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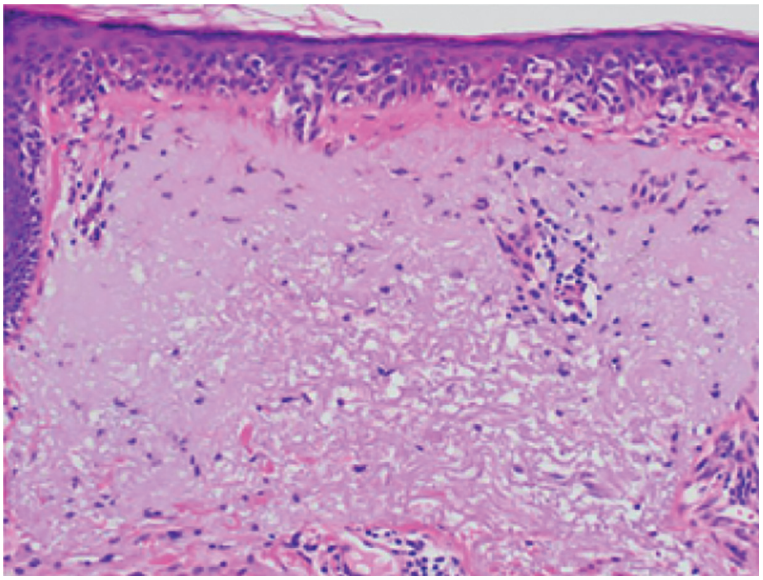

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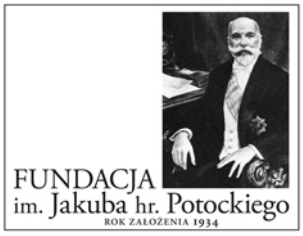

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Original article

Treatment of patients with primary cutaneous lymphomas – real-life data

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Background. Primary cutaneous lymphomas (PCL) comprise a heterogeneous group of neoplasms of mature lymphocytes with skin tropism. Although, by definition, these lymphomas are restricted to the skin at the time of diagnosis, during the course of the disease it may involve also lymph nodes and visceral organs. A close cooperation between a dermatologist and oncologist is required to ensure proper treatment. We present in a real-life data on treatment of patients with PCL between dermatology and oncology department.

Material and methods. 104 patients were registered in a joined database of Oncology Department of Oncology Centre in Bydgoszcz and Dermatology Department of Medical University in Toruń between 2007 and 2017. Due to different clinical and prognostic features data from MF/SS (44 patients), non-MF/SS CTCLs and CBCLs were presented separately.

Results. Median overall survival for patients with MF/SS was 76.7 months. Median follow-up time was 5 years.

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Key words: cutaneous lymphoma, follow-up data, mycosis fungoides, daily practice

Introduction

Primary cutaneous lymphomas (PCL) are rare extranodal non-Hodgkin lymphomas, 75% of them are derived from T lymphocytes (cutaneous T-cell lymphomas, CTCL) and 25% from B lymphocytes (cutaneous B-cell lymphomas, CBCL) [1–3].

CBCLs are divided into 3 subgroups: primary cutaneous follicle centre lymphoma (PCFCL), primary cutaneous marginal zone lymphoma (PCMZL), and primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCL, LT) [1–3]. CTCLs comprise a group of distinct entities with significantly varied clinical, histological and immunophenotypic features and prognoses.

The diagnosis and classification of PCL is based on histological assessment and immunohistochemical staining of an skin biopsy specimen. A prompt diagnosis is often difficult due to PCLs relative rarity and unspecific clinical presentations.

Mycosis fungoides (MF) and its leukemic phase, Sézary Syndrome (SS), is the most predominant subtype of CTCL ~53% [1–4]. MF can mimic different skin conditions, such as eczema, atopic dermatitis, psoriasis, and even other cutaneous lymphomas.

Histological findings are often unspecific and overlap with those of other inflammatory or non-neoplastic diseases so empirical treatment e.g. with topical steroids may hamper the diagnosis. MF has usually an indolent course and a good prognosis. Early-stage MF can be successfully managed by skin-directed therapy, advanced stages of MF and SS require systemic treatment modalities [4].

There is a relative scarcity of data regarding the treatment options of advanced stages CTCLs from non-dermatological units in Poland [5–8]. The aim of this paper is to present real-life clinical data on therapeutic collaboration between dermatological and oncological department. The data have been prepared within the framework of the Polish Lymphoma Research Group.

Methods

104 patients were diagnosed with PCL between 2007 and 2017 in Oncology Centre in Bydgoszcz and Dermatological Department of Medical University in Toruń.

The diagnosis of PCL was made when the clinical features were consistent with histological review and additional tests such as immunophenotyping. The PCL diagnosis was confirmed when lymphomatous infiltration was limited to the skin without any extracutaneous primary lesions found at the moment of diagnosis and subsequent 6 months of follow-up.

Initially, the patients with early stages of PCL were treated with skin-directed therapies such as PUVA or topical steroids. The first line of systemic therapy for advanced stages of PCL was either low-dose interferon alfa 2 beta (subcutaneous injection, 3 million units, 3 times per week) or low-dose methotrexate (orally, 20 mg per week). Subsequent treatment options varied widely depending on the patient's condition and drug availability.

Current paper focuses on the retrospective analysis of clinical data of unselected population of 44 patients diagnosed with MF/SS treated in years 2007–2013. 48 patients with MF/SS who were diagnosed after July 2014 were excluded from the analysis due to participation in the observational clinical trial (NCT 0232365). Due to distinct clinical features and prognosis, patients with non-MF/SS CTCLs and CBCLs are presented separately.

Statistical analysis comprised the calculation of overall survival, patients characteristics, previously applied treatment and coexisting comorbidities.

Results

The number of visits of the patients referred to the Dermatology Department in 2007–2017 with various dermatoses to confirm a suspicion of PCL are presented in table I. A confirmatory diagnosis of PCL was made in 104 patients. The data from 2006–2009 are not available due to technical reasons. The number of confirmed diagnosis of various types of PCL in 2007–2017 with ratio of non-MF PCLs to MF is presented in table II.

MF/SS was diagnosed in 92 patients (88.46% of PCLs); 44 subsequent patients treated in 2007–2013 were included into the analysis. The median follow-up time was 5 years.

Table II. The data from Oncologic Centre – the number of new patients with confirmed skin lymphoma. The proportion between more frequent type: MF (mycosis fungoides), SS (Sezary Syndrome) versus other types of skin lymphoma

Year	Number of new patients with confirmed skin lymphoma	Other type skin lymphoma/ /MF+SS
2007	6	1/5
2008	4	0/4
2009	5	0/5
2010	8	1/7
2011	14	2/12
2012	3	1/2
2013	15	2/13
2014	8	3/4
2015	16	2/14
2016	12	0/12
2017	13	0/12
All	104	12/92

These data comprise the whole 10 years period 2007–2017. Other skin lymphomas are represented both by B cell and T cell lymphoma: the details are shown in table III and IV. The number of diagnosed cutaneous lymphomas with respect to cases of dermatosis with a similar clinical picture (table I) was assessed to emphasise the scale of diagnostic needs in this area in everyday practice

Table I. The number of visits in a dermatologic department caused by dermatoses or inflammatory dermatoses in relation to the number of visits of the patients with CTCL between 2010 and 2017

Year	Allergic Contact dermatitis L23	Atopic skin dermatitis L20	Eczema L30	Parapsoriasis L41	Papulosquamous disorders L44	Contact dermatitis L24	All inflammatory dermatoses MF like
2010	441	147	203	29	16	46	246
2011	489	158	139	61	6	125	198
2012	529	98	251	48	16	116	186
2013	575	40	288	69	14	164	212
2014	636	63	323	92	21	137	190
2015	796	70	477	115	36	98	219
2016	1263	187	804	137	20	115	184
2017	1489	193	997	160	30	109	140

The data from 2006 to 2009 are not available due to technical reasons. The growing number of civilizational skin diseases resulting from this visits and diagnostic needs draws special attention

Table III. Clinical data of 44 MF/SS who started the treatment between 2007 to 2013, and have been follow up minimum 5 years

Woman	Men	Age <60	Age >61	MF primary	SS primary	Stage IIB	Stage III	A/B1/not known	B2
16	28	20	24	41	3	8	36	22/16/6	3

A – blood cytometry without any abnormalities, B1 – not clinically significant number of pathologic lymphocytes, B2 – significant number of pathologic lymphocyte

MF/SS was more prevalent in men (63%) and patients above 61 years (54%). Most patients (81.8%) were in stage III at the moment of the initiation of systemic treatment. The summary of clinical characteristics of the patients with MF/SS is presented in table III. The frequency of comorbidities and other coexisting dermatoses is shown in table IV. Alcohol use disorder was retrospectively diagnosed in 22.72% of all MF/SS patients. The summary of data regarding the first line of systemic treatment is presented in table V.

Interferon (INF) as the first-line treatment was used in 36 patients, methotrexate (MTX) was used in 8 patients. The median duration of treatment with interferon was 14 months and the median duration of treatment with methotrexate was 10 months. 23 patients received 2 lines of systemic therapy,

Table IV. The frequency of other skin diseases and comorbidities

Atopic dermatitis	Skin allergy not specified	Parapsoriasis	Other skin diseases	Cardio-vascular comorbidities	Diabetes	ZZA	Depression
44	27	17	40	24	23	10	17

ZZA – alcohol

Table V. The types of treatment and response

Interferon I line/ /months of therapy	Interferon RR	Methotrexate I line/ /months of therapy-medium value	Methotrexate RR	SAE-Interferon	SAE-Methotrexate
36 pts/3–120 Median: 14	CR–8, PR–28	8 pts/4–96 Median: 10	CR–2, PR–6	1 depression	1 infarctus

Death	Alive patients	Median OS	Pts treated to progression without interval/median OS	Pts treated to PR and after progression/median OS	Patients post SCT/ /with CR
14	30	76.7 months	23/4–144 mts, median: 74.3 mts	21/6–144 mts, median: 79.1 mts	4/4

pts – patients, SCT – stem cell transplantation

Table VI. CBCL and non MF/SS CTCL treatment details

CBCL type/gender	I line	II line	Maintenance	Observation only/ /medium number of visits per years	Efficacy/ /I line	Relapse	Time to II line
PCFCL/W	COP	No	No	No/7	CR	No	n.a
PCFCL/M	No	No	No	Yes/3	n.a	n.a	n.a
PCMZL/K	AC due to breast cancer	No	No	No/12	CR	No	n.a
PCDLBCL/W	R-CHOP	No	No	No/9	CR	No	n.a
FL/W	R-CVP	No	No	No/6	CR	No	n.a
FL/W	R-CVP	No	Yes	No/5	CR	No	n.a
DLBCL/W	R-CHOP mini	No	No	No/9	SD	Yes	n.a
LYP CD30+/W	MTX	n.a	No	No/12	CR	No	n.a
PCALCL ALK + CD 30+/M	Surgery	n.a	No	Yes/3	CR	No	n.a
PCALCL ALK–CD30+/W	MTX/surgery	ICE + SCT	No	n.a/12	CR	Yes	15
PCALCL ALK+ CD 30+/M	MTX	MTX	No	n.a/6	CR	No	n.a
LYP CD 30–/W	MTX/Interferon	Bexarotene	No	No/12	SD	Yes	56

CR – complete remission, PR – partial remission, SD – stabilisation, PD – progression, W – woman, M – man, PCFCL – primary cutaneous follicle centre lymphoma, PCMZL – primary cutaneous marginal zone lymphoma, PCDLBCL LT – primary cutaneous diffuse large B-cell lymphoma (leg type), PCALCL – primary cutaneous anaplastic large cel lymphoma, LYP – lymphomatoid papulosis, n.a – not applicable

15 patients – 3 lines and 15 patients – more than 3 lines (9 pts – 4 lines, 5 pts – 5 lines, 1 pt – 6 lines). The chemotherapy regimens used for relapsed or refractory disease beyond the second-line therapy were as follows: gemcytabine (10 pts), liposomal doxorubicin (11 pts), cytarabine (4 pts), pralatrexate (1 pt), bexarotene (8 pts).

Stem cell transplant (SCT) was performed in 4 patients after achieving remission after the use of romidepsin as an induction therapy (3 pts – allogeneic SCT, 1 pt – allogeneic SCT). 2 patients participated in Millennium clinical trial and received alisertib and pralatrexate. Overall survival data is presented in table VI.

There were 7 patients with CBCL. Patients with CBCL received rituximab-containing chemotherapy regimens. 1 patient with synchronic and breast cancer was treated with AC chemotherapy with subsequent breast-conserving surgery followed by radiotherapy.

5 patients have had a long-term remission. 2 patients with CBCLS died: 90 year old man due to a cardiovascular disease and 78-year old woman due to the disease progression; patients were diagnosed with lymphomatoid papulosis (CD30+ – 1 pts, CD30– – 1 pts) and 3 patients were diagnosed with primary cutaneous anaplastic large cell lymphoma CD30+ (ALK– – 1 pt, ALK+ – 2 pts).

A patient with PCALCL ALK+ received a complete remission after polychemotherapy and treatment was consolidated by allogeneic HCT. A patient with LyP CD30+, resistant to initial MTX and INF treatment, received bexarotene treatment with long-lasting partial remission despite the need for a significant dose reduction of bexarotene. Tables VI and VII presents clinical course and survival data CBCLS and non MF/SS lymphoma.

Discussion

CTCLs comprise a group of heterogeneous lymphomas with a varied clinical behaviour. Most CBCLs are indolent lymphomas that infrequently infiltrate extracutaneous sites, have a good prognosis and may be effectively managed with locally targeted therapies. The advanced stages of CBCLs require immunochemotherapy as other nodal non-Hodgkin lymphomas. The data presented in this paper regarding CBCLs and its clinical features are consistent with other reports [1, 2]. CTCL is the most dominant type of PCL. Most dominant subtypes of CTCL were MF/SS (44 pts), CD30+-lymphomas; other subtypes like PCALCL and LyP were rare (5 pts). Low-dose methotrexate is a frequent first line therapy for multifocal PCALCL with good clinical results and the rate of complete remission near 40% [9].

Two patients were MTX-resistant and required subsequent therapy. 1 patient was successfully managed by the surgical removal of a skin lesion.

Although MF is the most common type of PCL, the reports regarding treatment options is relatively sparse. A broad spec-

Table VII. Clinical data and overall survival in CBCL and patients with non MF/SS

Type PCL	Sex	Age	First visit	Other skin diseases	Comorbidities	Stage	Alive yes or no/OS	Date of death
PCFCL	W	59	V 2007	Any	(–)	T4N0M0 Symptoms B+	Yes/141 months	n.a
PCFCL	M	90	IV 2014	Any	Dementia	T4N1M0	No/14 months	June 2015
PCMZL	W	64	VII 2013	Any	Breast cancer	T3N0M0	Yes/67 months	n.a
PCDLBCL leg type	W	49	IX 2014	Any	Diabetes	I X A	Yes/54 months	n.a
PCFCL	W	59	X 2015	Any	(–)	T4N0M0	Yes/39 months	n.a
PCFCL	W	70	VII 2014	Any	Hypertension	T4N0M0	Yes/52 months	n.a
PCDLBCL leg type	W	78	III 2015	Any	Diabetes, hypretension	T4N1M0	No/8 months	October 2015
LYP CD30+	W	64	III 2010	Hypertension	Atopic dermatitis	T3N0M0	Yes/106 months	n.a
PCALCL ALK+ CD 30+	M	44	V 2011	Any	(–)	T1N0M0	Yes/91 months	n.a
PCALCL ALK– CD 30+	W	47	XI 2011	Any	(–)	T3N0M0	Yes/85 months	n.a
PCALCL AKL+CD30+	M	39	II 2012	Any	(–)	T3N0M0	Yes/82 months	n.a
LYP CD30–	W	72	I 2013	Coronary disease	Skin allergy not specified	T4N1M0	Yes/72 months	n.a

W – woman, M – man, PCFCL – primary cutaneous follicle centre lymphoma, PCMZL – primary cutaneous marginal zone lymphoma, PCDLBCL LT – primary cutaneous diffuse large B-cell lymphoma (leg type), OS – overall survival, PCALCL – primary cutaneous anaplastic large cell lymphoma, LYP – lymphomatoid papulosis, n.a – not applicable

trum of clinical features of MF may be initially missed and thus adequate therapeutic measures delayed.

Another problem regarding the treatment of MF is the limited access to novel drugs due to reimbursement decisions. Currently in Poland there is no access to treatment options like romidepsin and other HDAC inhibitors, denileukin diftitox, pegylated liposomal doxorubicin or extracorporeal photopheresis. [13–16]. For an early stage MF confined to the skin, the therapeutic concept is to control symptoms by use of skin-directed therapies e.g. topical agents such as corticosteroids, mechlorethamine, carmustine, retinoids, phototherapy, superficial radiotherapy, and total skin electron beam therapy. Due to chronic and recurrent nature of MF, in advanced stages, repeated systemic treatment are necessary for disease control [19, 20]. Possible systemic treatment options are: bexarotene, interferon-α, histone deacetylase inhibitors, denileukin diftitox, chemotherapy [13, 19, 21].

Single-agent chemotherapies with a high overall response rate (ORR) are as follows [13–16, 21]: pegylated liposomal doxorubicin (ORR = 88% in stage IA–IV 88%), gemcitabine (ORR = 70% in stage IIB–III), fludarabine (ORR = 55% in stage IIA–IV) [17]. Fludarabine can be substituted by cytarabine because of its favourable safety profile – it was used in 4 patients as salvage therapy. Allogeneic HCT is currently the curative treatment option advanced and resistant MF/SS for young and otherwise healthy patients [19, 21]. The median overall survival for advanced stage MF reported in literature (IIB–IVA) is 60 months [17, 21, 23]. In this study median OS was 75 months.

The aim of this analysis was to confront the treatment options recommended in professional guidelines with every-day practice. In the author's opinion, a limited access to the novel drugs and a small number of clinical trials in Poland make many of proposed treatment modalities a not viable option for the Polish population [17, 24]. Because of the rarity and a varied natural course of the MF, ranging from indolent to highly aggressive, the close cooperation between a dermatologist and an oncologist in important. In Poland there are formal limitations regarding which kind of treatment can be applied by a specific specialist [25]. Recently, radiotherapy has been more frequently used than in the past, but extracorporeal photopheresis is still not available because of reimbursement issues (the exception is GVHD after allo-SCT) [20].

The debate concerning the best way of treatment of these rare lymphoprolfierative disorders is necessary. Researchers hope that increased understanding of the pathogenesis of cutaneous lymphomas with identification of important molecular markers will lead to the development of new targeted therapies and a better effectiveness of the treatment [26].

Conflict of interests: none declared

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Apocrine breast carcinoma as an extremely rare breast cancer subtype – histopathological characteristics

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Apocrine carcinoma (AC) is a distinctive and rare type of malignancy, counted for 0.3–4% of all breast cancer cases. It does not have a particular clinical or radiological features, although it is characterized by the apocrine morphology, estrogen receptor-negative and androgen receptor-positive profile. In the present study, among 1122 patients with breast cancer only 5 of them were diagnosed with apocrine carcinoma (0.4%). All patients were above 50 years old (51–63, mean: 57). Tumor size varied from 1.4 cm to 3.8 cm with a mean size of 2.4 cm, while mean size of all 1122 studied cases counted for 1.9 cm. Two tumors were classified as high-grade (G3), 2 as G2, and 1 as G1. Four tumors out of 5 did not affect lymph nodes (pN0 stage), whereas 1 was classified as pN2 with 9/19 regional lymph nodes affected. This observation was consistent with the whole studied group, in which pN0 stage made up the largest percentage. Presented results suggest that AC is less frequent in premenopausal patients. AC tends to present as invasive malignancy without nodal involvement and is usually characterized by relatively less aggressive biological behavior compared to other histological types of breast cancer. Due to the fact that AC is definitely a rare type of breast cancer, modern medicine has still limited treatment options to offer. Further research needs to be conducted in order to develop target therapies for this carcinoma.

