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Original Contribution

Mesenchymal-epithelial transition factor (MET) immunoreactivity in positive sentinel nodes from patients with melanoma

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ABSTRACT

Objective: Patients with cutaneous melanoma and a positive sentinel node (SN) are currently eligible for adjuvant treatment with targeted therapy and immune checkpoint inhibitors. Near-infrared (NIR) fluorescence imaging could be an alternative and less invasive tool for SN biopsy to select patients for adjuvant treatment. One potential target for NIR is the mesenchymal-epithelial transition factor (MET). This study aimed to assess MET immunoreactivity in positive SNs and to evaluate its potential diagnostic, prognostic and therapeutic value.
Methods: In this retrospective study, positive SN samples from patients with primary cutaneous melanoma were collected to assess MET immunoreactivity. To this end, paraffin-embedded SNs were stained for MET (monoclonal antibody D1C2). A 4-point Histoscore was used to determine cytoplasmic and membranous immunoreactivity (0 negative/1 weak/2 moderate/3 strong). Samples were considered positive when ≥10% of the cancer cells showed MET expression (staining intensity ≥1). Patient and clinicopathological characteristics were used for descriptive statistics, binary logistic regression, and survival analyses.
Results: Positive MET immunohistochemistry was observed in 24 out of 37 samples (65%). No statistically significant associations were found between MET positivity and the following prognostic factors: Breslow thickness ($P = 0.961$), ulceration ($P = 1.000$), and SN tumor burden ($P = 0.792$). According to MET positivity, Kaplan-Meier curves showed no significant differences in survival.
Conclusion: This exploratory study found no evidence to support MET immunoreactivity in positive SNs as a possible diagnostic or prognostic indicator in patients with melanoma.

1. Introduction

Melanoma is the deadliest form of skin cancer, and its incidence is increasing [1,2]. However, the prognosis of patients with advanced melanoma has improved significantly over the past years since the introduction of systemic therapy with targeted therapies (i.e. BRAF/MEK inhibitors) and immune checkpoint inhibitors (ICIs, e.g. anti-PD1 and anti-CTLA-4) [3-7]. In patients with surgically resected stage III cutaneous melanoma, three pivotal phase III trials showed improved disease-free survival (DFS) for adjuvant treatment as compared to placebo [8-10]. As a result, patients with surgically resected stage III cutaneous melanoma have become eligible for adjuvant systemic therapy with BRAF/MEK inhibitors and anti-PD1.

To identify these patients for adjuvant treatment, sentinel node (SN) biopsy is currently essential for disease staging and to determine prognosis [11-13]. To visualize the SN, best practice dictates the pre-

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operative use of 99m-technetium for lymphoscintigraphy, followed by an injection of blue dye [14], leading to an SN identification rate of up to 99.6%.% [15]. However, this diagnostic tool causes radiation burden and blue discoloration of the skin, which can be long-lasting [16,17]. In addition, postoperative complications such as seroma, wound infection and lymphedema occur in approximately 1 out of 10 patients [16,18,19]. Moreover, the majority (70–85%) of SNs from patients with melanoma are histologically *negative* (i.e., without metastasis) [20-23]. Therefore, alternative diagnostic tools are required to identify SN metastasis non-invasively.

A promising non-invasive method to visualize the SN could be the near-infrared (NIR) technique [24,25], which can differentiate between malignant and benign proliferative tissue [26] and can image structures up to a few centimeters under the skin.[27] This method often employs fluorophores, such as specific tumor markers, as these can absorb specific NIR wavelengths [25,27,28]. A potential target for NIR is mesenchymal-epithelial transition factor (MET) [29], a proto-oncogene on the 7q31 locus encoding a transmembrane tyrosine kinase receptor [30]. In general, when bound and activated by hepatocyte growth factor (HGF), the HGF/MET signaling pathway plays a role in several normal cellular processes, such as cell proliferation, motility, and survival [31-34]. Abnormal activation of the HGF/MET signaling pathway is linked to malignant cell transformations, including the development and progression of melanoma [31,32,34-39]. Furthermore, it has been illustrated that high levels of MET expression in primary melanoma samples is associated with an overall poor clinical outcome [31,34,39-41].

To select patients with stage III melanoma for adjuvant systemic treatment non-invasively, the NIR technique targeting MET could be promising. To evaluate the feasibility of NIR targeting MET to detect SN metastases, the immunoreactivity of MET in SNs with melanoma metastases needed to be investigated. In this explorative study, we assessed MET expression in positive SNs from patients with melanoma and evaluated its prognostic and therapeutic value.

