DM, Stephenson T, Pruyers E, McCloud P, Tsigos A, Groome N, et al. Characterization of inhibin forms and their measurement by an inhibin alpha-subunit ELISA in serum from postmenopausal women with ovarian cancer. *J Clin Endocrinol Metab* 2002;87:816–24.
507. Robertson DM, Pruyers E, Burger HG, Jobling T, McNeillage J, Healy D. Inhibins and ovarian cancer. *Mol Cell Endocrinol* 2004;225:65–71.
508. Robertson DM, Burger HG, Fuller PJ. Inhibin/activin and ovarian cancer. *Endocr Relat Cancer* 2004;11:35–49.
509. Loyola A, Huang JY, LeRoy G, Hu S, Wang YH, Donnelly RJ, et al. Functional analysis of the subunits of the chromatin assembly factor RSF. *Mol Cell Biol* 2003;23:6759–68.
510. Shamay M, Barak O, Doitsh G, Ben-Dor I, Shaul Y. Hepatitis B virus pX interacts with HBXAP, a PHD finger protein to coactivate transcription. *J Biol Chem* 2002;277:9982–8.
511. Shamay M, Barak O, Shaul Y. HBXAP, a novel PHD-finger protein, possesses transcription repression activity. *Genomics* 2002;79:523–9.
512. Shih Ie M, Sheu JJ, Santillan A, Nakayama K, Yen MJ, Bristow RE, et al. Amplification of a chromatin remodeling gene, Rsf-1/HBXAP, in ovarian carcinoma. *Proc Natl Acad Sci U S A* 2005;102:14004–9.
513. Davidson B, Trope CG, Wang TL, Shih Ie M. Expression of the chromatin remodeling factor Rsf-1 is upregulated in ovarian carcinoma effusions and predicts poor survival. *Gynecol Oncol* 2006;103:814–9.
514. Mao TL, Hsu CY, Yen MJ, Gilks B, Sheu JJ, Gabrielson E, et al. Expression of Rsf-1, a chromatin-remodeling gene, in ovarian and breast carcinoma. *Hum Pathol* 2006;37:1169–75.
515. Stogios PJ, Downs GS, Jauhal JJ, Nandra SK, Prive GG. Sequence and structural analysis of BTB domain proteins. *Genome Biol* 2005;6:R82.
516. Nakayama K, Nakayama N, Davidson B, Sheu JJ, Jinawath N, Santillan A, et al. A BTB/POZ protein, NAC-1, is related to tumor recurrence and is essential for tumor growth and survival. *Proc Natl Acad Sci U S A* 2006;103:18739–44.
517. Wang J, Rao S, Chu J, Shen X, Levasseur DN, Theunissen TW, Orkin SH. A protein interaction network for pluripotency of embryonic stem cells. *Nature (Lond)* 2006;444:364–8.
518. Davidson B, Berner A, Trope CG, Wang TL, Shih IM. Expression and clinical role of the bric-a-brac tramtrack broad complex/poxvirus and zinc protein NAC-1 in ovarian carcinoma effusions. *Hum Pathol* 2007;38:1030–6.
519. Cheng L. Establishing a germ cell origin for metastatic tumors using OCT4 immunohistochemistry. *Cancer* 2004;101:2006–10.
520. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. *BMJ* 2004; 328:1490.
521. Cooner WH, Mosley BR, Rutherford CL, Jr., Beard JH, Pond HS, Terry WJ, et al. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. *J Urol* 1990;143: 1146–52.
522. Babaian RJ, Johnston DA, Naccarato W, Ayala A, Bhadkamkar VA, Fritsche HH Jr. The incidence of prostate cancer in a screening population with a serum prostate specific antigen between 2.5 and 4.0 ng/ml: relation to biopsy strategy. *J Urol* 2001;165:757–60.
523. Partin AW, Mangold LA, Lamm DM, Walsh PC, Epstein JI, Pearson JD. Contemporary update of prostate cancer staging nomograms (Partin Tables) for the new millennium. *Urology* 2001;58: 843–8.
524. Blackledge GR, Lowery K. Role of prostate-specific antigen as a predictor of outcome in prostate cancer. *Prostate Suppl* 1994;5:34–8.
525. Trapasso JG, deKernion JB, Smith RB, Dorey F. The incidence and significance of detectable levels of serum prostate specific antigen after radical prostatectomy. *J Urol* 1994;152:1821–5.
