



Emerging biomarkers in pulmonary metastasectomy

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Abstract: Pulmonary metastasectomy (PM) with curative intent is a routinely performed surgical procedure in a variety of end-stage malignant diseases with secondary spreading to the lungs. Current selection criteria for PM are solely based on few clinical factors without regarding the biology and behavior of the underlying malignant disease. Hence, great variation in outcome and recurrence patterns after PM is reported in the literature. In a variety of therapeutic options, prognostic and predictive biomarkers have become an integral part in planning targeted therapies. However, data on biomarkers in PM is sparse. This review summarizes recently reported tissue-based and circulating molecular markers in patients receiving PM.

Keywords: Pulmonary metastasectomy (PM); biomarkers; prognosis; selection criteria

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Introduction

Current management of patients with pulmonary metastases comprises various therapeutic approaches including chemotherapy, targeted therapy, external radiotherapy, radiofrequency ablation and pulmonary metastasectomy (PM) (1). Beside recent advances among all of these options, PM still seems to provide the best achievable long-term outcome for patients with lung metastasis (2-4). Currently established selection criteria for PM include (I) control of the primary tumor, (II) absence of extrathoracic metastasis, (III) resectability of all metastatic lesions with adequate postoperative pulmonary reserve and (IV) absence of alternative medical treatment options with lower morbidity (5). In carefully selected patients, 5-year survival rates up to 70% have been reported and clinical outcome after PM has been constantly improving over the last decades (6). However, there is a great variation in long-term prognosis with regard to recurrence and survival rates after PM (7,8). This variation might also be due to the limitations of currently used selection criteria, which are mainly based on

clinical factors without taking the biology of the primary tumor and the metastatic spread into account. Therefore, identifying patients who will benefit most from a surgical procedure in malignant disease with systemic spread remains a major obstacle.

Particularly in times of personalized medicine, knowledge on the tumor biology is essential for offering effective and evidence-based therapies. In many neoplastic diseases, prognostic and predictive biomarkers are becoming an integral part of guidelines and play an essential role in selecting candidates for individualized treatment algorithms (9). In context with PM, numerous biomarkers with remarkable prognostic and predictive potential have been reported over the last decades. However, the majority of these biomarkers have been identified in small series, from different centers and from different primary entities. Surveys amongst physicians on the current practice showed that the clinical application of these biomarkers in the setting of PM is still limited (10,11).

In 2013, our group summarized the evidence for molecular markers in patients with pulmonary metastasis (12). This review is an update to our previous review and summarizes

