The Tumor and Its Microenvironment as Complementary Sources of Cancer Biomarkers

22

Roopali Roy, Emily Man, Rama Aldakhlallah, Emma Rashes, and Marsha A. Moses

Abstract

The continuing success of the field of cancer research in elucidating the mechanisms that regulate solid tumor development, growth, and progression has provided an extraordinary opportunity to leverage this information to develop novel traditional and precision medicines for a variety of human cancers. Within this context, the discovery and validation of sensitive, accurate, and readily translatable cancer biomarkers have never been more essential. The expanding utility of such biomarkers includes, but is not limited to, risk assessment, early detection, determination of cancer status and stage, monitoring therapeutic efficacy, development of resistance and patient stratification and selection among other uses. Until relatively recently, it has been the tumor epithelial compartment that has been the focus and the source of the majority of cancer biomarkers despite the critical role of the tumor microenvironment (TME) in influencing cancer outcome. Here, we intentionally focus on the TME as a source of biomarkers for a wide variety of human cancers. We comprehensively review the key biological components of the TME and their importance in human cancers, we present and extensively discuss the validated TME-derived biomarkers to date including their applications and sample sources and we provide up-to-date information with respect to their current clinical status.

Take-Home Lessons

- Biomarkers are measurable in body fluids and tissues and serve as indicators of health and disease status.
- Common cancer biomarkers include proteins, nucleic acids, metabolites, lipids, extracellular vesicles/exosomes, microRNAs, immune cells and others.
- Biomarkers from solid tumors and the surrounding tumor microenvironment (TME) can serve as diagnostic, prognostic, and predictive tools for cancer.
- The complex TME affects tumor biology, development, progression, therapeutic response and resistance and may be a useful target for both cancer diagnostics and therapy.
- Clinical applications of markers from the tumor and its TME extend across early detection, risk assessment, patient stratification, recurrence prediction and therapeutic efficacy.

Introduction

The field of biomarker medicine has been foundational to the development of successful cancer therapeutics, diagnostics, and prognostics. Multiple approaches to biomarker discovery and validation continue to be utilized and have been extensively reviewed by our group and others [1–5]. As noted above, there are several unmet biomarker needs with respect to cancer detection and subsequent treatment including early and accurate disease detection, risk assessment, reliable monitoring of therapeutic efficacy and resistance and the ability to determine which patient populations will most benefit from a particular therapy, among other uses.

Emily Man and Rama Aldakhlallah contributed equally.

R. Roy · M. A. Moses (✉)
Vascular Biology Program, Boston Children's Hospital, Boston, MA, USA

Department of Surgery, Harvard Medical School and Boston Children's Hospital, Boston, MA, USA
e-mail: roopali.roy@childrens.harvard.edu; marsha.moses@childrens.harvard.edu

E. Man · R. Aldakhlallah · E. Rashes
Vascular Biology Program, Boston Children's Hospital, Boston, MA, USA

L. A. Akslen, R. S. Watnick (eds.), *Biomarkers of the Tumor Microenvironment*, https://doi.org/10.1007/978-3-030-98950-7_22

The important contribution of the TME to the development of cancer diagnostics and prognostics has been, to date, overshadowed by that of biomarkers derived from the tumor epithelium despite the fact that it is now well recognized that the complex microenvironment of solid tumors plays a crucial role in cancer development and progression. The TME is a dynamic and complex system composed of a variety of components that are produced and/or are recruited by the cancer cells during tumor progression. These components include the tumor vasculature, immune cells, fibroblasts, adipocytes, extracellular matrix (ECM) components, and a variety of secreted factors (Fig.22.1). The TME affects solid tumor biology, development and progression, as well as therapeutic response and resistance. For these reasons, an appreciation of the TME is essential to our understanding of tumor development and progression as well to the identification of novel

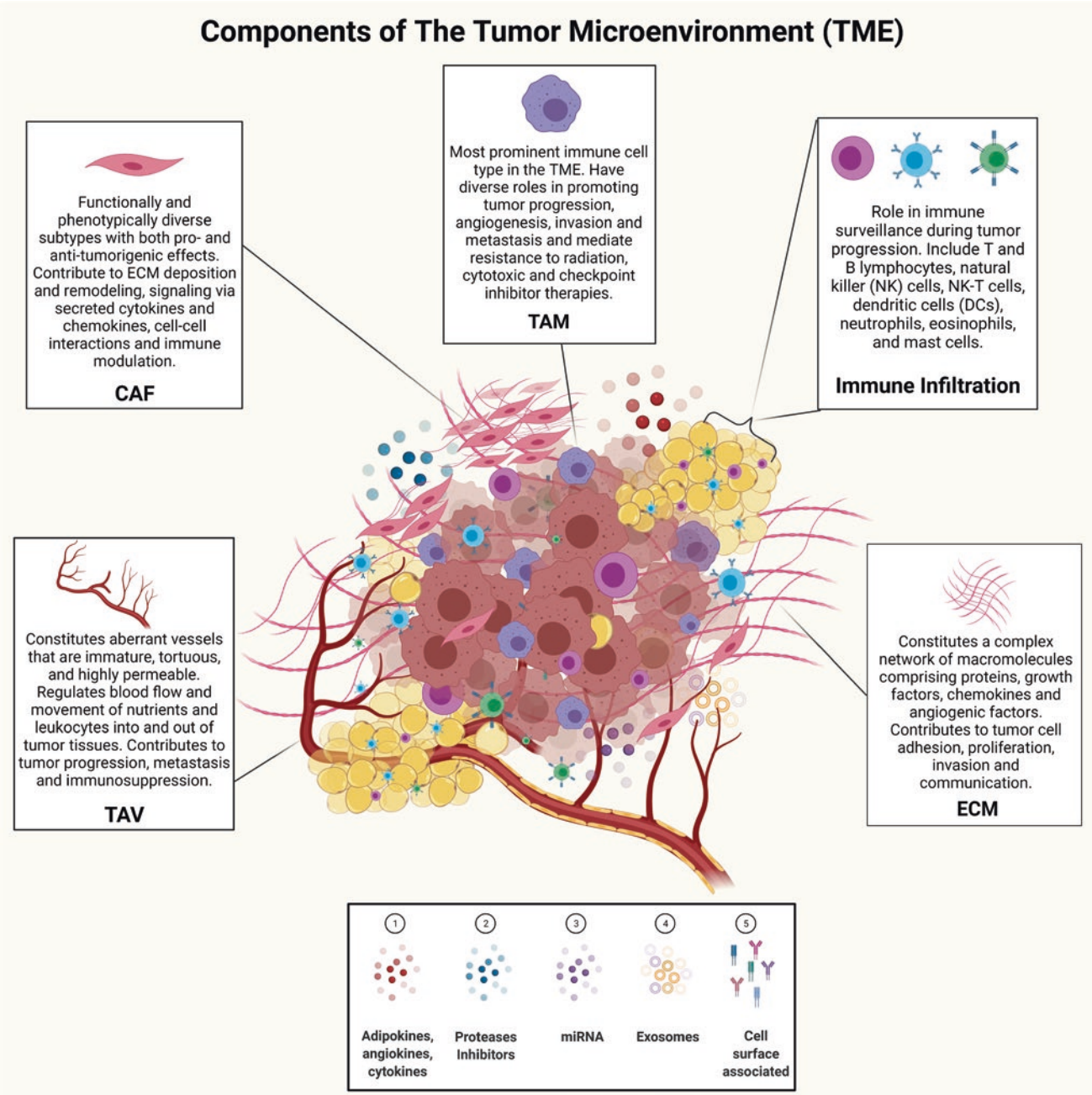

Fig. 22.1 The TME is a dynamic system containing a variety of components that are recruited and/or produced by the cancer cells during tumor establishment and maintenance including blood vessels (tumor vasculature; TAV), immune cells (Immune Infiltration), fibroblasts (CAF), adipocytes, extracellular matrix (ECM) components and regulators and a variety of secreted factors. The complex TME of solid tumors plays a crucial role in cancer development, progression and in modulating therapeutic responses and resistance mechanisms. Created with Biorender.com

and effective methods of cancer diagnosis and therapy. In addition, the targeting of the TME, its cellular components as well as TME-associated biomarkers represents potential therapeutic benefits for cancer patients as well.

The clinical necessity of accurate and easily translatable cancer biomarkers of all types is indisputable and the growing number of potential biomarker candidates associated with the TME, complemented by those derived from the tumor epithelium, hold the promise for early cancer detection, more instructive accurate monitoring of therapeutic efficacy and resistance and a number of other required elements of successful cancer diagnostics and prognostics. In this Chapter, we have provided a comprehensive overview of the predominant biological components of the TME with respect to their importance in human cancers and we present and extensively discuss current, validated TME-derived biomarkers across a large number of cancer types along with their sample sources, their potential clinical applications and the current status of those that have reached the stage of FDA clinical trial validation.

Fibroblasts

Cancer-associated fibroblasts (CAF) are key components of the TME and have diverse functions that include ECM deposition and remodeling, signaling, cell-cell interactions and immune modulation. CAFs can be phenotypically and functionally diverse with distinct subtypes that may have both pro- or anti-tumorigenic functions [6]. Soluble secreted factors from CAFs can influence tumor progression. For example, vascular endothelial growth factor (VEGF) expression by stromal cells has been shown to drive tumor angiogenesis [7]. Cytokines and chemokines produced by CAFs can also affect a range of immune cells such as leukocytes, T cells and macrophages and may exert both immunosuppressive or immunopromoting effects [8]. CAFs, therefore, may serve as both a source of biomarkers for cancer detection and prognosis as well as represent potential targets for therapeutic anticancer strategies.

Endothelium

The endothelium plays an important role in tumor initiation, progression, and metastasis. In normal tissues, an organized and efficient vascular endothelium regulates blood flow and the bidirectional movement of nutrients and leukocytes. In proliferating tumors, an overexpression of a variety of proangiogenic factors leads to the development of a distinct tumor-associated vasculature (TAV) characterized by disorganized aberrant vascular networks, immature and tortuous vessels and increased permeability [9–13]. Within the hypoxic, nutrient-deprived TME of a rapidly growing tumor, increased autophagy of the dysfunctional endothelial cells lining the blood vessels may further promote a pathological angiogenic cascade [14]. The TAV plays a significant role in tumor cell intravasation, an important component of metastasis and may also promote immunoevasion by actively suppressing the recruitment, adhesion, and activity of T cells to the tumor [15]. These unique features of the TAV have made it an attractive target of selective therapeutic intervention for a variety of cancers.

Immune Infiltration

Immune infiltration of tumors has been reported to contribute to the prediction of clinical outcomes. For several types of cancer, the composition of tumor-infiltrating immune cells (TIICs) can serve as novel targets for therapy as well as being important biomarkers for monitoring therapeutic response [16], predicting risk of recurrence and informing patient survival [17, 18]. TIIC include macrophages, T and B lymphocytes, natural killer (NK) cells, NK-T cells, dendritic cells (DCs), neutrophils, eosinophils, and mast cells.

Macrophages are the most prominent immune cell type in the TME and have diverse functions linked to cancer development, progression, and angiogenesis [19–21]. Tumor-associated macrophages (TAM) can augment, mediate and/or antagonize the antitumor activity of radiation therapy, cytotoxic agents, and checkpoint inhibitors [22]. TAMs have been shown to induce tumor angiogenesis [23–25] and support tumor migration, invasion, and proliferation through the production of various molecules, such as VEGF [26, 27]. Studies have also revealed that macrophages can assist circulating cancer cells in extravasating to distant sites, leading to metastases [28].

Extracellular Matrix (ECM)

The extracellular matrix (ECM) plays a key role in cancer development and progression and constitutes a versatile scaffold composed of a network of proteins such as glycosaminoglycans, fibrous proteins such as collagen, laminin, fibronectin, elastin, growth factors, angiogenic factors and chemokines that interact with cell surface receptors [29]. Importantly, the ECM influences tumor cell adhesion, proliferation, migration, and communication [19, 30]. The ECM is characterized by its biochemical composition as well as biophysical characteristics including topography, density, and elasticity. The ECM undergoes remodeling by tumor cells, CAFs, and other component cells of the TME as well as factors such as matrix metalloproteinases (MMPs) to release chemokines, growth and angiogenic factors to modify and

support the TME [1, 2, 31–34]. The ECM of both the stromal and epithelial compartments of a tumor is modified by the activity of ECM-degrading proteases such the MMPs and ADAMs (A Disintegrin And Metalloprotease), which themselves are controlled by their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMP) along with other factors [35–38].

Cancer Diagnostics and Prognostics

Here, we review the current status of biomarkers from the tumor and the tumor microenvironment (TME) with respect to cancer diagnosis, prognosis, disease progression, and therapeutic efficacy (Table 22.1). Studies have been included here if their sample numbers studied were $n = 50$ or greater with the exception of cancers where published sample sizes were limited as a function of lower prevalence of disease.

Breast Cancer

Breast cancer (BC) is the most common cancer in women in the USA (seer.cancer.gov) as well as globally [166]. In the early stages of breast cancer, cancer cells localize within the breast tissue and the tumors are identified as being "in situ" (e.g., ductal carcinoma in situ). Subsequently, in more advanced stages, cancer cells invade into the neighboring tissues and lymph nodes and, at the most malignant stage, metastasize to distant organs, such as bone, lung, and brain. Early detection and subtype-specific therapy for BC can significantly improve patient prognosis, with >90% 5-yr survival rates being observed for patients diagnosed with Stage I and II BC (seer.cancer.gov). However, survival is considerably lower for Stage IV disease (~26%) or for certain BC subtypes, ~77% for triple-negative breast cancer (TNBC) and ~75% for Her2+ BC, respectively [167]. Mammography and MRI approaches are the current gold standard for BC detection, however, the sensitivity and specificity of mammography can be low for young women and women with dense breast tissue [168]. Therefore, novel and effective methods of BC diagnosis and prognosis are urgently needed. We have previously reviewed the potential of MMP/ADAM biomarkers for the detection of BC [1, 2, 73]. Here we will focus on biomarkers originating from the breast tumor and its TME (Table 22.1).