526. Bjork T, Lilja H, Christensson A. The prognostic value of different forms of prostate specific antigen and their ratios in patients with prostate cancer. *BJU Int* 1999;84:1021–7.
527. Consensus statement: guidelines for PSA following radiation therapy. American Society for Therapeutic Radiology and Oncology Consensus Panel. *Int J Radiat Oncol Biol Phys* 1997;37:1035–41.
528. American Urological Association (AUA). Prostate-specific antigen (PSA) best practice policy. *Oncology (Williston Park)* 2000;14:267–8; 280.
529. Partin AW, Brawer MK, Subong EN, Kelley CA, Cox JL, Bruzek DJ, et al. Prospective evaluation of percent free-PSA and complexed-PSA for early detection of prostate cancer. *Prostate Cancer Prostatic Dis* 1998;1:197–203.
530. Screening for prostate cancer. American College of Physicians. *Ann Intern Med* 1997;126:480–4.
531. Aus G, Abbou CC, Pacik D, Schmid HP, Van PH, Wolff JM, Zattoni F. EAU guidelines on prostate cancer. *Eur Urol* 2001;40:97–101.
532. Prostate cancer: ESMO Clinical Recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2007;18 Suppl 2:ii36-ii37.
533. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Prostate cancer. Version 2. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp (Accessed May 2007).
534. Screening for prostate cancer: recommendation and rationale. U.S. Preventative Services Task Force. *Ann Intern Med* 2002;915–6.
535. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2008;149:185–91.
536. Gao CL, Rawal SK, Sun L, Ali A, Connelly RR, Banez LL, et al. Diagnostic potential of prostate-specific antigen expressing epithelial cells in blood of prostate cancer patients. *Clin Cancer Res* 2003;9:2545–50.
537. Khan MA, Sokoll LJ, Chan DW, Mangold LA, Mohr P, Mikolajczyk SD, et al. Clinical utility of proPSA and “benign” PSA when percent free PSA is less than 15%. *Urology* 2004;64:1160–4.
538. Steuber T, Niemela P, Haese A, Pettersson K, Erbersdobler A, Felix Chun KH, et al. Association of free-prostate specific antigen subfractions and human glandular kallikrein 2 with volume of benign and malignant prostatic tissue. *Prostate* 2005;63:13–8.

539. Haese A, Vaisanen V, Lilja H, Kattan MW, Rittenhouse HG, Pettersson K, et al. Comparison of predictive accuracy for pathologically organ confined clinical stage T1c prostate cancer using human glandular kallikrein 2 and prostate specific antigen combined with clinical stage and Gleason grade. *J Urol* 2005;173:752–6.
540. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004;363:1346–53.
541. Wolk A. The growth hormone and insulin-like growth factor I axis, and cancer. *Lancet* 2004;363:1336–7.
542. Fradet Y, Saad F, Aprikian A, Dessureault J, Elhilali M, Trudel C, et al. uPM3, a new molecular urine test for the detection of prostate cancer. *Urology* 2004;64:311–5.
543. Hessels D, Klein Gunnewiek JM, van OI, Karthaus HF, van Leenders GJ, van BB, et al. DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 2003;44:8–15.
544. Browne TJ, Hirsch MS, Brodsky G, Welch WR, Loda MF, Rubin MA. Prospective evaluation of AMACR (P504S) and basal cell markers in the assessment of routine prostate needle biopsy specimens. *Hum Pathol* 2004;35:1462–8.
545. Jiang Z, Wu CL, Woda BA, Iczkowski KA, Chu PG, Tretiakova MS, et al. Alpha-methylacyl-CoA racemase: a multi-institutional study of a new prostate cancer marker. *Histopathology* 2004;45:218–25.
546. Kumar-Sinha C, Shah RB, Laxman B, Tomlins SA, Harwood J, Schmitz W, et al. Elevated alpha-methylacyl-CoA racemase enzymatic activity in prostate cancer. *Am J Pathol* 2004;164:787–93.
547. Magi-Galluzzi C, Luo J, Isaacs WB, Hicks JL, de Marzo AM, Epstein JI. Alpha-methylacyl-CoA racemase: a variably sensitive immunohistochemical marker for the diagnosis of small prostate cancer foci on needle biopsy. *Am J Surg Pathol* 2003;27:1128–33.
548. Rubin MA, Zerkowski MP, Camp RL, Kuefer R, Hofer MD, Chinnaiyan AM, Rimm DL. Quantitative determination of expression of the prostate cancer protein alpha-methylacyl-CoA racemase using automated quantitative analysis (AQUA): a novel paradigm for automated and continuous biomarker measurements. *Am J Pathol* 2004;164:831–40.