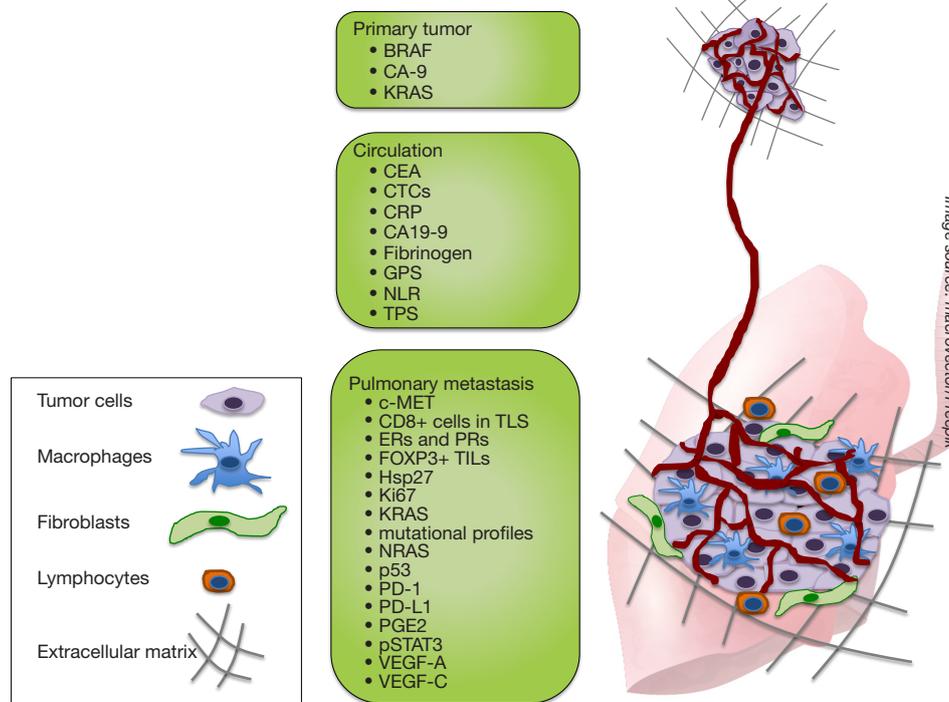


Figure 1 Summary of recently assessed biomarkers in pulmonary metastasectomy.

Table 1 Potential biomarkers detected in corresponding primary tumors

Biomarker	Cut-off	Impact	Sample size	Primary entity	Reference
BRAF	V600 mutation vs. wild-type	OS ↓	180	CRC	Renaud <i>et al.</i> (13)
CA-9	High vs. low expression	Recurrence ↓	44	CRC	Schweiger <i>et al.</i> (14)
KRAS	KRAS mutation vs. wild type	Recurrence ↓	494	CRC	Pereira <i>et al.</i> (15)
KRAS	KRAS mutation vs. wild type	OS ↓	75	CRC	Ghidini <i>et al.</i> (16)

↓, decreased. BRAF, v-raf murine sarcoma viral oncogene homolog B1; CA-9, Carbonic anhydrase IX; KRAS, Kirsten rat sarcoma viral oncogene homologue; OS, overall survival.

recently available literature on biomarkers in the context of PM since 2013. It provides comprehensive up-to-date summary for medical professionals treating patients with lung metastasis as well as for researchers in the field of translational oncology. Biomarkers were assigned to three different groups, depending on the origin of the sample: (I) primary tumor; (II) circulating markers and (III) pulmonary metastasis (Figure 1).

Biomarkers detected in corresponding primary tumors

Primary tumor biomarkers can be detected within resected tissue specimen of different primary entities (Table 1). They

may be especially useful when selecting between good and poor candidates for PM. In general, the concordance between histological markers in the primary tumors compared to pulmonary metastases is poor. This might be attributed to the evolution of tumor cells during the metastatic process (17).

BRAF

The v-raf murine sarcoma viral oncogene homolog B1 (BRAF) is a well-known human proto-oncogene on chromosome 7, encoding for the serin/threonine kinase called B-Raf. B-Raf plays a key-role in the mitogen-activated protein kinase (MAPK) signaling pathway,

regulating normal cellular growth and cell-division (18). Mutations of the B-raf cascade are commonly found in different human cancer types and already target of novel inhibitory drugs (19,20). Renaud *et al.* demonstrated the prognostic value of BRAF in patients with PM from metastatic colorectal cancer (mCRC) (13). In a cohort of 180 patients and subsequent PM, 19 patients had mutated BRAF (V600) in primary CRC specimen and showed significantly worse OS when compared to those patients with wild-type BRAF (5-year survival 0% *vs.* 100%).

Carbonic anhydrase IX (CA-9)

Hypoxia increases the expression of *CA-9*, an enzyme that catalyzes key-steps in metabolic processes and particularly in pH regulation. Overexpression of *CA-9* was reported to be associated with poor outcome in different malignancies (21-24). It was demonstrated that patients with a high expression of *CA-9* in resected primary CRC specimen had significantly earlier (23 *vs.* 37 months) spreading to the lung (14).