While BC is not considered to be highly immunogenic, the impact of the immune landscape of the TME on BC progression is currently an intense area of investigation. High levels of tumor-infiltrating lymphocytes (TILs) are most commonly found in HER2+ and TNBC tumors and are associated with a good prognosis and response to the anti-HER2

Table 22.1 Biomarkers of the tumor and its microenvironment

Cancer	Application	Biomarker	Sample Type	References
Breast	Prognostic	ADAM12, MMP-9	Tissue, urine	[2, 39–41]
		MMP-9	Tissue, serum	[42]
		CD68, CD163, MMP-9	Tissue	[43]
		PD-L1, CD8	Tissue	[44]
		TIL	Tissue	[45–47]
	Diagnostic	MMP-9, MMP-9/NGAL	Urine	[1, 48, 49]
		MMP-7, MMP-26, CA15-3	Plasma	[50]
		miR-99a-5p	Plasma	[51]
	Predictive	miR-18b, miR-103, miR-107, miR-652	Serum	[52]
Lung	Prognostic	*EGFR, BRAF-ALK, ROS1-RTK*	Tissue	[53]
	Diagnostic	TP63, keratin 5, CECAM6, SFTPB	Serum	[54]
		NSE	Serum	[55]
		CYFRA21-1	Plasma	[56]
	Predictive	IL-18	Serum	[57]
		ProGRP	Tissue	[58]
		LKB1	Tissue	[59]
Prostate	Prognostic	MMP-1, MMP-9, TIMP-2	Tissue	[2, 60]
		MMP-2, MMP-7, MMP-11	Serum	[61–63]
		ADAM15	Tissue	[64]
		Cav-1	Tissue	[65, 66]
		miR-205	Tissue	[67]
		IL-6, IL-8, TNF-α, CCL2	Serum	[68–70]
	Diagnostic	MMP-2, VEGF	Serum	[1, 48, 71, 72]
		β2M, PGA3, MUC3	Urine	[3, 73, 74]
		ADAM12	Serum, urine	[75]
		AR, PR	Tissue	[76]
		ASPN	Tissue	[77]
		SFRP4	Tissue	[78]

Table 22.1 (continued)

Cancer	Application	Biomarker	Sample Type	References
Pancreatic	Prognostic	Cav-1, FASN	Tissue	[79, 80]
		ADAM12	Tissue, serum, urine	[81–83]
	Diagnostic	VEGF	Blood	[84]
		MMP-7, CCN2, IGFBP2, TSP-2, sICAM1, TIMP-1, PLG	Plasma	[85]
		TFPI, TNC-FNII-C, CA19-9	Plasma	[86]
		LIF	Tissue, serum	[87]
		miR-3940/miR-8069	Urine	[88]
		MMP-2, TIMP-1	Urine	[2, 89]
		LYVE-1, REG1B, TFF1	Urine	[90]
Ovarian	Prognostic	TEM8	Tissue	[91]
		S100A1	Tissue	[92]
		YKL-40	Serum	[93]
	Diagnostic	MMP-2, MMP-9, NGAL	Urine, ascitic fluid	[2, 94, 95]
		ADAM17, ADAM12	Tissue, serum	[96–99]
		VEGF	Serum	[100]
		HE4	Serum, plasma	[101–104]
	Predictive	IL-6	Tissue, plasma	[105, 106]
		NLR	Blood	[107]
Liver	Prognostic	VASP	Tissue	[108]
		MMP-9	Tissue	[109]
		ST2	Serum	[110]
		VEGFR-1	Tissue	[111]
		GPC3, AFP	Cytoplasm	[112]
		MDK	Serum	[113]
		miR-18b	Tissue	[114]
	Diagnostic	GPC3, AFP	Cytoplasm	[112]
		miR-92a-3p, miR-107, miR-3126-5p	Tissue	[115]
	Predictive	VASP	Tissue	[109]
		MMP-2, MMP-9	Tissue	[109]
		sVEGFR-1, VEGF, bFGF, CD3, CD4, Treg, CD56, IL-6, s-MET	Plasma	[116]
		VEGF, Ang-2, bFGF	Plasma	[117]
		FGF19	Tissue	[118]
		MDK	Serum	[113]
		miR-18b, miR-92a-3p, miR-107, miR-3126-5p	Tissue	[114, 115]
Gastric	Prognostic	PAK6	Tissue	[119]
		ADAMTS-2	Tissue	[120]
		COL12A1	Tissue	[121]
		CD163	Tissue	[122]
		KLK10	Urine	[123]
	Diagnostic	MMP-9/NGAL, MMP-9, ADAM12	Urine	[124]
		TFF1, ADAM12, *H. pylori*	Urine	[125]
		miR-6807-5p, miR-6856-5p, *H. pylori*	Urine	[126]
		FAP-α	Tissue	[119]
	Predictive	Ki-67, TS, COX2, ERCC1, P21	Tissue	[119]
Kidney	Prognostic	TuM2PK	Plasma	[127]
		TGF-α, VEGFR-2, TNF-RII	Plasma	[128]
		CXCL7	Plasma	[129]
		CAIX	Tissue	[130, 131]
		MVD	Tissue	[132]
	Predictive	Ang-2, MMP-2	Serum, plasma	[127, 133, 134]
		MMP-9	Serum	[135]
		IL-8, IL-9, IL-2Rα, PDGF-AA, TNF-RI, TNF-α	Plasma	[128]
		CXCL10	Serum, plasma	[128, 133]

(continued)

Table 22.1 (continued)

Cancer	Application	Biomarker	Sample Type	References
Brain	Prognostic	OPN	Tissue, serum, plasma	[136]
		miR-340-5p, CD163, POSTN, LIBP1, HMGA-2	Tissue	[137]
		MMP-2, MMP-9, MMP-2/TIMP-1	Tissue, blood, cytoplasm	[138–140]
		AREG	Serum	[141]
	Diagnostic	BMP2, HSP70, CXC, CXCL10	Serum	[142]
		CD163, CD70, CD3	Tissue	[143]
	Predictive	MMP-9	Tissue	[138]
Brain (Pediatric)	Prognostic	CTC	Blood, CSF	[144]
	Diagnostic	MMP-2, MMP-9, MMP-9/NGAL, VEGF	Tissue, urine, CSF	[144, 145]
		bFGF, TIMP-3	Urine	[146]
		Neogenin, netrin-1	Urine	[147]
		Netrin-1, bFGF, MMP-3, TIMP-1	Urine	[148]
	Predictive	MMP-9, TIMP-1, MMP-13	Urine	[148]
Neuroblastoma (Pediatric)	Prognostic	*MYCN*	Blood	[149]
		USP17L5, SLC25A5, POF1B, RND3, KLC4, SLC12A1	Genetic	[150]
		Cell-free DNA	Plasma	[151, 152]
		TH, PHOX2B, DCX	Blood, bone marrow	[153]
		miR-29c, miR-342-3p, let-7b	Plasma	[154, 155]
		Catecholamines, VMA, HVA	Urine	[156]
		LDH, ferritin	Serum	[156, 157]
		NSE	Serum	[158]
	Predictive	*MYCN,* ALK, TrkB, GD2	Genetic	[159]
Wilms' Tumor (Pediatric)	Prognostic	Gain of 1q	Genetic	[160–162]
		Loss of 14q	Genetic	[160]
		11p15 loss of heterozygosity, *WT1* mutation	Genetic	[163]
		IL-6, STAT3	Tissue	[164]
	Predictive	PHB	Urine	[165]

Note: Studies were included if sample numbers were $n = 50$ or greater with the exception of cancers where published sample sizes were limited as a function of lower prevalence of disease

therapy, Trastuzumab [45–47]. For TNBC patients treated with nab-Paclitaxel (Abraxane) + Atezolizumab, programmed death-ligand 1 (PD-L1) expression on immune cells and tumor cells of both primary and metastatic BC have been reported to be linked to progression-free survival (PFS) and overall survival (OS) benefit [44]. Intratumoral CD8 expression and TIL positivity also predicted improved outcome in this study, suggesting that patients with a richer immune TME have a clinical benefit from this combination therapy [44]. TAMs are gaining interest as biomarkers of BC prognosis and therapeutic response. Immunofluorescence detection of the TAM markers CD68, CD163, and MMP-9 in two independent BC cohorts indicated that all three markers were expressed in TNBC. While CD68 positivity correlated with poor OS for TNBC, increased expression of CD163 in TAMs was associated with improved OS in the same group [43]. Interestingly, MMP-9 TAM expression was associated with worse OS in ER-positive but not ER-negative BC [43], suggesting that TAM-targeted therapies may be beneficial in the former group.

MicroRNAs (miRNAs) are a class of small noncoding RNA that can regulate gene expression and are known to be dysregulated at all stages of BC. Liquid biopsy-based detection of both cell-free and extracellular vesicle (EV)-associated miRNA may serve as biomarkers for the early detection, prognosis and therapy response for BC [169–171]. Plasma levels of miR-99a-5p were reported to be significantly higher in BC patients compared to healthy controls and demonstrated good diagnostic potential for early BC (Stage I and II) [51]. A four-miRNA serum signature (miR-18b, miR-103, miR-107, and miR-652) was associated with recurrence and reduced OS in TNBC patients [52], suggesting that this high-risk signature score may serve as a predictor of the presence of TNBC. Importantly, circulating miRNAs are currently being studied in multiple clinical trials as biomarkers for BC screening, early diagnosis and as sentinels of therapeutic efficacy for patients undergoing neoadjuvant treatment (clinicaltrials.gov).

MMPs are expressed by both tumor cells and stromal cells within the TME and are critical for tumor progression and metastasis [38, 172–174]. Plasma levels of MMP-7 and MMP-26 combined with the standard marker for BC, cancer antigen 15-3 (CA15-3), provided the highest diagnostic specificity for advanced BC (Stage III and IV), indicating that combined assessment of these circulating markers can improve the diagnostic utility of CA15-3 in determining dis-

ease progression [50]. Monitoring serum levels as well as immunohistochemistry (IHC) staining of tumors for MMP-9 expression during neoadjuvant chemotherapy for TNBC indicated that a decrease in MMP-9 levels after treatment was significantly associated with pathological complete response (PCR) in patients, illustrating the utility of MMP-9 detection to identify the subgroups of TNBC patients that are most likely to benefit from therapy [42]. We have previously reported that MMP-9 and MMP-9/NGAL complex [48, 49] and ADAM12 [39] are significantly upregulated in the urine of BC patients and may serve as predictive biomarkers of BC status and stage. We have also demonstrated that multiplexed analyses of urinary MMP-9 and ADAM12 can predict which patients are at an increased risk of developing BC [40]. ADAM12 expression in BC tissues has been reported to be closely related to Ki67 (cellular proliferation marker) and HER2 status in ER-positive tumors and high ADAM12 levels were reported to be associated with shorter OS [41]. In terms of translating these markers for use in BC management, MMPs, in particular, based on our work and others, MMP-2 and MMP-9 are currently or have been investigated in over forty FDA-approved clinical trials as biomarkers of therapeutic efficacy of a variety of BC therapies (clinicaltrials.gov).

Lung Cancer

Lung cancer (LC) is the second most commonly diagnosed malignancy of men and women and has the highest cancer-related mortality (seer.cancer.gov) [175]. LC comprises two major histological types: small cell (SCLC) and non-small cell lung cancer (NSCLC). In particular, NSCLC is the most prevalent form of lung cancer with a dismal 5-yr prognosis of ~15% [175]. Despite recent advances in surgery, radiation, chemotherapy, and targeted therapies, LC prognosis remains poor due to delayed diagnosis and the presence of locally advanced and metastatic disease [176]. Identification of NSCLC molecular subtypes including tumors with activating EGFR mutations, *BRAF-ALK* gene fusions and *ROS1-RTK* fusions have enabled targeted therapies that can improve OS in patients with metastatic disease [53]. However, these targeted therapies benefit only ~20% of LC patients and while initially effective may lead to eventual therapy resistance [53], underscoring the crucial importance of novel biomarkers for the diagnosis and prognosis of LC. We have previously reviewed the potential of MMP/ADAM biomarkers for the detection of LC [73], and here we will focus on biomarkers from the LC tumor and the TME (Table 22.1).

The TME plays a central role in the initiation and progression of primary lung cancers and is recognized as a viable target for anti-cancer therapies [177]. A panel of 4 exosomal mRNAs, including TP63, keratin 5, CEA cell adhesion molecule 6 (CECAM6) and surfactant protein B (SFTPB), isolated from the serum of LC patients has been reported to provide improved specificity and sensitivity compared to the individual mRNAs to differentiate between adenocarcinoma and squamous carcinoma, two subtypes of NSCLC [54]. The serum concentration of neuron-specific enolase (NSE) a glycolytic enzyme, was reported to be significantly higher in LC patients with bone metastasis compared to those without bone lesions [55], suggesting that NSE may be used to identify bone metastasis during primary LC diagnoses. Circulating cytokeratin 19 fragments (CYFRA21-1) levels have been reported to be an independent prognostic factor for all stages of LC as well as an indicator of metastasis [56]. The prognostic significance of blood CYFRA21-1 levels is currently being investigated via clinical trials to predict OS in NSCLC patients (clinicaltrials.gov).

Serum-based biomarkers from the tumor and the TME may also be used to determine prognosis or to monitor therapeutic efficacy in LC. For example, serum IL-18 has been reported to be an early biomarker for tumor response to Atezolizumab (anti-PD-L1) in NSCLC therapy [57]. Levels of progastrin-releasing peptide (ProGRP), which regulates gastric acid secretion, were shown to be elevated in most patients with SCLC and could aid in the diagnosis of suspicious lung nodules [58, 178], which is important for determining optimal treatment strategy. In addition, for LC patients with elevated baseline ProGRP levels, a reduction in ProGRP after chemotherapy has been reported to represent a lack of disease progression for SCLC suggesting that serum ProGRP may be useful in monitoring response to chemotherapy and might provide valuable prognostic information [58]. Liver kinase B1 (LKB1) is a key sensor for metabolic stress of the TME and is upregulated in hypoxia and during glucose deprivation, conditions that may arise during tumor anti-angiogenic therapy. LKB1 has been reported to be a potential predictive marker of sensitivity to Bevacizumab (Avastin) therapy for advanced NSCLC patients [59]. Moderate to intense LKB1 IHC staining of lung tumors was associated with lower risk of patient death, whereas negative LKB1 lung tissue staining correlated with the lack of clinical benefit from Bevacizumab treatment [59].

Manipulating the immune response to target tumor cells has revolutionized LC treatment resulting in prolonged OS in a subset of patients. Biomarkers such as PD-L1, tumor mutational burden and TILs in the TME may all serve as markers of response to immunotherapy in LC patients and have been extensively reviewed in the literature [179, 180]. A prognostic immune gene signature composed of 40 unique genes has been reported to be able to stratify low- and high-risk patients in terms of estimating OS in nonsquamous NSCLC [181]. A high immune score analyzed via PDL-1 IHC of tumors, and the presence of several immune cell types belonging to the adaptive immune system may also be predictive of prognosis after surgery for lung adenocarcinoma patients [182].