2. Materials and methods

2.1. Patient selection

This study was approved by the Ethics Committee. Human tissues and patient data were used according to "The Code for Proper Secondary Use of Human Tissue" and "The Code of Conduct for the Use of Data in Health Research" as stated by the Federation of Dutch Medical Scientific Societies [42,43]. Patients with cutaneous melanoma and a positive SN at the Erasmus MC Cancer Institute between 2000 and 2016 were randomly identified. Histopathological information of the SN tumor burden (i.e. diameter of metastasis and micro-anatomical localization in the SN) and of the primary melanoma (e.g. Breslow thickness, subtype, ulceration) was obtained from the pathology reports. Data on patient characteristics (e.g. age, gender) and follow-up (e.g. recurrence, survival) were retrieved from the medical records.

2.2. MET immunohistochemistry

The formalin-fixed paraffin-embedded (FFPE) SN samples from patients with primary cutaneous melanoma were collected at the Erasmus MC Cancer Institute pathology archives. The FFPE SN samples were deparaffinized and MET immunohistochemical analysis was done according to a standard protocol that was found to be reliable to determine MET immunohistochemistry. The protocol used the D1C2 antibody primarily directed against the C-terminus of MET (as well as the precursor and B-chain). Slides were incubated in the automated staining platform Benchmark Ultra (Ventana) Pre-treatment was performed with CC1 (EDTA pH 8.0) for 64 min at 100 °C. Then the primary antibody D1C2 (1:450; Cell Signaling Technology®; Leiden, the Netherlands), an antibody directed against the C-terminus of MET, was applied to the sample and incubated for 90 min at 37 °C. After the incubation, detection took place with the ultraView Universal DAB Detection Kit (Ventana Medical Systems, Inc.). Subsequently, the samples were contrasted with hematoxylin II for 20 min and a bluing reagent for 4 min. All controls gave satisfactory results. The immunohistochemical staining of MET in positive SN samples was examined simultaneously by two pathologists (T.K. and S.K.). The pathologists used an Olympus EX41 microscope to review the samples (×20 objective). Both pathologists had no access to information or knowledge of the patients' clinical outcomes before immunohistochemical evaluation for MET. Disagreements were resolved by discussion until consensus was reached.

2.3. Evaluation of MET immunohistochemical staining

In accordance with Cruz et al., samples with <10% of cancer cells showing immunoreactivity were regarded as (0) negative [40]. The samples were considered positive when at least 10% of the cancer cells showed immunoreactivity, categorized semi-quantitatively according to the following criteria: (1) weak; (2) moderate; (3) strong. The definitions of the cytoplasmic and membranous ordinal values and representative images of various staining intensities are illustrated in Fig. 2.

2.4. Statistical analysis

The differences between groups were calculated using $\chi 2$ tests, Fisher's exact tests or non-parametric Mann-Whitney *U* tests, as appropriate. Univariable binary logistic regression analyses were performed to determine associations with positive MET immunohistochemistry (i.e. staining intensity ≥1) and clinicopathological factors, including the following known prognostic factors: Breslow thickness, ulceration, and SN tumor burden [44,45]. The median follow-up length of the survivors was calculated from the date of SN biopsy until the last follow-up using the reverse Kaplan-Meier method. DFS was calculated from the date of SN biopsy to the date of first recurrence or death by any cause or the last follow-up. DMFS was calculated from the date of SN biopsy to the date of first distant recurrence or death by any cause or the last follow-up. OS was calculated from the date of SN biopsy to the date of death by any cause or the last follow-up. Follow-up was conducted according to standard Erasmus MC protocol which entails that in the first year, patients come for a routine follow-up every 3 months, in the second year every 6 months, and the third-fifth year once a year. The Kaplan–Meier method was used to estimate survival, and differences between groups were assessed using the log-rank test. All *P*-values were two-sided and $P < 0.05$ was considered statistically significant. SPSS (Statistical Package for Social Science) version 25.0 was used for all statistical analyses (IBM, Armonk, New York, USA).

3. Results

3.1. Patient selection

Forty-five patients with cutaneous melanoma and at least one positive SN were selected. MET immunohistochemistry was performed on one of the positive SNs of patients of whom tissue was available. This resulted in the inclusion of 37 patients. For the eight excluded patients, FFPE samples were unavailable or uninterpretable, either due to the absence of tumor tissue in the residual formalin-fixed paraffin-embedded (FFPE) or the presence of too much pigment. The median patient age was 58 years (interquartile range [IQR] 47–65), and most (62%) patients were females. The median SN tumor burden was 1.5 mm (IQR 0.8–3.9). In Table 1, all patient and tumor characteristics are summarized.