KRAS

As a part of the Ras family, the proto-oncogene KRAS encodes for a protein called K-ras that functions as an essential component in cell proliferation signaling. Mutations of KRAS can lead to constitutive overactivation of proliferation pathways, promoting tumorigenesis and metastasis (25-27). In a retrospectively analyzed cohort of 494 patients with mCRC and lung metastasis, 202 patients (41%) had KRAS mutations within the primary tumor tissue (15). Pereira *et al.* found that in case of a KRAS mutation the time to pulmonary metastasis was significantly decreased (15.2 *vs.* 22.4 months). Ghidini *et al.* further supplemented these findings by showing that patients with KRAS mutation in primary CRC specimen have significantly shorter (36.6 *vs.* 60.9 months) overall survival (OS) when undergoing PM (16). Renaud *et al.* supported these findings by demonstrating poor OS (5-year OS 44% *vs.* 100%) after PM in case of KRAS mutation in corresponding CRC specimen (13).

Circulating biomarkers

Circulating biomarkers are of increasing interest as they can be easily obtained from peripheral blood samples (Table 2). Circulating tumor markers e.g., CEA and CA 19-9 are already well established and have a number of clinical applications.

In patients with lung metastasis, they might play a role for predictive purposes when selecting patients for surgery as well as for analysis of recurrence patterns after PM.

CEA

The carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion during fetal development and therefore hardly measurable in blood levels of healthy adults. However, increased serum levels of CEA have been reported in several cancer types and were associated with poor prognosis (46-48). In case of CRC pulmonary metastasis, a number of reports with small sample sizes showed that increased (>5 and >6 ng/mL) pre-thoracotomy levels of CEA correlate with worsened OS and early recurrence after PM (28,31,32,34,35). These findings were confirmed in a large systematic review and meta-analysis including 2,925 patients by Gonzalez *et al.*, who found that an elevated (>5 ng/mL) pre-thoracotomy level of CEA represents a two-times higher risk of death (33). Furthermore, Sun *et al.* and Embun *et al.* pointed out that the prognostic value of CEA is even stronger when combined together with other discussed clinicopathological factors of poor survival (e.g., thoracic lymph node involvement, number of lung metastases, shorter disease-free survival after PM) (29,30). Interestingly, Osoegawa *et al.* highlighted that patients with elevated preoperative (>5 ng/mL) but normalized postoperative CEA levels have a better prognosis, compared to those patients whose CEA level remains high or even increase after PM (median survival time of 41.8 months compared with 28.1 or 15.7 months, respectively) (36).

Circulating tumor cells (CTCs)

CTCs are detectable in the blood stream of patients with malignant disease, especially in metastatic disease (49). They are extraordinary rare (on average one CTC per billion normal blood cells) and therefore hard to isolate, but seem to be a high-potential independent clinical prognostic marker in different cancers (50). Recently, two studies evaluating the role of CTCs in context with PM have been published. Hashimoto *et al.* showed, that 13 out of 79 enrolled mCRC patients had CTCs within preoperatively drawn peripheral blood samples (37). Patients with multiple CTCs (CTC count $\geq 2/7.5$ mL blood) had a significant shorter disease-free survival (8.6 *vs.* 19.8 months) and OS (median OS not reached *vs.* 37.8 months). Le *et al.* analyzed blood samples drawn