Prostate Cancer

Prostate cancer (PCa) is one of the most common malignancies in men and is associated with a high mortality rate when disease progresses to metastasis and acquires androgen and therapeutic resistance [3, 183]. Current prognostic factors of PCa include the Gleason score and prostate-specific antigen (PSA) which is secreted from prostate epithelial cells. However, serum levels of PSA are elevated in both benign and malignant prostate growth conditions resulting in limited sensitivity and specificity for PCa detection. Therefore, patients often have to rely on invasive prostate biopsies for a precise diagnosis. This highlights a clinical need for more accurate PCa biomarkers that differentiate between benign prostatic conditions and PCa. We have previously reported that MMPs, TIMPs, and other non-invasive biomarkers can predict the presence of early PCa as well as distinguish between PCa and benign prostatic hypertrophy (BPH) [2, 3, 73, 74]. Here we will focus on biomarkers from prostate tumors and the surrounding TME (Table 22.1).

Our group has reported that urinary MMP-2 and VEGF levels were significantly higher in PCa patients compared to age and sex-matched healthy controls and could aid in diagnosis and prediction of therapeutic response [1, 48, 71, 72]. We have also reported that elevated urinary levels of β_2 microglobulin (β2M), pepsinogen A3 (PGA3), and mucin 3 (MUC3) can distinguish between PCa and BPH [73, 74]. A meta-analysis has demonstrated that MMP-2 expression in tumor tissues of PCa patients was significantly higher compared to that in prostatic tissues of BPH patients and was also significantly associated with Gleason score and PCa clinical stage [61]. IHC studies have demonstrated that elevated expression of MMP-1 and MMP-9 in prostate tumor tissue was associated with better disease-free survival (DFS) [60]. Interestingly, the expression of TIMP-2, which inhibits MMP activity and may modulate endothelial cell proliferation and angiogenesis [35, 37], was upregulated in the normal adjacent tissues of PCa patients and was also associated with DFS [60]. Serum levels of MMP-7 have been reported to be significantly higher in PCa patients with metastatic disease, but there were no differences between patients with local disease and healthy controls [62]. MMP-11 has been shown to be strongly correlated with Gleason score, pathologic tumor stage, and shorter survival and as well as being associated with poor prognosis [63]. Several MMPs and their inhibitors are currently being investigated as biomarkers of therapeutic efficacy in FDA-approved clinical trials (clinical trials.gov). Additionally, ADAM family members have been reported to be significantly elevated in PCa patients compared to healthy controls. For example, increased ADAM15 expression in PCa tumor tissues was linked to high Gleason grade, advanced pathological tumor stage, positive nodal stage, surgical resection margin and PSA recurrence [64]. ADAM12 levels in serum and urine were significantly increased in patients with PCa compared to healthy controls, suggesting that ADAM12 might be a potential biomarker of PCa [75].

PCa tumor cells produce several inflammatory factors, which modify the TME and contribute to tumor cell growth, survival, invasion, and progression. Increased serum levels of IL-6, IL-8, tumor necrosis factor (TNF-α), and C-C Motif Chemokine Ligand 2 (CCL2) have been reported to be associated with accelerated progression and poor prognosis in PCa [68–70]. Importantly, cytokines including various interleukins, TNF-α, and other factors such as VEGF are being evaluated in clinical trials as biomarkers for a variety of therapeutic strategies for PCa (clinicaltrials.gov). Caveolin-1 (Cav-1) expression in PCa stroma has been reported to be inversely correlated with disease progression [65, 66]. Studies have shown that low stromal Cav-1 expression was associated with poor disease-specific survival (DSS), increased Gleason score and reduced relapse-free survival (RFS) [65, 66]. Furthermore, stromal expression of Cav-1 was found to be highest in non-malignant tissue [65]. These findings suggest that reduced stromal Cav-1 levels in the TME may contribute to cancer progression and may serve as a useful prognostic marker for PCa.

PCa markers originating in the TME have also been investigated in relation to the Gleason score. While progesterone receptor (PR) was expressed only in prostate stromal cells, androgen receptor (AR) and estrogen receptor (ER) were expressed in both epithelial and stromal cells. Interestingly, significant decreases have been reported in the expression of AR in tumor tissues (Gleason score 8) when compared with normal prostate tissues [76]. ER and AR-targeted therapies for PCa have been extensively studied in clinical trials (clinicaltrials.gov).

CAFs and other stromal cells in the TME have been reported to express asporin (ASPN), which regulates transforming growth factor β (TGFβ) and fibroblast growth factor 2 (FGF2) activity [184, 185]. ASPN expression in stroma was assessed by IHC and was significantly increased in tumors compared with benign prostate samples and correlated with biochemical recurrence or relapse following cancer treatment [77]. Secreted frizzled-related protein 4 (SFRP4), which regulates Wnt signaling, is upregulated in prostate tumors and has been reported to be associated with disease recurrence, poor prognosis and PCa aggressiveness. In a microarray study of PCa tissues, SFRP4 expression was also reported to be linked to high Gleason grade, lymph node metastasis, and a positive surgical margin [78], suggesting SFRP4 might have prognostic utility in specific types of PCa.

Another potential biomarker for PCa, miR-205, is a tumor suppressor and has been reported to be downregulated in PCa epithelium compared to adjacent normal tissues [67].

High expression of miR-205 in the normal epithelium of PCa patients was independently associated with biochemical relapse, and therefore, miR-205 may serve as a prognostic biomarker for PCa. In prostate tumors, miR-205 correlated positively with angiogenesis-related markers such as platelet-derived growth factor (PDGF)-D, PDGF-B, VEGF-A, VEGF-C, and VEGFR-2. A number of other microRNAs are currently being investigated as biomarkers for therapeutic response in PCa clinical trials (clinicaltrials.gov).

Pancreatic Cancer

Pancreatic cancer (PC) is a lethal malignancy associated with an extremely poor prognosis due to a combination of late diagnosis, frequent recurrence and resistance to current therapeutic modalities [167] (seer.cancer.gov). The majority (~82%) of PC patients present with regional or distant metastases at initial diagnosis making complete resection impossible, thereby leaving patients to rely predominantly on conventional chemotherapeutic options [186, 187], which results in drastically reduced patient survival (5-yr, ~10%). A lack of specific symptoms makes PC difficult to diagnose. Unfortunately, the only approved clinical PC biomarker, CA19-9, [188, 189] has moderate sensitivity and specificity for detecting PC [189–191], and in particular, a very low sensitivity for the detection of early PC [188, 192].

For these compelling reasons, there exists an intense interest in the development of accurate biomarkers for PC that can better detect disease and guide therapy. PC has a particularly active tumor TME characterized by desmoplastic fibrotic stroma, an abundant ECM, poor effector T cell infiltration and other immune-suppressive features. We have previously reviewed the potential of MMP/ADAM biomarkers for the detection of PC [2]. Here we will focus on biomarkers from the PC tumor and the TME (Table 22.1). CAFs have a crucial function in driving PC progression through paracrine interactions. Cav-1, a scaffolding protein, is expressed by fibroblasts of the desmoplastic PC stroma but not by stromal cells of the normal pancreas [79] and the co-expression of Cav-1 and fatty acid synthase (FASN) is reported to correlate with histologic grade and advanced tumor stage as well as lower survival in PC patients [79]. Loss of stromal Cav-1 expression was found to be associated with PC TNM stages, lymph node and distant metastasis and could predict poor clinical outcome for PC patients [80].

ADAM12 was reported to be upregulated in pancreatic CAFs compared to fibroblasts from normal pancreatic tissues [81] and is considered to be a marker of activated stroma. An analysis of patient tumors and serum samples from the Phase II MPACT trial found that high ADAM12 levels were significantly associated with poor outcome for PC patients [82]. Low ADAM12 levels associated with longer survival for PC patients who received nab-Paclitaxel (Abraxane) [82], suggesting that ADAM12 is a circulating biomarker for stromal activation with both prognostic and therapeutic significance. Ongoing clinical trials are assessing the relative abundance of stroma in metastatic PC tumor tissues and stromal markers such as ADAM12, in tissues and blood as a predictor of response to treatment and survival in PC patients (clinicaltrials.gov). We have previously reported that urinary ADAM12 levels could serve as significant independent predictor for distinguishing pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumors (pNET) patients from healthy controls [83]. Patient survival stratified by urinary ADAM12 levels indicated a significantly shorter OS for PDAC patients with high ADAM12 levels compared to patients with lower urinary ADAM12 [83]. We have reported that urinary levels of MMP-2 and its endogenous inhibitor TIMP-1 are significant independent predictors to distinguish PDAC patients from healthy controls [89]. Combined analysis of urinary MMP-2 and TIMP-1 resulted in a markedly improved accuracy [89] over using CA19-9 alone [193], the currently utilized biomarker for PDAC. In addition, urinary MMP-2 may predict the presence of pNET tumors, whereas TIMP-1 levels may differentiate between PDAC and pNET patient groups [89]. In an ongoing clinical trial, MMP-9 levels in pancreatic cyst fluid are being analyzed in patients with high-risk intraductal papillary mucinous neoplasm (IPMN) lesions who are at an increased risk of radiographic progression to PDAC (clinicaltrials.gov). Ongoing trials are also investigating the blood levels of MMP-7 and MMP-9 as surrogate markers of therapeutic efficacy for combined PRI-724 + Gemcitabine and proton beam therapies for PC patients, respectively (clinicaltrials.gov). VEGF is overexpressed in PC and reported to be a useful marker for poor prognosis in PC [194]. VEGF levels in portal blood were associated with tumor grade and correlate with tumor size for patients with PDAC [84]. Soluble stroma-based markers have also been investigated as potential biomarkers for PDAC. A biomarker panel of stroma-related proteins such as MMP-7, cellular communication network factor 2 (CCN2), insulin-like growth factor binding protein 2 (IGFBP2), thrombospondin-2 (TSP-2), soluble ICAM1 (sICAM1), TIMP-1, and plasminogen precursor (PLG) were evaluated in plasma samples and were reported to discriminate PDAC from healthy controls and from chronic pancreatitis [85].

Detection of early and localized PDAC has been shown to increase the 5-yr survival of PC patients to ~43% (Stage II) and ~50% (Stage I) from ~10.8% (5-yr overall survival) [195]. A 29-biomarker serum signature has been shown to discriminate between patients with Stage I and II PDAC from healthy controls and was subsequently validated in an independent case-control cohort [196]. Similarly, a plasma panel including tissue factor pathway inhibitor (TFPI),

tenascin C (TNC-FNII-C) and CA19-9 improved upon CA19-9 alone in discriminating early-stage PDAC compared to healthy controls as well as distinguishing early-stage PDAC from patients with benign pancreatic conditions such as diabetes and chronic pancreatitis [86]. PDAC is associated with pathogenic modifications to the peripheral nervous system that elevate metastatic capacity. IL6-related stem cell–promoting factor (LIF) has been reported to support PDAC-associated neural remodeling and is upregulated in PC tumor tissues compared to the healthy pancreas [87]. Compared to serum from patients with benign pancreatic conditions or healthy controls, the sera from PDAC patients contained elevated LIF levels which correlated with intratumoral nerve density, suggesting that LIF could be a candidate biomarker and a therapeutic target for PDAC-associated neural remodeling [87].

PC-derived exosomes have been explored for their potential application as cancer biomarkers [197, 198]. The miR-3940-5p/miR-8069 ratio was found to be elevated in the urinary exosomes of PDAC patients with early-stage disease [88] compared to that of healthy controls or chronic pancreatitis patients and when combined with CA19-9, were shown to be a useful tool for the diagnosis of PDAC [88]. A 3-protein (LYVE-1, REG1B, and TFF1) urinary biomarker panel has been reported to distinguish Stage I and II PDAC from healthy controls and when combined with plasma CA19-9, this panel achieved an increased accuracy [90, 199]. This 3-biomarker urine panel was recently combined with a logistic regression model to create the algorithm PancRISK score, which provided high sensitivity and specificity for the stratification of patients into normal or elevated risk categories [200]. The PancRISK score is being suggested for use in surveillance of individuals with a family history or genetic background for PC or at an increased risk due to benign diseases of the pancreas [200].

Ovarian Cancer

Epithelial ovarian cancer (OC) is the leading cause of death among women with gynecologic cancers. Despite advances in surgery and chemotherapy, 5-yr survival rates for OC have not significantly improved over the past few decades (seer.cancer.gov). The most common subtypes of OC include serous carcinomas, endometriosis carcinomas, mucinous carcinomas and clear cell carcinomas. Elevated serum levels of CA125 are widely used to detect OC. However, many patients with early-stage OC and a subset of patients with advanced OC have CA125 levels within the normal range (~35 U/mL), such that these women are left without reliable diagnostic biomarkers to detect OC status. We have previously reported that urinary MMP-2, MMP-9, and lipocalin-2 (NGAL) significantly discriminated between OC patients with normal CA125 levels compared to healthy controls and could represent an important predictor of the presence of OC in the ~30% of women for whom no reliable diagnostic test exists [2, 94]. Grounded in some of this work, several MMPs have been investigated as biomarkers of therapeutic efficacy in clinical trials for OC patients (clinicaltrials.gov). MMP-9 expression was significantly higher in the EVs isolated from the ascites of high-grade serous OC patients compared to patients with benign liver cirrhosis, suggesting the potential of this EV population to serve as a biomarker for high-grade serous OC [95]. Several members of the ADAMs family, specifically ADAM17, ADAM12, and ADAM9 are highly expressed in early and advanced OC tissues [96–99]. ADAM17 is a sheddase for activated leukocyte cell adhesion molecule (sALCAM) whose serum levels were found to be elevated in OC patients compared to healthy controls [97]. ADAM12 serum levels were associated with shorter PFS, OS, and tumor lymphatic and vascular invasion in an aggressive subtype of high-grade serous OC [98]. Finally, mutations in ADAMTS (A disintegrin and metalloproteinase with thrombospondin motifs) members have been significantly associated with an improved OS as well as PFS in OC patients without BRCA1/2 mutations [201].

Serum levels of CA125 and VEGF were reported to be significantly higher in patients with OC than in healthy controls [100]. In this study, the combination of CA125 and VEGF improved the specificity and sensitivity of detection of early-stage OC and was suggested to be more efficacious than CA125 alone. Similarly, the combination of human epididymis protein 4 (HE4) and CA125 has been shown to improve the sensitivity, specificity, and diagnostic power compared to CA125 alone [101] and has been reported to be increased in the serum of patients with OC compared with benign disease and healthy controls [102–104]. Elevated serum levels of HE4 and CA125 in patients with OC were also associated with worse PFS [103, 104]. The neutrophil-to-lymphocyte ratio (NLR) has been reported as a potential predictive marker of OC in patients with normal CA125 levels, and the combination of NLR and CA125 had greater specificity than CA125 alone [107].