3.2. Evaluation of MET immunohistochemical staining

Positive immunohistochemical MET staining (i.e. staining intensity ≥1) was observed in 24 out of 37 SN samples (65%). Of these positive

Table 1
Baseline patient and tumor characteristics by MET immunoreactivity. Samples were considered positive when ≥10% of the cancer cells showed MET expression (staining intensity ≥1, Histoscore 0–4), n (%) or median (IQR).

Characteristics	All patients (*n* = 37)	MET positive (*n* = 24)	MET negative (*n* = 13)	P-value
Patient characteristics				
Age	58 (47–65)	59 (47–65)	54 (46–69)	0.962
Sex				0.495
Male	14 (38)	8 (33)	6 (46)	
Female	23 (62)	16 (67)	7 (54)	
Tumor characteristics				
Breslow, mm	3.7 (2.5–5.5)	3.8 (2.0–5.5)	3.00 (2.6–5.7)	0.961
Location				0.151
Arm	4 (11)	2 (8)	2 (15)	
Leg	13 (35)	11 (46)	2 (15)	
Trunk	20 (54)	11 (46)	9 (70)	
Histology	n = 33	n = 22	n = 11	1.000
SSM	14 (42)	9 (41)	5 (46)	
NM	19 (58)	13 (59)	6 (55)	
Ulceration	n = 36		n = 12	1.000
Absent	16 (44)	11 (46)	5 (42)	
Present	20 (56)	13 (54)	7 (58)	
BRAF status	n = 32	n = 21	n = 11	1.000
Wild type	17 (53)	11 (52)	6 (55)	
Mutant	15 (47)	10 (48)	5 (46)	
Total no. of positive SNs	1 (1–2)	1 (1–3)	1 (1–2)	0.450
SN tumor burden, mm	1.5 (0.8–3.9)	1.3 (0.8–3.5)	1.5 (0.9–4.5)	0.792
SN tumor burden, subgroups	n = 36		n = 12	1.000
≤1.0 mm	14 (39)	9 (40)	5 (38)	
>1.0 mm	22 (61)	14 (60)	8 (62)	
Therapy				
CLND				0.602
No	4 (11)	2 (8)	2 (15)	
Yes	33 (89)	22 (92)	11 (85)	
Local therapy[a]				0.489
No	21 (57)	15 (62)	6 (46)	
Yes	16 (43)	9 (38)	7 (54)	
Systemic therapy[b]				0.446
No	28 (76)	17 (71)	11 (85)	
Yes	9 (24)	7 (29)	2 (15)	
Outcome				
Recurrence				0.793
No	16 (43)	11 (46)	5 (39)	
Yes	21 (57)	13 (54)	8 (61)	
Type of first recurrence				1.000
Locoregional[c]	11 (55)	7 (58)	4 (50)	
Distant	9 (45)	5 (42)	4 (50)	
Status				0.497
NED	19 (51)	14 (58)	5 (38)	
AWD	2 (5)	1 (4)	1 (8)	
DOC	1 (3)	0	1 (8)	
DOD	15 (41)	9 (38)	6 (46)	

Abbreviations: AWD, alive with disease; CLND, completing lymph node dissection; DOC, death other cause; DOD, death of disease; IQR, interquartile range; MET, mesenchymal-epithelial transition factor; NED, no evidence of disease; NM, nodular melanoma; SN, sentinel node; SSM, superficial spreading melanoma.

[a] Surgical excision and/or radiotherapy.

[b] Chemotherapy and/or immunotherapy and/or targeted therapy.

[c] Locoregional recurrence includes satellites, in-transit metastases, and regional lymph node metastases.

samples, 17 (71%) showed both cytoplasmic and membranous immunoreactivity. Only cytoplasmic MET-expression was found in five samples (21%), only membranous MET-expression in two samples (8%).

3.3. MET correlation with clinicopathological features and prognostic significance

In univariable binary logistic regression, no association between MET immunoreactivity of the SN and the standard clinicopathological features of primary melanoma was observed, including Breslow thickness ($P = 0.886$), ulceration ($P = 0.813$), and SN tumor burden ($P = 0.696$) (see Table 2). Kaplan-Meier curves (in years) are presented in Fig. 1, with a median follow-up of 85 months (IQR 57–140). Presence of MET expression in the SN was not found to be prognostic for DFS ($P = 0.675$), distant metastasis-free survival (DMFS, $P = 0.280$), and overall survival (OS, $P = 0.395$).

4. Discussion

The current study examined the MET immunoreactivity in patients with cutaneous melanoma with positive SNs and evaluated its potential diagnostic and prognostic value. MET immunoreactivity was observed in 65% of the positive SNs, with a wide range of expression in both intensity and cytoplasmic and/or membranous localization. However, MET immunoreactivity was neither associated with primary tumor characteristics or SN tumor burden, nor with survival.