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Key words: apocrine breast carcinoma, breast cancer, estrogen receptor, progesterone receptor, HER2

Introduction

According to estimations performed in 2017 by American Cancer Society, breast cancer (BC) is expected to reach 30% of all new cancers diagnosed among women [1]. Apocrine carcinoma (AC) is one of the rarest histological types of this malignancy, comprising from 0.3% to 4% of all cases [2]. ACs are usually estrogen receptor-negative (ER–), progesterone receptor-negative (PR–), but in 100% of cases androgen receptor-positive (AR+). This observation leads to extensive interest in possible treatment encompassing androgen antagonists [3]. On the other hand, about 30% of AC cases are HER-positive, which enables another treatment possibility with monoclonal antibodies targeting this protein. Consequently, 70% of cases without HER2 expression might be proposed as a subtype of triple-negative breast cancer (TNBC). However, this connection may be misleading due to the fact that AC is characterized by AR positive expression being unparalleled in TNBC [3, 4].

Aim of the study

The aim of the study was to assess histological grade (G), tumor size (pT), regional lymph node status (pN) and expression of ER, PR and HER2 in apocrine carcinoma of the breast in comparison to other studied types of breast cancer.

Material and methods

The material consisted of 1122 tissue blocks derived from patients suffering from breast cancer. The analyzed material

came from biopsies, excisional biopsies and modified radical mastectomies. Histological and immunohistochemical studies were performed. Apocrine carcinoma was detected in 5 out of 1122 samples. Analyzed samples were fixed in 10% buffered formalin phosphate. After 24-hours fixation, materials were dehydrated and then paraffin blocks were cut into sections 4 µm thick. Preparations stained with hematoxylin and eosin were used for defining type of neoplasm according to WHO classification and grading. Immunohistochemical staining was performed for assessing expression of ER, PR and HER2. Evaluation of these markers was performed as follows: ER and PR were categorized as negative – (0%), low positive – (1–10%); nuclear staining in >10% of tumor cells was considered positive both for ER and PR. HER2 expression was determined using HerceptTest™ (Code: K5204, DAKO, Santa Clara, United States).

Results

Five cases of apocrine carcinoma represented 0.4% of all 1122 studied breast cancer samples. All 5 patients were above 50 years old (51–63; mean: 57). Four tumors out of 5 were detected in the left breast. 2 samples were classified as high grade (G3), 2 as G2, and 1 as G1. Tumor size varied from 1.4 cm to 3.8 cm as follows: 1.4 cm (pT1c), 1.7 cm (pT1c), 2 samples – 2.5 cm (pT2), 3.8 cm (pT2). Mean size was 2.4 cm, whereas mean size of all 1122 studied samples reached 1.9 cm. Four out of 5 studied cancers presented without nodal involvement (pN0), while only 1 was classified as pN2 with metastases in 9/19 lymph nodes. This investigation was in consistency with the whole analyzed group, in which pN0 comprised the largest percentage. Distant metastases (M) were not observed in any of the investigated AC cases. As far as immunohistochemistry is concerned, none of 5 analyzed cases expressed ER or PR. Positive expression of HER2 was detected in only 1 sample, which accounted for 20% of studied AC cases. Detailed data on the examined AC cases are presented in the table I.

Discussion

Breast cancer is the most common malignancy among women worldwide [5]. ER, PR and HER2 are commonly used for diagnosing process and choosing appropriate treatment options for particular types of breast cancer. However, AR detected in 100% of ACs is an emerging potential target for accurate diagnosis and targeted treatment [6].

AC is specific for the group of postmenopausal women [3, 7–9]. This was confirmed in the present study, as the mean age of all diagnosed women was 57 years. Given tumor size, all cases were presented in low stage – none of them reached pT3 stage. Similar results were obtained by Mills et al. (2016) [3] – the majority of tumors were assessed as pT1 or pT2 making up 90% of studied cases – and by Seo et al. (2015) [7] – all lesions were described in the range between 1.2 cm and 2.2 cm. As far as nodal involvement is concerned, the vast majority of analyzed tumors were assessed pN0, as in 4 out of 5 cases regional lymph nodes involvement has not been observed. Interestingly, the opposite results were achieved by Liu et al. (2015) [6] – they described molecular apocrine breast cancer (defined as each BC presenting ER–/PR–/AR+ profile) with tendency to affect more lymph nodes than other studied types of BC. However, so defined apocrine cancer did not exhibit all the histopathological traits that were characteristic of classical ACs. Moreover, they detected a close association between lymph node invasion and expression of gross cystic disease fluid protein 15 (GCDFP15) in AC patients. This molecule is regulated by AR and was proposed as a negative marker for AC patients outcome due to significant correlation with shorter disease-free survival (DFS) and overall survival (OS). Unfortunately, as mentioned above, those results cannot have a strict reference into a histologically defined apocrine carcinoma, because both groups of AC, defined molecularly and histologically, are not completely synonymous.

Some studies describe AC without HER2 expression as triple-negative apocrine carcinoma (TNAC) [9]. In the present study 4 out of 5 ACs presented HER2 negativity. In the study con-

Table I. Detailed histopathological characteristics of analyzed apocrine breast cancer cases

Age	Material	Side	Maximal diameter (cm)	Necrosis	Tumor size (pT)	Regional lymph nodes status (pN)	Regional lymph nodes involved	Metastasis (M)	Tumor stage (TNM)	Grade (G)	ER expression	PR expression	HER expression
60	Postoperative material	Left	2.5	–	2	2	9/19	0	IIIA	2	–	–	2+
63	Postoperative material	Left	1.4	–	1c	0	0/18	0	IB	1	–	–	0
56	Postoperative material	Left	1.7	–	1c	0	0/20	0	IB	2	–	–	0
57	Postoperative material	Left	3.8	+	2	0	0/3	0	IIA	3	–	–	0
51	Postoperative material	Right	2.5	–	2	0	0/16	0	IIA	3	–	–	0

ducted by Meattini et al. (2018) [9], TNAC were characterized by lower Ki-67 index and better survival in comparison to group of non-apocrine triple negative tumors. Their multivariate analysis demonstrated that the only significant factor for OS was the histology of TNAC. On the other hand, Vranic et al. (2010) [10] highlighted the necessity of considering apocrine breast cancers as molecularly diverse group and distinguishing pure apocrine carcinomas and apocrine-like carcinomas. According to their study, pure apocrine carcinomas predominantly belong to either HER2 overexpressing or TNBC groups, whereas apocrine-like carcinomas to luminal phenotype (both A and B). What is more, study results suggested that EGFR expression may by essential for the proper identification of apocrine carcinomas when considering doubtful HER2-positive APc.

Numerous studies have proved the prognostic potential of AR expression in BC and its association with more favorable prognosis. Moreover, several experiments discovered a crosstalk between AR and ER by inhibiting each other's activity [11]. Due to emerging role of AR in BCs, new therapies are consequently implemented. One of them encompasses bicalutamide – a nonsteroidal antiandrogen originally used in the treatment of prostate cancer [12]. The study conducted by Huang et al. (2017) [13] revealed the mechanism responsible for effective treatment with bicalutamide usage. This nonsteroidal antiandrogen was proved to block androgen-stimulated oncogenic AR and Wnt/β-catenin signaling and as an effect to inhibit the growth of AR+/ER– breast cancer.

Conclusions

AC is a distinctive and rare type of breast cancer. It has a tendency to affect older population of females. Although tumors are usually diagnosed in larger average size, they tend to present without regional lymph nodes involvement. Slightly different results concerning characteristics of BC subgroup might be explained by small groups incorporated into different analysis. Owing to AR positive expression, new therapies are developed for more specific treatment, however further research is still needed to develop target therapies for this malignancy. Associations described in the present study should be investigated further, as the group of patients was small, even though representing a rare histological subtype of breast cancer.

Conflict of interests: none declared

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Review article

Akademia Czerniaka

Management of melanoma metastases in the brain

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The basic principle for the diagnosis of melanoma metastases in the brain should be the management of multidisciplinary teams including at least a neurosurgeon, radiotherapist and clinical oncologist experienced in the treatment of melanoma and melanoma metastases in the CNS. Detection of brain lesions is associated with poor prognosis; metastases in the brain are the cause of death in 20–50% patients, and symptomatic tumours are a direct cause of death in about 90% patients. Treatment of melanoma with CNS metastases may include local management and/or systemic and symptomatic treatment. In the last 5 years, 10 new advanced melanoma drugs have been registered in Europe. Two-drug therapy anti-PD-1 and anti-CTLA-4 (nivolumab with ipilimumab) is the treatment of choice for asymptomatic melanoma metastases in the brain, while in the presence of *BRAF* mutations and asymptomatic metastases systemic treatment with BRAFi and MEKi may be the first-choice treatment.

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Key words: melanoma, brain, metastases, radiotherapy, immunotherapy, BRAF inhibitors, MEK inhibitors

Introduction

In terms of the frequency of metastases in the brain, melanoma is the third most common malignant tumour after breast and lung cancer. It is estimated that in the course of advanced melanoma in about 50–60% patients the disease will spread to the brain (including about 75% patients with multiple metastases, often initially asymptomatic). In autopsy about 75% of patients have metastases in the brain. At the moment of diagnosis of melanoma, 7% of patients have metastases in the brain. In 3% of patients with diagnosed metastatic lesions in the brain, the primary lesion cannot be found. It should be noted that only in 8–46% melanoma patients metastatic tumours in the brain are found *in vivo*, and in 94% of them they are the direct cause of death.

In the latest classification of melanoma severity according to American Joint Committee on Cancer (AJCC; eighth edition) metastases in the brain were distinguished as a separate, last category in the fourth stage of melanoma severity – M1d. [1]. The risk of metastases in the brain increases with the grade of melanoma [2]. Currently, there are no predictive possibilities to determine the risk of metastases in the central nervous system (CNS) in patients with melanoma. However, it is known that some factors contribute to a higher risk of metastases in the CNS (primary lesion in the head and neck, increased activity of lactate dehydrogenase (LDH), ulceration in the primary lesion, mutations in the *BRAF, NRAS* and *PTEN* genes) [3].

The occurrence of brain lesions is associated with poor prognosis. Metastases in this part of the CNS contribute to death in 20–50% patients and symptomatic tumours are the direct cause of death in about 90% patients. Historical data show that the overall survival (OS) median after the diagnosis of brain metastasis is within 5–7 months, whereas in patients with symptoms of the disease treated with whole brain radiotherapy (WBRT), which is currently rarely used, the OS median

was 2–5 months. In patients undergoing surgical treatment or stereotactic radiosurgery (SRS)/radiosurgery, the median overall survival was twice as long [4].

The aim of this paper is to present multidisciplinary guidelines for diagnostic and therapeutic management in patients with melanoma with brain metastases, as it is currently the greatest challenge in the care of advanced stage melanoma.

New therapies introduced into everyday clinical practice have made the current management of metastatic melanoma little in common with clinical practice 5 years ago. More and more often metastases in the brain are diagnosed before their symptoms appear, after routine brain imaging (magnetic resonance imaging – MRI and/or computed tomography – CT) during the follow-up or qualification of the patient for systemic treatment. Advanced techniques of stereotactic radiotherapy play a fundamental role in local treatment. In the last 5 years 10 new drugs for advanced melanoma therapy have been registered in Europe: vemurafenib, dabrafenib, trametinib, cobimetinib, binimetinib, encorafenib, ipilimumab, nivolumab, pembrolizumab and talimogen laherparepvec (T-VEC). In Poland, 7 new drugs are currently available under drug programmes: vemurafenib, cobimetinib, dabrafenib, trametinib, ipilimumab, pembrolizumab and nivolumab. For both pembrolizumab/nivolumab and combined therapy with BRAF (BRAFi) and MEK (MEKi) inhibitors, in the whole group of patients with metastatic melanoma with the presence of *BRAF* mutation, the median OS based on clinical data is now about 2 years (i.e. about 4 times longer than 5 years ago). Perhaps the best results can be achieved with dual-drug immunotherapy (anti-CTLA-4 and anti-PD-1), as shown by the preliminary results of studies, or other combined therapies (e.g. T-VEC + pembrolizumab) or even iBRAFi, MEKi with anti-PD-1 or anti-PD-L1. Therefore, whenever metastases in the brain are confirmed, it is necessary to investigate the presence of *BRAF* gene mutation in the fixed material (if this has not been previously evaluated) [5, 6].

The basic and post-metastatic management of melanoma in the brain should be carried out in multidisciplinary teams, whose members have experience in the diagnosis and treatment of melanoma. Such a team should include at least: neurosurgeon, radiotherapist and clinical oncologist [7].

Diagnostics

Objective and subjective symptoms of CNS metastases may be subtle and difficult to recognize. They depend, among other things, on the number, size and location of metastases. Metastases are most often formed in the telencephalon, then (about 15% of them), are located in the cerebellum and (about 5%) in the brain stem. The most common symptoms of these lesions are:

- headaches, sometimes accompanied by nausea and/or vomiting,
- epileptic seizures,
- speech, comprehension and vision disturbances,
- numbness,
- mobility disorders.

The occurrence of clinical symptoms of metastases in the CNS is associated with worse treatment outcome. Melanoma patients in stage I and II are less likely to develop metastases in the CNS than patients in stage III and IV patients [8]. In younger patients the risk of late metastases in CNS in case of thicker primary lesions is higher [9]. Based on data from retrospective analysis carried out in a large multi-centre S0008 study, the risk of metastases in the CNS in patients with melanoma at the stage of IIIB and IIIC is 15% – they were found mainly during the first 3 years after surgery[10]. The time from the treatment of the primary lesion can be relatively long and can be as long as 3–4 years (median) [11].

Therefore, in patients with melanoma at III and IV stage of advancement, it is important to detect metastases in CNS on the basis of control imaging tests, despite the absence of clinical symptoms. Performing MRI of CNS during the evaluation of disease progression after the diagnosis of melanoma in the fourth stage should be the standard of management. In patients with melanoma at the stage of IIIC and higher without tumour symptoms, CT or MRI of the CNS should be considered [6]. In the case of patients with objective and/or subjective symptoms, even of minor severity, which indicate the possibility of CNS lesions, MRI should be performed [12]. It is the most sensitive in terms of metastasis detection in CNS and has an advantage over contrasting CT. Unfortunately, MRI is less accessible and more expensive, so it can be considered a necessary complementary study in patients:

- with confirmed CNS metastases – to obtain the information necessary to determine the further course of action (number and/or location of lesions) and
- with clinical symptoms with no change in contrasting CT [13].

It should be emphasized that metastases of melanoma in the CNS are characterized by a tendency to occur in the plural and a tendency to bleed [14].

Therapeutic management

The therapeutic management depends on the clinical situation and includes systemic, local (radiotherapy/SRS and/or surgery) or symptomatic treatment. In the treatment of melanoma metastases in the CNS, apart from clinical symptoms, numerous parameters related to the disease and the patients themselves play an important role, such as:

- number, size and location of metastases,
- presence and control of lesions outside the CNS,
- previous treatment of melanoma and its outcome,
- the presence of a mutation in the *BRAF* gene,
- the patient's general condition, his or her age,
- comorbidities and their treatment.

In the symptomatic treatment of melanoma metastases in the CNS, anti-swelling drugs are used, mainly glucocorti-

costeroids, but also diuretics (loop diuretics, mannitol). In the event of an epileptic seizure, antiepileptic treatment should be instituted, bearing in mind interactions with other drugs used in the patient, including glucocorticosteroids.

Tables I and II summarize two prognostic scales used in patients with CNS metastases, where the recursive partitioning analysis (RPA) scale refers to all neoplasms and the diagnosis-specific graded prognostic assessment (DS-GPA) scale to melanoma patients only.

It should be remembered, however, that these scales were developed before the introduction of new systemic therapies for the treatment of generalised melanoma. Updated scales also include the status of *BRAF* gene mutation and the presence of metastases outside the brain.

The pattern of management in patients with melanoma with CNS metastases is presented in figure 2.

Local treatment of melanoma metastases in the brain

In patients with symptomatic metastatic melanoma lesions in the brain, the expected survival without treatment is 2–3 months, and only 13% of OS patients will survive longer than one year (better prognosis in patients under 65 years of age and with Karnofsky Performance Scale (KPS) >70 points). Prognosis is affected by the removal or irradiation of all metastatic lesions. Leaving one of several lesions causes the prognosis to be the same as in the absence of treatment [16].

Table I. RPA (recursive partitioning analysis; n = 1200) [15]

	Class I	Class II	Class III
KPS	≥70	≥70	<70
Primary lesion	Controlled	Active	Active
Age	<65	65	Any
Extracranial disease	No	Present	Present
Incidence	15%	65%	20%
Median OS (months)	7.1	4.2	2.3

Table II. Prognostic assessment of the survival of melanoma patients with brain metastases – DS-GPA scale (diagnosis-specific graded prognostic assessment) [16]

KPS (points)	<70	70–80	90-100
Number of metastases within the CNS	3	2–3	1
Points	0	1	2

Division based on the sum of the number of points awarded for KPS and the number of metastases (including the patient's age: >60 years – 0, 50–60 years – 0.5 and <50 years – 1.0)

DS-GPA	0–1.0	1.5–2.0	2.5–3.0	3.5–4.0
Median OS (months)	3.4	4.7	8.8	13.2

The median survival rate of all patients with melanoma was 6.74 months (range 3.38–13.32 months; n = 481)

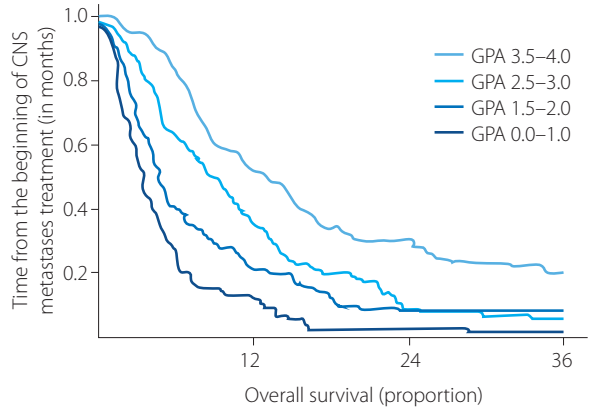


Figure 1. Kaplan-Meier survival curves for individual groups on the GPA scale [16]

There are still no clear predictive factors for the occurrence of melanoma metastases in the CNS. It is known, however, that certain factors are associated with increased risk. These include, but are not limited to:

- primary lesion within the head and neck,
- increased LDH activity,
- ulceration in the primary lesion,
- molecular changes in *BRAF, NRAS* and *PTEN* [3].

In patients with metastases in the brain, mutations in the *BRAF* gene occur in 24–58% cases and in 23% in the *NRAS* gene.

Surgical treatment

Eligibility criteria for surgical treatment of melanoma metastases in the brain (Evidence Based Medicine [EBM], 2010, level 1):

- newly discovered, single lesions up to 4,
- the size of the lesion prevents SRS (diameter greater than 3 cm),
- the location of the lesion is surgically accessible,
- symptomatic tumours causing:
  - neurological deficit and/or
  - increased intracranial pressure due to its volume and/or accompanying haemorrhage and/or secondary obstruction of the fluid pathways leading to hydrocephalus (lesions in the posterior cranial fossa),
- efficiency according to KPS >70, age <65 years,
- progression after prior stereotactic irradiation.

Objectives of surgical treatment:

- histological verification of the lesion,
- radical excision of all lesions, which affects OS (no justification for biopsy) – in case of multiple tumours, hybrid therapy (resection of large, surgically accessible lesions in combination with SRS for smaller tumours located in deep brain structures) is possible,
- improvement or stabilization of neurological condition (occurrence of new neurological deficits shortens OS by 4 months),
- enabling further oncological treatment,
- resection of symptomatic radionecrosis after SRS.

Radiotherapy

Stereotactic radiation therapy (radiosurgery)

Stereotactic irradiation is the delivery of a biologically high dose of radiation to a precisely defined small volume with a significant drop in the dispersed dose outside the target area. Treatment can be performed with one fractional dose (radiosurgery) or 3–5 fractions (fractionated stereotactic radiotherapy). Irradiation can be carried out with equipment designed for such treatment (Gamma Knife, Cyber Knife, EDGE), as well as with conventional linear accelerators equipped with high-resolution leaf collimators. The prescribed total dose and the choice of fractionation scheme depends on the location of metastatic lesions and their volume.