Several potential biomarkers have been identified in the TME of OC. IL-6 signaling plays a significant role in carcinogenesis across a variety of solid tumors, including OC and is expressed by ovarian tumor and stromal cells. IHC staining of IL-6 was reported to be significantly higher in malignant tumors and was associated with shorter PFS in OC [105]. IL-6 has also been reported to be predictive of a therapeutic advantage of Bevacizumab for PFS and OS compared with the placebo group [106]. IL-6 in addition to other interleukins and VEGF have been investigated as predictive biomarkers in clinical trials for OC (clinicaltrials.gov).

Tumor endothelial marker 8 (TEM8) is highly expressed in the OC TME and is associated with poor prognosis and

tumor-associated angiogenesis. Expression of TEM8 was elevated in the malignant tumor tissues and the borderline tumor tissues with atypical epithelial proliferation compared to normal ovarian tissues and was significantly associated with the International Federation of Gynecology and Obstetrics (FIGO) stages, lymph node metastasis, and poor prognosis in OC patients [91]. S100 calcium-binding protein A1 (S100A1) is a calcium-binding protein belonging to the family of S100 proteins that are reported to be implicated in the crosstalk between tumor cells and stroma and is highly expressed in OC tissues [202]. Compared with healthy fallopian tubes and ovarian tissues, S100A1 expression was significantly increased in OC tissues and correlated with lymph node metastasis, FIGO stage and tumor grade [92]. The chitinase-like glycoprotein, YKL-40, is secreted by various cell types including tumor cells and TAMs in the TME and may represent a novel marker for OC. Preoperative YKL-40 serum levels in early-stage OC were significantly elevated compared with healthy controls [93]. YKL-40 serum levels were also reported to be significantly associated with stage and worse prognosis [93].

Liver Cancer

Liver cancer is the sixth most diagnosed cancer as well as the third leading cause of cancer-related death worldwide (GLOBOCAN)(gco.iarc.fr). Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor in the USA (seer.cancer.gov). Although screening for liver disease has increased over time, insufficient diagnostic and monitoring tools and the lack of consistent screening limits treatment options for patients. Early diagnosis is essential to improve OS for HCC patients and provide better treatment options [203]. Curative treatments are available for patients with early-stage diagnosis, which has in turn increased HCC survival rates by ~70% [204]. Developing and identifying robust diagnostic and prognostic markers is essential for the early detection of HCC and for predicting clinical outcomes. Here we discuss biomarkers of the liver tumor and its TME that play a role in the diagnostic, prognostic, and therapeutic efficacy in HCC patients (Table 22.1). Vasodilator-stimulated phosphorylation (VASP) is a regulator of actin cytoskeleton and cell migration, and has been reported to be overexpressed in HCC and is indicative of poor prognosis as it promoted aggressive phenotype and metastasis [109]. Although VASP was reported to be an independent factor in predicting survival of HCC patients, the downstream effect of VASP overexpression resulted in upregulated MMP-2 and MMP-9 levels, which were associated with increased migration and invasion of HCC cells in these studies [109]. Upregulated expression of ADAMTS5 in tumor tissues has been reported to be associated with poor prognosis and worse OS for HCC patients [108]. A recent study demonstrated a correlation between angiogenic and immune biomarkers of the liver TME and time of progression (TTP) during combination Sorafenib and FOLFOX treatment in HCC patients [116]. Shorter TTP was found to be associated with high plasma levels of sVEGFR-1, VEGF, bFGF, circulating CD3+, CD3+CD4+Treg and CD56 for Sorafenib treatment alone. There was an increase in CD56, IL-6 and soluble Met (s-Met) plasma levels for the combination treatment suggesting that these markers may be useful to monitor therapeutic efficacy for HCC [116]. Similarly, circulating biomarkers have been evaluated as sentinels of therapeutic efficacy for Cediranib (panVEGFR RTK inhibitor) in HCC patients [117]. Increased plasma levels of VEGF, angiopoietin-2 (Ang-2) and bFGF were associated with poor outcome, whereas an increase in IFN-γ was significantly associated with longer PFS in this study [117], suggesting that proangiogenic and inflammatory factors may serve as potential biomarkers of anti-VEGF therapy in HCC [117]. Currently, MMPs, chemokines, and immune infiltrates are being investigated via clinical trials as tools to monitor clinical efficacy and response to different treatment interventions for HCC such as Axitinib, Lenvatinib, and Nivolumab (clinicaltrials.gov).

ST2 is a member of the interleukin-1 receptor family, and is the receptor for IL-33. While serum IL-33 levels remained unchanged, soluble ST2 was a significant predictor of OS in HCC [110]. Fibroblast growth factor 19 (FGF19) was significantly associated with larger tumor size and higher score according to the Barcelona Clinic Liver Cancer staging (BCLC) system. FGF19 can be an effective predictor of early recurrence and poor prognosis of HCC, which makes it a potential preventive target for HCC patients [118]. In a study designed to assess the clinicopathological features associated with progression and poor differentiation, strong expression of VEGFR-1 in HCC patient tissues was reported to be a prognosticator factor for RFS and OS. This study concluded that after curative resections, high expression of VEGFR-1 in HCC tissues resulted in diminished RFS and OS [111]. Glypican-3 (GPC3), an oncofetal protein, is overexpressed in ~84% of HCC tissues, where it was found to be mainly localized in the cytoplasm. High expression of GPC3 was found to correlate with multiple tumors, high serum alpha-fetoprotein (AFP) levels, and late TNM stage. High GPC3 expression was an independent risk factor for both tumor recurrence and OS in HCC patients who have normal AFP levels and has been reported to be a prognostic biomarker and a good predictor for differing clinical outcomes for HCC patients [112]. Midkine (MDK) is a heparin-binding growth factor involved in cell growth and invasion during HCC progression. MDK levels have been shown to be elevated in patients with early-stage HCC as well as those with untreated or recurrent HCC. In addition, serum MDK has better diagnostic performance in the detection of HCC in AFP negative HCC [113].

In HCC cell lines from human tissue samples, miR-18b overexpression has been associated with tumor progression, metastatic potential and a poor prognosis for HCC. miR-18b downregulated its target gene trinucleotide repeat containing 6B (TNRC6B) and the decreased TNRC6B expression in turn promoted the metastatic potential of HCC cells [114]. A panel of four serum miRNAs (miR-16-2-3p, 92a-3p, 107, and 3126-5p) were analyzed as potential biomarkers for the early detection of HCC. Logistic regression analysis identified three miRNAs (miR-92a-3p, 107, and 3126-5p) to be significantly changed in early-stage HCC patients. The combination of the 3-miRNA panel and AFP allowed for a better and more effective way in identifying the early stages of HCC in low-AFP level patients compared to the healthy patients [115]. Multiple clinical trials have investigated the high expression of FGF19, VEGFR-1, GPC3 in HCC patients as biomarkers to monitor the therapeutic efficacy of targeted therapies. Other current clinical trials have utilized the usefulness of combining biomarkers with AFP for early detection and therapy surveillance of HCC (clinicaltrials.gov).

Gastric Cancer

Gastric Cancer (GC) represents a global health concern as it is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide (GLOBOCAN)(gco.iarc.fr) [205]. Characterized as having a highly aggressive nature, it is often diagnosed at an advanced stage when the gastric wall tumor invasion and metastasis have already occurred [206]. The standard treatment for early-stage GC is endoscopic resection which has fewer complications and provides a much better life quality for GC patients compared to partial or total gastrectomy procedures typically used to treat more advanced stage GC [206]. Biomarker-based stratification of Stage II or III GC patients who could benefit from adjuvant chemotherapy is currently needed [207]. For example, 5-Fluorouracil (5-Fu) and Oxaliplatin are commonly used for GC management, however, the administration of such drugs to all Stage II and III GC patients is ineffective [208], since many patients relapse after this initial treatment with acquired resistance to the 5-Fu based chemotherapy [208]. For these reasons, a more accurate diagnostic method is needed to correctly classify patients who may be sensitive to 5-Fu chemotherapy and to provide more effective treatment options. The expression of cyclin-dependent kinase inhibitor (p21)-activated kinase 6 (PAK6) was reported to correlate with an aggressive phenotype in GC patients as well as chemoresistance to 5-Fu chemotherapy [119]. Chemotherapy score, a support vector machine (CS-SVM) classifier, is used to distinguish subgroups of Stage II and III GC patients [119]. The addition of Ki-67, thymidylate synthase (TS), cyclooxygenase 2 (COX2), excision repair cross-complementing gene 1 (ERCC1), and P21 to the chemotherapy score has been reported to effectively identify a small subset of GC patients that would benefit from 5-Fu chemotherapy [119]. With the increased understanding of the relationship between biomarkers and disease, many of these markers have been investigated as potential therapeutic and monitoring targets in clinical trials. COX2 inhibition has been used in clinical trials as a therapeutic target as well as to measure treatment prognosis and response (clinicaltrials.gov). In addition, current clinical trials are evaluating Ki-67, TS, ERCC1, and p21 as biomarkers of clinical outcomes and treatment response for GC (clinicaltrials.gov).

MMPs have been found to be elevated in patients with GC compared to healthy controls. Urinary levels of MMP-9/NGAL complex and ADAM12 were significantly elevated in the GC patients compared with healthy controls [124]. IHC of GC tissues demonstrated significant upregulation of MMP-9, lipocalin-2, and ADAM12 expression compared with adjacent normal tissues [124]. Additionally, urinary kallikrein 10 (uKLK10) was significantly elevated in inoperable GC compared to operable GC patients and uKLK10 levels were positively associated with tumor stage and GC progression [123]. Interestingly, both the high urinary levels and increased pathological expression of KLK10 were associated with shorter DFS in this study [123].

Urinary trefoil factor 1 (TFF1) and ADAM12 have been reported to be independent diagnostic biomarkers for GC as well. A panel combining TFF1, ADAM12, *and Helicobacter pylori* (*H. pylori*) was reported to significantly distinguish between healthy controls and GC patients with excellent accuracy [125]. A similar panel of markers including urinary miR-6807-5p, miR-6856-5p, and *H. pylori* indicated excellent accuracy in distinguishing between healthy patients and Stage I GC patients [126]. ADAMTS-2 is a procollagen enzyme and a member of the larger ADAMTS family. ADAMTS-2 expression was higher in GC cells and CAFs compared to normal gastric tissues and was reported to be associated with OS [120].

Collagen type XII α1 chain (*COL12A1*) is a member of the fibril-associated collagen family and has a tumor-promoting role in human cancer making it a potential prognostic indicator as well as therapeutic targeting candidate. Elevated expression of COL12A1 levels, through immunoreactivity scoring, has been associated with GC invasiveness, clinical metastasis and aggressive clinical features [121].

TAMs play a significant role in tumor progression and angiogenesis. Based on the analysis of infiltrating TAMs in the stroma and tumor margins, increased CD163+ TAMs were associated with tumor progression and depth of invasion in GC [122]. Fibroblast activation protein alpha (FAP-α)

expression has been reported to be upregulated in GC tumor tissues of patients with adverse clinical-pathological characteristics, diffuse histological subtypes, advanced pathological stage and poor survival [119].

Kidney Cancer

Renal cell carcinoma (RCC) is the most common type of kidney cancer and encompasses several subtypes. The most common subtype (~70% of cases) is clear cell RCC (ccRCC). Although the standard of care for ccRCC has improved significantly over the past few decades with the emergence of new treatments [209], there remains a need for biomarkers to detect RCC, monitor resistance, and predict therapeutic efficacy of these treatments. Several cytokines and angiogenic factors in the plasma of patients with non-ccRCC have been identified as potential prognostic markers. Ang-2 is expressed by the tumor endothelium and has been reported to be elevated in the plasma of patients with advanced RCC compared to those with benign disease [127]. Higher preoperative plasma levels of Ang-2 were also associated with shorter DFS [133]. Elevated preoperative levels of Ang-2 and M2 Pyruvate kinase (TuM2PK), a dimeric form of the M2 isoform of pyruvate kinase implicated in oncogenesis and overexpressed in tumor cells, were correlated with increased tumor size and advanced grade [127], suggesting potential clinical value for the detection of RCC. Ang-2 is currently being investigated as a potential biomarker for therapeutic response in a clinical trial for advanced solid renal tumors (clinicaltrials.gov).

Several studies have identified biomarkers originating from the TME as predictors of therapeutic response in kidney cancer (Table 22.1). Serum Ang-2 and MMP-2 were identified as relevant baseline biomarkers of Sunitinib activity in advanced RCC. Lower Ang-2 and higher MMP-2 pretreatment serum levels were significantly associated with therapeutic response and are potential baseline efficacy markers for Sunitinib treatment in advanced RCC [134]. Elevated serum levels of MMP-9 before BNC105P monotherapy of patients with ccRCC were associated with improved PFS [135]. In addition, high plasma levels of IL-8, PDGF-AA, TGF-α, and VEGFR-2 were independently associated with reduced OS; whereas high plasma levels of IL-2 receptor alpha (IL-2Rα) chain, TNF-RI, TNF-RII, and TNF-α were associated with reduced PFS in addition to OS in RCC [128]. IL-8, IL-9, IL-2Rα, PDGF-AA, TNF-RI and TNF-α were also associated with poor response to Sunitinib treatment [128]. TGFs and TNFs are secreted by macrophages and inflammatory cells in the TME. C-X-C motif chemokine ligand 10 (CXCL10), a chemokine with known anti-angiogenic and immune-stimulatory properties, has been shown to enhance T cell and NK-cell activity, and may be a prognostic biomarker of RCC. CXCL10 serum and plasma levels increased during Sunitinib and Sorafenib treatment, and higher baseline levels were associated with worse OS and DFS [128, 133]. Another chemokine, CXCL7, is involved in inflammation and angiogenesis and generates autocrine and paracrine loops that impact the TME. Metastatic ccRCC patients with CXCL7 plasma levels above the baseline of 250 ng/ml had significantly longer PFS [129], suggesting that baseline plasma levels of CXCL7 may predict the therapeutic efficacy of Sunitinib or other anti-angiogenic drugs targeting the VEGF/VEGFR axis in RCC. Tissue biomarker carbonic anhydrase IX (CAIX) is a transmembrane enzyme induced by hypoxia that has also been proposed to have prognostic value for RCC. Expression of CAIX in RCC tissue has been reported to be inversely associated with tumor stage, tumor grade, and worse DSS, PFS, and OS [130, 131], suggesting that renal tissue CAIX expression may have prognostic utility in RCC. Importantly, MMP-2 and MMP-9 as well as IL-6, IL-2, CAIX, and chemokines have been or are currently being investigated as biomarkers in clinical trials for response to treatment for RCC (clinicaltrials.gov).