Since MET expression is upregulated in several malignancies [46,47], it has become a target for the development of imaging probes, showing promising results [26,48]. As our data showed that MET was not present in 35% of the positive SN samples, it is not conceivable that MET could serve as a sensitive diagnostic tracer for NIR to identify positive SNs in patients with melanoma who are currently eligible for SN evaluation (i.e., patients with ≥T1b cutaneous melanoma) non-invasively [49]. With MET immunoreactivity in two-thirds of positive SNs, one-third of positive SNs would be missed if MET would be used as a target for NIR. Whilst the current golden standard to identify the SN with lymphoscintigraphy and blue dye is successful in the vast majority of patients [15,50], comorbidities associated with SN biopsy are not negligible [16,19]. Moreover, this procedure cannot differentiate between SNs with or without metastasis prior to surgery, as the pathological examination is required. In order to identify a subgroup of

Table 2
Univariable binary logistic regression for positive MET immunoreactivity.

Characteristics	n	Univariable OR (95% CI)	P-value
Patient characteristics			
Age	37	0.99 (0.94–1.04)	0.643
Sex			
Female	23	Reference	
Male	14	0.58 (0.15–2.32)	0.445
Tumor characteristics			
Breslow, mm	36	0.81 (0.04–15.32)	0.886
Location			
Trunk	20	Reference	
Arm	4	0.82 (0.10–7.02)	0.855
Leg	13	4.50 (0.79–25.77)	0.091
Histology			
NM	19	Reference	
SSM	14	0.83 (0.19–3.58)	0.803
Ulceration			
Present	20	Reference	
Absent	16	0.84 (0.21–3.43)	0.813
BRAF status			
Mutant	17	Reference	
Wild type	15	0.92 (0.21–3.96)	0.907
No. of positive SNs	37	1.87 (0.66–5.28)	0.235
SN tumor burden, mm	36	0.73 (0.15–3.53)	0.696
SN tumor burden, subgroups			
>1.0 mm	22	Reference	
≤1.0 mm	14	1.03 (0.26–4.16)	0.968

Abbreviations: CI, confidence interval; IQR, interquartile range; MET, mesenchymal-epithelial transition factor; NM, nodular melanoma; SN, sentinel node; SSM, superficial spreading melanoma.

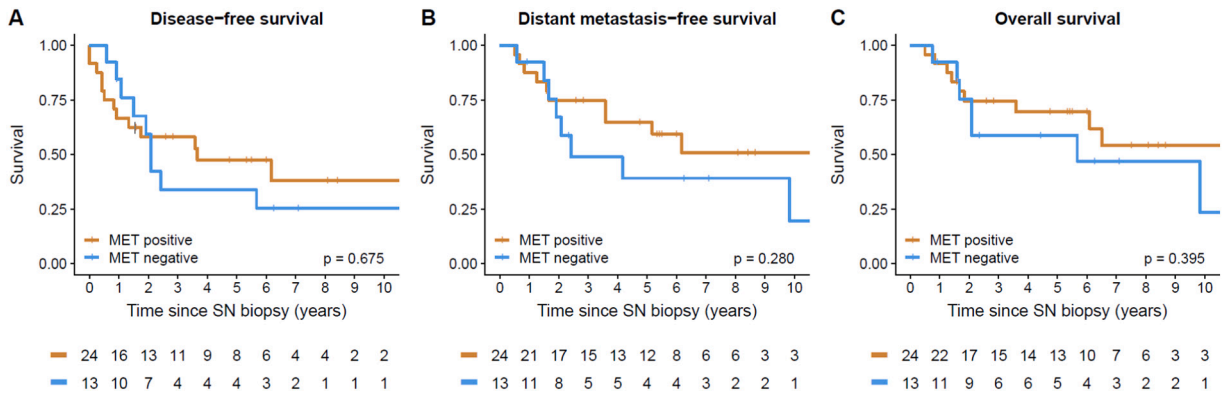

Fig. 1. Kaplan-Meier curves, comparing (A) DFS, (B) DMFS and (C) OS of patients with MET positive versus MET negative SN metastases. Samples with <10% of cancer cells showing immunoreactivity were regarded as (0) negative. The samples were considered positive when at least 10% of the cancer cells showed immunoreactivity.