Table 2 Potential circulating biomarkers

Biomarker	Cut-off	Impact	Sample size	Primary entity	Reference
CEA	>6 ng/mL	DFS ↓	84	CRC	Cho <i>et al.</i> (28)
CEA	>5 ng/mL	OS ↓	522	CRC	Embun <i>et al.</i> (29)
CEA	>5 ng/mL	OS ↓	154	CRC	Sun <i>et al.</i> (30)
CEA	>5 ng/mL	OS ↓	33	CRC	Hachimaru <i>et al.</i> (31)
CEA	>5 ng/mL	OS ↓	93	CRC	Suzuki <i>et al.</i> (32)
CEA	>5 ng/mL	OS ↓	2,925	CRC	Gonzalez <i>et al.</i> (33)
CEA	>5 ng/mL	DFS, OS ↓	59	CRC	Yokoyama <i>et al.</i> (34)
CEA	>5 ng/mL	DFS, OS ↓	785	CRC	Okumura <i>et al.</i> (35)
CEA	>5 ng/mL (pre- and postoperative)	OS ↓	87	CRC	Osoegawa <i>et al.</i> (36)
CTCs (peripheral)	≥2 cells/7.5 mL blood	DFS, OS ↓	79	CRC	Hashimoto <i>et al.</i> (37)
CTCs (pulmonary vein)	Presence vs. absence	Thoracic lymph node involvement	24	CRC	Le <i>et al.</i> (38)
CRP	≥0.5 mg/dl	DFS ↓	52	CRC	Ghanim <i>et al.</i> (39)
CRP	>10 mg/L	OS ↓	88	CRC	Li <i>et al.</i> (40)
CRP	≥0.5 mg/dL	OS ↓	23	Urinary bladder/upper urinary tract cancer	Nakawaga <i>et al.</i> (41)
CRP	>2 (preoperative) and >84 mg/L (postoperative)	OS ↓	846	Different epithelial tumors, sarcomas and melanomas	Pastorino <i>et al.</i> (42)
CA19-9	>28 IU/mL	OS ↓	75	CRC	Vodicka <i>et al.</i> (43)
Fibrinogen	>325 mg/dL	Recurrence, OS ↓	51	CRC	Ghanim <i>et al.</i> (39)
GPS	1/2 vs. 0	OS ↓	51	CRC	Ghanim <i>et al.</i> (39)
GPS	1/2 vs. 0	OS ↓	132	CRC	Kobayashi <i>et al.</i> (44)
NLR	>4,05	Recurrence, OS ↓	574	CRC	Renaud <i>et al.</i> (45)
NLR	>4	Recurrence, OS ↓	47	CRC	Ghanim <i>et al.</i> (39)
TPS	>140 IU/L	Recurrence, OS ↓	75	CRC	Vodicka <i>et al.</i> (43)

↓, decreased. CEA, carcinoembryonic antigen; CTCs, circulating tumor cells; CRP, C-reactive protein; CA19-9, carbohydrate antigen 19-9; GPS, Glasgow Prognostic Score; NLR, neutrophil-to-lymphocyte ratio; TPS, tissue polypeptide specific antigen; DFS, disease-free-survival; OS, overall survival.

intraoperatively from the pulmonary vein in 24 patients undergoing PM with curative intent (38). They could isolate more CTCs in pulmonary venous blood samples than in peripherally taken blood samples at the same time. However, pulmonary venous CTC count did not correlate with clinical outcome results, but with the presence of positive thoracic lymph nodes.

C-reactive protein (CRP)

CRP is an established and widely used acute phase protein

in current clinical routine. Elevated preoperative CRP serum levels were reported to predict poor outcome in different thoracic malignancies (51,52). In small sample sized cohorts including mCRC and urothelial carcinoma patients undergoing PM with curative intent, those patients with high preoperative CRP levels (≥0.5 or ≥1 mg/dL) suffered from significantly earlier tumor recurrence and worse OS (39-41). These findings were complemented in recent work including 846 consecutive patients with resectable lung metastasis from different primary malignancies. Pastorino *et al.* found out that elevated

preoperative levels and postoperative levels of CRP together predict poor survival after PM, compared to low pre- and postoperative CRP levels (5-year OS 43% *vs.* 57%) (42).

Fibrinogen

Similarly to CRP, fibrinogen is another acute phase protein that had been recently investigated for its prognostic impact in context with PM. 78.4% of the assessed mCRC patients had elevated serum levels (cut-off 325 mg/dL) of fibrinogen one day before PM (39). High levels of fibrinogen predicted early tumor recurrence and were significantly associated with decreased OS (HR 10.745, 95% CI: 1.737–66.475).