Microvessel density (MVD) is a commonly used measurement of tumor angiogenesis. High MVD in primary RCC nephrectomy tissues was significantly associated with improved OS [132]. High MVD also correlated with lower prognostic factor Fuhrman grade, clear cell histology, and absence of necrosis but not with gender, age, sarcomatoid features, lymphovascular invasion, or tumor size, suggesting high MVD may be indicative of better prognosis of RCC [132]. MVD is also being explored as a RCC biomarker for therapeutic efficacy in ongoing clinical trials (clinicaltrial.gov).

Brain Cancer

Brain and central nervous system (CNS) cancers are the tenth leading cause of mortality for adults in the USA and survival rates decrease with age (www.cancer.nets). The main brain tumor types are gliomas which include astrocytomas, oligodendrogliomas, brain stem gliomas along with non-glioma tumors such as meningiomas, primary CNS lymphomas, and medulloblastomas [210]. Gliomas are the most common primary intracranial malignant tumors in adults with a high recurrence rate [211]. Astrocytomas are diagnosed in adults as infiltrating tumors that spread to surrounding tissue in the brain and originate from the astrocytes that form the supportive tissue of the brain [211]. Glioblastoma is a form of high-grade astrocytoma that arises with no prior clinical history of precursor neoplasia or abnormal growth of tissue [212].

The expression of MMP-2 and MMP-9 has been demonstrated to correlate with tumor grade of primary and recurrent gliomas, making them key players in the progression and invasiveness of tumors [138, 139]. Overexpression of membrane-associated MMP-2 correlated with tumor grade and OS in glioblastoma and astrocytoma compared to normal brain tissue [140]. Multiplexing MMP-2 and TIMP-1 resulted in a positive correlation between the two proteins allowing for a stronger prognostic impact [140]. The benefits of these markers specifically MMP-2 and MMP-9 have been shown in a number of current clinical trials along with neuroimaging to evaluate disease status and therapeutic efficacy (clinicaltrials.gov).

Amphiregulin (AREG), which stimulates cell growth, survival, and migration, is upregulated in the serum of patients with glioma and has been shown to associate with a worse survival prognosis [141]. Osteopontin (OPN) mediates cancer progression and regulates processes such as immune response, cell adhesion and migration. OPN levels have been reported to be higher in tissue, plasma, and serum in high-grade glioma patients compared to those with low-grade glioma and is related to OS [136]. Another study has reported that multiplexing bone morphogenic protein 2 (BMP2), heat shock 70-kDA protein (HSP70) and CXCL10 resulted in better specificity and sensitivity to accurately distinguish between GBM patients and healthy controls [142]. miRNAs expressed by the TME in glioblastoma have been shown to be involved in disease progression and may prove to be important biomarkers of this disease. For example, the downregulation of the miR-340-5p has been correlated with the density of TAMs which are associated with poor prognosis. Additionally, patients with low miR-340-5p expression, high CD163, periostin (POSTN), LIBP1 and high mobility group A (HMGA-2) levels were associated with a poor prognosis and shorter OS [137]. In terms of the immunological markers, GBM patients exhibited, in tissue, a decreased expression of CD163 and CD70 while CD3 immunoreactivity increased in tumor cells and blood vessels [143].

To monitor the efficacy of Bevacizumab treatment in patients with recurrent GBM, a new approach was developed termed "TME mapping." This approach consists of multiparametric magnetic resonance imaging (MRI) along with methods to visualize oxygen metabolism in the TME. TME mapping allowed for the classification of five different TME compartments and has been used to monitor the tumor biology and treatment efficacy for GBM [213].

Pediatric Cancers

In this section, we will discuss biomarkers for pediatric brain cancer, neuroblastoma and Wilms' tumor (Table 22.1) as they are among the most common pediatric solid tumors.

Brain Cancer

Brain cancer is the most common solid tumor in pediatric patients with one of the highest mortality rates. The highest rates of pediatric BC are found in the USA with an incidence rate of between 1.15 to 5.14 cases per 100,000 children [214]. We have previously reported the significant upregulation of MMP-2, MMP-9, MMP-9/NGAL complex, and VEGF in urine of patients with brain tumors. MMP activity was reduced after surgery, demonstrating that MMPs can be both diagnostic markers and markers of tumor recurrence. We confirmed that these proteins originate in the brain tumor tissues as elevated MMPs were observed in cerebrospinal fluid (CSF) and brain tumor tissue [145, 215]. Other urinary biomarkers such as bFGF and TIMP-3 have been reported as successful diagnostic markers in detecting juvenile pilocytic astrocytoma (JPA) with high accuracy [146]. MMPs, VEGF, bFGF, thrombospondin, TNF-α, IL-12 and IL-8 in blood and urine have been investigated as CNS tumor biomarkers in clinical trials for radiation therapy (clinicaltrials.gov). Additionally, neogenin and netrin-1 have been identified as urinary biomarkers for diffuse intrinsic pontine glioma (DIPG), a pediatric brain tumor representing a major clinical challenge [147]. Urinary biomarkers were evaluated in a pediatric brain tumor consortium (PBTC) clinical trial of Veliparib and radiation therapy followed by Veliparib and Temozolomide (TMZ) in DIPG patients. High levels of netrin-1, bFGF, MMP-3, and TIMP-1 could distinguish DIPG patients compared to healthy controls [148]. Additionally, in the same study other biomarkers significantly predicted survival (MMP-9), progression-free survival (TIMP-1) and correlation with baseline tumor volume (MMP-13) [148]. Circulating tumor cells (CTCs) have also been detected in blood and CSF samples and could be useful markers for tumor surveillance [144]. CTCs in CSF may be used to determine tumor staging in both adult and pediatric brain cancers [216]. Diagnosis of brain cancer is currently heavily reliant on radiographic studies where sedation is required for the pediatric population [215]. Therefore, biomarker discovery and validation would lead to better diagnostic tools as well as a reduction in the use of sedation and its risks [144, 215].

Neuroblastoma

Neuroblastoma (NB) is the second most common solid tumor in pediatric patients. One in 100,000 children in the USA is diagnosed with NB each year (seer.cancer.gov). NB develops from neural crest cells and is a cancer of the peripheral sympathetic nervous system, typically found within the adrenal medulla. Therapeutics for NB are currently chosen based on tumor gene expression, disease stage and age. Therapeutic

targets include *MYCN*, anaplastic lymphoma kinase (ALK), tropomyosin receptor kinase B (TrkB) and disialoganglioside (GD2), a surface antigen in NB tumor cells [159]. *MYCN* oncogene amplification is currently the most powerful prognostic biomarker known in NB. Circulating *MYCN* concentration in the blood of NB patients has been reported to decrease after chemotherapy treatment [149]. It has been reported that there was a significant difference in the TME of *MYCN*-amplified (*MYCN* -A) and non-amplified (*MYCN* -NA) NB tumors, differing in the levels of stromal inflammatory cells and immunosuppressive activity [154]. Importantly, *MYCN,* ALK, TrkB, and GD2 are currently being investigated as therapeutic targets in clinical trials for pediatric NB (clinicaltrials.gov). For NB, expression of two genes *USP17L5* and *SLC25A5* in tumor tissues has been reported to correlate with low OS in patients with an older diagnostic age [150]. In the same study, expression of four genes, including *POF1B*, *RND3*, *KLC4*, and *SLC12A1,* was reported to be upregulated in patients with a younger age at diagnosis and correlated with a higher OS [150]. Plasma cell-free DNA was found to be a marker of tumor burden [151] and prognosis in NB [152]. Elevated levels of mRNAs including tyrosine hydroxylase (TH), PHOX2B, and doublecortin (DCX) in bone marrow and peripheral blood at diagnosis strongly predicted worse event-free survival (EFS) and OS in patients with Stage 4 NB [153].

MicroRNA from EVs originating from the TME is important for tumor cell communication in NB and have the potential to serve as biomarkers of tumor aggressiveness and therapy response for NB [154]. Exosomal miRNA expression has been reported to be a prognostic marker for high-risk NB patients and correlated with disease aggression. A 3-exosomal miRNA signature (miR-29c, miR-342-3p, let-7b) was identified that could predict EFS and differentiate between good and poor responders to induction chemotherapy for NB [155]. Urinary metabolites, such as catecholamines, vanillylmandelic acid (VMA), and homovanillic acid (HVA), were first recognized as NB biomarkers in the 1970s. At diagnosis, VMA and HVA levels were upregulated in ~90–95% of NB patients and a low VMA/HVA ratio indicated poor prognosis [156]. Various serum proteins such as lactate dehydrogenase (LDH), neuron-specific enolase (NSE), and ferritin were also recognized as biomarkers for NB. Serum NSE has been reported to be a useful marker for advanced NB, wherein elevated NSE levels were associated with a poor outcome in patients and returned to normal after therapy [158]. NB tumor burden has been estimated by tracking serum LDH levels in patients [156]. For NB patients >18 months of age with metastatic disease, serum LDH and ferritin levels have been shown to be significant predictors of EFS and OS [157]. Ferritin is a useful prognostic biomarker for NB since tumor cells express glycosylated ferritin, whereas healthy cells secrete non-glycosylated ferritin. Serum ferritin has been reported to distinguish between NB disease stages with significantly higher levels present in Stage IV (metastases to bone) patients compared to Stage IVS (metastases to liver, skin, or bone marrow but not to bone) NB patients [156]. LDH and ferritin have both been studied in clinical trials as pediatric NB biomarkers for predicting treatment success (clinicaltrials.gov).

Wilms' Tumor

Wilms' tumor (WT) is the fourth most common pediatric cancer [217]. Renal tumors afflict 600 pediatric patients per year in the USA, and ~90% of these patients have WT [218]. Long-term survival rates for WT are over 90%, however, ~50% of patients who relapse ultimately die from this disease. With more effective prognostic biomarkers, the identification of patients who have a greater chance of relapse would facilitate earlier and perhaps more aggressive treatment as would the identification of patients with a greater chance of survival who might be treated with less aggressive treatment with lower morbidity [218]. Multiple studies have linked the gain of chromosome 1q to worse EFS and OS in tumor subsets of patients with intermediate-risk localized disease or non-anaplastic localized disease making this a good prognostic biomarker for WT [160, 161]. Additionally, gain of 1q was significantly correlated with an increased risk of recurrence in WT with absence of anaplasia, i.e., favorable histology WT [162]. Loss of chromosome 14q was also found to be related to worse EFS in WT [160]. 11p15 loss of heterozygosity and *WT1* mutation were both significantly related to relapse in very low-risk Wilms tumors weighing <550 gm and were classified as Stage I favorable histology WTs in children younger than 24 months of age (patients who do not undergo chemotherapy). All patients with the *WT1* mutation also had 11p15 loss of heterozygosity [163]. A correlation has been noted between tumor progression and prognosis and IL-6 and signal transducer and activator of transcription 3 (STAT3) expression in WT [164]. IL-6 and STAT3 were reported to be upregulated in invasive and metastatic WT compared to non-invasive and non-metastatic WT. IL-6 expression was correlated with DFS and OS, whereas STAT3 was correlated with DFS alone [164]. Prohibitin (PHB), a protein that regulates cellular proliferation has been reported to be a predictive marker for tumor stage in WT. Urinary PHB levels were significantly upregulated in patients with recurrent disease and might therefore serve as a WT marker. PHB might also serve as an important biomarker of drug resistance given that the overexpression of PHB limited mitochondrial apoptosis and led to resistance to certain chemotherapy drugs [165].

Concluding Remarks/Summary

The studies reviewed above highlight the importance of the TME as a rich source of viable biomarkers for a wide variety of human cancers and support a renewed effort to exploit this important tumor component as a potentially powerful theranostic target.

Acknowledgements The authors gratefully acknowledge the support of the NIH R21 CA253051, the Breast Cancer Research Foundation, the Karp Family Foundation, the Goodman and Kaplan Families, the Michael B. Rukin Charitable Foundation, and the Brad and Tracy Stevens Family Foundation.