(A)

Staining intensity	Classification	Cytoplasmic	Membranous
0	Negative	No (<10%) cytoplasmic staining	No (<10%) membranous staining
1	Positive	Weak cytoplasmic staining	Weak complete OR
			Weak/moderate/strong incomplete membranous staining
2	Positive	Moderate cytoplasmic staining	Moderate complete membranous staining
3	Positive	Strong cytoplasmic staining	Strong complete membranous staining

(B)

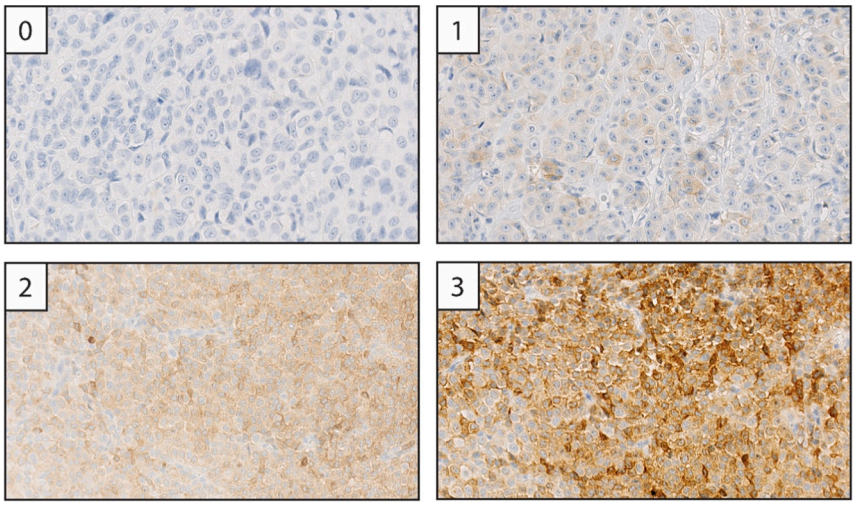

Fig. 2. MET immunohistochemistry on melanoma positive SN tissue. (A) The 4-point Histoscore to determine cytoplasmic and membranous immunoreactivity. (B) Photographs representing the defined staining intensities observed using D1C2 (×20 objective).

patients where MET could serve as a fluorophore for positive SN detection with NIR, it would be interesting to correlate the presence of MET in the primary tumor to the MET status in the SN.

Although limited, previous studies focusing on primary cutaneous melanoma, suggest that MET expression is related to a poorer outcome [36,51] and survival [40]. Cruz and colleagues found that membranous MET overexpression in the primary melanoma was statistically significant associated with more aggressive behavior (resulting in poor clinical outcome), whereas cytoplasmic MET overexpression alone was not [40]. In line with this study by Cruz et al., most SN samples in the current study showed cytoplasmic and membranous staining of MET. However, MET positivity in SNs from our melanoma cohort was not associated with known pathological parameters associated with poor prognosis, such as Breslow depth, ulceration, and SN tumor burden [44,45]. In contrast to previous studies indicating a worse clinical outcome in patients with MET overexpression [31,39-41], positive SN MET expression was neither associated with SN tumor burden nor with survival. Hence, MET expression in SNs with melanoma metastases does not appear reliable for NIR detection to stratify patients who could benefit from adjuvant treatment.

While the prognostic impact of MET activation remains unclear, it has been suggested that the HGF/MET signaling pathway could serve as a therapeutic target in the treatment of melanoma [52-54]. This targeted approach would be similar to patients with BRAF mutant (mt) melanoma; although its prognostic impact is controversial [55], the introduction of BRAF inhibitors in patients with BRAF-mt melanoma (present

in 40–60% of patients [56,57]) has led to revolutionary changes in the treatment of advanced metastatic melanoma [58,59]. The combination of BRAF/MEK inhibitors further improved these outcomes in patients with BRAF-mt advanced melanoma [4] and also showed improved DFS rates in the adjuvant setting [60]. However, their efficacy is not yet satisfactory. This is due to the fact that patients with melanoma may either have an innate resistance to these targeted drugs, or acquire some form of resistance early on in the treatment regime [59,61,62]. Previous studies have shown that the presence of MET can lead to bypass signaling and resistance to targeted therapies [63], suggesting that level of MET expression may be used to predict resistance to BRAF inhibitors in patients with melanoma [64-67]. Therefore, it might be useful to determine MET expression to identify patients who will or will not benefit from BRAF/MEK inhibitors, preferably prior to treatment commencement (in both the advanced and adjuvant setting). Preliminary results of selective MET inhibitors in patients with elevated MET expression showed promising results [67,68]. To evaluate the potential of MET inhibitors as an adjunct or even alternative treatment to established therapeutic strategies, further clinical research is needed.