To achieve high local efficacy, a total dose should be administered that is more than 100 Gy after conversion to a biologically effective dose (BED). The efficacy of SRS in the treatment of small metastases of melanoma in the brain has been confirmed in many studies and is similar to that achieved by metastasectomy. The most important is the appropriate qualification of patients for treatment, which should be carried out in multidisciplinary teams.

The rules for qualifying for the SRS are as follows:

- the general condition of the patient: WHO 0–2,
- a single metastasis with a diameter of <3 cm,
- the number of metastases >1 where the total volume of the healthy brain irradiated with 12 Gy dose does not exceed 10 cm^3,
- no progression of changes outside the CNS or availability of potentially effective systemic treatment,
- irradiation of the postoperative bed [17, 18],
- possible local repeated irradiation after progression has been detected,
- life expectancy >6 months.

Recently, the indications for SRS have been extended; it was originally reserved for patients with no more than 3 metastases [22–24]. Ideally, the number of lesions should not exceed 5, but none of them should exceed 3 cm in diameter. However, a cautious qualification of patients who do not meet these assumptions is possible [19].

Nowadays, the number of metastases is of lesser importance and a limitation for stereotactic radiation is the volume of all lesions and the volume of the brain receiving a total dose of 12 Gy [25, 26]. It has been demonstrated that a healthy brain volume of more than 10 cm^3 receiving a 12 Gy dose is associated with a high risk of radionecrosis. In such clinical situations, reduction of the therapeutic dose or disqualification of the patient from stereotactic irradiation and qualification for WBRT should be considered, especially in the presence of multiple metastases. If properly qualified, local efficacy of SRS (no progression in irradiated volume) is achieved at 90–95% patients with melanoma [20, 21]. Moreover, in half of the patients a radiologically significant tumour response is observed [20]. The local efficacy is closely linked to the location of the lesion and its size.

Whole brain radiotherapy

Melanoma is considered to be a radiation-resistant neoplasm and sensitive only to higher fractional doses. Fractionation schemes used to irradiate the whole brain (whole brain radiotherapy, WBRT; 5 × 4 Gy, 10 × 3 Gy) do not provide a biological dose that allows for long-term control of the disease within the CNS. In addition, WBRT is associated with neurological toxicity. The deterioration of the quality of life of patients is caused mainly by cognitive dysfunction [27, 28]. Therefore, the WBRT should be reserved exclusively for patients:

- with a predicted short survival time,
- in poor general condition: WHO 3–4,
- disqualified from a surgery and SRS,
- with a large volume of neoplastic lesions within the CNS,
- with their rapid progression and in case of lack of possibility of effective systemic treatment,
- with metastases in the meninges, in good general condition.

Patients in very poor general condition (performance status: WHO 4) with symptoms of brain oedema that do not yield to anti-oedematous treatment should be disqualified from any form of radiotherapy. The management of choice is then symptomatic treatment, such as effective anti-oedema and antiepileptic management, as well as treatment of symptoms often associated with progression within the CNS.

The results of phase III study published in 2019 indicate that WBRT as a supplementary treatment after local treatment of melanoma metastases within the CNS does not improve the results of the therapy. The whole brain radiotherapy should therefore be reserved for patients disqualified from local treatment.

Systemic treatment

Systemic treatment is the basis of the management of patients with disseminated melanoma, including patients with brain metastases. Similarly as in the case of molecularly targeted therapy (BRAF and MEK inhibitors [BRAFi and MEKi]), the use of immunotherapy, including anti-CTL-A4 and anti-PD-1 drugs, significantly improves the prognosis of melanoma patients with metastases to the CNS. More and more long-term remissions are observed in patients responding to immunotherapy [29]. Depending on the previous treatment, the presence of V600 *BRAF* mutation and the patient's condition and clinical situation, appropriate systemic therapy should be implemented, in most cases supplemented by local treatment. In a situation of a few small metastases in the CNS, exclusive systemic treatment remains an option. Blood-brain barrier is not important for the activity of new drugs used in the therapy of melanoma.

Molecularly targeted therapy

The efficacy of molecularly targeted drugs (BRAF/MEK inhibitors) in patients with brain metastases of skin melanoma has been confirmed in several prospective clinical studies. The first clinical trials conducted exclusively in this group of patients evaluated the effectiveness of BRAFi used in monotherapy. In the largest study, including as many as 172 patients with asymptomatic metastases, the efficacy of dabrafenib (study phase II BREAK-MB) was assessed. The patients participating in the study were divided into two groups based on the previous local treatment due to brain metastases (without prior local treatment *vs.* progression after prior local treatment). The intracranial response rates were 39.2% and 30.8%, respectively. The median OS in both groups was more than 8 months [2]. In a similar clinical trial on the use of vemurafenib in 146 patients with skin melanoma with brain metastases (phase II trial), the intracranial response rate was 18% regardless of previous local treatment. The median OS was about 9 months [30]. If we take into account the assessment of responses by an independent review committee (IRC), the intracranial response rates in both studies were very similar (about 18%). These studies also showed a relatively high percentage of disease control (about 70–80%). This is due to the fact that in the majority of patients the reduction of metastatic lesions in the brain was observed, but only in some of them did it meet the criterion of partial response.

A difficult clinical situation is the presence of symptomatic metastases in the brain. This stage of disease is associated with particularly poor prognosis (median OS 3–4 months). The only clinical trial that included only this group of patients concerned the use of vemurafenib in monotherapy [31]. It was a study with a small number of patients: 24 patients not eligible for neurosurgery were included, after previous treatment of brain metastases and requiring the use of glucocorticosteroids to control symptoms. The percentage of intracranial responses was 16% and the median OS – 5.3 months. During treatment, a reduction in pain symptoms was observed, as well as improvement of patients' performance status, and reduction of the need for glucocorticoids. Unfortunately, the effect of the treatment was short-lived and the disease progressed rapidly.

The improvement of targeted treatment results was brought about by the combination therapy of BRAFi with MEKi. The only prospective clinical study evaluating the activity of this therapy in patients with metastases in the brain is phase II of COMBI-MB using dabrafenib and trametinib [32]. 125 patients with performance status 0–2 according to Eastern Cooperative Oncology Group (ECOG) with or without prior treatment of local metastases in the brain were enrolled to the study. The intracranial response rate was 56–59%, regardless of the previous local treatment and presence of symptomatic metastases. Longer duration of response was observed in patients with asymptomatic brain metastases. The median duration of the response was, however, considerably shorter than that observed in phase III clinical trials without the participation of patients with brain metastases (about 6 months *vs.* 12–14 months) [33–35]. No significant differences in treatment tolerance were observed. The most common side effects were fever and gastrointestinal disorders.

The results of the studies mentioned above confirm the activity of BRAFi/MEKi in patients with brain metastases. The response to treatment is rapid, and the reduction in tumour lesions occurs in the majority of patients. This is not only important for improvement of OS in this group of patients with poor prognosis, but also to improve the quality of life. This is particularly true for patients with symptomatic brain metastases. Unfortunately, the above data also indicate a short-term therapeutic effect of targeted treatment. Resistance in this group of patients appears faster than in patients without metastases in the brain. Therefore, in order to improve treatment outcome, attempts are currently being made to combine BRAFi/MEKi with other kinase inhibitors or immunotherapy. The results of BRAFi/MEKi tests in patients with melanoma with CNS metastases are presented in table III.

Radiotherapy in combination with targeted therapy

High initial BRAFi/MEKi activity in patients with melanoma with brain metastases has slightly changed the approach to the use of radiotherapy. The increasingly widespread use of SRS gives a high percentage of local disease control. However, it has not been shown to protect against further spread of the disease within the CNS and therefore, with the exception of patients with isolated brain metastases, has little effect on OS. Therefore, radiotherapy is often used only during the treatment of BRAFi/MEKi.

The data on the purposefulness of combining BRAFi drugs with simultaneous irradiation are contradictory. On the one hand, the potential benefits of such a strategy in terms of sensitisation of melanoma cells to radiotherapy after BRAFi, as described in in *vitro* studies, are pointed out [36]. On the other hand, the radiation-sensitising BRAFi action can lead to increased side effects, which has been confirmed by several described case studies of significant skin toxicity during simultaneous use of a combination of irradiation with these drugs, also WBRT. So far, a similar radiosensitizing effect has not been described after the simultaneous use of BRAFi with MEKi. There is no clear evidence of an increased risk of neurotoxicity, haemorrhage or brain radiation necrosis in the combination of targeted treatment with radiotherapy [37–39]. The combination of targeted treatment with radiosurgery to the CNS area gives fewer side effects than the combination with conventional radiotherapy. For conventional radiotherapy, the most common adverse reaction is skin toxicity (more severe with vemurafenib) [40].

Irradiation during targeted therapy increases the risk of dermatitis in degree II and III. As the severity of inflammation

Table III. Studies on the effectiveness of molecularly targeted therapy in the treatment of melanoma with metastases in the CNS

Study	Characteristics of patients	Number of patients	PFS (median, months)	OS (median, months)
Phase II study [30] (NCT01378975) vemurafenib	Previously untreated CNS metastases	90	3.7	8.9
	Previously treated, CNS metastases	56	4.0	9.6
Pilot study [31] (NCT01253564) vemurafenib	Previously treated, symptomatic metastases in CNS	24	3.9	5.3
Phase II study BREAK-MB [2] (NCT01266967) dabrafenib	CNS metastases without prior treatment	89	~4 [a]	~8 [a]
	Progression after prior local treatment	83	~4 [a]	~8 [a]
Phase II study COMBI-MB [32] (NCT02039947) dabrafenib + trametinib	Asymptomatic CNS metastases without prior local treatmentECOG PS 0–1	76	5.6	10.8
	Asymptomatic CNS metastases; prior local treatment ECOG PS 0–1	16	7.2	24.3
	Asymptomatic metastases with/without prior local treatment ECOG PS 0–1	16	4.2	10.1
	Symptomatic metastases with/without prior local treatment ECOG PS 0–2	17	5.5	11.5

[a] Median refers to patients with the presence of *BRAF* V600E mutation

depends on the irradiation dose, doses ≥4 Gy for conventional radiotherapy are not recommended. It is currently recommended to stop using BRAFi and MEKi at least 3 days before the irradiation and to re-activate the drugs at the earliest 3 days after its completion [37]. The exception is SRS for CNS, in which case a sufficient break in the use of BRAFi and MEKi before and after radiotherapy is one day.

Immunotherapy

Immunotherapy is the primary option in patients with melanoma with CNS metastases in the absence of V600 mutation in the *BRAF* gene. In patients with *BRAF* mutation, the choice of immunotherapy or treatment with BRAFi from MEKi depends on the clinical situation.

In an open-label phase II of clinical trial with ipilimumab (NCT00623766), the highest response rates were observed in asymptomatic patients who did not receive steroids. On the basis of immune related response (IRR) criteria, the median progression-free survival (PFS) of CNS lesions was 1.9 months in the asymptomatic group *vs.* 1.2 months in a group requiring glucocorticosteroids due to clinical symptoms of CNS metastases, and OS, respectively, 7.0 *vs.* 3.7 months [41]. In the CheckMate 204 (NCT02320058) study with nivolumab and ipilimumab, which enrolled patients with at least one CNS lesion, the primary endpoint was intracranial clinical benefit rate (CBR) – a complex endpoint including complete response (CR), partial response (PR) and stable disease (SD) for more than 6 months. The intracranial objective response rate (ORR) was 55% and CR was 21%. Extracranial responses were similar to those observed in the CNS, and the PFS rate at six months of treatment was 67%. The results of this study confirm that similarly to the treatment of extracranial disease, in patients with CNS metastases it is possible to obtain a similar response to the treatment of CNS lesions [41]. In 2019, updated CheckMate 2004 results from two cohorts of patients were presented. The A cohort included persons without neurological symptoms, not taking steroids (a cohort of patients with asymptomatic brain metastases), and the B cohort included persons with neurological symptoms – regardless of whether they received steroids or not. Patients from both groups received nivolumab (NIVO) at a dose of 1 mg/kg of body weight + ipilimumab (IPI) at a dose of 3 mg/kg b.w., every 3 weeks, 4 doses followed by NIVO at a dose of 3 mg/kg b.w. every 2 weeks – to the onset of disease progression or toxicity of treatment. In cohort A after the follow-up period of 20.6 months CBR amounted to 58.4%, while in cohort B after the follow-up period of 5.2 months it amounted to 22.2%. Level III and IV treatment-related adverse events were observed in 54% of patients in cohort A and 56% of patients in cohort B. Level III and IV nervous system related adverse events occurred in 7% and 17% of patients, respectively. Similarly, in the Australian ABC study (NCT02374242), in which the efficacy of nivolumab versus nivolumab plus ipilimumab in melanoma patients with brain metastases (n = 79) was investigated, the efficacy of immunotherapy was demonstrated, including the advantage of dual therapy in melanoma patients with asymptomatic brain metastases. In this study, the patients were assigned to three cohorts: cohort A (n = 36, a group of asymptomatic patients without local treatment due to brain metastases, receiving ipilimumab with nivolumab); cohort B (n = 27, group of asymptomatic patients without local treatment due to metastases to the CNS, receiving nivolumab); and cohort C (n = 16, patients after local treatment

due to brain metastases failure and symptomatic patients with brain metastases and patients with leptomeningeal disease, receiving nivolumab). Complete responses to treatment were observed in 17% of patients in cohort A, 12% in cohort B, and none in cohort C [42]. In the CheckMate 204 study and in the ABC study, grade 3 and 4 treatment-related adverse events in patients receiving dual therapy occurred in 52% and 54% of patients, respectively.

In asymptomatic patients, the efficacy and good tolerance of immunotherapy were confirmed by the clinical trials presented. The response rate for ipilimumab was 16% and for nivolumab and pembrolizumab about 20%. In the study of the combination of anti-PD-1 and anti-CTLA-4 in the group of asymptomatic patients, further significant improvement in treatment results was achieved. In patients with symptomatic metastases the clinical response rate was also significant and amounted to 16.7%. In the situation of the availability of combination therapy with anti-PD-1 plus anti-CTLA-4 (nivolumab with ipilimumab) and in the case of good performance status of the patient, this combination is the treatment of choice for asymptomatic melanoma patients with brain metastases.

The results of clinical studies with immunotherapy in patients with melanoma brain metastases are summarised in table IV.

Combination of radiotherapy with immunotherapy

There are more and more reports related to beneficial effect of combining radiotherapy with immunotherapy. The studies published so far show a significant increase in the percentage of the phenomenon called abscopal effect (response of untreated lesions to local treatment of other lesions) after radiotherapy was added to immunotherapy [46]. This is explained by local stimulation of the immune system and enhancement of the antigenic effect, where dendritic cells probably play a major role. There are many clinical trials underway in which radiotherapy and immunotherapy are combined. There are no contraindications for combining SRS/WBRT with immunotherapy, the decision should be made at a multidisciplinary meeting for each patient individually. Attention should be paid to the accompanying radiotherapy prophylactic anti-oedema treatment in the form of high doses of glucocorticoids that can reduce the efficacy of immunotherapy. According to current recommendations, the indications for the use of glucocorticosteroids in anti-oedema treatment during SRS are significantly limited.

The combination of immunotherapy or molecularly targeted therapy with SRS seems to be generally well tolerated, as demonstrated by studies and analyses conducted so far. In 2016, the results of the retrospective analysis done in the subgroup of patients participating in two prospective studies with nivolumab for unresectable or metastatic disease were published [47]. The analysis included 26 patients treated with melanoma and treated with SRS due to CNS metastases, including patients with CNS metastases diagnosed and treated with SRS within 6 months of treatment with nivolumab (before, after or during immunotherapy). A total of 73 CNS lesions were identified in these patients. The primary endpoint of the analysis was treatment tolerability, and secondary endpoints were intracranial disease control and extracranial disease

Table IV. Studies on the effectiveness of immunotherapy in the treatment of patients with melanoma with CNS metastases

Treatment	Patients	Characteristics of patients	IC DCR	IC ORR	IC DOR (months)	mPFS (months)	mOS (months)
IPI CA184–042 [41]	51 (A) 21 (B)	Asymptomatic Symptomatic	24% 10%	16% 5%	–	1.4 1.2	7.0 3.7
IPI + fotemustine NIBIT-M1 [43]	20	Asymptomatic	50%	40%	30.3	4.5	12.7
Pembrolizumab (NCT02085070) [44]	18	Untreated or progressive brain metastases	44%	22%	–	–	NR
NIWO: ABC; CA209–170 [42] (NCT02374242)	27 (B) 16 (C)	Asymptomatic, no local treatment (B) Prior treatment or symptomatic (C)	20% 19%	20% 6%	NR NR	2.5 2.3	18.5 5.1
NIWO + IPI: ABC; CA209–170	36 (A)	Asymptomatic, no local treatment (A)	57%	46%	NR	NR	NR
NIWO + IPI: CheckMate 204 [45] (NCT02320058)	75	Asymptomatic, prior treatment, ≤3 metastases	60%	55%	NR	NR	–

NR – not reached

control as well as OS. The majority of metastases were treated with single-fraction radiosurgery, only 12 CNS lesions were treated with fractionated SRS. In one patient headaches of grade 2 were observed, which disappeared after steroids were applied. No other neurological complications associated with the treatment were observed. In case of 8 CNS lesions (11%) failure of treatment in the form of increase of their volume by at least 20% was observed. Local control rates after six and 12 months were, respectively, 91% and 85%. The median OS was 12.0 months from the beginning of treatment with nivolumab and 11.8 months from SRS.

In 2017 a systematic review devoted to the evaluation of the tolerance of combined immunotherapy or molecularly targeted therapy with SRS was published. In the overview six retrospective studies and two case studies of patients treated with SRS and ipilimumab were included. Based on the analysis of these data, combination therapy with ipilimumab and SRS for intracranial lesions can be considered as a safe method of treatment [48].

New systemic treatment methods

Due to the often short-term or insufficient response to systemic treatment of melanoma patients with CNS metastases after immunotherapy or molecularly targeted therapy, attempts are now being made to combine BRAF/MEK inhibitors with other kinase inhibitors or immunotherapeutic agents. The objective is to improve treatment outcomes. One such study is the TRIDeNT study using nivolumab in combination with dabrafenib and/or trametinib, which may involve patients with metastases to the CNS and patients with melanoma with leptomeningeal metastases (NCT02910700) [49].

Monitoring patients after local treatment of CNS metastases and management in case of progression

Patients undergoing surgery or SRS should be monitored by performing a brain magnetic resonance imaging to quickly detect possible progression within the CNS. The first MRI should be performed within one month after surgery/SRS, and the next every 2–3 months. The imaging test results should be interpreted with caution, especially in patients undergoing immunotherapy due to the possibility of pseudoprogression and changes after treatment, which can be difficult to distinguish from disease progression. Metastases of melanoma in the CNS increase the risk of new metastases in the CNS, hence the need to monitor the CNS by means of MRI [6]. In about 50% of patients new metastatic lesions or progression of metastases previously treated (relapse in the tumour bed, progression after SRS/WBRT) will be detected [50]. However, these are not situations disqualifying from further therapy. In such a situation, one of the rescue methods of local treatment (surgery, SRS, WBRT) can usually be applied after the patient's case has been discussed at a multidisciplinary meeting [51–53]. After confirmation of the progression of CNS lesions after SRS or radiotherapy, while retaining the previously described eligibility criteria for neurosurgical treatment, surgical treatment remains the therapy of choice. Despite the introduction of modern neuroimaging techniques, it may be difficult to determine whether the observed progression is secondary to active neoplastic process or secondary to radionecrosis. In doubtful cases, the treatment of choice should be resection of the lesion, because apart from oncological indications, the removal of dead tissues has an antioedematous effect.