References

1. Yang J, Roy R, Jedinak A, Moses MA. Mining the Human Proteome: Biomarker Discovery for Human Cancer and Metastases. Cancer J. 2015;21(4):327–36.
2. Roy R, Morad G, Jedinak A, Moses MA. Metalloproteinases and their roles in human cancer. Anat Rec (Hoboken). 2020;303(6):1557–72.
3. Jedinak A, Loughlin KR, Moses MA. Approaches to the discovery of non-invasive urinary biomarkers of prostate cancer. Oncotarget. 2018;9(65):32534–50.
4. Sohel MMH. Circulating microRNAs as biomarkers in cancer diagnosis. Life Sci. 2020;248:117473.
5. Srivastava S, Wagner PD. The early detection research network: a national infrastructure to support the discovery, development, and validation of cancer biomarkers. Cancer Epidemiol Prev Biomark. 2020;29(12):2401–10.
6. Liu T, Han C, Wang S, Fang P, Ma Z, Xu L, et al. Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. J Hematol Oncol. 2019;12(1):1–15.
7. Ito T, Ishii G, Chiba H, Ochiai A. The VEGF angiogenic switch of fibroblasts is regulated by MMP-7 from cancer cells. Oncogene. 2007;26(51):7194–203.
8. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174–86.
9. Folkman J. Tumor angiogenesis: a possible control point in tumor growth. Ann Intern Med. 1975;82(1):96–100.
10. Huang J, Guo P, Moses MA. Rationally designed antibody drug conjugates targeting the breast cancer-associated endothelium. ACS Biomater Sci Eng. 2019;6(5):2563–9.
11. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol. 2002;20(21):4368–80.
12. Nagy JA, Benjamin L, Zeng H, Dvorak AM, Dvorak HF. Vascular permeability, vascular hyperpermeability and angiogenesis. Angiogenesis. 2008;11(2):109–19.
13. Majidpoor J, Mortezaee K. Angiogenesis as a hallmark of solid tumors-clinical perspectives. Cell Oncol. 2021:1–23.
14. Schaaf MB, Houbaert D, Mece O, Agostinis P. Autophagy in endothelial cells and tumor angiogenesis. Cell Death Differ. 2019;26(4):665–79.
15. Schaaf MB, Garg AD, Agostinis P. Defining the role of the tumor vasculature in antitumor immunity and immunotherapy. Cell Death Dis. 2018;9(2):115.
16. Zhang SC, Hu ZQ, Long JH, Zhu GM, Wang Y, Jia Y, et al. Clinical implications of tumor-infiltrating immune cells in breast cancer. J Cancer. 2019;10(24):6175–84.
17. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29(8):1093–102.
18. Stoll G, Zitvogel L, Kroemer G. Immune infiltrate in cancer. Aging (Albany NY). 2015;7(6):358–9.
19. Arneth B. Tumor microenvironment. Medicina (Kaunas) 2019;56(1).
20. Ngambenjawong C, Gustafson HH, Pun SH. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. Adv Drug Deliv Rev. 2017;114:206–21.
21. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity. 2014;41(1):49–61.
22. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017;14(7):399–416.
23. Ono M, Torisu H, Fukushi J-i, Nishie A, Kuwano M. Biological implications of macrophage infiltration in human tumor angiogenesis. Cancer Chemother Pharmacol. 1999;43(1):S69–71.
24. Sunderkötter C, Steinbrink K, Goebeler M, Bhardwaj R, Sorg C. Macrophages and angiogenesis. J Leukoc Biol. 1994;55(3):410–22.
25. Chen Y, Song Y, Du W, Gong L, Chang H, Zou Z. Tumor-associated macrophages: an accomplice in solid tumor progression. J Biomed Sci. 2019;26(1):78.
26. Hughes R, Qian BZ, Rowan C, Muthana M, Keklikoglou I, Olson OC, et al. Perivascular M2 macrophages stimulate tumor relapse after chemotherapy. Cancer Res. 2015;75(17):3479–91.
27. Osterberg N, Ferrara N, Vacher J, Gaedicke S, Niedermann G, Weyerbrock A, et al. Decrease of VEGF-A in myeloid cells attenuates glioma progression and prolongs survival in an experimental glioma model. Neuro-Oncology. 2016;18(7):939–49.
28. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141(1):39–51.
29. Eble JA, Niland S. The extracellular matrix in tumor progression and metastasis. Clin Exp Metastasis. 2019;36(3):171–98.
30. Walker C, Mojares E, Del Rio Hernandez A. Role of extracellular matrix in development and cancer progression. Int J Mol Sci. 2018;19(10).
31. Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, et al. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. Proc Natl Acad Sci. 2000;97(8):3884–9.
32. Yan L, Borregaard N, Kjeldsen L, Moses MA. The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL): modulation of MMP-9 activity by NGAL. J Biol Chem. 2001;276(40):37258–65.
33. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci. 2012;125(Pt 23):5591–6.
34. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol. 2009;27(31):5287.
35. Fernández CA, Butterfield C, Jackson G, Moses MA. Structural and functional uncoupling of the enzymatic and angiogenic inhibitory activities of tissue inhibitor of metalloproteinase-2 (TIMP-2): loop 6 is a novel angiogenesis inhibitor. J Biol Chem. 2003;278(42):40989–95.
36. Moses MA. The regulation of neovascularization by matrix metalloproteinases and their inhibitors. Stem Cells. 1997;15(3):180–9.
37. Fernandez CA, Roy R, Lee S, Yang J, Panigrahy D, Van Vliet KJ, et al. The anti-angiogenic peptide, loop 6, binds insulin-like growth factor-1 receptor. J Biol Chem. 2010;285(53):41886–95.

38. Shimoda M, Ohtsuka T, Okada Y, Kanai Y. Stromal metalloproteinases: crucial contributors to the tumor microenvironment. Pathol Int. 2021;71(1):1–14.
39. Roy R, Wewer UM, Zurakowski D, Pories SE, Moses MA. ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. J Biol Chem. 2004;279(49):51323–30.
40. Pories SE, Zurakowski D, Roy R, Lamb CC, Raza S, Exarhopoulos A, et al. Urinary metalloproteinases: noninvasive biomarkers for breast cancer risk assessment. Cancer Epidemiol Prev Biomark. 2008;17(5):1034–42.
41. Ma B, Ma Q, Jin C, Wang X, Zhang G, Zhang H, et al. ADAM12 expression predicts clinical outcome in estrogen receptor-positive breast cancer. Int J Clin Exp Pathol. 2015;8(10):13279.
42. Wang R-X, Chen S, Huang L, Shao Z-M. Predictive and prognostic value of matrix metalloproteinase (MMP)-9 in neoadjuvant chemotherapy for triple-negative breast cancer patients. BMC Cancer. 2018;18(1):1–8.
43. Pelekanou V, Villarroel-Espindola F, Schalper KA, Pusztai L, Rimm DL. CD68, CD163, and matrix metalloproteinase 9 (MMP-9) co-localization in breast tumor microenvironment predicts survival differently in ER-positive and-negative cancers. Breast Cancer Res. 2018;20(1):1–10.
44. Emens LA, Molinero L, Loi S, Rugo HS, Schneeweiss A, Diéras V, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer: biomarker evaluation of the IMpassion130 study. JNCI: J Natl Cancer Inst 2020;113(8):1005–16.
45. Okabe M, Toh U, Iwakuma N, Saku S, Akashi M, Kimitsuki Y, et al. Predictive factors of the tumor immunological microenvironment for long-term follow-up in early stage breast cancer. Cancer Sci. 2017;108(1):81–90.
46. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50.
47. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs working group 2014. Ann Oncol. 2015;26(2):259–71.
48. Moses MA, Wiederschain D, Loughlin KR, Zurakowski D, Lamb CC, Freeman MR. Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res. 1998;58(7):1395–9.
49. Fernández CA, Yan L, Louis G, Yang J, Kutok JL, Moses MA. The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients. Clin Cancer Res. 2005;11(15):5390–5.
50. Piskór BM, Przylipiak A, Dąbrowska E, Sidorkiewicz I, Niczyporuk M, Szmitkowski M, et al. Plasma concentrations of Matrilysins MMP-7 and MMP-26 as diagnostic biomarkers in breast cancer. J Clin Med. 2021;10(7):1436.
51. Garrido-Cano I, Constâncio V, Adam-Artigues A, Lameirinhas A, Simón S, Ortega B, et al. Circulating miR-99a-5p expression in plasma: a potential biomarker for early diagnosis of breast cancer. Int J Mol Sci. 2020;21(19):7427.
52. Sahlberg KK, Bottai G, Naume B, Burwinkel B, Calin GA, Børresen-Dale A-L, et al. A serum microRNA signature predicts tumor relapse and survival in triple-negative breast cancer patients. Clin Cancer Res. 2015;21(5):1207–14.
53. Mayekar MK, Bivona TG. Current landscape of targeted therapy in lung cancer. Clin Pharmacol Ther. 2017;102(5):757–64.
54. Cao B, Wang P, Gu L, Liu J. Use of four genes in exosomes as biomarkers for the identification of lung adenocarcinoma and lung squamous cell carcinoma. Oncol Lett 2021;21(4):1-.
55. Zhou Y, Chen W-Z, Peng A-F, Tong W-L, Liu J-M, Liu Z-L. Neuron-specific enolase, histopathological types, and age as risk factors for bone metastases in lung cancer. Tumor Biol. 2017;39(7):1010428317714194.
56. Zhang L, Liu D, Li L, Pu D, Zhou P, Jing Y, et al. The important role of circulating CYFRA21-1 in metastasis diagnosis and prognostic value compared with carcinoembryonic antigen and neuron-specific enolase in lung cancer patients. BMC Cancer. 2017;17(1):1–14.
57. Netterberg I, Li CC, Molinero L, Budha N, Sukumaran S, Stroh M, et al. A PK/PD analysis of circulating biomarkers and their relationship to tumor response in atezolizumab-treated non-small cell lung cancer patients. Clin Pharmacol Therap. 2019;105(2):486–95.
58. Muley T, Zhang X, Holdenrieder S, Korse CM, Zhi X-y, Molina R, et al. A continuous responder algorithm to optimize clinical management of small-cell lung cancer with progastrin-releasing peptide as a simple blood test. Tumor Biol 2020;42(9):1010428320958603.
59. Bonanno L, De Paoli A, Zulato E, Esposito G, Calabrese F, Favaretto A, et al. LKB1 expression correlates with increased survival in patients with advanced non–small cell lung cancer treated with chemotherapy and bevacizumab. Clin Cancer Res. 2017;23(13):3316–24.
60. Ozden F, Saygin C, Uzunaslan D, Onal B, Durak H, Aki H. Expression of MMP-1, MMP-9 and TIMP-2 in prostate carcinoma and their influence on prognosis and survival. J Cancer Res Clin Oncol. 2013;139(8):1373–82.
61. Xie T, Dong B, Yan Y, Hu G, Xu Y. Association between MMP-2 expression and prostate cancer: a meta-analysis. Biomed Rep. 2016;4(2):241–5.
62. Szarvas T, Becker M, Vom Dorp F, Meschede J, Scherag A, Bankfalvi A, et al. Elevated serum matrix metalloproteinase 7 levels predict poor prognosis after radical prostatectomy. Int J Cancer. 2011;128(6):1486–92.
63. Nonsrijun N, Mitchai J, Brown K, Leksomboon R, Tuamsuk P. Overexpression of matrix metalloproteinase 11 in Thai prostatic adenocarcinoma is associated with poor survival. Asian Pac J Cancer Prev. 2013;14(5):3331–5.
64. Burdelski C, Fitzner M, Hube-Magg C, Kluth M, Heumann A, Simon R, et al. Overexpression of the a disintegrin and metalloproteinase ADAM15 is linked to a small but highly aggressive subset of prostate cancers. Neoplasia. 2017;19(4):279–87.
65. Ayala G, Morello M, Frolov A, You S, Li R, Rosati F, et al. Loss of caveolin-1 in prostate cancer stroma correlates with reduced relapse-free survival and is functionally relevant to tumour progression. J Pathol. 2013;231(1):77–87.
66. Hammarsten P, Dahl Scherdin T, Hagglof C, Andersson P, Wikstrom P, Stattin P, et al. High Caveolin-1 expression in tumor stroma is associated with a favourable outcome in prostate cancer patients managed by watchful waiting. PLoS One. 2016;11(10):e0164016.
67. Nordby Y, Richardsen E, Ness N, Donnem T, Patel HRH, Busund LT, et al. High miR-205 expression in normal epithelium is associated with biochemical failure - an argument for epithelial crosstalk in prostate cancer? Sci Rep. 2017;7(1):16308.
68. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res. 2000;6(7):2702–6.
69. Sharma J, Gray KP, Harshman LC, Evan C, Nakabayashi M, Fichorova R, et al. Elevated IL-8, TNF-alpha, and MCP-1 in men with metastatic prostate cancer starting androgen-deprivation therapy (ADT) are associated with shorter time to castration-resistance and overall survival. Prostate. 2014;74(8):820–8.