549. Goessl C, Muller M, Heicappell R, Krause H, Straub B, Schrader M, Miller K. DNA-based detection of prostate cancer in urine after prostatic massage. *Urology* 2001;58:335–8.
550. Ntais C, Polycarpou A, Ioannidis JP. Association of GSTM1, GSTT1, and GSTP1 gene polymorphisms with the risk of prostate cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2005;14:176–81.
551. Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zochbauer-Muller S, Farinas AJ, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. *Clin Cancer Res* 2002;8:514–9.
552. Tokumaru Y, Harden SV, Sun DJ, Yamashita K, Epstein JI, Sidransky D. Optimal use of a panel of methylation markers with GSTP1 hypermethylation in the diagnosis of prostate adenocarcinoma. *Clin Cancer Res* 2004;10:5518–22.
553. Straub B, Muller M, Krause H, Goessl C, Schrader M, Heicappell R, Miller K. Molecular staging of pelvic surgical margins after radical prostatectomy: comparison of RT-PCR for prostate-specific antigen and telomerase activity. *Oncol Rep* 2002;9:545–9.
554. Vicentini C, Gravina GL, Angelucci A, Pascale E, D'Ambrosio E, Muzi P, et al. Detection of telomerase activity in prostate massage samples improves differentiating prostate cancer from benign prostatic hyperplasia. *J Cancer Res Clin Oncol* 2004;130:217–21.
555. Ylikoski A, Pettersson K, Nurmi J, Irjala K, Karp M, Lilja H, et al. Simultaneous quantification of prostate-specific antigen and human glandular kallikrein 2 mRNA in blood samples from patients with prostate cancer and benign disease. *Clin Chem* 2002;48:1265–71.
556. Thomas GV, Horvath S, Smith BL, Crosby K, Lebel LA, Schrage M, et al. Antibody-based profiling of the phosphoinositide 3-kinase pathway in clinical prostate cancer. *Clin Cancer Res* 2004;10:8351–6.
557. Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di CA, Xiao A, et al. Pten dose dictates cancer progression in the prostate. *PLoS Biol* 2003;1: E59.
558. Shaffer DR, Viale A, Ishiwata R, Leversha M, Olgac S, Manova K, et al. Evidence for a p27 tumor suppressive function independent of its role regulating cell proliferation in the prostate. *Proc Natl Acad Sci U S A* 2005;102:210–5.
559. Gao H, Quyang X, Banach-Petrosky W, Borowsky AD, Lin Y, Kim M, et al. A critical role for p27kip1 gene dosage in a mouse model of prostate carcinogenesis. *Proc Natl Acad Sci U S A* 2004;101:17204–9.
560. Pollack A, DeSilvio M, Khor LY, Li R, Al-Saleem TI, Hammond ME, et al. Ki-67 staining is a strong predictor of distant metastasis and mortality for men with prostate cancer treated with radiotherapy plus androgen deprivation: Radiation Therapy Oncology Group Trial 92–02. *J Clin Oncol* 2004;22:2133–40.
561. Qian J, Hirasawa K, Bostwick DG, Bergstralh EJ, Slezak JM, Anderl KL, et al. Loss of p53 and c-myc overrepresentation in stage T(2–3)N(1–3)M(0) prostate cancer are potential markers for cancer progression. *Mod Pathol* 2002;15:35–44.
562. Han KR, Seligson DB, Liu X, Horvath S, Shintaku PI, Thomas GV, et al. Prostate stem cell antigen expression is associated with gleason score, seminal vesicle invasion and capsular invasion in prostate cancer. *J Urol* 2004;171:1117–21.
563. Prostate Cancer Foundation (PCF). Report to the nation on prostate cancer. <http://www.prostatecancerfoundation.org/guide/> (Accessed November 2008).
564. Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, Drake JC, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. *J Clin Oncol* 1994;12:2640–7.
565. Boland CR. Clinical uses of microsatellite instability testing in colorectal cancer: an ongoing challenge. *J Clin Oncol* 2007;25:754–6.
566. Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, Wolmark N, Allegra CJ. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. *J Clin Oncol* 2007;25:767–72.
567. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919–32.
568. Rowley PT. Inherited susceptibility to colorectal cancer. *Annu Rev Med* 2005;56:539–54.
569. Hendriks YM, de Jong AE, Morreau H, Tops CM, Vasen HF, Wijnen JT, et al. Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. *CA Cancer J Clin* 2006;56:213–25.