Glasgow Prognostic Score (GPS)

The GPS is a systemic inflammation-based score (composed of serum elevation of CRP and decrease in albumin concentration) that has been reported to be significant as a prognostic indicator in various types of malignancies (53). Two recently published studies investigated the impact of GPS in context with PM (39,44). Kobayashi *et al.* reported that patients with elevated GPS scores had a significantly worse 5-year post-metastasectomy survival (13% *vs.* 46%) (44). Ghanim *et al.* confirmed this observation and found elevated GPS score to be an independent indicator of poor OS (39).

Neutrophil-to-lymphocyte ratio (NLR)

There is increasing evidence that neutrophils play a determining role in cancer progression and metastasis (54). Furthermore, they are able to suppress the anti-tumor effects of human lymphocytes (55). Consequently, an elevated serum NLR has been shown to associate with an adverse OS in numerous malignant diseases (56). In a recently published work by Renaud *et al.*, 5-year survival of the 574 included patients undergoing PM with high NLR prior to surgery was 25%, compared to 83% in case of a low preoperative NLR (45). Furthermore, a high NLR was associated with a significantly decreased 5-year pulmonary-recurrence free survival (6% *vs.* 49%). Supporting these findings, Ghanim *et al.* presented poor OS and early recurrence in case of elevated preoperative NLR in a small cohort of 47 mCRC patients (39).

Carbohydrate antigen 19-9 (CA 19-9)

CA 19-9 is a glycoprotein currently used as a tumor-

associated prognostic marker in the management of different gastrointestinal malignancies, especially of pancreatic cancer (57). In context with PM, Vodicka *et al.* found that CRC patients with pre-operatively elevated serum levels of CA 19-9 (cut-off >28 IU/mL) had a significantly higher risk of death (95% hazard ratio: 3.2, 95% confidence interval: 1.2–8.4) after surgical resection of pulmonary nodules (43).

Tissue polypeptide specific antigen

In contrast to other established biomarkers correlating with tumor burden, the tissue polypeptide specific antigen (TPS) particularly reflects the activity and proliferation-rate of cancer cells. Interestingly, a recently published work by Vodicka *et al.* delivered first results on the prognostic value of TPS in patients undergoing PM (43). High preoperative values of TPS (cut-off 140 IU/L) were associated with poor OS and early recurrence after PM.

Biomarkers detected in pulmonary metastasis

Biomarkers that are available within pulmonary metastasis specimen can be identified either after PM with curative intent or by a biopsy with diagnostic intent. Interestingly, they represent the largest group of biomarkers analyzed in context with PM within the last years (*Table 3*). In context to other non-surgical treatment modalities (e.g., radiofrequency-ablation or radiation) for pulmonary metastasis, it underlines the important role of surgery in providing adequate histological material for precise analysis and individualized patient management. Beyond the removal of all pulmonary nodules by means of a metastasectomy with curative intent, diagnostic metastasectomies are frequently performed to aid further treatment planning.

c-MET and STAT3

The transmembrane tyrosine-protein kinase receptor Met (*c-MET*) and its downstream signaling mediator called signal transducer and activator of transcription 3 (*STAT3*) have been shown to play a key-role in cancer (71). In a cohort of 51 analyzed mCRC patients, immunohistochemical overexpression of *c-MET* and *STAT3* was associated with decreased median OS compared to patients with low-expression (30 *vs.* 65 months, 24 *vs.* 65 months, respectively) (58). Furthermore, overexpression