70. Archer M, Dogra N, Kyprianou N. Inflammation as a driver of prostate cancer metastasis and therapeutic resistance. Cancers (Basel). 2020;12(10).
71. Chan LW, Moses MA, Goley E, Sproull M, Muanza T, Coleman CN, et al. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: a longitudinal study of protein kinetics throughout tumor progression and therapy. J Clin Oncol. 2004;22(3):499–506.
72. Roy R, Louis G, Loughlin KR, Wiederschain D, Kilroy SM, Lamb CC, et al. Tumor-specific urinary matrix metalloproteinase fingerprinting: identification of high molecular weight urinary matrix metalloproteinase species. Clin Cancer Res. 2008;14(20):6610–7.
73. Jia D, Roy R, Moses MA. MMPs in Biology and Medicine. Matrix metalloproteinase biology. 2015:183.
74. Jedinak A, Curatolo A, Zurakowski D, Dillon S, Bhasin MK, Libermann TA, et al. Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. BMC Cancer. 2015;15(1):1–9.
75. Bilgin Dogru E, Dizdar Y, Aksit E, Ural F, Sanli O, Yasasever V. EMMPRIN and ADAM12 in prostate cancer: preliminary results of a prospective study. Tumour Biol. 2014;35(11):11647–53.
76. Gevaert T, Van Eycke YR, Vanden Broeck T, Van Poppel H, Salmon I, Rorive S, et al. Comparing the expression profiles of steroid hormone receptors and stromal cell markers in prostate cancer at different Gleason scores. Sci Rep. 2018;8(1):14326.
77. Rochette A, Boufaied N, Scarlata E, Hamel L, Brimo F, Whitaker HC, et al. Asporin is a stromally expressed marker associated with prostate cancer progression. Br J Cancer. 2017;116(6):775–84.
78. Bernreuther C, Daghigh F, Moller K, Hube-Magg C, Lennartz M, Lutz F, et al. Secreted frizzled-related protein 4 (SFRP4) is an independent prognostic marker in prostate cancers lacking TMPRSS2: ERG fusions. Pathol Oncol Res. 2020;26(4):2709–22.
79. Witkiewicz AK, Nguyen K, Dasgupta A, Kennedy EP, Yeo CJ, Lisanti MP, et al. Co-expression of fatty acid synthase and caveolin-1 in pancreatic ductal adenocarcinoma: implications for tumor progression and clinical outcome. Cell Cycle. 2008;7(19):3021–5.
80. Shan T, Lu H, Ji H, Li Y, Guo J, Chen X, et al. Loss of stromal caveolin-1 expression: a novel tumor microenvironment biomarker that can predict poor clinical outcomes for pancreatic cancer. PLoS One. 2014;9(6):e97239.
81. Yu J, Walter K, Omura N, Hong S-M, Young A, Li A, et al. Unlike pancreatic cancer cells pancreatic cancer associated fibroblasts display minimal gene induction after 5-aza-2′-deoxycytidine. PLoS One. 2012;7(9):e43456.
82. Veenstra V, Damhofer H, Waasdorp C, van Rijssen L, van de Vijver M, Dijk F, et al. ADAM12 is a circulating marker for stromal activation in pancreatic cancer and predicts response to chemotherapy. Oncogenesis. 2018;7(11):1–11.
83. Roy R, Dagher A, Zurakowski D, Kulke M, Moses MA. Abstract A53: ADAM12 contributes to the malignant potential of pancreatic cancer and may serve as a non-invasive biomarker for its detection. AACR; 2016.
84. Hogendorf P, Durczynski A, Kumor A, Strzelczyk J. Pancreatic head carcinoma and vascular endothelial growth factor (VEGF-A) concentration in portal blood: its association with cancer grade, tumor size and probably poor prognosis. Arch Med Sci. 2014;10(2):288–93.
85. Resovi A, Bani MR, Porcu L, Anastasia A, Minoli L, Allavena P, et al. Soluble stroma-related biomarkers of pancreatic cancer. EMBO Mol Med. 2018;10(8).
86. Balasenthil S, Huang Y, Liu S, Marsh T, Chen J, Stass SA, et al. A plasma biomarker panel to identify surgically resectable early-stage pancreatic cancer. JNCI: J Natl Cancer Inst. 2017;109(8):djw341.
87. Bressy C, Lac S, Nigri J, Leca J, Roques J, Lavaut M-N, et al. LIF drives neural remodeling in pancreatic cancer and offers a new candidate biomarker. Cancer Res. 2018;78(4):909–21.
88. Yoshizawa N, Sugimoto K, Tameda M, Inagaki Y, Ikejiri M, Inoue H, et al. miR-3940-5p/miR-8069 ratio in urine exosomes is a novel diagnostic biomarker for pancreatic ductal adenocarcinoma. Oncol Lett. 2020;19(4):2677–84.
89. Roy R, Zurakowski D, Wischhusen J, Frauenhoffer C, Hooshmand S, Kulke M, et al. Urinary TIMP-1 and MMP-2 levels detect the presence of pancreatic malignancies. Br J Cancer. 2014;111(9):1772–9.
90. Radon TP, Massat NJ, Jones R, Alrawashdeh W, Dumartin L, Ennis D, et al. Identification of a three-biomarker panel in urine for early detection of pancreatic adenocarcinoma. Clin Cancer Res. 2015;21(15):3512–21.
91. Wang CX, Xiong HF, Wang S, Wang J, Nie X, Guo Q, et al. Overexpression of TEM8 promotes ovarian cancer progression via Rac1/Cdc42/JNK and MEK/ERK/STAT3 signaling pathways. Am J Transl Res. 2020;12(7):3557–76.
92. Tian T, Li X, Hua Z, Ma J, Liu Z, Chen H, et al. S100A1 promotes cell proliferation and migration and is associated with lymph node metastasis in ovarian cancer. Discov Med. 2017;23(127):235–45.
93. Dupont J, Tanwar MK, Thaler HT, Fleisher M, Kauff N, Hensley ML, et al. Early detection and prognosis of ovarian cancer using serum YKL-40. J Clin Oncol. 2004;22(16):3330–9.
94. Coticchia CM, Yang J, Moses MA. Ovarian cancer biomarkers: current options and future promise. J Natl Compr Cancer Netw. 2008;6(8):795–802.
95. Reiner AT, Tan S, Agreiter C, Auer K, Bachmayr-Heyda A, Aust S, et al. EV-associated MMP9 in high-grade serous ovarian cancer is preferentially localized to annexin V-binding EVs. Dis Markers. 2017;2017:9653194.
96. Yagi H, Miyamoto S, Tanaka Y, Sonoda K, Kobayashi H, Kishikawa T, et al. Clinical significance of heparin-binding epidermal growth factor-like growth factor in peritoneal fluid of ovarian cancer. Br J Cancer. 2005;92(9):1737–45.
97. Carbotti G, Orengo AM, Mezzanzanica D, Bagnoli M, Brizzolara A, Emionite L, et al. Activated leukocyte cell adhesion molecule soluble form: a potential biomarker of epithelial ovarian cancer is increased in type II tumors. Int J Cancer. 2013;132(11):2597–605.
98. Cheon DJ, Li AJ, Beach JA, Walts AE, Tran H, Lester J, et al. ADAM12 is a prognostic factor associated with an aggressive molecular subtype of high-grade serous ovarian carcinoma. Carcinogenesis. 2015;36(7):739–47.
99. Ueno M, Shiomi T, Mochizuki S, Chijiiwa M, Shimoda M, Kanai Y, et al. ADAM9 is over-expressed in human ovarian clear cell carcinomas and suppresses cisplatin-induced cell death. Cancer Sci. 2018;109(2):471–82.
100. Robati M, Ghaderi A, Mehraban M, Shafizad A, Nasrolahi H, Mohammadianpanah M. Vascular endothelial growth factor (VEGF) improves the sensitivity of CA125 for differentiation of epithelial ovarian cancers from ovarian cysts. Arch Gynecol Obstet. 2013;288(4):859–65.
101. Kristjansdottir B, Levan K, Partheen K, Sundfeldt K. Diagnostic performance of the biomarkers HE4 and CA125 in type I and type II epithelial ovarian cancer. Gynecol Oncol. 2013;131(1):52–8.
102. Lakshmanan M, Kumar V, Chaturvedi A, Misra S, Gupta S, Akhtar N, et al. Role of serum HE4 as a prognostic marker in carcinoma of the ovary. Indian J Cancer. 2019;56(3):216–21.
103. Sandri MT, Bottari F, Franchi D, Boveri S, Candiani M, Ronzoni S, et al. Comparison of HE4, CA125 and ROMA algorithm in women with a pelvic mass: correlation with pathological outcome. Gynecol Oncol. 2013;128(2):233–8.
104. Hamed EO, Ahmed H, Sedeek OB, Mohammed AM, Abd-Alla AA, Abdel Ghaffar HM. Significance of HE4 estimation in com-

parison with CA125 in diagnosis of ovarian cancer and assessment of treatment response. Diagn Pathol. 2013;8:11.
105. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, et al. Interleukin-6 as a therapeutic target in human ovarian cancer. Clin Cancer Res. 2011;17(18):6083–96.
106. Alvarez Secord A, Bell Burdett K, Owzar K, Tritchler D, Sibley AB, Liu Y, et al. Predictive blood-based biomarkers in patients with epithelial ovarian cancer treated with carboplatin and paclitaxel with or without bevacizumab: results from GOG-0218. Clin Cancer Res. 2020;26(6):1288–96.
107. Zhang H, Huo Q, Huang L, Cheng Y, Liu Y, Bao H. Neutrophil-to-lymphocyte ratio in ovarian cancer patients with low CA125 concentration. Biomed Res Int. 2019;2019:8107906.
108. Li C, Xiong Y, Yang X, Wang L, Zhang S, Dai N, et al. Lost expression of ADAMTS5 protein associates with progression and poor prognosis of hepatocellular carcinoma. Drug Des Devel Ther. 2015;9:1773–83.
109. Liu Z, Wang Y, Dou C, Xu M, Sun L, Wang L, et al. Hypoxia-induced up-regulation of VASP promotes invasiveness and metastasis of hepatocellular carcinoma. Theranostics. 2018;8(17):4649–63.
110. Bergis D, Kassis V, Ranglack A, Koeberle V, Piiper A, Kronenberger B, et al. High serum levels of the Interleukin-33 receptor soluble ST2 as a negative prognostic factor in hepatocellular carcinoma. Transl Oncol. 2013;6(3):311–8.
111. Li T, Zhu Y, Qin CY, Yang Z, Fang A, Xu S, et al. Expression and prognostic significance of vascular endothelial growth factor receptor 1 in hepatocellular carcinoma. J Clin Pathol. 2012;65(9):808–14.
112. Fu SJ, Qi CY, Xiao WK, Li SQ, Peng BG, Liang LJ. Glypican-3 is a potential prognostic biomarker for hepatocellular carcinoma after curative resection. Surgery. 2013;154(3):536–44.
113. Vongsuvanh R, van der Poorten D, Iseli T, Strasser SI, McCaughan GW, George J. Midkine increases diagnostic yield in AFP negative and NASH-related hepatocellular carcinoma. PLoS One. 2016;11(5):e0155800.
114. Murakami Y, Tamori A, Itami S, Tanahashi T, Toyoda H, Tanaka M, et al. The expression level of miR-18b in hepatocellular carcinoma is associated with the grade of malignancy and prognosis. BMC Cancer. 2013;13:99.
115. Zhang Y, Li T, Qiu Y, Zhang T, Guo P, Ma X, et al. Serum microRNA panel for early diagnosis of the onset of hepatocellular carcinoma. Medicine (Baltimore). 2017;96(2):e5642.
116. Goyal L, Zheng H, Abrams TA, Miksad R, Bullock AJ, Allen JN, et al. A phase II and biomarker study of sorafenib combined with modified FOLFOX in patients with advanced hepatocellular carcinoma. Clin Cancer Res. 2019;25(1):80–9.
117. Zhu AX, Ancukiewicz M, Supko JG, Sahani DV, Blaszkowsky LS, Meyerhardt JA, et al. Efficacy, safety, pharmacokinetics, and biomarkers of cediranib monotherapy in advanced hepatocellular carcinoma: a phase II study. Clin Cancer Res. 2013;19(6):1557–66.
118. Hyeon J, Ahn S, Lee JJ, Song DH, Park CK. Expression of fibroblast growth factor 19 is associated with recurrence and poor prognosis of hepatocellular carcinoma. Dig Dis Sci. 2013;58(7):1916–22.
119. Jiang Y, Liu W, Li T, Hu Y, Chen S, Xi S, et al. Prognostic and predictive value of p21-activated kinase 6 associated support vector machine classifier in gastric cancer treated by 5-fluorouracil/oxaliplatin chemotherapy. EBioMedicine. 2017;22:78–88.
120. Jiang C, Zhou Y, Huang Y, Wang Y, Wang W, Kuai X. Overexpression of ADAMTS-2 in tumor cells and stroma is predictive of poor clinical prognosis in gastric cancer. Hum Pathol. 2019;84:44–51.
121. Jiang X, Wu M, Xu X, Zhang L, Huang Y, Xu Z, et al. COL12A1, a novel potential prognostic factor and therapeutic target in gastric cancer. Mol Med Rep. 2019;20(4):3103–12.
122. Park JY, Sung JY, Lee J, Park YK, Kim YW, Kim GY, et al. Polarized CD163+ tumor-associated macrophages are associated with increased angiogenesis and CXCL12 expression in gastric cancer. Clin Res Hepatol Gastroenterol. 2016;40(3):357–65.
123. Shimura T, Ebi M, Yamada T, Yamada T, Katano T, Nojiri Y, et al. Urinary kallikrein 10 predicts the incurability of gastric cancer. Oncotarget. 2017;8(17):29247–57.
124. Shimura T, Dagher A, Sachdev M, Ebi M, Yamada T, Yamada T, et al. Urinary ADAM12 and MMP-9/NGAL complex detect the presence of gastric cancer. Cancer Prev Res. 2015;8(3):240–8.
125. Shimura T, Dayde D, Wang H, Okuda Y, Iwasaki H, Ebi M, et al. Novel urinary protein biomarker panel for early diagnosis of gastric cancer. Br J Cancer. 2020;123(11):1656–64.
126. Iwasaki H, Shimura T, Yamada T, Okuda Y, Natsume M, Kitagawa M, et al. A novel urinary microRNA biomarker panel for detecting gastric cancer. J Gastroenterol. 2019;54(12):1061–9.
127. Gayed BA, Gillen J, Christie A, Pena-Llopis S, Xie XJ, Yan J, et al. Prospective evaluation of plasma levels of ANGPT2, TuM2PK, and VEGF in patients with renal cell carcinoma. BMC Urol. 2015;15:24.
128. Bilen MA, Zurita AJ, Ilias-Khan NA, Chen HC, Wang X, Kearney AY, et al. Hypertension and circulating cytokines and angiogenic factors in patients with advanced non-clear cell renal cell carcinoma treated with sunitinib: results from a phase II trial. Oncologist. 2015;20(10):1140–8.
129. Dufies M, Giuliano S, Viotti J, Borchiellini D, Cooley LS, Ambrosetti D, et al. CXCL7 is a predictive marker of sunitinib efficacy in clear cell renal cell carcinomas. Br J Cancer. 2017;117(7):947–53.
130. Samberkar S, Rajandram R, Mun KS, Samberkar P, Danaee M, Zulkafli IS. Carbonic anhydrase IX immunohistochemistry has potential to predict renal cell carcinoma outcomes: a systematic review and meta-analyses. Malays J Pathol. 2019;41(3):233–42.
131. Zhao Z, Liao G, Li Y, Zhou S, Zou H, Fernando S. Prognostic value of carbonic anhydrase IX immunohistochemical expression in renal cell carcinoma: a meta-analysis of the literature. PLoS One. 2014;9(11):e114096.
132. Jilaveanu LB, Puligandla M, Weiss SA, Wang XV, Zito C, Flaherty KT, et al. Tumor microvessel density as a prognostic marker in high-risk renal cell carcinoma patients treated on ECOG-ACRIN E2805. Clin Cancer Res. 2018;24(1):217–23.
133. Xu W, Puligandla M, Manola J, Bullock AJ, Tamasauskas D, McDermott DF, et al. Angiogenic factor and cytokine analysis among patients treated with adjuvant VEGFR TKIs in resected renal cell carcinoma. Clin Cancer Res. 2019;25(20):6098–106.
134. Motzer RJ, Hutson TE, Hudes GR, Figlin RA, Martini JF, English PA, et al. Investigation of novel circulating proteins, germ line single-nucleotide polymorphisms, and molecular tumor markers as potential efficacy biomarkers of first-line sunitinib therapy for advanced renal cell carcinoma. Cancer Chemother Pharmacol. 2014;74(4):739–50.
135. Pal S, Azad A, Bhatia S, Drabkin H, Costello B, Sarantopoulos J, et al. A phase I/II trial of BNC105P with everolimus in metastatic renal cell carcinoma. Clin Cancer Res. 2015;21(15):3420–7.
136. Zhao M, Xu H, Liang F, He J, Zhang J. Association of osteopontin expression with the prognosis of glioma patient: a meta-analysis. Tumour Biol. 2015;36(1):429–36.
137. Liu Y, Li X, Zhang Y, Wang H, Rong X, Peng J, et al. An miR-340-5p-macrophage feedback loop modulates the progression and tumor microenvironment of glioblastoma multiforme. Oncogene. 2019;38(49):7399–415.
138. Zhou W, Yu X, Sun S, Zhang X, Yang W, Zhang J, et al. Increased expression of MMP-2 and MMP-9 indicates poor prognosis in glioma recurrence. Biomed Pharmacother. 2019;118:109369.
139. Könnecke H, Bechmann I. The role of microglia and matrix metalloproteinases involvement in neuroinflammation and gliomas. Clin Dev Immunol. 2013.