Leptomeningeal metastases

Prognosis in this group of patients is poor, the survival time usually does not exceed a few weeks. Data on the effectiveness of modern systemic treatment in the case of metastases to the meninges are limited and scientific evidence-based standards of management are lacking. Results of recently published retrospective analyses indicate that molecularly targeted therapy and immunotherapy may improve prognosis in these patients [54, 55]. A phase I clinical trial (NCT03025256) is currently being conducted using nivolumab, intravenous and intrathecal, in patients with leptomeningeal disease.

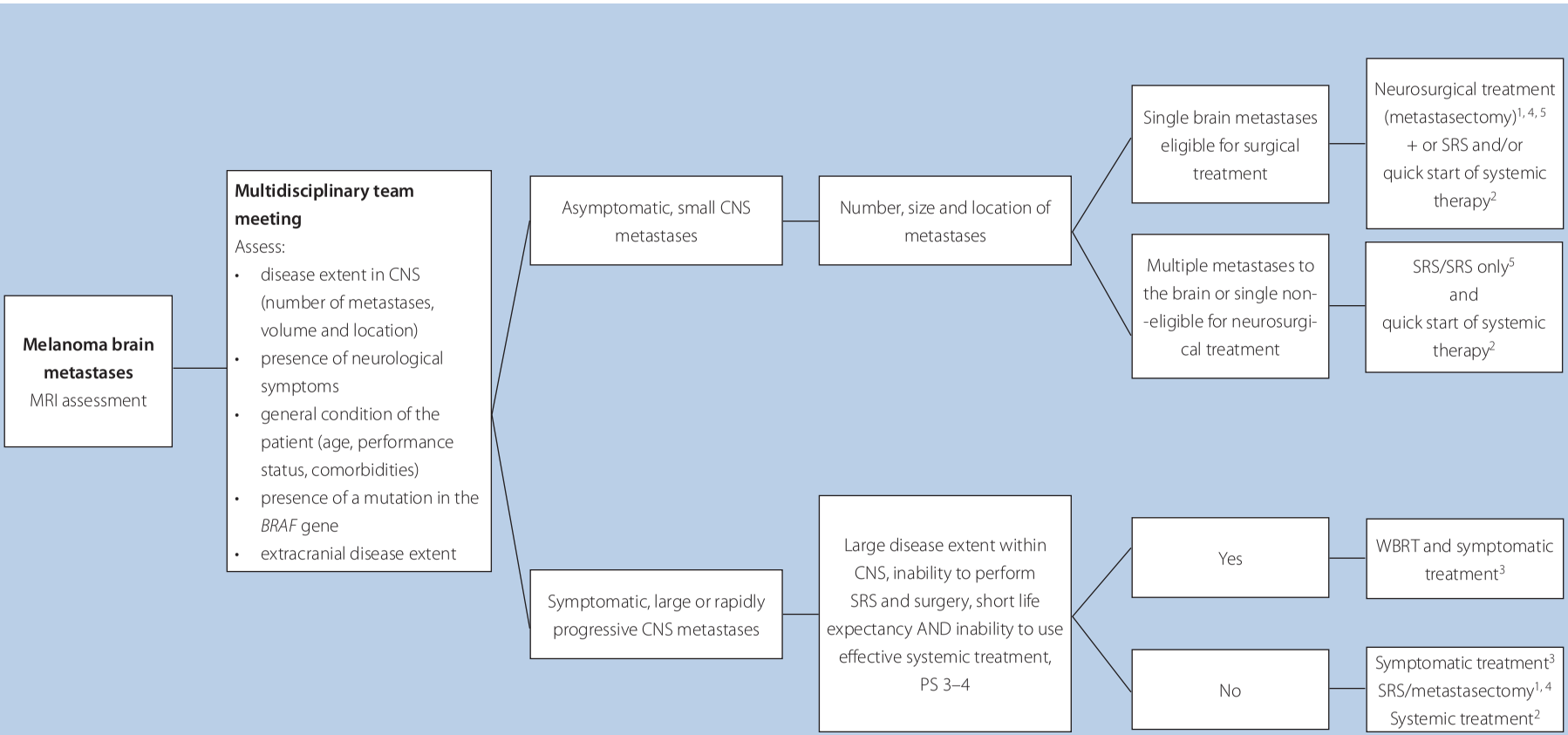
The data concerning the systemic use of IL-2 are encouraging; the 1-, 2- and 5-year survival rates in the group of 43 patients were 36%, 26% and 13% respectively. However, in view of the increased toxicity, Il-2 is not considered as a standard procedure [56].

Radiotherapy in the form of WBRT including meninges up to C2 level is a palliative treatment and should be used only in a selected group of patients (good performance status, active systemic treatment).

Summary

The basic and binding principle for the diagnosis of melanoma metastases in the brain should be the management carried within multidisciplinary teams including at least a neurosurgeon, radiotherapist and clinical oncologist experienced in the treatment of melanoma and melanoma metastases in the CNS. Predictive factors of metastases in CNS in melanoma patients have not been determined yet. Detection of brain lesions is associated with poor prognosis; metastases in the brain are the cause of death in 20–50% patients, and symptomatic tumours are a direct cause of death in about 90% patients. Historical data indicated the median OS after the diagnosis of brain metastasis was within 5–7 months. Nowadays, more and more often metastases in the brain are detected at the asymptomatic stage using routine brain imaging during patient follow-up or staging evaluation before systemic treatment.

Treatment of melanoma with CNS metastases includes, depending on the clinical situation, local and/or systemic treatment and symptomatic treatment. Advanced SRS techniques currently play a key role in local treatment. In the last 5 years, 10 new advanced melanoma drugs have been registered



CNS – central nervous system; SRS – radiosurgery; WBRT – whole brain irradiation

[1] SRS as an alternative to surgery for surgically inaccessible lesions or patients not eligible for surgical treatment.

[2] Available options include immunotherapy (nivolumab combined with ipilimumab preferred, of possible) or BRAF inhibitors with MEK inhibitors in patients with a confirmed *BRAF* mutation; simultaneous radiotherapy with BRAF inhibitors may increase the toxicity of treatment. The treatment with BRAFi + MEKi should be considered first of all in patients with the *BRAF* V600 mutation, especially when it is necessary to implement the treatment with the aim to rapidly reduce the tumour mass; in patients with brain metastases and extracranial disease the systemic treatment may be initiated during or after local treatment, bearing in mind the increased risk of simultaneous or sequential therapy with BRAFi and radiotherapy.

[3] Antioedematous and/or anti-epileptic treatment, if necessary.

[4] Adjuvant tumour bed radiosurgery is not recommended except for: leaving a tumour residue, excision of isolated CNS metastasis, without extracranial disease.

[5] In selected patients, quick start of systemic therapy and close observation of the disease within the brain should be considered (the first MR one month after the beginning of treatment, then every 2–3 months), and local intervention should be considered in the case of CNS progression.

Figure 2. Algorithm for management of patients with melanoma CNS metastases

in Europe. Thanks to the introduction of modern systemic treatment, the median OS on the basis of clinical trial data is currently about 2 years. If anti-PD-1 and anti-CTLA-4 (nivolumab with ipilimumab) are available as well as if the patient is in good condition, this is the procedure of choice for asymptomatic melanoma metastases in the brain, while in the case of *BRAF* mutations and asymptomatic metastases, systemic BRAFi and MEKi treatment can be the first-choice treatment. In every case of melanoma metastases in the brain, individual multidisciplinary assessment of the patient with neurosurgeon, radiotherapist and clinical oncologist is necessary. The summary of management in patients with melanoma with CNS metastases is presented in figure 2.

Conflict of interests: Piotr Rutkowski has received honorariums for lectures and Advisory Board from Novartis, BMS, MSD, Roche, Amgen, Pierre Fabre.

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Review article

Akademia Czerniaka

New developments in the perioperative treatment of melanomas with locoregional advancement

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Surgical intervention is the treatment of choice for patients with melanomas. However, the prognoses of the patients with melanomas at the IIC–IV stage, even after a complete resection of the lesions, is very diverse and, to a great degree, connected with a high risk of disease recurrence. The positive results of the studies in this area have resulted in systemic adjuvant therapy becoming the standard for patients in this group. New methods of systemic treatment – both the molecularly targeted treatment with BRAF and MEK inhibitors (dabrafenib with trametinib) and anti-PD-1 immunotherapy (nivolumab or pembrolizumab) – are already registered in the United States and the European Union. Also the results of the studies concerning the use of preoperative systemic treatment in patients with loco-regionally advanced melanomas seem to be very promising.

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Key words: melanoma, adjuvant treatment, preoperative treatment, BRAF inhibitors, MEK inhibitors, immunotherapy, anti-PD-1

Introduction

Surgical intervention is the treatment of choice in patients with melanomas. However, the prognoses of patients with melanomas at the IIC–IV stage, even after a complete resection of the lesions, is very diverse and, to a great degree, connected with a high risk of disease recurrence [1–5]. Currently, systemic adjuvant treatment after surgical intervention in the group of patients with a high risk of disease recurrence, has become the treatment standard. A novel approach to the treatment of melanomas with locoregional advancement are studies concerning the implementation of a systemic preoperative treatment. Given the combination of the surgical intervention and conservative treatment, the core binding standard should be the management of the disease by multi-specialist teams whose members are experienced in the diagnostics and treatment of patients with melanomas with locoregional and systemic spread.

Neoadjuvant treatment

Neoadjuvant treatment has been growing in significance in cases of borderline resectable tumours or locally advanced locoregional stage III metastases. The results of the II phase trials published in 2018–2019 point out that the use of combined preoperative treatment with BRAF and MEK inhibitors (with the presence of the *BRAF* mutation) or anti-PD-1 immunotherapy in combination with z anti-CTLA-4 treatment, leads to a response to therapy in a significant of patients whilst complete pathological remissions are connected with better prognoses.

The report of Amaria et al. [6] presents the results of neoadjuvant treatment with dabrafenib and trametinib applied in patients with resectable III and IV stage melanomas (with the exception of the metastases in the brain and bones) with the presence of the *BRAF* mutation. This treatment was carried out within II phase clinical trials with a random patient selection.

Seven patients were randomly selected for a standard surgical intervention with possible adjuvant treatment whilst 14 patients – for preoperative treatment with dabrafenib with trametinib, and then (after the resection) for an adjuvant treatment for up to one year. The trial was prematurely terminated on account of a significantly longer event-free survival (EFS) in the neo-/adjuvant arm in comparison with the standard treatment arm. After a follow-up period with the median follow-up of 18.6 months, the rate of patients who survived in the arm which underwent neo-/adjuvant treatment (71%; 10/14) was significantly larger than the rate of patients surviving in the group treated according to standard methods (0). The median EFS was 19.7 months *vs*. 29 months respectively ($p < 0.001$). The neo-/adjuvant treatment with dabrafenib with trametinib was well tolerated. A radical surgical intervention in this group was performed in 12 patients and in 7 cases (58%) a complete pathological response was observed, which also gave better prognoses.

Similar results were obtained in the II phase trial, NeoCombi [7], in which patients in the IIB–C stage with confirmed *BRAF* mutation, received dabrafenib with trametinib for 12 weeks before the resection of metastases and 40 weeks after the surgery. The study comprised 35 patients and in 30 of them (86%), the response to the preoperative treatment, according to the RECIST criteria, was observed, whilst in 17patients (49%) a pathological complete response (pCR) was found. The rate of the 2-year progression-free survival was 43.4%, with better results observed in the group of patients with the complete pathological response.

Two other studies evaluated the use of preoperative immunotherapy. The first of them [8] concerned the application of preoperative nivolumab (up to 4 doses) in comparison with ipilimumab together with nivolumab (up to 3 doses) in 23 patients with resectable stage III or IV melanomas. The treatment with ipilimumab with nivolumab was connected with a high response rate (73%; pCR 45%), yet with significant toxicity (73% adverse events [AE] with grade 3.), whilst monotherapy with nivolumab gave much fewer responses (25%, pCR 25%), yet its toxicity was low (8% AE in stage 3.). The latter study, [9] OpACIN-neo, evaluated the best regime with the use of nivolumab in connection with ipilimumab with the use of randomisation:

- in group A: 2 ipilimumab cycles 3 mg/kg body weight, plus nivolumab 1 mg/kg body weight every 3 weeks;
- in group B: 2 ipilimumab cycles 1 mg/kg body weight, plus nivolumab 3 mg/kg body weight, every 3 weeks;
- in group C: 2 ipilimumab cycles 3 mg/kg body weight, every 3 weeks and then 2 ipilimumab cycles 3 mg/kg body weight, every 2 weeks.

The study comprised 86 patients in stage III of the disease with clinically confirmed metastases in regional lymph nodes. Within the first 12 weeks, immune-related adverse events (irAE), grade 3–4 were found in 40% of patients in group A, 20% in group B, and 50% in group C. Objective radiological response to treatment was obtained in 63% of patients in group A, 57% in group B, and 35% in group C. A pathological response was found in a larger rate of patients than a radiological response. In 57% of patients in group B, pCR was confirmed. None of these groups obtained the median event-free survival (EFS) or median relapse free survival (RFS).

During the follow-up period, symptoms of a relapse were observed in none of the patients. The B regimen seems to be the most preferred option for further studies.

Moreover, in some patients neoadjuvant treatment seems to be more efficient than adjuvant therapy (which might be connected with the activity of the immune system). What is more, this is a short lasting therapy and, as such is cost-effective. This type of treatment allows also for a better prognostic/predictive evaluation and personalisation of the follow-up examinations after therapy; in particular no complete histopathological response was obtained and a patient might require adjuvant treatment (e.g. radiotherapy or targeted treatment with *BRAF* and *MEK* inhibitors after preoperative immunotherapy). In the entire studied patient population group, within the neoadjuvant treatment (table I), the rate of complete pathological remissions was 41% (38% after immunotherapy and 47% after molecularly targeted treatment). This strategy, however, requires further research with the participation of randomly selected patients and a comparison with postoperative adjuvant treatment.

Table I. The most important clinical trial concerning neoadjuvant treatment of melanoma with locoregional spread

Clinical trial	Treatment regime	n	pCR (%)	Median RFS (months)	Median follow-up (months)
Amaria *Lancet Oncol* 2018 [6]	Dabrafenib/trametinib	21	58	19.7	18.6
Long *Lancet Oncol* 2019 [7]	Dabrafenib/trametinib	35	49	23.0	27.0
Blank *Nat Med* 2018 [10]	Ipilimumab + nivolumab	10	33	NR	32
Amaria *Nat Med* 2018 [8]	Nivolumab/ipilimumab + nivolumab	12 11	25 45	NR NR	20
Huang *Nat Med* 2019 [11]	Pembrolizumab	30	19	NR	18
Rozeman *Lancet Oncol* 2019 [9]	Ipilimumab + nivolumab	86	57	NR	8.3

pCR – complete remission in histopathological assessment, RFS – recurrence free survival, NR – not reached

Adjuvant radiotherapy

In individualised cases, after surgical treatment of patients with high-risk melanomas, it is possible to use adjuvant radiotherapy (RTH) – the dosage pattern comprises a hypofractionation at 3–8 Gy/fraction or conventional fractionation depending on the location of the lesions. Indications for adjuvant RTH after resection of the primary tumour may comprise:

- a diagnosis of the desmoplastic melanoma resected with narrow margins,
- the presence of "positive" surgical margins (especially after the resection of local relapse) with the lack of any possibility of radicalisation of surgical intervention,
- the presence of satellite foci,
- sever neurotropism, or:
- location in the area of head and neck (note: RTH is the ole method of treatment and may be applied in the cases extensive lentigo malignant melanoma (LMM).

In the case of the resection of local lesions and lymphadenectomy in the case of metastases in regional lymph nodes, the indications for adjuvant RTH may comprise:

- the presence of extracapsular infiltration of the tumour,
- the involvement of ≥4 lymph nodes (IIIC stage),
- metastases diameter >3 cm,
- metastases were found in the lymph nodes of the neck (from 2 lymph nodes involved with metastases or in the case of metastases size of at least 2 cm),
- a relapse after a previous resection [1, 2, 4, 12].

The results of one completed study with a random selection of patients in which the value of the adjuvant radiotherapy was evaluated (48 Gy in 20 fractions) after a lymphadenectomy in the case of a high risk of melanoma relapse, confirmed the improvement of the local control after the radiation therapy. At the same time, no effect from radiotherapy on the overall survival was observed. That said, an increase in the frequency of distant locoregional complications and a deterioration of the patient's quality of life were observed. These results suggest that the use of adjuvant radiotherapy should be limited [13, 14]. It must also be stressed that there are no indications for RTH undertaken after the completion of a lymphadenectomy (CLND) resulting from the positive result of a sentinel node biopsy.

Systemic adjuvant therapy

Currently, systemic adjuvant therapy is a standard treatment in clinical practice for patients after a radical resection of primary lesions and a lymphadenectomy, whilst adjuvant radiotherapy might be considered solely in the strictly defined cases described above. The results of recently published clinical trials point to an improvement in therapy results through the use of immunotherapy with immune system checkpoint inhibitors as well as combined treatment with BRAF and MEK inhibitors [1–4].

Interferon

For many years, apart from interferon (IFN), no other agents had been effective in the treatment of high risk skin melanomas. Interferon (mainly alfa-2b IFN only in monotherapy) used for adjuvant treatment of patients with melanomas (for a highly selected group) leads to (in a repetitive way) prolongation of the relapse-free survival (RFS) in the majority of patients (table I) [4, 15–19]. However, evidence for the improvement of overall survival (OS) as a result of the use of IFN is much weaker and more controversial. In 10 out of 17 evaluated studies, an improvement in RFS was observed, and the recent results of meta-analysis point to a decrease of the risk of relapse by 17–18% (relative risk [hazard ratio, HR]: 0.82–0.83; $p < 0.0001$) after the use of IFN in adjuvant treatment. Evidence for an improvement in OS comes mostly from meta-analyses and translates into an OS improvement of about 3% within 5 years within the entire patient group. The use of IFN in adjuvant treatment in all patients with high risk melanomas is therefore not justified (especially given a significant toxicity of the treatment) and thus becomes only some option in selected patients.

On the basis of the positive results of one of the three studies carried out by the Eastern Cooperative Oncology Group (ECOG): ECOG 1684, Interferon alfa-2b (IFN α-2b) administered in high doses was registered in the United States and the European Union for the treatment of melanomas in IIB–III stage, whilst in small doses it was registered in the European Union for patients in stage II of the disease. The basis for the registration was the prolongation of the overall survival in a 7-year follow-up period, which, however, was not confirmed after a longer period of time (12 years). The results of the metanalyses show that the basic group for which the adjuvant treatment with IFN is beneficial are patients with an ulcerated primary focus of melanoma, in particular those with metastases which are not clinically overt (former terminology: micro-metastases) and not with clinically overt metastases observed in the enlarged lymph nodes (former terminology: macro-metastases) [17, 18].

Currently, the results of the 18081 study of the European Organisation for Research and Treatment of Cancer are expected, concerning the evaluation of the use of the pegylated IFN form in the treatment of patients after resection of a primary skin melanoma with ulceration without metastases in the regional lymph nodes (the study recruitment was discontinued).

The most frequent adverse effects of IFN comprise of flu-like symptoms, fever, fatigue, neutropenia, hepatoxicity and depression. Some part of the IFN toxicity profile changes within the course of treatment. Together with the length of the treatment, the flu-like symptoms recede whilst others reported adverse events remaining unchanged or even increasing with the length of treatment (mainly: fatigue, anorexia, symptoms of depression/anxiety).

Immunotherapy with immune system checkpoint inhibitors

In 2015 the results of the study became available concerning the use of adjuvant therapy with anti-CTLA-4 antibody (ipilimumab) after a lymphadenectomy due to metastases in the regional lymph nodes (III stage). 951 patients were enrolled in the study, and they were randomised to the group with a high dose of ipilimumab: 10 mg/kg of body mass for 3 weeks and then every 3 months up to 3 years (n = 476) or to the placebo group (n = 476). With the median follow-up period of 2.7 years, 234 events in relation to the RFS in the group with ipilimumab were observed in comparison with 294 events in the group with placebo; the median RFS was 26.1 months versus 17.1 months, respectively (p = 0.0013). The improvement of RFS concerned both the patients with macro-, and micro-metastases (definitions according to the TNM classification binding at that time) in the lymph nodes, and the effect of the adjuvant treatment was more significant with the ulceration of primary focus. In the group treated with ipilimumab, adverse effects occurred in 54% patients with 3–4 toxicity grade in comparison with 25% of patients receiving the placebo. On account of the complications connected with the administration of ipilimumab, 5 patients (1%) died. Adverse effects led to permanent discontinuation of the therapy in 52% patients who had started treatment with ipilimumab [20].