570. Duffy MJ, van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *Eur J Cancer* 2007;43:1348–60.
571. Van Cutsem EJKW. ESMO minimum clinical recommendations for diagnosis, adjuvant treatment and follow-up of primary colon cancer. *Ann Oncol* 2005;16:i16–7.
572. Tveit KM, Kataja VV. ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of rectal cancer. *Ann Oncol* 2005;16(Suppl 1):i20–1.
573. Van Cutsem EJ. Colon cancer: ESMO clinical recommendations for diagnosis, adjuvant treatment of follow-up of primary colon cancer. *Ann Oncol* 2007;18(Suppl 2):ii21–2.
574. Van Cutsem EJ. Advanced colorectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up of rectal cancer. *Ann Oncol* 2007;18(Suppl 2):ii25–6.
575. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Colon cancer. Version 1. 2007. http://www.nccn.org/professionals/physician_gls/PDF/colon.pdf (Accessed April 2007).
576. Duffy MJ. Estrogen receptors: role in breast cancer. *Crit Rev Clin Lab Sci* 2006;43:325–47.
577. Mirza AN, Mirza NQ, Vlastos G, Singletary SE. Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Ann Surg* 2002;235:10–26.
578. Ravdin PM, Green S, Dorr TM, McGuire WL, Fabian C, Pugh RP, et al. Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study. *J Clin Oncol* 1992;10:1284–91.
579. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol* 2003;21:1973–9.
580. Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, et al. The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. *Oncologist* 2003;8:307–25.
581. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783–92.

582. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673–84.
583. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659–72.
584. Foekens JA, Look MP, Peters HA, van Putten WL, Portengen H, Klijn JG. Urokinase-type plasminogen activator and its inhibitor PAI-1: predictors of poor response to tamoxifen therapy in recurrent breast cancer. *J Natl Cancer Inst* 1995;87:751–6.
585. Meijer-van Gelder ME, Look MP, Peters HA, Schmitt M, Brunner N, Harbeck N, et al. Urokinase-type plasminogen activator system in breast cancer: association with tamoxifen therapy in recurrent disease. *Cancer Res* 2004;64:4563–8.
586. Harbeck N, Kates RE, Look MP, Foekens JA. Pooled analysis (n=8,377) evaluates predictive impact of uPA and PAI-1 for response to adjuvant therapy in breast cancer. In: *Journal of Clinical Oncology, 2004 ASCO Annual Meeting proceedings (post-meeting edition)*. 2004 Jul 15; 22(14S):(Abstract 523).
587. Foekens JA, Look MP, Bolt-de VJ, Meijer-van Gelder ME, van Putten WL, Klijn JG. Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. *Br J Cancer* 1999;79:300–7.
588. Ferrandina G, Scambia G, Bardelli F, Benedetti PP, Mancuso S, Messori A. Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: a meta-analysis. *Br J Cancer* 1997;76:661–6.
589. Ravdin PM, Tandon AK, Allred DC, Clark GM, Fuqua SA, Hilsenbeck SH, et al. Cathepsin D by western blotting and immunohistochemistry: failure to confirm correlations with prognosis in node-negative breast cancer. *J Clin Oncol* 1994;12:467–74.
590. Elledge RM, Allred DC. Prognostic and predictive value of p53 and p21 in breast cancer. *Breast Cancer Res Treat* 1998;52:79–98.
591. Pharoah PD, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br J Cancer* 1999;80:1968–73.
592. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001;1:233–40.
593. Colozza M, Azambuja E, Cardoso F, Sotiriou C, Larsimont D, Piccart MJ. Proliferative markers as prognostic and predictive tools in early breast cancer: where are we now? *Ann Oncol* 2005;16:1723–39.
594. Michels JJ, Marnay J, Delozier T, Denoux Y, Chasle J. Proliferative activity in primary breast carcinomas is a salient prognostic factor. *Cancer* 2004;100:455–64.
595. Cheung KL, Graves CR, Robertson JF. Tumour marker measurements in the diagnosis and monitoring of breast cancer. *Cancer Treat Rev* 2000;26:91–102.
596. Molina R, Filella X, Alicarte J, Zanon G, Pahisa J, Munoz M, et al. Prospective evaluation of CEA and CA 15.3 in patients with locoregional breast cancer. *Anticancer Res* 2003;23:1035–41.