Table 3 Potential biomarkers detected in pulmonary metastasis

Biomarker	Cut-off	Impact	Sample size	Primary entity	Authors
c-MET	IHC Score >100	OS ↓	51	CRC	Schweiger <i>et al.</i> (58)
CD8+ cells in TLS	Low-expression	OS ↓	57	CRC	Schweiger <i>et al.</i> (59)
ERs and PRs	Positive expression	OS ↑	81	Breast cancer	Meimarakis <i>et al.</i> (60)
FOXP3+ TILs	Over-expression	OS ↑	57	CRC	Schweiger <i>et al.</i> (59)
Hsp27 in stroma	Over-expression	Recurrence, OS ↓	51	CRC	Schweiger <i>et al.</i> (61)
Ki67	Staining area of ≥ 0.33	OS ↓	29	Osteosarcoma	Matsumoto <i>et al.</i> (62)
KRAS	Mutation vs. wild type	Recurrence, OS ↓	44	CRC	Schweiger <i>et al.</i> (63)
KRAS	exon 2 codon 12 vs. 13	Recurrence, OS ↓	150	CRC	Renaud <i>et al.</i> (64)
EGFR, GNAQ, KIT, MET and PTPN11*	Mutation vs. wildtype	Recurrence ↓	47	CRC	Schweiger <i>et al.</i> (65)
PDGFRA, SMARCB1, and TP53*	Mutation vs. wildtype	OS ↓	47	CRC	Schweiger <i>et al.</i> (65)
NRAS	Mutation vs. wild type	Recurrence, OS ↓	25	Melanoma	Ulivieri <i>et al.</i> (66)
p53	Over-expression	OS ↓	88	CRC	Li <i>et al.</i> (67)
PD-1	Over-expression	OS ↓	53	CRC	Kollmann <i>et al.</i> (68)
PD-L1	Over-expression	OS ↓	26	Head and neck squamous cell cancer	Okada <i>et al.</i> (69)
PGE2	Over-expression	Recurrence ↓	53	CRC	Lang <i>et al.</i> (70)
pSTAT3	Evidence of nuclear staining in >15% of cancer cells	Recurrence, OS ↓	51	CRC	Schweiger <i>et al.</i> (58)
VEGF-A	Staining area of ≥ 0.56	OS ↓	29	Osteosarcoma	Matsumoto <i>et al.</i> (62)
VEGF-C	Positive vs. negative staining	OS ↓	29	Osteosarcoma	Matsumoto <i>et al.</i> (62)

↓, decreased; ↑, increased. c-MET, tyrosine-protein kinase Met; CD8+ cells in TLS, CD8+ cytotoxic T-lymphocytes in tertiary-lymphoid structures; ERs, estrogen receptors; PRs, progesterone receptors; FOXP3+ TILs, FOXP3+ regulatory tumor infiltrating lymphocytes; Hsp27, Heat shock protein 27; KRAS, Kirsten rat sarcoma viral oncogene homologue; EGFR, epidermal growth factor; GNAQ, gene coding for the guanine nucleotide-binding protein Gq subunit alpha; KIT, gene coding for the tyrosine kinase c-KIT; MET, gene coding for c-MET; PTPN11, gene coding for the tyrosine-protein phosphatase non-receptor type 11; PDGFRA, platelet-derived growth factor receptor A; SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1; NRAS, neuroblastoma RAS viral oncogene homolog; TP53, tumor protein p53; PD-1, programmed cell death protein-1; PD-L1, programmed cell death 1 ligand-1; PGE2, prostaglandin E2; pSTAT3, signal transducer and activator of transcription 3; VEGF-A, vascular endothelial growth factor A; VEGF-C, vascular endothelial growth factor C. *, coincident mutation detected by targeted next-generation sequencing.

of *STAT3*—but not *c-MET*—was associated with early tumor recurrence after PM (10 vs. 19 months).

KRAS

The rate of KRAS mutations in patients with lung metastasis is 46%, which exceeds the rate of KRAS mutations in the overall cohort of patients with CRC. Furthermore, patients with KRAS mutation suffered from early recurrence of lung metastasis after PM (63). Renaud *et al.* evaluated and

compared the impact of different KRAS mutation subtypes on clinical outcome. Especially mutations in exon 2 and codon 13 resulted in early pulmonary recurrence (56 vs. 78 months) and poor OS (54 vs. 82 months) after PM (64). Furthermore, it could be shown that patients with KRAS exon 2 codon 12 mutations seem to be good candidates for perioperative bevacizumab treatment. In the presence of codon 12 mutation, OS (101 vs. 55 months) as well as time to recurrence (70 vs. 24 months) were significantly higher when bevacizumab was part of the treatment regimen (72).