The median follow-up period in this study was 5.3 years. The results pointed to a significant improvement after adjuvant therapy with high doses of ipilimumab, both with regards to the relapse free survival period as well as the distant metastasis free survival and OS. The rate of the 5-year OS in the group receiving ipilimumab was 65.4% in comparison with 54.4% in the group with the placebo, the hazard ratio (HR) for death was z 0.72; 95.1% and the confidence interval (CI) 0.58–0.88; p = 0.001) [21].

The preliminary results of another study (E1609) showed a similar efficacy of a lower dose of ipilimumab (3 mg/kg of body mass) with lower toxicity. The EORTC 18071 study resulted in the registration of ipilimumab in the United States in the adjuvant treatment of patients with melanomas after a lymphadenectomy on account of the metastases in regional lymph nodes. However, the practical application of this therapy is limited because of its high toxicity and the fact that the trials with the anti-PD-1 antibodies (nivolumab and pembrolizumab) and kinase inhibitors gave more beneficial results (table II).

The study CheckMate 238 with a random selection of patients in clinical IIIB, IIIC and IV stages after resection of metastases, showed that after one year of treatment with po nivolumab, recurrence-free survival improved by 10% in comparison to treatment with ipilimumab; nivolumab showed a lower toxicity than ipilimumab (18-month RFS: 65% *vs.* 53%) [22]. This was the only study where patients after the resection of distant metastases were included. Moreover, there was an improvement in the distant metastases free survival (DMFS): HR 0.73). Adverse events, in the 3 or 4 grade, connected with the treatment were observed in 14.4% of patients receiving nivolumab in comparison with 45.9% in the group treated with ipilimumab [23]. The update of the data from 2018 with the longer follow-up period confirmed the beneficial effects of nivolumab in the year-long adjuvant treatment irrespective of the PD-L1 expression status and BRAF mutation in reference to RFS (HR 0.66) and DMFS (HR 0.76) [17]. Nivolumab is currently registered for adjuvant treatment in the United States and the European Union.

The results of the Keynote-054/EORTC 1325 study with the participation of 1019 patients point to a decrease in the risk of recurrence (HR for RFS 0.57) and DMFS after one year of adjuvant treatment with pembrolizumab in comparison with the placebo in the group of patients in stage III, characterised

Table II. The summary of the most recent clinical studies concerning adjuvant treatment after the resection of melanoma with high recurrence risk

	EORTC 18071 ipilimumab *vs.* placebo	**BRIM-8 vemurafenib *vs.* placebo**	**COMBI-AD dabrafenib + trametinib *vs.* placebo**	**CheckMate 238 IPI *vs.* NIVO**	**EORTC 1325/ /Keynote 054 pembrolizumab *vs.* placebo**
Author	Eggermont 2015 [21] Eggermont 2016 [22]	Maio 2018 [29]	Long 2017 [26] Hauschild 2018 [27]	Weber 2017 [22, 23]	Eggermont 2018 [24]
Population	IIIA (>1 mm), IIIB, IIIC	IIC, IIIA, IIIB, IIIC	IIIA (>1 mm), IIIB, IIIC	IIIB, IIIC, IV	IIIA (>1 mm), IIIB, IIIC
BRAF mutation	?	100%	100%	41%/43%	
RFS	41% *vs.* 30% (5 years)	82% *vs.* 63% (12 months); 62% *vs.* 53% (24 months); 79% *vs.* 58% (12 months); 46% *vs.* 47% (24 months) IIIC; 84% *vs.* 66% (12 months); 72% *vs.* 56% (24 months) IIC–IIIB	67% *vs.* 44% (2 years) HR = 0,47; 58% *vs.* 39% (3 years)	66% *vs.* 53% (18 months); 62.6% *vs.* 50.2% (24 months) HR 0.66 HR 0.65	HR 0.57; difference after 18 months 18.2%: 71.4% *vs.* 53.2%
OS	65% *vs.* 54% (5 years) HR = 0.72	BD	91% *vs.* 83% (2 years); 86% *vs.* 77% (3 years) HR = 0.57	BD	

OS – overall survival , RFS – recurrence free survival, BD – no data

with a higher risk (i.e. stage IIIA with the micro-metastasis size >1 mm, IIIB and IIIC) [24]. A re-classification with reference to a new classification of stage III according to AJCC (eighth edition) confirms the benefits with respect to RFS (test for interaction: p = 0.68) after one year of treatment with pembrolizumab in comparison with the placebo (excluding IIIA stage), respectively:

- IIIB stage (79.0% *vs.* 65.5%; HR 0.59 [99% CI 0.35–0.99]),
- IIIC stage (73.6% *vs.* 53.9%; HR 0.48 [99% CI 0.33–0.70]),
- IIID stage (50.0% *vs.* 33.3%; HR 0.69 [0.24–2.00]) [25].

Currently there is an ongoing study comparing the use of nivolumab and the combination of nivolumab with ipilimumab in adjuvant treatment (CheckMate 915).

Molecularly targeted therapy

Adjuvant therapy with the use of dabrafenib with trametinib in the group of high risk stage III patients with *BRAF* mutation, showed an improvement of RFS (HR 0.47), DMFS (HR 0.51; 91% *vs.* 70% after one year, 77% *vs.* 60% after 2 year and 71% *vs.* 57% after 3 years) and OS (HR 0.57) in comparison with the placebo. In this study (COMBI-AD), dabrafenib in combination with trametinib were used for a year in comparison with placebo (IIIA stage with the metastasis size >1 mm, IIIB/C) [26]. The benefits in treatment with dabrafenib in combination with trametinib were observed in all the analysed subgroups. The update of the data from the 4-year follow-up periods confirm the benefits of treatment with dabrafenib in combination with trametinib (RFS: 54%; HR: 0.49; DFS: 67%; HR: 0.53) [27]. Moreover, the model evaluating the cure rate of patients treated with adjuvant therapy in this case makes up as much as 17% [27]. The safety profile of dabrafenib in combination with trametinib was compliant with the profile observed in the study comprising patients with melanoma in stage IV, but the entire treatment was relatively well tolerated (although 26% patients discontinued treatment) [28].

Formally, a "positive" clinical study BRIM-8 [29] also concerned the application of vemurafenib in monotherapy in adjuvant treatment (in comparison with the placebo). This treatment was applied in stage IIC–III patients treated for melanoma after resection (this has so far been the only study comprising patients with stage II melanoma). The median disease-free survival (DFS) was 23.1 months in the group treated with vemurafenib, in comparison with 15.4 months in the group with the placebo (HR 0.8; p = 0.026), yet this effect was limited solely to the subgroup with tumour stage IIC–IIIA–IIIB, and was not observed in patients with more advanced melanomas (IIIC). At the same time, we can observe from the current experiments carried out with patients with metastatic melanomas with the BRAF mutation , that monotherapy with BRAF inhibitors is not the optimal treatment method in comparison with the combined treatment with BRAF and MEK inhibitors for these patients.

Conclusions

The summary of the results of systemic adjuvant treatment with the use of immunotherapy after the resection of high-risk melanoma is presented in table II. Other methods of immunotherapy (e.g. interleukin 2), vaccinations or cytotoxic medication do not have any application in post-operative adjuvant treatment [1, 4, 5, 30].

To sum up, in accordance with Polish and American recommendations [2, 4, 31] adjuvant treatment with anti-PD-1 immunotherapy with (nivolumab or pembrolizumab) or combined treatment BRAF and MEK inhibitors (dabrafenib in combination with trametinib for the patient population with the *BRAF* mutation) has become a new therapeutic standard for patients after resection of melanomas with a high recurrence risk (resection stages IIIA–IV). This, in turn, leads to the fact that the cases of all patients with melanomas in stages from IIIA to IV should be discussed at multi-specialist team meetings so as to guarantee patients optimal, modern, and as effective as possible treatment options. Additionally, it must be remembered that high risk melanomas should be included into prospective clinical trials concerning new methods of adjuvant treatment.

Conflict of interests: Piotr Rutkowski has received honorariums for lectures and Advisory Board from Novartis, BMS, MSD, Roche, Amgen, Pierre Fabre.

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Review article

Akademia Czerniaka

New pathomorphological classification of melanomas

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Melanoma is a neoplasm whose biology we are getting to know better and better. The consequence of this is the latest edition of "WHO Classification of Skin Tumours 4th edition, 2018". The division presented in this paper takes into account the character of growth and location of melanoma, but also results from the analysis of the most frequent mutations occurring in this neoplasm. The assessment of the stage of melanoma progression, based on two most important prognostic microscopic features, i.e. the depth of infiltration and the presence or absence of ulceration, remains valid.

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Key words: melanoma, TNM classification, mutations

Melanoma, from a clinical and pathological point of view, makes up a heterogenous disease entity. The basis for the classification of this tumour was worked out by W.H. Clark [1] and V.J. McGovern [2] in the 1970s. The classification which they proposed presents melanoma as a melanocytic lesion, which, within its development, undergoes progression. The first stage is defined as melanoma in situ. This type of melanoma is characterised by the presence of atypical melanocytes:

- melanocytes, located on the entire thickness of the epidermis, are irregularly placed, creating pagetoid-like groups (superficial spreading melanoma in situ); or:
- in the basal layers of epidermis, placed in a linear and lentiginal way (lentigo melanoma in situ).

In the case of melanoma in situ and/ or accompanying melanocyte infiltration in the upper layers of the skin, the prognosis is defined as very good. The phase of melanoma growth is defined as the radial growth phase (RGP). It precedes the melanoma's progression into the skin, a process which consists in transgressing the basal membrane of the epidermis and an infiltration into the lower skin layers with the creation of a nodule. This stage is defined as the vertical growth phase (VGP). It is connected with the progression of a lesion and has a poor prognosis.

The creators of the most recent classification of skin tumours, the "WHO Classification of Skin Tumours 4th edition, 2018" [3] point to two basic types of melanoma: melanomas with the radial phase and those which since their onset begin to develop in a vertical way. The first group comprises: a superficial spreading melanoma and a malignant lentigo. The other group comprises nodular melanoma, which has only a vertical phase and naevoid melanoma, which usually does not have a radial phase.

The above listed melanoma groups differ from one another in terms of their ontogenetic mechanisms, and the genetic changes occurring within them as well as their clinical picture. The main ontogenetic mechanism is the damage caused by the UV radiation connected with exposure to the sun (or artificial UV radiation). High-energy UVB rays, which make up 5% of the radiation which reaches the Earth, penetrate the skin, damaging the epidermis and causing tumours.

The most recent WHO classification proposes the division into the skin melanomas based on the factors which cause the disease:

- significant damage to the skin resulting from a cumulative dose of sun radiation (high cumulative skin damage, high-CSD melanoma);
- and slight damage to the skin caused by a small or sporadic UV exposure (low cumulative skin damage, low-CSD melanoma).

The first group of melanomas contains a large number of punt mutations; in particular the mutations in the following genes are typical: *NF1*, *NRAS*, *BRAF* (other than p. V600E), KIT (MAPK activation pathway), TP53. Melanomas of the skin which have been chronically exposed to sun (high-CSD melanoma/melanocytic tumours in chronically sun-exposed skin) comprise: lentigo malignant melanomas and desmoplastic melanomas. In the low-CSD melanomas, the mutation in the codon 600 *BRAF* gene is dominating (*BRAF* p. V600E). This group is also comprised of the low-CSD melanoma/superficial spreading melanoma.

There is also a group of melanomas that have no connection to UV exposure. These are: acral melanoma, mucosal melanoma, uveal melanomas and Spitz melanomas. In these melanomas, the following gene mutations are detected: *HRAS* (Spitz melanoma), *KIT*, *NRAS*, *BRAF*, *HRAS*, *KRAS*, *ALK*, *NTRK3* (acral melanoma), *KIT*, *NRAS*, *KRAS* (mucosal melanoma) and *GNAQ*, *GNA11*, *CYSLTR2* (uveal melanoma).

Moreover, in all three types of these melanomas, there is a mutation of the CDKN2A gene, coding p16 protein which performs the function of a tumour suppressor within a cell. The loss of p16 expression in the immunohistochemical reaction is a proof of the presence of melanoma, and this is why the quantification of this protein is used in histopathological deferential diagnosis [4, 5]. A detailed list of genetic changes occurring in specific types of melanocyte proliferations is presented in figure 1.

Apart from the above-listed mutations, high-CSD melanomas contain a very high mutation burden, whilst, for example, in acral melanomas and mucosal melanomas the mutation burden is low, and in uveal melanomas – even lower. Amongst many genetic anomalies, the mutations of the *BRAF* V600E and *C-KIT* genes have predictive significance – and for this reason the tissue material containing the cell pattern of the primary or metastatic melanoma is assessed with regards to the presence of these mutations.

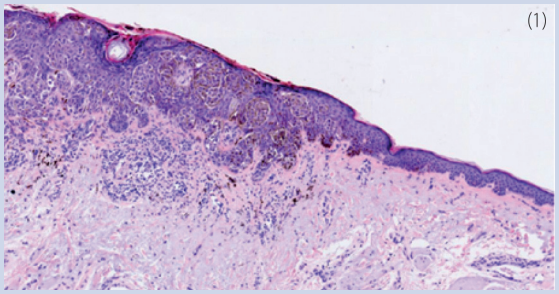
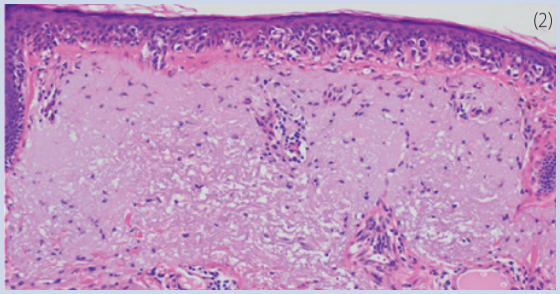
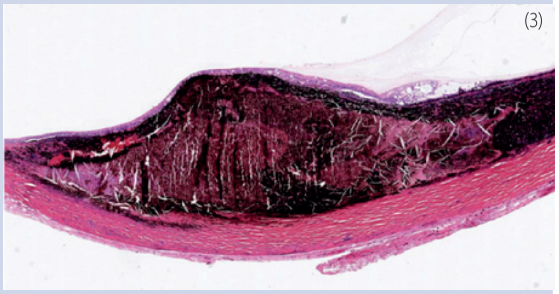
Another difficult group with respect to diagnostics comprises melanocytic lesions of the Spitz type, with their malignant form being Spitz melanoma/malignant Spitz tumour). A malignant form of melanocytic proliferations of the skin of the limbs is subungual melanomas of the limbs. Other types which have been distinguished are mucosal melanomas/genital, oral, sino-nasal melanomas), including mucosallentiginous melanomas and mucosalnodular melanomas. Melanomas

Table I. Division of the tumours arising from melanocytes according to the WHO classification from 2018

Melanocytic tumours in intermittently sun-exposed skin
low-CSD melanoma (superficial spreading melanoma)
simple lentigo and lentiginous melanocytic naevus
junctional naevus
compound naevus
dermal naevus
dysplastic naevus
naevus spilus
special-site naevi (of the breast, axilla, scalp and ear)
halo naevus
Meyerson naevus
recurrent naevus
deep penetrating naevus
pigmented epithelioid melanocytoma
combined naevus, including combined BAP1-inactivated naevus/ melanocytoma
Melanocytic tumours in chronically sun-exposed skin
lentigo maligna melanoma
desmoplastic melanoma
Spitz tumours
malignant Spitz tumour (Spitz melanoma)
Spitz naevus
pigmented spindle cell naevus (reed naevus)
Melanocytic tumours in acral skin
acral melanoma
acral naevus

Genital and mucosal melanocytic tumours
mucosal melanomas (genital, oral, sinonasal) mucosal lentiginous melanoma mucosal nodular melanoma
genital naevus
Melanocytic tumours arising in blue naevus
melanoma arising in blue naevus
blue naevus NOS
cellular blue naevus
mongolian spot
naevus of Ito
naevus of Ota
Melanocytic tumours arising in congenital naevi
melanoma arising in giant congenital naevus
congenital melanocytic naevus
proliferative nodules in congenital melanocytic naevus
Ocular melanocytic tumours
uveal melanoma epithelioid cell melanoma spindle cell melanoma, type A spindle cell melanoma, type B
conjunctival melanoma melanoma NOS
conjunctival primary acquired melanosis with atypia/melanoma in situ
conjunctival naevus
Nodular, naevoid and metastatic melanomas
nodular melanoma
naevoid melanoma
metastatic melanoma

Figure 1. Melanoma classification on the basis of the WHO recommendation from 2018 with reference to genetic changes

	Low UV exposure				High UV exposure				Low/ no/ varied UV exposure			
Type of malignant lesion	Low-CSD melanoma/superficial spreading melanoma				Melanomas chronically UV-exposed skin/ lentigo malignant melanomas	Desmoplastic melanoma	Malignant Spitz tumour (Spitz melanoma)	Subungual melanoma	Mucosal melanoma	Melanoma with congenital naevus	Melanoma with blue naevus	Uveal melanoma
	low-CSD melanoma/ superficial spreading melanoma – vertical growth phase [1]	combined BAP1-inactivated naevus/ melanocytoma	deep penetrating naevus	pigmented epithelioid melanocytoma								
Examples of histopathological images	 (1)				 (2)				 (3)			
Most frequent genetic changes												
BRAF p.V600e	+									+		
BRAF		+	+	+		+	+	+	+	+		
BRAF non-p.V600e					+							
NRAS	+	+	+		+	+		+	+			
HRAS							+	+				
KIT					+			+	+			
NF1					+	+		+	+			
CDKN2A	+				+		+	+	+			
TP53 and *PTEN*	+				+							
BAP1		+									+	+
TERT	+				+	+		+				
GNAQ, GNA11, CSLTR2											+	+

The most frequently occurring genetic changes are colour-coded: red – mutation of acquiring function, blue – mutation of the loss of function, purple – rearrangement, grey – promotor mutation, orange – amplification

can also be a malignant form of tumour arising in the blue naevus or rising in giant congenital naevus. A separate group is made by ocular melanocytic tumours, comprised of uveal melanomas and conjunctival melanomas. The last group is comprised of nodular melanomas, naevoid melanomas, and metastatic melanomas. The current classification of melanocytic proliferations is presented in table I.

The above classification specifies melanocytic proliferations in a traditional way, dividing them into benign and malignant lesions. Yet, as is the case with other tumours (for examples ovarian tumours or soft tissue carcinomas), the authors of the current classification "legalise" the terms which were previously used by dermatologists to describe the lesions with uncertain malignancy potential. This is the outcome of a belief that it is not always possible to definitely determine the potential of a lesion malignancy on the basis of morphologic features, immuno-profiling, and genetic changes.

The WHO classification from 2018 presents definitions and terms used for the description of melanocytic tumours of uncertain malignant potential (MELTUMP). Atypical melanocyte proliferation in the skin means that a lesion has the potential for vertical growth (tumorigenic), yet there are no definite criteria which would allow one to determine whether this lesion is benign or malignant. Also superficial atypical melanocytic proliferations of unknown significance *(*SAMPUS*)* were defined as atypical melanocytic proliferations localised in the epidermis and upper layer of the skin. Such a lesion cannot be definitely specified on the basis of a microscopic image, neither can the melanoma radial phase be excluded. In other words, SAMPUS is an atypical proliferation of pigment cells with the thickness of 0.8 mm, without ulceration in which deep maturation and symmetry are difficult to determine (which is understandable); also this proliferation lacks other typical morphological features typical for melanoma, such as mitotic activity. From a practical point of view, the therapeutic approach in both forms of melanocytic lesions is identical and consists of enlarging the surgical margin (the so-called wide resection of the scar). A differential diagnosis of SAMPUS is very difficult, especially when a skin specimen does not contain the entire lesion, is not optimally fixed or if there are some features of regression. It must be remembered that both "over-diagnosing" and "under-diagnosing" melanoma may lead to serious legal consequences for a pathomorphologist.