597. Gion M, Boracchi P, Dittadi R, Biganzoli E, Pelloso L, Mione R, et al. Prognostic role of serum CA15.3 in 362 node-negative breast cancers. An old player for a new game. *Eur J Cancer* 2002;38:1181–8.
598. Ebeling FG, Stieber P, Untch M, Nagel D, Konecny GE, Schmitt UM, et al. Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. *Br J Cancer* 2002;86:1217–22.
599. Duffy MJ, Duggan C, Keane R, Hill AD, McDermott E, Crown J, O'Higgins N. High preoperative CA 15-3 concentrations predict adverse outcome in node-negative and node-positive breast cancer: study of 600 patients with histologically confirmed breast cancer. *Clin Chem* 2004;50:559–63.
600. Dnistrian AM, Schwartz MK, Greenberg EJ, Schwartz DC. BR29.29 as a marker in breast cancer. *J Tumor Marker Oncol* 1995;10:91–7.
601. Chan DW, Beveridge RA, Muss H, Fritsche HA, Hortobagyi G, Theriault R, et al. Use of Truquant BR radioimmunoassay for early detection of breast cancer recurrence in patients with stage II and stage III disease. *J Clin Oncol* 1997;15:2322–8.
602. Molina R, Zanon G, Filella X, Moreno F, Jo J, Daniels M, et al. Use of serial carcinoembryonic antigen and CA 15.3 assays in detecting relapses in breast cancer patients. *Breast Cancer Res Treat* 1995;36:41–8.
603. Soletormos G, Nielsen D, Schioler V, Mouridsen H, Dombrowsky P. Monitoring different stages of breast cancer using tumour markers CA 15-3, CEA and TPA. *Eur J Cancer* 2004;40:481–6.
604. Molina R, Jo J, Filella X, Zanon G, Pahisa J, Munoz M, et al. c-erbB-2 oncoprotein, CEA, and CA 15.3 in patients with breast cancer: prognostic value. *Breast Cancer Res Treat* 1998;51:109–19.
605. van Dalen A. TPS in breast cancer—a comparative study with carcinoembryonic antigen and CA 15-3. *Tumour Biol* 1992;13:10–7.
606. van Dalen A, Heering KJ, Barak V, Peretz T, Cremaschi A, Geroni P, et al. Treatment response in metastatic breast cancer: A multicenter study comparing UICC criteria and tumor marker changes. *Breast* 1996;5:82–8.
607. Carney WP, Leitze K, Ali S, Neumann R, Lipton A. HER-2/neu diagnostics in breast cancer. *Breast Cancer Res* 2007;9:207.
608. Li J, Zhang Z, Rosenzweig J, Wang YY, Chan DW. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clin Chem* 2002;48:1296–304.
609. Vlahou A, Laronga C, Wilson L, Gregory B, Fournier K, McGaughey D, et al. A novel approach toward development of a rapid blood test for breast cancer. *Clin Breast Cancer* 2003;4:203–9.
610. Braun S, Pantel K, Muller P, Janni W, Hepp F, Kantenich CR, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med* 2000;342:525–33.
611. Wiedswang G, Borgen E, Karesen R, Kvalheim G, Nesland JM, Qvist H, et al. Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. *J Clin Oncol* 2003;21:3469–78.
612. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med* 2005;353:793–802.
613. Chen SL, Hoehne FM, Giuliano AE. The prognostic significance of micrometastases in breast cancer: A SEER population-based analysis. *Ann Surg Oncol* 2007;14:3378–84.
614. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. *Lancet* 1990;335:1565–8.
615. Cserni G, Amendoeira I, Apostolikas N, Bellocq JP, Bianchi S, Bussolati G, et al. European Working Group for Breast Cancer Screening Pathology. Pathological work-up of sentinel lymph nodes in breast cancer: review of current data to be considered for the formulation of guidelines. *Eur J Cancer* 2003;39:1654–67.
616. Wilke LG, Giuliano A. Sentinel lymph node biopsy in patients with early-stage breast cancer: status of the National Clinical Trials. *Surg Clin North Am* 2003;83:901–10.
617. Cristofanilli M, Hayes DF, Budd GT, Ellis M, Stopeck A, Reuben JM, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *N Engl J Med* 2005;23:1420–30.
618. Smerage JB, Hayes DF. The measurement and therapeutic implications of circulating tumour cells in breast cancer. *Br J Cancer* 2006;94:8–12.