Tumor infiltrating lymphocytes (TILs) and tertiary lymphoid structures (TLS)

Subsets of TILs and so-called TLS are frequently found in the microenvironment as well as the stroma of numerous human cancers. TILs and TLS reflect an active immune response of the host against the tumor and seem to affect clinical outcome and survival (73,74). In a cohort of 57 CRC patients undergoing resection of pulmonary nodules, the density and distribution of TILs and TLS was investigated (59). High presence of FOXP3+ regulatory T-lymphocytes—which are known to have immune modulatory function—at the invasive front of the tumor was associated with decreased OS after PM (35 *vs.* 65 months). Moreover, low numbers of CD8+ cytotoxic T-lymphocytes—which are known for their anti-tumor effect—in surrounding TLS correlated with impaired OS (median survival not reached *vs.* 35 months).

Programmed cell death protein-1 (PD-1) and programmed cell death 1 ligand-1 (PD-L1)

PD-1 and its ligand PD-L1 are profoundly studied immune checkpoints and play a crucial role in suppressing endogenous activity of lymphocytes. Tumors try to escape immune surveillance by up-regulation and overexpression PD-1 and PD-L1. This mechanism is currently used as a target for specific antibodies blocking this pathway and thus supporting the anti-tumor effects of endogenous lymphocytes. Recently, it could be shown that PD-1 and PD-L1 are highly present in TILs of mCRC pulmonary metastasis specimen (68). Furthermore, overexpression of PD-1 by TILs was associated with poor OS (35 *vs.* 78 months) after PM. Another study presented by Okada *et al.* investigated 26 patients undergoing PM from primary head and neck squamous cell carcinoma. The median 5-year OS for patients with high PD-L1 expression was 16.7%, compared to 72.5% in case of low PD-L1 expression (69). Moreover, high PD-L1 expression was an independent prognostic factor for OS in multivariate analysis.

TP53

One of the most commonly studied tumor-suppressor genes is the TP53, coding for the protein p53 that plays an essential role cell cycle regulation and apoptosis of damaged cells. However, studies investigating the impact of p53 expression on outcome after PM failed to find a correlation

(75,76). In a recent study by Li *et al.*, overexpression of p53 was associated with impaired OS (46.1 *vs.* 62.6 months) however, level of significance was lost in multivariate analysis (67).

Comprehensive mutational profiles

Targeted next generation sequencing (tNGS) has become a routinely used tool of predictive molecular pathology in various epithelial entities. In 2016, Kovaleva and colleagues reported on various gene mutations found by tNGS in formalin-fixed and paraffin embedded CRC and lung metastasis specimen (77). Moreover, our group retrospectively analyzed the mutational profiles of 47 CRC patients undergoing PM using tNGS in primary tumor and pulmonary metastasis specimens (65). Cox regression model identified simultaneous mutation of TP53, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (SMARCB1) and platelet-derived growth factor receptor A (PDGFRA) as being prognostic for poor OS after PM. Moreover, a coincident mutation of *EGFR* (gene coding for the epidermal growth factor), *GNAQ* (gene coding for the guanine nucleotide-binding protein Gq subunit alpha), *KIT* (a gene coding for the tyrosine kinase c-KIT), *MET* (a gene coding for the tyrosine kinase c-MET) and *PTPN11* (a gene coding for the tyrosine-protein phosphatase non-receptor type 11) predicted early tumor recurrence after PM.

Prostaglandin-E2 (PGE2)

PGE2 is an inflammatory mediator, which regulates physiological—e.g., vasodilatation or muscle relaxation—but also pathophysiological—e.g., inflammation—processes. An overexpression of PGE2 has been reported in various malignancies (19), whereas its prognostic value remains elusive. In a cohort of 53 CRC patients undergoing PM, PGE2 was evident in 100% of the investigated metastasis specimen (70). Moreover, overexpression of PGE2 correlated with a significantly favorable tumor recurrence after PM (17 *vs.* 13 months). However, in multivariate analysis PGE2 could not be confirmed as a prognostic factor.