In the case of MELTUMP, there is always a chance that this is an atypical malignant proliferation of melanocytes which is potentially capable of producing metastases, and even life--threatening for a patient. To sum up, the term, "uncertain significance" in reference to the lesions of the SAMPUS or IAMPUS type (intraepidermal atypical melanocytic proliferation of uncertain significance) means only the possibility of a relapse or progression whilst the term "uncertain malignancy potential" in the case of MELTUMP means that a malignant course of the disease cannot be excluded. A differential diagnosis of MELTUMP always comprises a melanoma and histopathological assessment and should always contain a statement that, for example, this is "a lesion intermediate between a blue naevus and melanoma arising from a blue naevus".

The above diagnoses are descriptive and provisional, which means that one must always try to establish a precise and definite histopathological diagnosis. In a differential diagnosis, apart from a thorough microscopic assessment of the specimen routinely dyed with haematoxylin-eosin, the authors of the most recent classification recommend the use of immunohistochemical reactions, including HMB45, Ki-67 and p16. Immunohistochemical loss of the p16 protein usually signifies melanoma, yet in some cases, in which CDKN2A deletion does not occur within the melanoma ontogenetic pathway, a strongly preserved reaction with p16 is seen [7]. *BRAF* and *NRAS* gene mutations, frequently present in melanoma, are unfortunately also present in benign lesions. Therefore they do not have any diagnostic significance.

According to the 8th edition of the classification of pathological stages of melanoma (pTNM) worked out by the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) from 2017, the assessment of a melanoma stage is based on two microscopic properties with the largest prognostic significance, i.e. the depth of infiltration and the presence of ulceration. The evaluation of an additional factor in pT1, i.e. mitotic activity in the vertical phase (present in the previous, 7th edition of the classification pTNM/AJCC/UICC) was abandoned. It must be emphasised that the correlation between mitotic activity and the frequency of metastases formation in lymph nodes was shown [6]. Yet, in comparison with the previous edition, the presence of the figures of cellular division in the vertical phase of thin melanomas does not mean the change of tumour stage from pT1a to pT1b. In spite of this, mitotic activity still remains a significant prognostic factor and should form a part of the histopathological diagnosis. Currently the "demarcation point" for thin melanomas is regarded to be a depth of 0.8 mm and with a lack of ulceration. From a clinical point of view, these lesions are treated as locally advanced and do not require the sentinel node procedure to be performed.

Table II. Primary melanoma staging with regards to T feature

pT	Lesion depth according to Breslow's classification
pT1a	Infiltration depth ≤0.8 mm, no ulceration
pT1b	Infiltration depth ≤0.8 mm, ulceration (+) or infiltration to the depth of 0.8–1 mm
pT2a	Infiltration depth >1–2 mm, ulceration (–)
pT2b	Infiltration depth >1–2 mm, ulceration (+)
pT3a	Infiltration depth >2–4 mm, ulceration (–)
pT3b	Infiltration depth >2–4 mm, ulceration (+)
pT4a	Infiltration depth >4 mm, ulceration (–)
pT4b	Infiltration depth >4 mm, ulceration (+)

Table II presents the stages of the primary melanoma according to the 8th edition of pTNM AJCC/UICC classification from 2017.

According to this classification, the pN stage specifies the melanoma metastases in lymph nodes (irrespective of their size and the number of tumour cells), microsatellite foci, satellite or in-transit metastases in lymph node(s) above 0 (pN > 0). In order to make a credible evaluation of lymph node status, at least six lymph nodes must be assessed. Not finding the melanoma metastasis in a lower number of the examined lymph nodes must also be classified as pN0 (like in the case of the evaluation of six or more lymph nodes). If no complete lymphadenectomy was performed, the histopathological report should contain a note that the classification is based only on the microscopic assessment of the sentinel node(s) – for example: pN0 (sn).

The current classification of the pN stage distinguishes the patients with clinically occult metastases. Such lesions, in a situation when no microsatellite or satellite foci or in transit metastases are found, are classified as N1a, N2a, N3a stage – depending on the number of the lymph nodes. When the above satellite foci or in transit metastases are present, yet without the metastases in the lymph nodes, the pN stage is qualified as N1c, N2c, N3c respectively – depending on the number of lymph nodes involved. But in the case of clinically evident metastases in the lymph nodes and without the presence of microsatellite or satellite foci or in transit metastases, the pN stage is evaluated to be pN1b, pN2b, pN3b – depending on the number of lymph nodes involved. In the 7th and 8th classification of TNM AJCC/UICC [8], the N stage is evaluated differently. However, the detection of a distant melanoma metastasis in a microscopic assessment is marked with the M1 symbol – as in the previous classifications.

The most recent WHO classification of skin cancers, similarly to the previous editions, emphasises the role of microscopic assessment. This classification presents detailed criteria, definitions, and terms used in the daily histopathological practice of assessing skin cancers, including melanoma. The pTNM AJCC/UICC classification, takes into consideration the role of the pathomorphological examination. This classification specifies the tumour stages based on significant prognostic factors. The update of the histopathological WHO classification and of the pTNM AJCC/UICC stages is the outcome of the developments in the studies of melanoma pathogenesis and epidemiological data.

All the microscopic parameters (apart from the histological type of melanoma) which have prognostic significance and which are useful for the selection of the treatment method, and which need to be obligatorily evaluated and stated in the histopathological report comprise: the depth of infiltration, the presence of ulceration and microsatellite or satellite foci or in-transit metastases. They determine the pTNM AJCC/UICC tumour stage.

Significant progress in access to new therapeutic methods of targeted therapies has been made in recent years; this has contributed to the increase in the importance of molecular tests – not only in the understanding of the process of oncogenesis, but also in the detection of predictive factors in personalised therapies. Therefore, a pathomorphological report should consider significant microscopic prognostic factors and predictive molecular markers.

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Review article

Akademia Czerniaka

Management of metastases in regional lymph nodes in melanoma patients in 2019

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For several years, the standard of management in case of melanoma metastases in regional lymph nodes was to remove an adequate node group. In 2016 and 2017, the results of two large, well-designed clinical trials with randomization and a control group were published, which changed the current management. The authors of DeCOG-STL study came to the conclusion that withdrawal from completion lymph node dissection in the case of a small melanoma metastasis in a sentinel lymph node (metastasis diameter ≤1 mm) is not associated with a worsening of the 3-years' survival chance (both in terms of overall survival and survival time to the occurrence of distant metastases). The results of MSTL-II study were similar. Based on the results of both studies presented above, in 2018 the American Society of Clinical Oncology (ASCO) and the Society of Surgical Oncology (SSO) presented joint recommendations concerning, among others, current indications for completion lymph node dissection in SNB positive melanoma patients.

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Key words: melanoma, SNB, completion lymphadenectomy

Introduction

For several years, the standard of management in case of melanoma metastases in regional lymph nodes (both clinically/cytologically confirmed and by means of sentinel lymph node biopsy) was to remove an adequate node group. For example, the recommendations of Sociedad Española de Oncología Médica (SEOM) formulate this principle as follows: "lymph nodes must be completely removed when there is a metastasis in a sentinel lymph node or when there is a clinical finding of metastasis (i.e. degree IIIB or IIIC) [1]." SEOM described the strength of this recommendation as A (strong) and the evidence base as 2A. Therefore, the scientific premises for such a procedure at the time of publication of the recommendation did not raise any doubts. This strategy was unanimously confirmed by the recommendations of other organizations, including the national recommendations of the Polish Society of Clinical Oncology [2].

In 2016 and 2017, the results of two large, well-designed clinical trials with randomization and a control group were published, which changed the current management and resulted in the content modification of clinical recommendations, both global and national [3, 4]. The above mentioned studies were based on data available in the medical literature suggesting that completion lymph node dissection (CLND) – i.e. lymphadenectomy following the confirmation of metastasis in a sentinel lymph node – in a certain group of patients does not bring any additional benefit in terms of total survival time compared to therapeutic lymph node dissection (TLND) [5]. Moreover, it has been observed in both small and large groups of patients that clinical practice differs significantly from the academic canon in the case of e.g. metastases in the sentinel lymph node [6, 7]. For example, in a group of approximately 125 000 melanoma patients undergoing a sentinel lymph node biopsy in the USA (2002–2012), metastasis in this node was

found in approximately 25 000 patients. However, completion lymph node dissection was performed only in about 13 000 patients, which accounted for slightly more than half (56%) of all patients in whom the procedure should be performed according to the commonly accepted indications [7].

DeCOG-SLT study

The first study, which changed clinical practice, was designed in Germany and conducted at 41 skin cancer treatment centers there, between 2006 and 2014 [3]. The study included 483 patients with melanoma of the trunk or a limb with a metastasis in the sentinel lymph node (selection criteria are presented in table I).

The study participants were randomly assigned to two compared groups: 242 patients were qualified for completion lymph node dissection and 241 for follow-up with strict ultrasound control of the relevant nodal group. It should be emphasized that about 2/3 of the participants had a small metastasis in the sentinel lymph node – a diameter ≤1 mm. The median of the follow-up period was 35 months. The percentage of patients surviving 3 years without distant metastases was 77.0% (90% confidence interval – CI: 71.9–82.1) in the group of patients under follow-up and 74.9% (95% CI: 69.5–80.3) in the group of patients undergoing completion lymph node dissection. The total percentage of patients surviving 3 years was 81.7% (90% CI: 76.8–86.6) in the observation group and 81.2% (95% CI: 76.1–86.3) in the completion lymph node dissection group. The small percentage differences between the two endpoints were not significant. The authors of the study – noting its weakness resulting from insufficient number of participants in relation to the intended number (underpowered) – came to the conclusion that withdrawal from completion lymph node dissection in the case of a small lesions of melanoma metastasis in a sentinel lymph node (metastasis diameter ≤1 mm) is not associated with a worsening of the 3-years' survival chance (both in terms of overall survival and survival time to the occurrence of distant metastases). In a non-inferiority study, this conclusion seems to be justified [3].

MSLT-II study

The second of studies mentioned above, MSLT-II, was conducted mainly in the USA between 2004 and 2014 with a similar patient group as in the German study. A significant difference between the two studies was the fact that MSLT-II also included patients with scalp and neck melanoma [4]. The study was multi-center in nature, it was conducted with randomization and a control group. The objective of the study was to compare the results of completion lymph node dissection after excision of sentinel node containing melanoma metastasis with exclusive follow-up (without completion lymph node dissection). It is worth noting that the median size of the metastatic lesion in the sentinel lymph node was about 0.65 mm in study participants and in over 2/3 of patients the size of the metastatic lesion did not exceed 1 mm. After 3 years there were no significant differences between the compared groups in terms of survival time, including melanoma specific survival (86.13% *vs.* 86.12%; p = 0.43). The authors of this study observed a borderline significance (p = 0.05) in terms of the percentage of patients surviving 3 years without symptoms of the disease in favor of the group undergoing completion lymph node dissection (68% *vs.* 63%), which resulted from better local control after that time in the group of patients undergoing lymphadenectomy (92% *vs.* 77%; p < 0.001). At the same time, the authors demonstrated several times higher

Table I. Selection criteria for the DeCOG-SLT study

Inclusion criteria	Exclusion criteria
Primary skin melanoma of the trunk or limb	Melanoma located within the head and neck
Patient age: 18–75 years	Satellite tumors/*in transit*
Melanoma thickness according to Breslow ≥1 mm	M1
SLB + (micrometastasis and isolated neoplastic cells)	Macrometastasis

SLB + – positive result of sentinel node biopsy; M1 – current distant metastases (M parameter according to TNM)

Table II. Results of DeCOG-SLT and MSLT-II studies, in which the CLND was compared with exclusive follow-up after a sentinel node biopsy and metastasis confirmation

Study	Number of patients	Median time of observation	Results (follow-up *vs.* CLND)
Leiter et al. DeCOG-SLT [3]	483	34 months	OS HR 1.02, p = 0.95 10-year OS 62.6% *vs.* 61.9% RFS HR 0.959 DMFS HR 1.19 10-year DMFS 55.8% *vs.* 55.5%
Faries et al. MSLT-II [4]	1755	43 months	MSS HR 1.08, p = 0.42 DMFS HR 1.1 follow up 63% *vs.* DFS CLND 68%

CLND – completion lymph node dissection; DFS – disease-free survival; DMFS – distance metastases-free survival; HR – hazard ratio; OS – overall survival; RFS – relapse-free survival; MSS – microsatellite stability

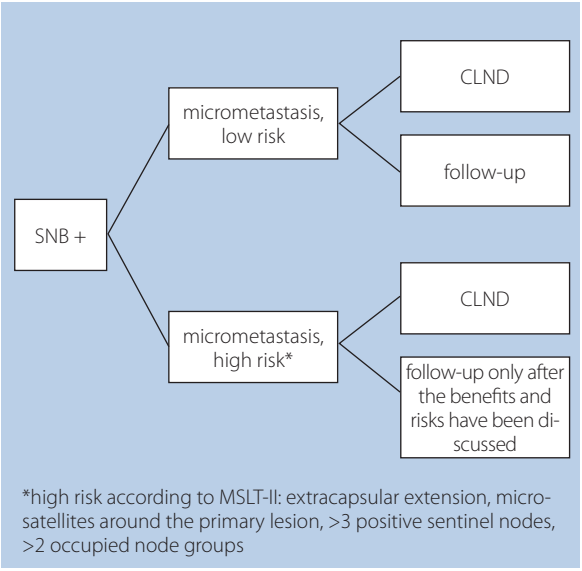

Figure 1. Management in case of positive results of sentinel node biopsy in melanoma patients (based on ASCO and SSO 2018 common recommendations [8])

risk of lymphedema in the group undergoing lymphadenectomy compared to the group undergoing only sentinel node biopsy and follow-up (24.1% *vs.* 6.3%; $p < 0.001$). The authors of the MSLT-II study concluded that completion lymph node dissection increases the percentage of local control, but does not improve survival by taking into account the cause of death. However, it contributes to a significant increase in the incidence of a serious complication, which the limb's lymphoedema. Therefore, they recommended limiting the indications to completion lymph node dissection in patients with clinical characteristics that corresponded to the characteristics of the study participants (mainly low metastatic mass in the sentinel lymph node) [4].

Table II presents a summary of the results of both studies – DeCOG-SLT and MSLT-II. Both cited studies confirmed the basic prognostic role of sentinel node biopsy.

Summary

Based on the results of both studies presented above, in 2018 the American Society of Clinical Oncology (ASCO) and the Society of Surgical Oncology (SSO) presented joint recommendations concerning, among others, current indications for completion lymph node dissection [8]. The course of action suggested in these recommendations is presented in figure 1.

However, clinical follow-up as a management option may be used only in patients with a small metastatic lesion in a sentinel lymph node (metastasis diameter does not exceed 1 mm), not burdened with other prognostic factors that may increase the risk of melanoma metastases in non-sentinel lymph nodes (metastatic lesion diameter in a sentinel lymph node, number of occupied sentinel lymph nodes, thickness/presence of ulceration in the primary lesion) [9].

Also in the Polish recommendations on melanoma published in 2017 and 2019, the follow-up with a strict ultrasound monitoring of the lymphatic flow area after a sentinel lymph node biopsy, which confirmed the presence of a small melanoma metastasis, was presented as an acceptable course of action [10, 11]. The authors of joint ASCO and SSO recommendations emphasize that in the case of follow-up, strict ultrasound supervision over regional lymph nodes is necessary every 4–6 months (strength of recommendation according to ASCO – strong) [8].

In clinical practice, the role of completion lymph node dissection is gradually reduced and individualized, however, each patient who has not undergone this procedure must be subject to strict supervision, including ultrasound evaluation of regional lymphatic flow every 3-4 months. Moreover, patients should be consulted with regard to the possibility of implementing systemic complementary treatment [11]. This issue is described in another article in this issue of *Nowotwory*.

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Review article

Merkel cell carcinoma (MCC) – neuroendocrine skin cancer

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Merkel cell carcinoma (MCC) is a rarely occurring skin cancer of high malignancy. It develops, most probably, from the neuroendocrine cells (Merkel's cells). The most frequent location of this cancer is the skin of the head and neck (44–48% of cases), and then in the skin of the upper limbs (about 19% of cases) and then the lower limbs (16–20% of cases). The aetiology of this cancer is unknown, yet some role in its pathogenesis is played by ultraviolet light and immunosuppression. The basis of therapy in cases with locoregional spread is surgical intervention, whilst in more advanced cases, an effective systemic treatment is possible with the use of molecularly targeted therapies. This paper presents the current treatment possibilities in patients with Merkel cell carcinoma.

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Key words: Merkel cell carcinoma, diagnostics, surgical treatment, systemic treatment

Introduction

Merkel cell carcinoma (MCC) is a rarely occurring skin cancer of high malignancy. It develops, most probably, from the neuroendocrine cells (Merkel's cells) [1, 2].

The incidence of MCC is low, evaluated to be 0.25––0.32/100 000 people per year. The prevalence is higher among men than women (at a ratio of 1.5:1). This cancer is markedly more common in representatives of the white race than in other races. The risk of developing MCC increases with age – the frequency of MCC in patients below 50 years of age is very low. The most frequent location of this cancer is the skin of the head and neck (44–48% of cases), the skin of the upper limbs (about 19% of cases and then the lower limbs (16–20% of cases) [3, 4]. Merkel cell carcinoma very rarely develops within the mucous membranes. There are also patients in whom – though with a lack of detectable primary focus – the metastases of Merkel cell carcinoma are found in the lymph nodes [5]. According to some findings, such cases may account for 10%–15% of all MCC cases. Observational studies in the USA population seem to suggest that the incidence of Merkel cell carcinoma is increasing, which may be connected with the ageing population and be an outcome of developments in histopathological diagnostics [6].

Aetiology

The aetiology of this cancer is unknown though there are well identified factors which predispose for MCC. These factors comprise first and foremost:

- Exposure to ultraviolet irradiation (UV) – whether natural or artificial, e.g. after treatment for psoriasis with the use of phototherapy and psolaren ultraviolet A – PUVA) [7, 8];
- Immunocompromising diseases, such as:
  a) HIV/AIDS infection (the risk of developing MMC is increased 11-fold) [9],
  b) immunosuppression after an organ transplant (the risk of developing MMC is increased 5-fold) [10, 11],
  c) chronic lymphocytic leukaemia;
- Some viral infections, with the most significant being a *polyoma* infection – the type characteristic for MCC: Merkel cell polyomavirus (MCPyV) [12, 13]. The role of MCPyV in the pathogenesis of MCC is unclear. Viral DNA is detected in 60–80% people affected with MCC. At the same time,

in people with the confirmed presence of the virus, longer overall survival is observed in comparison with the group of patients without the viral infection [12, 14].

Diagnostics

Merkel cell carcinoma (MCC) most frequently takes the form of a relatively rapidly expanding tumour or solid infiltration on the skin, often of a red to violet colour. Ulceration is rare. Sometimes the tumour spreads quickly through the pathways of local lymphatic vessels, which in turn leads to the development of satellite foci. The tumour is not usually accompanied by any disorders – it is painless in the majority of cases [15]. This unspecific clinical picture has the effect that MCC is rarely suspected before the result of a histopathological assessment of material from an excisional biopsy or specimen.

In English-language publications, a mnemotechnical acronym has been proposed to facilitate MCC diagnostics – AEIOU:

A – asymptomatic;
E – expanding rapidly;
I – immunosuppressed;
O – older than 50 years;
U – UV-exposed skin.

Only 7% of MCC patients meet all the above criteria, but in about 90% of patients, at least 3 of these criteria can be found [15].