Although this is the first study addressing the presence of MET in positive SNs from patients with cutaneous melanoma, the current study has limitations. The fact that this study did not demonstrate a statistically significant association between MET positivity in SNs positive for melanoma and survival could be attributed to the fact that sample size was limited. Another explanation for the failure to observe this in the SNs, while this has been observed in the primary melanoma, could be attributed to the fact that the immunohistochemical environment in SNs is different than in primary melanoma tissue [69]. Since the Erasmus MC Cancer Institute is an academic tertiary referral hospital, the vast majority of patients were referred for SN biopsy after primary diagnosis. Therefore, primary melanoma tissue was not available for comparison. Although this study did not find a significant association between MET immunoreactivity and prognostic pathological features such as Breslow thickness, ulceration, and SN tumor burden, further investigation is needed. To further evaluate MET expression in patients with stage II-III cutaneous melanoma, matched samples of primary melanoma and SNs (both positive and negative) should be investigated.

5. Conclusions

This exploratory study illustrated that the expression of MET in positive SNs from patients with melanoma was not detected in a third of all samples, suggesting that the use of MET as a diagnostic tracer for non-invasive NIR is currently limited. In addition, this study found no evidence to support MET immunoreactivity in positive SNs as a possible prognostic indicator. Since MET was present in two-thirds of patients, MET could serve as a therapeutic target for targeted therapy in a selection of patients with cutaneous melanoma, similar to BRAF/MEK inhibitors in patients with BRAF-mt melanoma.

Declaration of competing interest

A.V. is an advisory board member for Bristol-Myers Squibb, Ipsen, Merck Sharp & Dohme, Novartis, Pierre Fabre, Pfizer, Roche, Eisai, Merck and Sanofi. The other authors declare no conflicts of interest.

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References

[1] Ossio R, Roldan-Marin R, Martinez-Said H, Adams DJ, Robles-Espinoza CD. Melanoma: a global perspective. Nat Rev Cancer 2017;17:393–4.
[2] MacKie RM, Hauschild A, Eggermont AM. Epidemiology of invasive cutaneous melanoma. Ann Oncol 2009;20(Suppl. 6). vi1-7.
[3] Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
[4] Robert C, Grob JJ, Stroyakovskiy D, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. N Engl J Med 2019;381:626–36.
[5] Hamid O, Robert C, Daud A, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. Ann Oncol 2019;30:582–8.
[6] Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2019;381:1535–46.
[7] Zhu Z, Liu W, Gotlieb V. The rapidly evolving therapies for advanced melanoma—towards immunotherapy, molecular targeted therapy, and beyond. Crit Rev Oncol Hematol 2015;99:91–9.
[8] Dummer R, Hauschild A, Santinami M, et al. Five-year analysis of adjuvant dabrafenib plus trametinib in stage III melanoma. N Engl J Med 2020;383:1139–48.
[9] Ascierto PA, Del Vecchio M, Mandalá M, et al. Adjuvant nivolumab versus ipilimumab in resected stage IIIB-C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial. Lancet Oncol 2020;21:1465–77.
[10] Eggermont AMM, Blank CU, Mandala M, et al. Longer follow-up confirms recurrence-free survival benefit of adjuvant pembrolizumab in high-risk stage III melanoma: updated results from the EORTC 1325-MG/KEYNOTE-054 trial. J Clin Oncol 2020;38:3925–36.