Estrogen receptors (ERs) and progesterone receptors (PRs)

The presence and therapeutic implication of steroid hormone

receptors in human breast cancer had been recognized over 40 years ago (78). It has since become clear that the course of human breast cancer is dependent upon estrogen and/or progesterone triggered signals, mediated through ERs and PRs. Meimarakis and colleagues investigated 81 patients could show that the presence of ERs and/or PRs in pulmonary metastasis tissue from breast cancer significantly correlated with improved OS (127 *vs.* 27 months) after PM (60).

NRAS

The neuroblastoma RAS viral oncogene homolog (NRAS) is closely related to KRAS. NRAS encodes for a protein called N-ras that also plays an important role in regulating cell division. Mutations of NRAS have been detected in many types of human cancer (79). Olivieri *et al.* could show that NRAS mutations are evident in a subset of resected lung metastases and are predictive for poor clinical outcome (66). Patients with mutated NRAS had early relapse of lung metastasis (30 *vs.* 53 months) and impaired OS (37 *vs.* 68 months) after PM from primary melanoma.

Heat-shock protein 27

Heat shock proteins represent a family of highly conserved proteins induced in response to various physiological and environmental insults, helping cells to survive. Among all heat shock proteins, Hsp27, Hsp7 and Hsp 90 are the most commonly studied subtypes. During physiological conditions—e.g., wound healing or keloid formation—activated fibroblasts express heat-shock protein 27 (Hsp27), which plays a crucial role for fibroblast motility and angiogenesis. However, expression of Hsp27 was reported to be high in cancer tissue and to strongly correlate with outcome (80,81). Addressing the prognostic value in PM, our group has previously investigated metastasis specimens of CRC patients and found high expression of Hsp27 in cancer-associated stroma of lung metastases (61). Moreover, extensive stromal Hsp27 expression was associated with decreased OS (31 *vs.* 52 months) and early tumor recurrence (10 *vs.* 19 months) after PM.

VEGF-A and VEGF-C

Subtypes of the vascular endothelial growth factor (VEGF) are well-known mediators in cancer-associated angiogenesis (82). However, one recent work investigated the prognostic value of two VEGF-subtypes on clinical

outcome in context with PM (62). Patients with a low VEGF-A expression (defined as positive staining area <56%) and a negative VEGF-C staining in metastatic lesions from primary osteosarcoma showed significantly better OS in multivariate analysis (hazard ratio: 0.162, 95% confidence interval: 0.045–0.576 and hazard ratio: 0.058, 95% confidence interval: 0.008–0.421, respectively).

Ki67

Ki-67 is an established marker for determining the proliferation activity of normal and cancer cells. It has been shown that expression of Ki-67 is significantly higher in more aggressive and poorly differentiated tumor types. Different expression levels of Ki-67 strongly correlate with outcome in various cancer types, and moreover, there is increasing evidence that it may be an effective target for future cancer therapies (83). In context of PM, Matsumoto *et al.* investigated 29 osteosarcoma patients undergoing resection of pulmonary metastasis and found significant association between Ki-67 expression and long-term survival (62). Particularly, patients with high expression (≥ 0.33 positive staining for Ki67) had a significantly poorer OS (<60 months), compared to patients without expression of Ki67 in their pulmonary specimen (163 months).

Conclusions

Despite encouraging long-term survival rates after PM, there is a wide-variation of outcomes in terms of recurrence and remission (84). It seems that selection criteria—based on clinical factors—fail to differentiate between patients who benefit from a surgical resection and patients with rapid progression of disease. Several prognostic biomarkers have been investigated over the last decades and current available evidence was summarized in this review. Knowledge on the impact of these factors might help for the decision-making progress when selecting candidates for PM and for the development of future treatment strategies.

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