The clinical picture and a short interview suggestive of a lesion of a malignant nature, may be an indication for an excisional biopsy performed in accordance with generally binding principles. Microscopic assessment of the excised tumour allows for diagnosis. The pathological diagnosis is facilitated by immunohistochemistry. The histopathological image of the lesion shows a small cell cancer (often the expression of cytokeratin 20 and neuroendocrine markers and a lack of TTF-1 expression characteristic of small cell lung cancer [SCLC] are observed; PD-L1 expression is present in about 50% of cases).

In order to evaluate the stage of the disease in the cases where a Merkel cell carcinoma tissue pattern is found, it is recommended to perform a physical examination and imaging diagnostics. Depending on individual indications, these would be an X-ray, computed tomography (CT), magnetic resonance (MR), possibly in conjunction with pathological or cytological diagnostics (fine needle aspiration biopsy) of the suspicious foci.

In some cases, where the histopathological diagnosis is doubtful and there is a suspicion of an extra-dermal primary focus of the cancer (skin metastases of the tumours other than MCC, e.g. SCLC), there may be some indications to expand the diagnostics process with positron-emission tomography (PET) in conjunction with CT.

Clinical stages, prognosis

Currently the eighth edition of the tumour classification, as established by the American Joint Committee on Cancer (AJCC) is in use. This classification is based on typical TNM criteria (tumour-node-metastases) (table I and II) [5, 16–18]. It seems, however, that the factors with the largest prognostic value are the primary tumour size, the presence of metastases at the moment of diagnosis and the scope of the involvement of lymph nodes.

The ten-year overall survival in MCC patients is estimated to be 65% in women and 50.5% in men (57% on average for

Table I. Classification of MCC stages (2017)

Primary tumour (T)	
TX	Primary tumour not possible for evaluation
T0	No presence of primary tumour (e.g. node metastases with unknow primary focus)
Tis	cancer *in situ*
T1	Maximum tumour diameter up to 2 cm
T2	Tumour diameter above 2 cm up to 5 cm inclusive
T3	Maximum tumour diameter above 5 cm
T4	Tumour infiltration to the bones, muscles, fascia or cartilage
Regional lymph nodes (N)	
NX	Regional lymph nodes not possible for evaluation
N0	No metastases in regional lymph nodes
N1	Metastases in regional lymph node(s)
N1a (sn)	Micro-metastases (detected in the sentinel node biopsy)
N1a	Clinically not detectable metastasis found in lymphadenectomy
N1b	Macro-metastases (found in clinical or radiological assessment), confirmed in microscopic evaluation
N2	Metastases *in transit* without the metastases in regional lymph nodes
N3	Metastases *in transit* with the metastases in regional lymph nodes
Distant metastases (M)	
M0	No metastases
M1	Metastases in distant organs (other than regional lymph nodes)
M1a	Metastases in the skin, subcutaneous tissue and lymph nodes
M1b	Lung metastases
M1c	Other locations of metastases

Table II. Pathological stages/prognostic groups

Stage	T	N	M
0	Tis	N0	M0
I	T1	N0	M0
IIA	T2–T3	N0	M0
IIB	T4	N0	M0
IIIA	T0	N1b	M0
IIIA	Each T	N1a (sn)/ N1a	M0
IIIB	Each T	N1b–N3	M0
IV	Each T	Each N	M1

all patients). Depending on the size of the primary tumour, the 10-year survival rate is as follows:

- for tumours with a 2 cm diameter or smaller – 61%
- larger than 2 cm – only 39% [5]

5-year survival is the following:

- 37% – for patients with locoregional spread (stage IIIb)
- 16% – for patients with distant metastases [19].

Treatment

The basis of therapy in cases with a locoregional spread is surgical intervention. The treatment of an MCC should be carried out in highly specialised centres [17, 20, 21].

Clinical stage I and II

Where there is a lack of detectable metastases in the regional lymph nodes, a sentinel node biopsy should be considered with a wide scar excision (up to a margin of at least 1–2 cm). This is prompted by the observation that metastases in the sentinel nodes occur in 25–35% of patients even when clinical symptoms of metastases are not present. The risk of developing micro-metastases increases significantly in patients with a primary focus with a diameter measuring 1cm or more [22, 23].

The majority of recommendations suggest that local surgical treatment should be combined with radiotherapy, although the efficiency of such an approach has not been confirmed in randomised trials. However, the recently published results of a meta-analysis of the available observations suggest that radiotherapy slightly improves the overall survival rate and significantly affects the locoregional control of the tumour. The results of the meta-analysis show that patients with an MCC in stage T2 or later benefit from the combination of surgery with radiotherapy [24].

Clinical stage III

The presence of metastases in regional lymph nodes (both micro- and macro-metastases; stage III) is an indication of the resection of the regional lymph nodes.

In spite of the lack of evidence coming from studies with patient randomisation, the majority of retrospective analyses point to an improvement of loco-regional control and patient survival after the application of adjuvant radiotherapy to the bed created after the resection of regional lymph nodes (at a dose of 50–60 Gy) [25, 26].

Some authors postulate that in patients with a massive involvement of the lymph nodes, chemotherapy should also be considered. However, no typical systemic therapy in this group of patients has been established – the treatment can be carried out both preoperatively and postoperatively. In some centres, lymphadenectomy in these patients is performed between chemotherapy cycles. The published data do not allow, however, for a definitive conclusion as to whether systemic therapy affects the improvement of overall survival in this group [26–28].

Preliminary results from the application of checkpoint inhibitors in preoperative treatment of an MCC seem to be promising. In 2018, the results of a I/II phase trial of the use of nivolumab in neoadjuvant treatment of MCC patients in stage IIa–IV (CheckMate 358) were published. The trail comprised 29 adult patients who had not been previously systemically treated for an MCC. In the majority of patients, the presence of polyoma virus (MCPyV; 71.4%) was found. The PD-L1 expression was established in 20 patients and in 30% of them the expression was on the level of at least 1%. The patients received a nivolumab infusion at a dose of 240 mg on day 1 and 15 (counting from the commencement of therapy), and then on day 29, surgery was performed. Out of 27 patients who underwent surgery, 9 received post-operative radiotherapy and 1 patient received nivolumab for one year on account of the progression of the disease. After a median follow-up period of 67.1 weeks, in 40% of the 25 patients, radiological assessment revealed a decrease of the lesions by about 30%. No correlation between the treatment response and MCPyV status and PD-L1 expression was found. Although the radiological assessment revealed only one complete response, in the pathological assessment, a complete pathological response was found in 47% patients and a major pathological response (≤10% of live tumour cells) in 18% patients. In some patients, the response which was achieved allowed for surgery with a smaller scope. At the same time, no median progression free survival (PFS) or median overall survival (OS) were gained. The rates of progression free survival after 6 and 12 months were 92.1 and 72.6% respectively. The survival rates after 18 and 24 months were 100 and 75% respectively. In none of the patients with complete or major pathological response was disease recurrence observed.

The drug safety profile was compliant with the results seen in other clinical trials. No adverse events of grade 5 or severe were observed. In none of the patients qualified for surgical intervention was it necessary to postpone the surgery on account of poor tolerance for the systemic treatment [29].

Currently there is a multi-centre, phase III, double blinded, placebo-controlled clinical trial being carried out with the objective to evaluate the efficacy of avelumab in the adjuvant treatment of MCC patients after surgical treatment (with or without radiotherapy) with clinically confirmed metastases in regional lymph nodes (NCT03271372). The patients are randomised (ratio 1:1) either to a group receiving avelumab at a dose of 10 mg/kg of body mass or to a placebo group. The primary endpoint is recurrence free survival [30].

Clinical stage IV

In cases of advanced disease, the treatment is palliative. In patients who are in a satisfactory condition, palliative chemotherapy might be considered although there is no data which could confirm the effect of such treatment on overall survival. Additionally, the justification for immunotherapy sho-

uld be evaluated [17, 31] – provided that it is available – as there are data pointing to its efficacy. On account of the high activity of immune system checkpoint inhibitors (anti-PD-1 and anti-PD-L1) in the treatment of metastatic MCC, current recommendations suggest the application of these drugs as treatment of choice (a fact which has been confirmed by phase II clinical trials) [32].

Many observations point to the chemosensitivity of MCC (although the response does not exceed 8–10 months and the rate of long-term overall survival stands at 0–18%). The most frequently used therapeutic regimes are chemotherapy with cisplatin, doxorubicin and vincristine or etoposide as well as 5-fluorouracil or cyclophosphamide. In cases where it is justified, palliative surgical interventions and/or radiotherapy may also be applied.

In 2019, the results of a retrospective analysis of treatment patterns was applied to patients with newly diagnosed MCC, treated between October 2013 and January 2018. Out of 120 patients treated systemically within the first line of treatment, 17%, 45% and 38% patients were treated with checkpoint inhibitors, chemotherapy applied according to the NCCN guidelines or another type of chemotherapy respectively. The most frequently used chemotherapy patterns were carboplatin with etoposide and cisplatin with etoposide. Only 33% patients systemically treated in the first line commenced the second line of treatment [33].

Moreover, the results of clinical studies into the use of avelumab, pembrolizumab and nivolumab in the treatment of advanced MCC have been published.

A single-arm second phase clinical trial, JAVELIN Merkel 200, showed the efficacy of avelumab in the treatment of MCC with metastases after the failure of systemic chemotherapy; avelumab was administered at a dose of 10 mg/kg of body mass intravenously every two weeks until the moment of progression or unacceptable toxicity. The objective response rate (ORR) was 31.8% (95% confidence interval (CI): 21.9–43.1%; 28 patients), including 8 complete responses (9%) and 20 partial responses (23%). Additionally, in 9 patients (10%) disease stabilisation was observed [34]. The treatment responses had a lasting effect and, at the moment of analysis, they persisted in 23 (82%) patients. The length of the response was at least 6 months in 92% of cases. The median PFS was 2.7 months (95% CI: 1.4–6.9), and the rate of patients free from disease progression after 6 months was 40%. The PFS curves reached *plateau*. The survival rate after 6 months was 69% (95% CI: 58–78), and the median OS – 11.3 months (95% CI: 7.5–14.0). Objective responses were obtained in the following patients:

- 20 out of 58 patients (34.5%) with PD-L1 expression,
- 3 out of 16 patients (18.8%) PD-L1 (–),
- 12 out of 46 patients (26.1%) MCPyV (+),
- 11 out of 31 patients (35.5%) MCPyV (–).

More responses were obtained in patients who had previously undergone only one line of treatment. Avelumab was generally well tolerated. Treatment related adverse events occurred in 62 (70%) out of 88 patients. Updated results with median follow-up periods of 18 months and 24 months published in 2018, confirm the efficacy of avelumab for this indication. On the basis of an analysis of the data from 88 patients followed up for 29.2 months (24.8–38.1) it was observed that the median OS was 12.6 months (95% CI: 7.5–17.1), with the 2-year survival rate being 36% (50% survival after 1 and 39% after 1.5 years). The median treatment period was 3.9 months (0.5–36.3). The rate of confirmed ORR was 33.0% (95% CI: 23.3–43.8; CR observed in 11.4% patients) and this remained on the same level as in the case of the analyses carried out after one year and 1.5 years of follow-up. The median response period was not reached (2.8–31.8 months; 95% CI: 18.0 – not reached). The long-term responses to avelumab treatment determine stable PFS values in evaluations after 1 year of observation (29%), after 1.5 years (29%) and after 2 years of follow-up (26%). Clinical activity persisted irrespectively of PD-L1 expression status and the presence of polyoma virus. The tolerance profile of avelumab was consistent with those already existant. In 67 patients (76.1%) treatment related adverse events were observed and in 10 patients (11.4%) they were at least 3 grade. In 20 patients (22.7%) adverse events related to immunological activity of avelumab were observed. No deaths connected with the treatment occurred [35, 36].

The second phase trial, JAVELIN Merkel 200, also resulted in the registration for the first line of treatment of advanced MCC. The data concerning the survival of these patients, published in 2018, point to a mean survival rate of 49.9 months (6.3; 179.4) with the one year and five year survival rates being 66% and 23% respectively [37]. So far no predictive factors of avelumab treatment response of MCC patients have been established [38].

In 2017, during the annual conference of the American Society of Clinical Oncology (ASCO), the preliminary results of part of the second phase trial with the use of avelumab (JAVELIN Merkel 200) in the first line of treatment of advanced MCC were presented [39]. In 16 patients, after a follow-up period of at least 3 months, the response rate was 62.5% (in 10 patients 3 complete remissions and 7 partial remissions were observed), and all these responses persisted at the moment of the last evaluation. The updated results of part B of this trial confirmed that 77.8% (14 out of 18) treatment responses persisted and the response duration in 83% cases was longer than 6 months (95% CI: 46–96%) [40]. In 29 patients, the safety of the therapy was evaluated: adverse events with minimum toxicity grade 3 occurred in 5 patients (17.2%), and this was the reason for the termination of the treatment (2 patients developed reactions related to the administration of the drug, such as increased activity of aspartate aminotransferase and alanine aminotransferase, cholangitis, paraneoplastic syndrome and gait disorders). According to recently updated analysis, in 8 patients in total there were grade 3 adverse events related to the immunology system (20.5%).

A second phase clinical trial published in 2016 showed the efficacy of pembrolizumab (anti-PD-1 antibody) in the treatment of the stage IIIB–IVC MCC patients, who were systemic treatment naïve [41]. This was a multi-centre clinical trial (Cancer Immunotherapy Trials Network-09/Keynote-017), which enrolled 50 patients with advanced MCC. They received pembrolizumab at a dose of 2 mg/kg of body mass every 3 weeks for up to 2 years. The median age of the subjects was 70.5 years. In 64% the tumour was MCPyV(+). The efficacy evaluation was performed on the basis of RECIST 1.1. criteria; the ORR totalled 56% (CR 24%, PR 32%; 95% CI: 41.3–70.0%): the ORR in the patients in the group MCPyV(+) was 59%, whilst in those in the group MCPyV(–) it was 53%, with a median follow-up of 14.9 months (range 0.4–36.4 months). Among the 28 patients in whom a treatment response was observed, the median response duration was not reached (range 5.9–34.5 months). The PFS ratio after 24 months was 48.3% with a median PFS of 16.8 months, whilst OS rate after 24 months was 68.7%, and the median OS was not reached. The presence of polyoma virus did not correlate with ORR, PFS or OS. Some trend for better results concerning PFS and OS was observed in patients with PD-L1 expression. Treatment related adverse events ≥G3 were found in 28% of patients (14 out of 50) and in 14% (7 out of 50) these events required the termination of the treatment. One treatment related death occurred [42].

Similarly, in the avelumab trial, a tendency towards greater treatment response was observed where the number of previous treatment lines was smaller. This shows (taking into consideration the pembrolizumab trials), that immunotherapy in MCC should be the treatment of choice in the first line of therapy. In all the presented trials, the responses were found in both MCPyV-positive and negative patients, and, as was confirmed, this type of treatment may also be applied to elderly patients (a fact which is vital given that this disease develops mostly among people of advanced age).

Currently, immunotherapy with anti-PD-1/anti-PD-L1, in accordance with Polish and international recommendations, makes for standard systemic treatment in patients with unresectable/metastatic MCC [32, 43], and avelumab, registered for this indication in the European Union, is available in Poland as part of the Emergency Access to Therapy programme (Ratunkowy Dostęp Terapii Lekowej) in conjunction with a positive opinion on the part of AOTMiT (the Agency for Health Technology Assessment and Tariff System).

Additionally, preliminary results of the first and second phase trials with nivolumab administered in a group of 22 patients with MCC. In these people, the ORR rate was 68% after the 26-week follow-up period (with a scope of 5–35 weeks) and it was slightly larger in patients who had not been systemically treated previously (71%, n = 14), in comparison with those who had been previously treated (63%, 1 or 2 lines of previous treatment, n = 8) [44].

The treatment of local relapses and recurrences in regional lymph nodes

The most frequent recurrence form is a local relapse. This affects about 30% of patients treated surgically (postoperative radiotherapy decreases this rate to about 11%) [45].

Local relapses may be treated like a primary MCC with correct reference to the clinical stage (I–III). If possible, the tumour foci should be resected with a margin of healthy tissues, with adjuvant radiotherapy, provided that this was not applied for the treatment of the primary focus. Disease recurrence makes for a bad prognosis, and for this reason systemic adjuvant treatment should also be considered even though there still is no evidence for its effectiveness.

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Review article

Long-term responses to molecularly targeted treatment and immunotherapy – groups of patients, management

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The use of immune checkpoint inhibitors and BRAF and MEK protein inhibitors in patients with advanced melanoma resulted in the overall survival median exceeding 2 years. For ipilimumab and anti-PD-1 antibodies, the percentage of 5-year survival is about 20% and 35%, respectively. Better results are obtained by patients treated in the first-line treatment. The most effective option seems to be the combined use of anti-CTLA-4 antibody with anti-PD antibody – in this case the percentage of 4-year overall survival was 53%. The 5-year overall survival rate of patients treated with BRAF/MEK inhibitors is 34%. Patients with a early stage of disease and normal lactate dehydrogenase concentration before systemic treatment are more likely to benefit from treatment.

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Key words: ipilimumab, pembrolizumab, nivolumab, dabrafenib, trametinib, long-term survival, melanoma

Introduction

In 2011, two new drugs were approved, which changed the prognosis of patients with advanced melanoma – ipilimumab [1] and vemurafenib [2]. Both drugs became representatives of new groups of drugs – immune checkpoints inhibitors and BRAF protein inhibitors. Less than a decade ago, the median overall survival rate for patients with advanced melanoma was 6–8 months, and the chance of 5- year survival ranged from 5% to 10% [3]. Molecularly targeted drugs and immunotherapy currently allow to reach the median total survival of more than 2 years.

Immunotherapy

Ipilimumab

In 2015, data on 3-year survival in patients treated with ipilimumab in phase II and phase III studies were published. 1861 patients were included in the analysis; 1257 patients received ipilimumab in the second or subsequent lines. The majority of patients [n = 965] received 3 mg/kg of body weight; 706 patients received 10 mg/kg of body weight; the remaining 190 patients received ipilimumab in a different dose. All patients received at least 4 doses of the drug at three-week intervals. In some studies, patients may have received maintenance treatment or may have been re-treated inductively after the progression of the disease. Overall survival (OS) was 11.4 months (95% confidence interval – CI): 10.7–12.1 months) with a 3-year OS percentage estimated at 22% (95% CI: 20%–24%). The median of the follow-up period was 11 months. Ten percent of patients were followed-up for at least 50 months. The maximum follow-up time was impressive and it was 119 months. The overall survival curve flattened at about 3 years after the start of treatment (fig. 1).

Longer overall survival was observed in patients receiving ipilimumab in the first line of treatment (median 13.5 months) compared to patients previously receiving systemic treatment (median 10.7 months). The 3-year survival rate for these groups was 26% and 20% respectively.

No significant differences in overall survival were observed in patients depending on ipilimumab doses.

To this group 2985 patients from the program of extended access to ipilimumab (EAP) (4846 patients in total) were

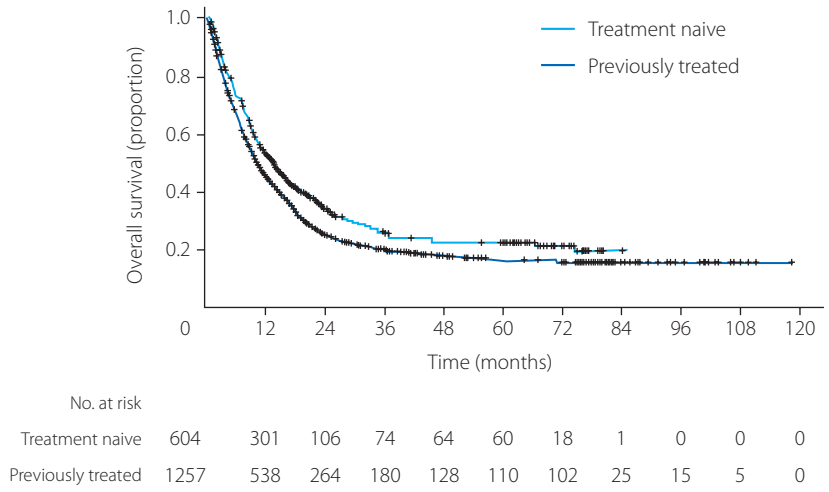

Figure 1. Long-term survival in patients treated with ipilimumab depending on the treatment line [4]

added. In this program, ipilimumab was also used to treat patients who would not meet the criteria for inclusion in the majority of clinical trials: patients with efficiency level 2 on the ECOG scale, patients with metastases in the brain, patients with melanoma of mucous membranes and eyeballs. The OS median for the entire group was 9.5 months with a 3-year total survival rate of 21%.