619. Tanner M, Isola J, Wiklund T, Erikstein B, Kellokumpu-Lehtinen P, Malmstrom P, et al. Topoisomerase IIalpha gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian Breast Group Trial 9401. *J Clin Oncol* 2006;24:2428–36.
620. Mano MS, Rosa DD, De Azambuja E, Ismael GF, Durbecq V. The 17q12–q21 amplicon: Her2 and topoisomerase-IIalpha and their importance to the biology of solid tumours. *Cancer Treat Rev* 2007;33:64–77.
621. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Genetic/familial high-risk assessment: breast and ovarian cancer. Version 1.2008. http://www.nccn.org/professionals/physician_gls/PDF/genetics_screening.pdf (Accessed November 2008).
622. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Breast cancer. Version 2, 2008. http://www.nccn.org/patients/patient_gls/_english/_breast/contents.asp (Accessed August 2008).
623. Fiskén J, Leonard RC, Roulston JE. Immunoassay of CA125 in ovarian cancer: a comparison of three assays for use in diagnosis and monitoring. *Dis Markers* 1989;7:61–7.
624. Bridgewater JA, Nelstrop AE, Rustin GJ, Gore ME, McGuire WP, Hoskins WJ. Comparison of standard and CA-125 response criteria in patients with epithelial ovarian cancer treated with platinum or paclitaxel. *J Clin Oncol* 1999;17:501–8.
625. van der Burg ME, Lammes FB, Verweij J. The role of CA 125 in the early diagnosis of progres-

- sive disease in ovarian cancer. *Ann Oncol* 1990;1:301–2.
626. Rustin GJ, Nelstrop AE, McClean P, Brady MF, McGuire WP, Hoskins WJ, et al. Defining response of ovarian carcinoma to initial chemotherapy according to serum CA 125. *J Clin Oncol* 1996;14:1545–51.
627. Rustin GJ, Nelstrop AE, Tuxen MK, Lambert HE. Defining progression of ovarian carcinoma during follow-up according to CA 125: a North Thames Ovary Group Study. *Ann Oncol* 1996;7:361–4.
628. Camilleri-Broet S, Hardy-Bessard AC, Le TA, Paraiso D, Levrel O, Leduc B, et al. HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: a multicenter study of the GINECO group. *Ann Oncol* 2004;15:104–12.
629. Singer G, Rebmann V, Chen YC, Liu HT, Ali SZ, Reinsberg J, et al. HLA-G is a potential tumor marker in malignant ascites. *Clin Cancer Res* 2003;9:4460–4.
630. Sehouli J, Akdogan Z, Heinze T, Konsgen D, Stengel D, Mustea A, Lichtenegger W. Preoperative determination of CASA (Cancer Associated Serum Antigen) and CA-125 for the discrimination between benign and malignant pelvic tumor mass: a prospective study. *Anticancer Res* 2003;23:1115–8.
631. Sutphen R, Xu Y, Wilbanks GD, Fiorica J, Grendys EC Jr, LaPolla JP, et al. Lysophospholipids are potential biomarkers of ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:1185–91.
632. Chambers SK, Gertz RE, Jr., Ivins CM, Kacinski BM. The significance of urokinase-type plasminogen activator, its inhibitors, and its receptor in ascites of patients with epithelial ovarian cancer. *Cancer* 1995;75:1627–33.
633. Coppola D, Szabo M, Boulware D, Muraca P, Alsarraj M, Chambers AF, Yeatman TJ. Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies. *Clin Cancer Res* 2004;10:184–90.
634. Brakora KA, Lee H, Yusuf R, Sullivan L, Harris A, Colella T, Seiden MV. Utility of osteopontin as a biomarker in recurrent epithelial ovarian cancer. *Gynecol Oncol* 2004;93:361–5.
635. Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res* 2003;63:3695–700.
636. Scholler N, Crawford M, Sato A, Drescher CW, O'Briant KC, Kiviati N, et al. Bead-based ELISA for validation of ovarian cancer early detection markers. *Clin Cancer Res* 2006;12:2117–24.
637. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. *Gynecol Oncol* 2008;108:402–8.
638. Baron-Hay S, Boyle F, Ferrier A, Scott C. Elevated serum insulin-like growth factor binding protein-2 as a prognostic marker in patients with ovarian cancer. *Clin Cancer Res* 2004;10:1796–806.
639. NCCN [National Comprehensive Cancer Network] clinical practice guidelines in oncology. Ovarian cancer. Version 1, 2007. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp (Accessed June 2007).