[11] Verver D, van Klaveren D, van Akkooi ACJ, et al. Risk stratification of sentinel node–positive melanoma patients defines surgical management and adjuvant therapy treatment considerations. Eur J Cancer 2018;96:25–33.
[12] Balch CM, Gershenwald JE. Clinical value of the sentinel-node biopsy in primary cutaneous melanoma. N Engl J Med 2014;370:663–4.
[13] Gershenwald JE, Scolyer RA, Hess KR. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017;67:472–92.
[14] Morton DL, Cochran AJ, Thompson JF, et al. Sentinel node biopsy for early-stage melanoma - accuracy and morbidity in MSLT-I, an international multicenter trial. Ann Surg 2005;242:302–13.
[15] Faries MB, Morton DL. Surgery and sentinel lymph node biopsy. Semin Oncol 2007;34:498–508.
[16] Moody JA, Ali RF, Carbone AC, Singh S, Hardwicke JT. Complications of sentinel lymph node biopsy for melanoma – a systematic review of the literature. Eur J Surg Oncol 2016;43:270–7.
[17] Gumus M, Gumus H, Jones SE, Jones PA, Sever AR, Weeks J. How long will I be blue? Prolonged skin staining following sentinel lymph node biopsy using intradermal patent blue dye. Breast Care (Basel) 2013;8:199–202.
[18] Lucci A, McCall LM, Beitsch PD, et al. Surgical complications associated with sentinel lymph node dissection (SLND) plus axillary lymph node dissection compared with SLND alone in the American College of Surgeons oncology group trial Z0011. J Clin Oncol 2007;25:3657–63.
[19] Faries MB, Thompson JF, Cochran AJ, et al. Completion dissection or observation for sentinel-node metastasis in melanoma. N Engl J Med 2017;376:2211–22.
[20] Oude Ophuis CM, van Akkooi AC, Rutkowski P, et al. Effects of time interval between primary melanoma excision and sentinel node biopsy on positivity rate and survival. Eur J Cancer 2016;67:164–73.
[21] Parrett BM, Accortt NA, Li R, et al. The effect of delay time between primary melanoma biopsy and sentinel lymph node dissection on sentinel node status, recurrence, and survival. Melanoma Res 2012;22:386–91.
[22] van Akkooi AC, de Wilt JH, Verhoef C, et al. High positive sentinel node identification rate by EORTC melanoma group protocol. Prognostic indicators of metastatic patterns after sentinel node biopsy in melanoma. Eur J Cancer 2006;42:372–80.
[23] Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. N Engl J Med 2014;370:599–609.
[24] Galema HA, Meijer RPJ, Lauwerends LJ, et al. Fluorescence-guided surgery in colorectal cancer; a review on clinical results and future perspectives. Eur J Surg Oncol 2021. In press.
[25] Hernot S, van Manen L, Debie P, Mieog JSD, Vahrmeijer AL. Latest developments in molecular tracers for fluorescence image-guided cancer surgery. Lancet Oncol 2019;20:e354–67.
[26] Ren HZ, Cheung S, Churg A. C-MET immunohistochemistry for differentiating malignant mesothelioma from benign mesothelial proliferations. Hum Pathol 2020;105:31–6.
[27] Vahrmeijer AL, Frangioni JV. Seeing the invisible during surgery. Br J Surg 2011;98:749–50.
[28] Lee JYK, Cho SS, Stummer W, et al. Review of clinical trials in intraoperative molecular imaging during cancer surgery. J Biomed Opt 2019;24:1–8.
[29] Esfahani SA, Heidari P, Kim SA, Ogino S, Mahmood U. Optical imaging of mesenchymal epithelial transition factor (MET) for enhanced detection and characterization of primary and metastatic hepatic tumors. Theranostics 2016;6:2028–38.
[30] Zhou Y, Song KY, Giubellino A. The role of MET in melanoma and melanocytic lesions. Am J Pathol 2019;189:2138–48.