140. Ramachandran RK, Sorensen MD, Aaberg-Jessen C, Hermansen SK, Kristensen BW. Expression and prognostic impact of matrix metalloproteinase-2 (MMP-2) in astrocytomas. PLoS One. 2017;12(2):e0172234.
141. Urbanaviciute R, Skauminas K, Skiriute D. The evaluation of AREG, MMP-2, CHI3L1, GFAP, and OPN serum combined value in astrocytic glioma patients' diagnosis and prognosis. Brain Sci. 2020;10(11).
142. Elstner A, Stockhammer F, Nguyen-Dobinsky TN, Nguyen QL, Pilgermann I, Gill A, et al. Identification of diagnostic serum protein profiles of glioblastoma patients. J Neuro-Oncol. 2011;102(1):71–80.
143. Rahman M, Kresak J, Yang C, Huang J, Hiser W, Kubilis P, et al. Analysis of immunobiologic markers in primary and recurrent glioblastoma. J Neuro-Oncol. 2018;137(2):249–57.
144. Madlener S, Gojo J. Liquid biomarkers for pediatric brain tumors: biological features, advantages and perspectives, J Pers Med. 2020;10(4).
145. Smith ER, Manfredi M, Scott RM, Black PM, Moses MA. A recurrent craniopharyngioma illustrates the potential usefulness of urinary matrix metalloproteinases as noninvasive biomarkers: case report. Neurosurgery. 2007;60(6):E1148–9. discussion E9
146. Pricola Fehnel K, Duggins-Warf M, Zurakowski D, McKee-Proctor M, Majumder R, Raber M, et al. Using urinary bFGF and TIMP3 levels to predict the presence of juvenile pilocytic astrocytoma and establish a distinct biomarker signature. J Neurosurg Pediatr. 2016;18(4):396–407.
147. Sesen J, Driscoll J, Shah N, Moses-Gardner A, Luiselli G, Alexandrescu S, et al. Neogenin is highly expressed in diffuse intrinsic pontine glioma and influences tumor invasion. Brain Res. 2021;1762:147348.
148. Baxter PA, Su JM, Onar-Thomas A, Billups CA, Li XN, Poussaint TY, et al. A phase I/II study of veliparib (ABT-888) with radiation and temozolomide in newly diagnosed diffuse pontine glioma: a pediatric brain tumor consortium study. Neuro-Oncology. 2020;22(6):875–85.
149. Combaret V, Audoynaud C, Iacono I, Favrot MC, Schell M, Bergeron C, et al. Circulating MYCN DNA as a tumor-specific marker in neuroblastoma patients. Cancer Res. 2002;62(13):3646–8.
150. Diviney A, Chobrutskiy BI, Zaman S, Blanck G. An age-based, RNA expression paradigm for survival biomarker identification for pediatric neuroblastoma and acute lymphoblastic leukemia. Cancer Cell Int. 2019;19:73.
151. Wang X, Wang L, Su Y, Yue Z, Xing T, Zhao W, et al. Plasma cell-free DNA quantification is highly correlated to tumor burden in children with neuroblastoma. Cancer Med. 2018.
152. Chicard M, Boyault S, Colmet Daage L, Richer W, Gentien D, Pierron G, et al. Genomic copy number profiling using circulating free tumor DNA highlights heterogeneity in neuroblastoma. Clin Cancer Res. 2016;22(22):5564–73.
153. Viprey VF, Gregory WM, Corrias MV, Tchirkov A, Swerts K, Vicha A, et al. Neuroblastoma mRNAs predict outcome in children with stage 4 neuroblastoma: a European HR-NBL1/SIOPEN study. J Clin Oncol. 2014;32(10):1074–83.
154. Blavier L, Yang RM, DeClerck YA. The tumor microenvironment in neuroblastoma: new players, new mechanisms of interaction and new perspectives. Cancers (Basel). 2020;12(10).
155. Morini M, Cangelosi D, Segalerba D, Marimpietri D, Raggi F, Castellano A, et al. Exosomal microRNAs from longitudinal liquid biopsies for the prediction of response to induction chemotherapy in high-risk neuroblastoma patients: a proof of concept SIOPEN study. Cancers (Basel). 2019;11(10).
156. Trigg RM, Shaw JA, Turner SD. Opportunities and challenges of circulating biomarkers in neuroblastoma. Open Biol. 2019;9(5):190056.
157. Morgenstern DA, Potschger U, Moreno L, Papadakis V, Owens C, Ash S, et al. Risk stratification of high-risk metastatic neuroblastoma: a report from the HR-NBL-1/SIOPEN study. Pediatr Blood Cancer. 2018;65(11):e27363.
158. Zeltzer PM, Marangos PJ, Evans AE, Schneider SL. Serum neuron-specific enolase in children with neuroblastoma. Relationship to stage and disease course. Cancer. 1986;57(6):1230–4.
159. Pastor ER, Mousa SA. Current management of neuroblastoma and future direction. Crit Rev Oncol Hematol. 2019;138:38–43.
160. Segers H, van den Heuvel-Eibrink MM, Williams RD, van Tinteren H, Vujanic G, Pieters R, et al. Gain of 1q is a marker of poor prognosis in Wilms' tumors. Genes Chromosomes Cancer. 2013;52(11):1065–74.
161. Chagtai T, Zill C, Dainese L, Wegert J, Savola S, Popov S, et al. Gain of 1q as a prognostic biomarker in Wilms tumors (WTs) treated with preoperative chemotherapy in the International Society of Paediatric Oncology (SIOP) WT 2001 trial: a SIOP renal Tumours biology consortium study. J Clin Oncol. 2016;34(26):3195–203.
162. Gratias EJ, Jennings LJ, Anderson JR, Dome JS, Grundy P, Perlman EJ. Gain of 1q is associated with inferior event-free and overall survival in patients with favorable histology Wilms tumor: a report from the children's oncology group. Cancer. 2013;119(21):3887–94.
163. Perlman EJ, Grundy PE, Anderson JR, Jennings LJ, Green DM, Dome JS, et al. WT1 mutation and 11P15 loss of heterozygosity predict relapse in very low-risk wilms tumors treated with surgery alone: a children's oncology group study. J Clin Oncol. 2011;29(6):698–703.
164. Zhang LJ, Liu W, Gao YM, Qin YJ, Wu RD. The expression of IL-6 and STAT3 might predict progression and unfavorable prognosis in Wilms' tumor. Biochem Biophys Res Commun. 2013;435(3):408–13.
165. Ortiz MV, Ahmed S, Burns M, Henssen AG, Hollmann TJ, MacArthur I, et al. Prohibitin is a prognostic marker and therapeutic target to block chemotherapy resistance in Wilms' tumor. JCI Insight 2019;4(15).
166. Sharma R. Global, regional, national burden of breast cancer in 185 countries: evidence from GLOBOCAN 2018. Breast Cancer Res Treat. 2021;187(2):557–67.
167. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin. 2019;69(5):363–85.
168. Roganovic D, Djilas D, Vujnovic S, Pavic D, Stojanov D. Breast MRI, digital mammography and breast tomosynthesis: comparison of three methods for early detection of breast cancer. Bosn J Basic Med Sci. 2015;15(4):64.
169. Nassar FJ, Nasr R, Talhouk R. MicroRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. Pharmacol Ther. 2017;172:34–49.
170. Ozawa PMM, Jucoski TS, Vieira E, Carvalho TM, Malheiros D, Ribeiro EMdSF. Liquid biopsy for breast cancer using extracellular vesicles and cellfree microRNAs as biomarkers. Transl Res. 2020;223:40–60.
171. Morad G, Moses MA. Brainwashed by extracellular vesicles: the role of extracellular vesicles in primary and metastatic brain tumour microenvironment. J Extracell Vesic. 2019;8(1):1627164.
172. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. 2006;25(1):9–34.
173. MacDougall JR, Matrisian LM. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. Cancer Metastasis Rev. 1995;14(4):351–62.

174. Roy R, Zhang B, Moses MA. Making the cut: protease-mediated regulation of angiogenesis. Exp Cell Res. 2006;312(5):608–22.
175. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.
176. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553(7689):446–54.
177. Altorki NK, Markowitz GJ, Gao D, Port JL, Saxena A, Stiles B, et al. The lung microenvironment: an important regulator of tumour growth and metastasis. Nat Rev Cancer. 2019;19(1):9–31.
178. Molina R, Augé JM, Bosch X, Escudero JM, Viñolas N, Marrades R, et al. Usefulness of serum tumor markers, including progastrin-releasing peptide, in patients with lung cancer: correlation with histology. Tumor Biol. 2009;30(3):121–9.
179. Bodor JN, Boumber Y, Borghaei H. Biomarkers for immune checkpoint inhibition in non-small cell lung cancer (NSCLC). Cancer. 2020;126(2):260–70.
180. Bianco A, Perrotta F, Barra G, Malapelle U, Rocco D, De Palma R. Prognostic factors and biomarkers of responses to immune checkpoint inhibitors in lung cancer. Int J Mol Sci. 2019;20(19):4931.
181. Li B, Cui Y, Diehn M, Li R. Development and validation of an individualized immune prognostic signature in early-stage nonsquamous non-small cell lung cancer. JAMA Oncol. 2017;3(11):1529–37.
182. Öjlert ÅK, Halvorsen AR, Nebdal D, Lund-Iversen M, Solberg S, Brustugun OT, et al. The immune microenvironment in non-small cell lung cancer is predictive of prognosis after surgery. Mol Oncol. 2019;13(5):1166–79.
183. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
184. Awata T, Yamada S, Tsushima K, Sakashita H, Yamaba S, Kajikawa T, et al. PLAP-1/Asporin positively regulates FGF-2 activity. J Dent Res. 2015;94(10):1417–24.
185. Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, et al. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nat Genet. 2005;37(2):138–44.
186. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet. 2004;363(9414):1049–57.
187. Borazanci E, Dang CV, Robey RW, Bates SE, Chabot JA, Von Hoff DD. Pancreatic cancer: "a riddle wrapped in a mystery inside an enigma". Clin Cancer Res. 2017;23(7):1629–37.
188. Steinberg W. The clinical utility of the CA 19-9 tumor-associated antigen. Am J Gastroenterol. 1990;85(4):350–5.
189. Goggins M. Molecular markers of early pancreatic cancer. J Clin Oncol. 2005;23(20):4524–31.
190. Yeo TP, Hruban RH, Leach SD, Wilentz RE, Sohn TA, Kern SE, et al. Pancreatic cancer. Curr Probl Cancer. 2002;26(4):176–275.
191. Ozkan H, Kaya M, Cengiz A. Comparison of tumor marker CA 242 with CA 19-9 and carcinoembryonic antigen (CEA) in pancreatic cancer. Hepato-Gastroenterology. 2003;50(53):1669–74.
192. Goggins M. Identifying molecular markers for the early detection of pancreatic neoplasia. Semin Oncol. 2007;34(4):303–10.
193. Sandblom G, Granroth S, Rasmussen IC. TPS, CA 19-9, VEGF-A, and CEA as diagnostic and prognostic factors in patients with mass lesions in the pancreatic head. Ups J Med Sci. 2008;113(1):57–64.
194. Pistol-Tanase C, Raducan E, Dima SO, Albulescu L, Alina I, Marius P, et al. Assessment of soluble angiogenic markers in pancreatic cancer. 2008;2(5):447–55.
195. Matsuno S, Egawa S, Fukuyama S, Motoi F, Sunamura M, Isaji S, et al. Pancreatic cancer registry in Japan: 20 years of experience. Pancreas. 2004;28(3):219–30.
196. Mellby LD, Nyberg AP, Johansen JS, Wingren C, Nordestgaard BG, Bojesen SE, et al. Serum biomarker signature-based liquid biopsy for diagnosis of early-stage pancreatic cancer. J Clin Oncol. 2018;36(28):2887.
197. Jin H, Wu Y, Tan X. The role of pancreatic cancer-derived exosomes in cancer progress and their potential application as biomarkers. Clin Transl Oncol. 2017;19(8):921–30.
198. Madhavan B, Yue S, Galli U, Rana S, Gross W, Müller M, et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer. 2015;136(11):2616–27.
199. Debernardi S, O'Brien H, Algahmdi AS, Malats N, Stewart GD, Plješa-Ercegovac M, et al. A combination of urinary biomarker panel and PancRISK score for earlier detection of pancreatic cancer: a case–control study. PLoS Med. 2020;17(12):e1003489.
200. Blyuss O, Zaikin A, Cherepanova V, Munblit D, Kiseleva EM, Prytomanova OM, et al. Development of PancRISK, a urine biomarker-based risk score for stratified screening of pancreatic cancer patients. Br J Cancer. 2020;122(5):692–6.
201. Liu Y, Yasukawa M, Chen K, Hu L, Broaddus RR, Ding L, et al. Association of somatic mutations of ADAMTS genes with chemotherapy sensitivity and survival in high-grade serous ovarian carcinoma. JAMA Oncol. 2015;1(4):486–94.
202. Chen H, Xu C, Jin Q, Liu Z. S100 protein family in human cancer. Am J Cancer Res. 2014;4(2):89–115.
203. Dimitroulis D, Damaskos C, Valsami S, Davakis S, Garmpis N, Spartalis E, et al. From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J Gastroenterol. 2017;23(29):5282–94.
204. Choi DT, Kum HC, Park S, Ohsfeldt RL, Shen Y, Parikh ND, et al. Hepatocellular carcinoma screening is associated with increased survival of patients with cirrhosis. Clin Gastroenterol Hepatol. 2019;17(5):976–87 e4.
205. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
206. Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumour Biol. 2017;39(7):1010428317714626.
207. Jiang Y, Xie J, Huang W, Chen H, Xi S, Han Z, et al. Tumor immune microenvironment and chemosensitivity signature for predicting response to chemotherapy in gastric cancer. Cancer Immunol Res. 2019;7(12):2065–73.
208. Noh SH, Park SR, Yang HK, Chung HC, Chung IJ, Kim SW, et al. Adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): 5-year follow-up of an open-label, randomised phase 3 trial. Lancet Oncol. 2014;15(12):1389–96.
209. Duda DG. Molecular biomarkers of response to antiangiogenic therapy for cancer. ISRN Cell Biol 2012;2012.
210. Perkins A, Liu G. Primary brain tumors in adults: diagnosis and treatment. Am Fam Physician. 2016;93(3):211–7.
211. Mesfin FB, Al-Dhahir MA. Gliomas. Treasure Island (FL): StatPearls; 2021.
212. Caren H, Pollard SM, Beck S. The good, the bad and the ugly: epigenetic mechanisms in glioblastoma. Mol Asp Med. 2013;34(4):849–62.
213. Stadlbauer A, Roessler K, Zimmermann M, Buchfelder M, Kleindienst A, Doerfler A, et al. Predicting glioblastoma response to bevacizumab through MRI biomarkers of the tumor microenvironment. Mol Imaging Biol. 2019;21(4):747–57.
214. Subramanian S, Ahmad T. Childhood brain tumors. Treasure Island (FL): StatPearls; 2021.

215. Smith ER, Zurakowski D, Saad A, Scott RM, Moses MA. Urinary biomarkers predict brain tumor presence and response to therapy. Clin Cancer Res. 2008;14(8):2378–86.
216. Le Rhun E, Seoane J, Salzet M, Soffietti R, Weller M. Liquid biopsies for diagnosing and monitoring primary tumors of the central nervous system. Cancer Lett. 2020;480:24–8.
217. Leslie SW, Sajjad H, Murphy PB. Wilms tumor. Treasure Island (FL): StatPearls; 2021.
218. Cone EB, Dalton SS, Van Noord M, Tracy ET, Rice HE, Routh JC. Biomarkers for Wilms tumor: a systematic review. J Urol. 2016;196(5):1530–5.