[31] Czyz M. HGF/c-MET signaling in melanocytes and melanoma. Int J Mol Sci 2018;19:3844.
[32] Demkova L, Kucerova L. Role of the HGF/c-MET tyrosine kinase inhibitors in metastasic melanoma. Mol Cancer 2018;17:26.
[33] Vergani E, Vallacchi V, Frigerio S, et al. Identification of MET and SRC activation in melanoma cell lines showing primary resistance to PLX4032. Neoplasia 2011;13:IN17.
[34] Jubb AM, Ribas A, Sosman JA, et al. Impact of MET expression on outcome in BRAFV600E/K advanced melanoma. Histopathology 2013;63:351–61.
[35] Timothy RW, Jane F, Yibing Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 2012;487:505–9.
[36] Easty DJ, Gray SG, O'Byrne KJ, O'Donnell D, Bennett DC. Receptor tyrosine kinases and their activation in melanoma. Pigment Cell Melanoma Res 2011;24:446–61.
[37] Ravid S, Teppei M, Kevin S, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature 2012;487:500–4.
[38] De Herdt MJ, Willems SM, van der Steen B, et al. Absent and abundant MET immunoreactivity is associated with poor prognosis of patients with oral and oropharyngeal squamous cell carcinoma. Oncotarget 2016;7:13167–81.
[39] Riker AI, Enkemann SA, Fodstad O, et al. The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. BMC Med Genet 2008;1:13.
[40] Cruz J, Reis-Filho JS, Silva P, Lopes JM. Expression of c-met tyrosine kinase receptor is biologically and prognostically relevant for primary cutaneous malignant melanomas. Oncology 2003;65:72–82.
[41] Natali PG, Nicotra MR, Di Renzo MF, et al. Expression of the c-Met/HGF receptor in human melanocytic neoplasms: demonstration of the relationship to malignant melanoma tumour progression. Br J Cancer 1993;68:746–50.
[42] Grobbee DE, van Veen EB, Coebergh JWW, Oosterhuis JW. Code for proper secondary use of human tissue in the Netherlands. Federation of Medical Scientific Societies; 2011.
[43] The Council of the Federation of Medical Scientific S. Code of conduct for the use of data in health research. 2004.
[44] van der Ploeg APT, van Akkooi ACJ, Haydu LE, et al. The prognostic significance of sentinel node tumour burden in melanoma patients: an international, multicenter study of 1539 sentinel node-positive melanoma patients. Eur J Cancer 2014;50:111–20.
[45] Verver D, Rekkas A, Garbe C, et al. The EORTC-DeCOG nomogram adequately predicts outcomes of patients with sentinel node-positive melanoma without the need for completion lymph node dissection. Eur J Cancer 2020;134:9–18.
[46] Comoglio PM, Trusolino L, Boccaccio C. Known and novel roles of the MET oncogene in cancer: a coherent approach to targeted therapy. Nat Rev Cancer 2018;18:341–58.
[47] Gentile A, Trusolino L, Comoglio PM. The met tyrosine kinase receptor in development and cancer. Cancer Metastasis Rev 2008;27:85–94.
[48] Liang M, Yang M, Wang F, et al. Near-infrared fluorescence-guided resection of micrometastases derived from esophageal squamous cell carcinoma using a c-met-targeted probe in a preclinical xenograft model. J Control Release 2021;332:171–83.
[49] National Comprehensive Cancer Network, Fort Washington U. NCCN clinical practice guidelines in oncology (NCCN guidelines): cutaneous melanoma, version 2 (2021). Available at: NCCN.org; 2021. Accessed Oct 1.
[50] Valsecchi ME, Silbermins D, de Rosa N, Wong SL, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in patients with melanoma: a meta-analysis. J Clin Oncol 2011;29:1479–87.
[51] Yeh I, Botton T, Talevich E, et al. Activating MET kinase rearrangements in melanoma and spitz tumours. Nat Commun 2015;6:7174.
[52] Chattopadhyay C, Ellerhorst JA, Ekmekcioglu S, Greene VR, Davies MA, Grimm EA. Association of Activated c-met with NRAS-mutated human melanomas: a possible avenue for targeting. Int J Cancer 2012;131:E56–65.
[53] Surriga O, Rajasekhar VK, Ambrosini G, Dogan Y, Huang R, Schwartz GK. Crizotinib, a c-met inhibitor, prevents metastasis in a metastatic uveal melanoma model. Mol Cancer Ther 2013;12:2817–26.
[54] Ohara M, Saito K, Kageyama K, et al. Dual targeting of CDK4/6 and cMET in metastatic uveal melanoma. Cancers (Basel) 2021;13.
[55] Ny L, Hernberg M, Nyakas M, et al. BRAF mutational status as a prognostic marker for survival in malignant melanoma: a systematic review and meta-analysis. Acta Oncol 2020;59:833–44.
[56] Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54.
[57] Colombino M, Capone M, Lissia A, et al. BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. J Clin Oncol 2012;30:2522–9.
[58] Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–16.
[59] McArthur GA, Chapman PB, Robert C, et al. Safety and efficacy of vemurafenib in BRAFV600E and BRAFV600K mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. Lancet Oncol 2014;15:323–32.
[60] Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. N Engl J Med 2017;377:1813–23.
[61] Bouattour M, Raymond E, Qin S, et al. Recent developments of c-met as a therapeutic target in hepatocellular carcinoma. Hepatology 2018;67:1132–49.
[62] Wilson TR, Fridlyand J, Yan Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 2012;487:505–9.
[63] Miller MA, Oudin MJ, Sullivan RJ, et al. Reduced proteolytic shedding of receptor tyrosine kinases is a post-translational mechanism of kinase inhibitor resistance. Cancer Discov 2016;6:382–99.
[64] Organ SL, Tsao M-S. An overview of the c-MET signaling pathway. Ther Adv Med Oncol 2011;3:S19.
[65] Danilkovitch-Miagkova A, Zbar B. Dysregulation of met receptor tyrosine kinase activity in invasive tumors. J Clin Invest 2002;109:863–7.
[66] Mo H-N, Liu P. Targeting MET in cancer therapy. Chronic Dis Transl Med 2017;3:148–53.
[67] Daud A, Kluger HM, Kurzrock R, et al. Phase II randomised discontinuation trial of the MET/VEGF receptor inhibitor cabozantinib in metastatic melanoma. Br J Cancer 2017;116:432–40.
[68] Krepler C, Xiao M, Sproesser K, et al. Personalized preclinical trials in BRAF inhibitor-resistant patient-derived xenograft models identify second-line combination therapies. Clin Cancer Res 2016;22:1592–602.
[69] Essner R, Kojima M. Dendritic cell function in sentinel nodes. Oncology (Williston Park) 2002;16:27–31.