Among 88 patients who survived at least 4 years and were treated in studies CA 184-007, CA 184-008 and CA 184-022, 35 (40%) obtained an objective response, 29 (33%) disease stabilization and in 22 (25%) there was a progress in the disease. Therefore, the lack of an objective answer did not prejudge the short-term survival.

This data confirms observations from other studies included in this analysis, as well as coincides with observations from clinical practice [4].

In 2015, an analysis of the long-term survival o patients who were treated in the third phase of the study CA 184-024 (dacarbazine + ipilimumab 10 mg/kg of body weight) *vs.* dacarbazine + placebo) was published. The study showed significantly longer OS in the group treated with ipilimumab and dacarbazine than in the group treated with dacarbazine in monotherapy: 11.2 months *vs.* 9.1 months (hazard ratio [HR] 0.72, $p < 0.001$).

502 patients were treated with 250 ipilimumab with dacarbazine and 252 with dacarbazine. After 5 years, 40 patients receiving ipilimumab and 20 patients receiving monotherapy still lived (fig. 2).

The percentage of 5-year overall survival for combined therapy was 18.2%, and for dacarbazine 8.8%. Responses to treatment were assessed using modified WHO criteria. In the group of patients receiving ipilimumab with dacarbazine, complete response (CR) was observed in 7.5%, while partial response (PR) was observed in 42.5% of patients. In the group of patients receiving dacarbazine in monotherapy, no complete responses were observed, and partial responses were observed in 35% of treated patients.

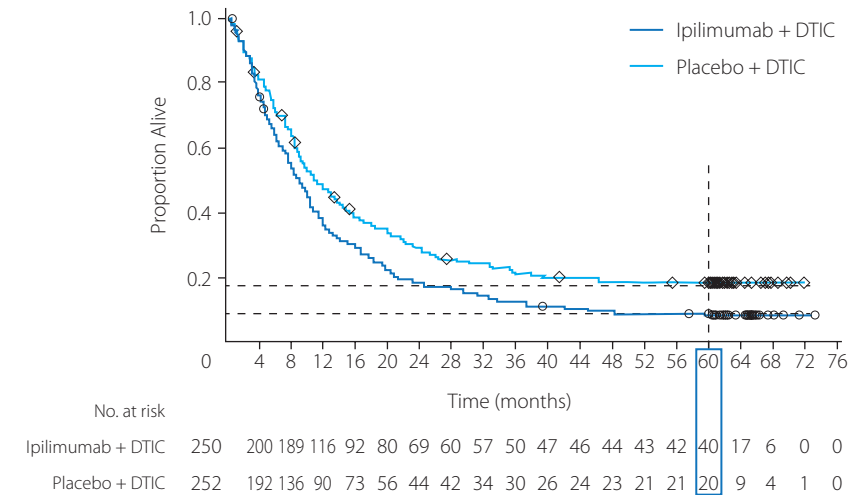

Figure 2. Long-term survival in patients treated with ipilimumab compared with dacarbazine [5]

Longer overall survival was observed in patients with objective response to treatment. In the group treated with ipilimumab with dacarbazine, median OS was not achieved in patients with objective response to treatment. In the group of patients where no response was achieved, the median OS was 14.3 months (HR 0.28; 95% CI: 0.16–0.47). Similarly, better survival rates were observed in patients with objective response to dacarbazine in monotherapy compared to patients with no response to treatment. The median OS was 20.2 and 12.3 months respectively (HR 0.51; 95% CI: 0.32–0.84) [5].

Two studies on patients treated with ipilimumab in phase I and II studies are interesting in terms of long-term survival. The first one describes 177 patients [6], who were treated in 3 studies, for which recruitment was conducted in the years 2003–2005. Ipilimumab was administered in combination with gp100 peptide, high-dose interleukin 2 or alone. The drug was administered in different patterns. The analysis presents data of 15 patients with complete response (CR). At the time of publication, 14 patients were alive. The time to obtain CR was very different – from 3 to even 70 months. The longest duration of CR was 99 months. In 1 patient the disease progressed after 42 months of complete response.

The data of 18 patients who were alive at the time of the analysis and who did not obtain CR were also presented. For 3 of them, a partial response has been maintained for 56, 66 and 71 months. In 6 patients metastasectomy or other local treatment, including radiation therapy or percutaneous radio--frequency ablation (RFA) was chosen. The remaining patients underwent other systemic treatment – chemotherapy, targeted therapy, biological treatment, immunotherapy.

The percentage of 5-year overall survival; for the three analysed studies was 13%, 25% and 23% respectively.

The second analysis concerns 733 patients treated with ipilimumab in 6 phase II studies. They received ipilimumab at a dose of 0.3, 3 or 10 mg/kg of body weight. In the group of patients who were previously treated, the percentage of 5-year overall survival was 12.3%, 12.3–16.5% and 15.5–28.4%, respectively. The percentage of 5-year overall survival in the group of patients untreated earlier was 26.8% for those receiving 3 mg/kg of body weight and 21.4–49.5% for those receiving 10 mg/kg of body weight [7].

In 2017, a retrospective analysis of 1034 patients treated under the European extended access programme (EURO--VOYAGE) was published.

The OS median was 6.8 months, with 3- and 4-year survival rates of 10.9% and 8%, respectively. The patient survival in this group was much shorter than in other studies. The reason for such results was the wider criteria for inclusion in the extended access program than in clinical trials [8].

According to the available data, patients in the first-line treatment received a greater benefit from ipilimumab treatment. Since 2014, when the results of anti-PD-1 antibodies tests were published, it has been known that they are a better choice for patients than ipilimumab. Data on the efficacy of ipilimumab after progression during anti-PD-1 treatment are limited, however, this drug remains an option in the second line in patients without *BRAF* mutations.

Anti-PD-1 antibodies

In the first line of treatment of patients with advanced melanoma, anti-PD-1 antibodies, i.e. pembrolizumab [9] and nivolumab [10], are currently the preferred choice.

Pembrolizumab

In 2019, the analysis of long-term survival of patients treated in the phase Ib of the open-label study KEYNOTE -001 was published. The study included 655 patients with advanced melanoma, including 8 patients with diagnosis of advanced untreated melanoma of the eyeball. Most patients (n = 496) had previously received systemic treatment (205 received one line of treatment, 178 received two lines of treatment, 113 received 3 or more lines of treatment). The percentage of 5-year PFS was 21% for the whole population; and 29% for patients treated in line 1. The median of the follow-up period was 55 months. The longest response lasts for 66 months. The percentage of 5-year survival in the whole group was 34%, and 41% in the subgroup treated in the first line. The OS median for the whole population was 23.8 months (95% CI: 20.2–30.4), whereas for patients treated in the first line it was 38.6 months (95% CI: 27.2 – not reached) (fig. 3).

Complete response to treatment was obtained in 16% of patients, partial response in 25% of patients, and stabilization of the disease in 24% of patients. At the time of analysis of the response data, 93 patients (89%) still had a complete response and 102 (63%) had a partial response. The protocol assumed that pembrolizumab could be discontinued in patients after a good response to treatment. Among the patients who ended CR treatment in this way, 67 patients were observed, and 5 patients were treated with PR. Only 7 of these patients developed progression after discontinuation of pembrolizumab (6 CR, 1 PR); 90% of the responses were still present. Of these 7 patients, 4 received pembrolizumab again. One patient obtained CR again, one SD; 2 patients experienced further progression of the disease [11] (fig. 4).

KEYNOTE-006 was the second study to analyse the long--term survival in patients with advanced melanoma. In this phase III study patients were randomized to 3 arms:

- pembrolizumab 10 mg/kg of body weight every 2 weeks,
- pembrolizumab 10 mg/kg of body weight every 3 weeks,
- ipilimumab 3 mg/kg of body weight (4 applications).

Patients could receive only one line of treatment beforehand. Pembrolizumab was administered for a maximum of 2 years.

The OS median for both arms of pembrolizumab was 32.7 months (95% CI: 24.5–41.6), for ipilimumab was 15.9 months (95% CI: 13.3–22.0), (HR 0.73, 95% CI: 0.61–0.88, p = 0.00049].

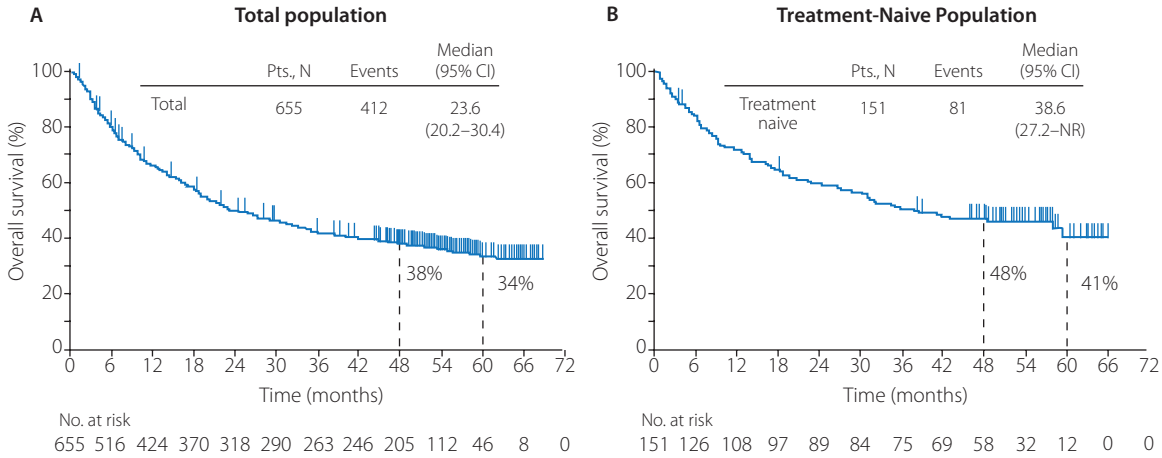

Figure 3. Long-term survival in the whole population (A) and in the previously untreated group (B) [11]

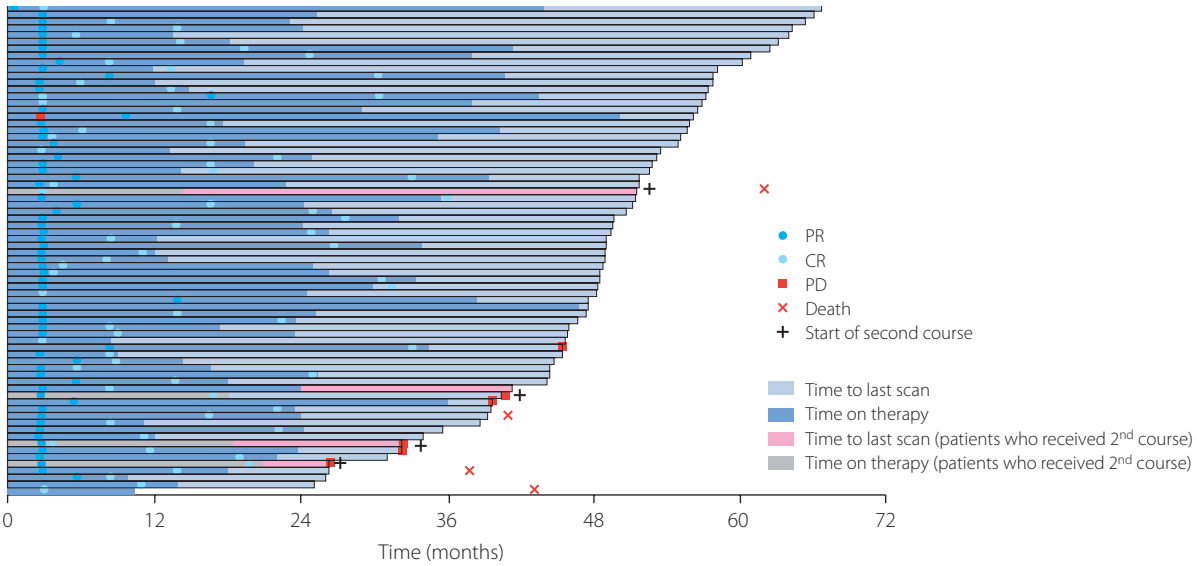

Figure 4. Analysis of the response in 72 patients who discontinued treatment with pembrolizumab in a clinical trial [11]

The percentage of 5-year overall survival was 38.7% for both groups of pembrolizumab and 31% for ipilimumab group. Patients who received pembrolizumab or ipilimumab in the first line had better results – median OS in these groups was 38.7 months (95% CI: 27.3–50.7) and 17.1 months (95% CI: 13.8–26.2).

Pembrolizumab used in the second line of treatment allowed to reach the median OS at the level of 23.5 months (95% CI: 16.8–34.2). The OS median for ipilimumab used in the 2nd line was 13.6 months (95% CI: 10.7–22.0).

For 2 years 103 patients (19%) received pembrolizumab; 21 of them obtained CR, 69 – PR and 13 – SD as the best response. After the median observation period at the end of pembrolizumab 34.2 months, the studied percentage of 2-year PFS was 78.4% (95% CI: 68.3–85.6). The percentage of 2-year and 3-year OS was 95.9% (95% CI: 89.4–98.4) and 93.8% (95% CI: 86.7–97.2).

In the group of patients who ended treatment with pembrolizumab after 2 years, the median time to progression of the disease was 33.3 months after the end of pembrolizumab administration. Thirteen patients in whom the disease progressed after the end of treatment received pembrolizumab again. In this group the percentage of CR was 23%, PR – 31% and SD – 15%. In one patient the disease progressed further and in 2 patients the response to treatment was not assessed yet [12, 13].

Nivolumab

One of the first studies of long-term survival was the analysis of patients participating in the first phase of the study CA209–003. The percentage of 5-year survival in the group of patients treated for advanced melanoma was 34% – a similar result was obtained in patients treated with pembrolizumab [14].

Recently, the long-term results of Checkmate-067 have been published. It was a phase III study in which patients were randomly assigned to one of the three arms:

- nivolumab,
- ipilimumab or
- nivolumab and ipilimumab.

Nivolumab in monotherapy or in combination with ipilimumab significantly improved PFS and OS compared to ipilimumab in monotherapy. After at least 48 months of observation the median OS was not achieved for the group treated with ipilimumab and nivolumab (95% CI: 38.2 – not reached) In the remaining arms, the median OS for nivolumab and ipilimumab was 36.9 (95% CI: 28.3 – not achieved) months and 19.9 (95% CI: 16.9–24.6) months.

The percentage of 4-year overall survival was 53% for combined therapy, 46% for nivolumab and 30% for ipilimumab. Both groups treated with nivolumab achieved significantly longer overall survival compared to those treated with ipilimumab. No statistically significant differences in overall survival between nivolumab and ipilimumab and nivolumab were found (HR 0.84, 95% CI: 0.67–1.05). An interesting observation from this analysis was the comparison of total survival depending on the initial concentration of lactate dehydrogenase (LDH). In the group of patients in whom LDH concentration exceeded the upper limit of norm by more than 2 times, the percentage of 4-year overall survival was as high as in the group of patients in whom LDH concentration exceeded the upper limit of norm: 28% in patients receiving nivolumab with ipilimumab, 14% in patients receiving nivolumab and only 7% in patients receiving ipilimumab [15].

Anti-PD-1 antibodies

For anti-PD-1 antibodies the overall survival curves after 3 years reach a plateau at about 40%. The problem with treating patients with immunotherapy with anti-PD-1 antibodies in monotherapy or in combination with anti-CTLA-4 antibodies is to determine the optimal duration of treatment. PET-TK examination, in some cases in combination with biopsy, may be a helpful tool in deciding to discontinue immunotherapy [16, 17].

Another problem is the relapse of the disease after an earlier discontinuation of immunological treatment after a long-term response. Available data confirm the need for this type of treatment again due to the possibility of a further response [18]. In Poland, however, current records of drug programs with anti-PD-1 antibodies do not allow for the possibility of re-treatment with anti-PD-1 in patients with advanced melanoma.

Achieving a long-term response to treatment is desirable for any patient who is struggling with a deadly disease. One may feel that the patients who get these results are constantly satisfied, but they have to struggle with a constant fear of relapse. There are currently no data on potential long-term adverse effects on cognitive capacity or emotional sphere in this group of patients [19].

Treatment with BRAF or MEK inhibitors

One of the analyses concerning the long-term survival of patients with advanced melanoma with *BRAF* V600 mutation treated with BRAF inhibitors is a study concerning the second phase (randomized) of BRF113220 study [20]. Part C of the study included 162 patients who were assigned to one of three groups (54 patients in each group) treated with:

- dabrafenib as monotherapy (D),
- dabrafenib 150 mg/day + trametinib 1 mg/day (D + T 150/1) or
- dabrafenib 150 mg/day + trametinib 2 mg/day (D + T 150/2).

The percentage of 4-year and 5-year PFS was 13% (95% CI: 5–25) in the arm D + T 150/2, 9% and 3% (95% CI: 0–11%) in the arm with monotherapy. The percentage of 4-year and 5-year OS was 30% (95% CI: 18–43%) and 28% (95% CI: 17–41%) respectively for D + T 150/2 and 23% (95% CI: 13-35%) and 21% (95% CI: 11–33%) for D with monotherapy. The percentage of 5-year overall survival was similar in patients treated with D + T 150/1 and D + T 150/2 (33% *vs.* 28%).

Further anticancer treatment resulted in a higher number of patients on the D arm in monotherapy than in the D + T 150/2 arm. The most frequent subsequent treatment regimens were immunotherapy (37% *vs.* 43% for D + T 150/2 and D respectively as monotherapy) and targeted treatment (24% *vs.* 87%).

A complete response to treatment according to Response Evaluation Criteria in Solid Tumors (RECIST) was reported in 9 (17%) patients in the D + T 150/2 arm and 2 (4%) patients in the monotherapy arm. In the subgroup of patients with CR in the D + T 150/2 arm, the percentage of 4- and 5-year OS was 56% and 44%, respectively, at the median OS 53.4 months.

In the subgroup of patients with normal LDH levels, the percentage of 4-year and 5-year survival in the D + T 150/2 arm was 48% (95% CI: 30–64%) and 45% (95% CI: 27–61%). In the monotherapy arm, the values were 31% (95% CI: 15–50%) and 26% (95% CI: 11–45%). Patients with normal LDH levels, in whom the cancer developed in three (or less) locations, benefited most from treatment [21, 22]. The percentage of 4-year and 5-year OS was 57% (95% CI: 32–76%) and 51% (95% CI: 27–71%) respectively for D + T 150/2 and 42% (95% CI: 15–67%) and 31% (95% CI: 8–58%) for the arm treated with dabrafenib.

Five-year survivals were analysed in a group of 563 patients who received dabrafenib and trametinib in two phase III studies: COMBI-d (211 patients) and COMBI-v (352 patients) [23]. The percentage of 4- and 5-year progression-free survival was 21% and 19%, respectively. In patients with normal LDH levels at initiation of treatment, the 5-year PFS percentage was 25%, compared to 8% in patients with LDH levels above the upper limit of normal.

Previous analysis, including the data of patients treated in the above two studies and one phase II study (617 patients in total), allowed us to identify a subgroup reaching a significan-

tly longer time to progression of the disease. There were 216 patients (38%) who had normal LDH levels at the beginning of treatment and at locations of the disease [21]. This group reached a 5-year PFS of 31%. The OS median for the whole population was 25.9 months (95% CI: 22.6–31.5). The percentage of 4- and 5-year overall survival was 37% and 34%, respectively. Patients with normal LDH concentration had better prognosis than patients with elevated LDH concentration. The percentage of 5-year OS was 43% and 16% respectively. In the subgroup of patients with normal LDH concentration and limited to

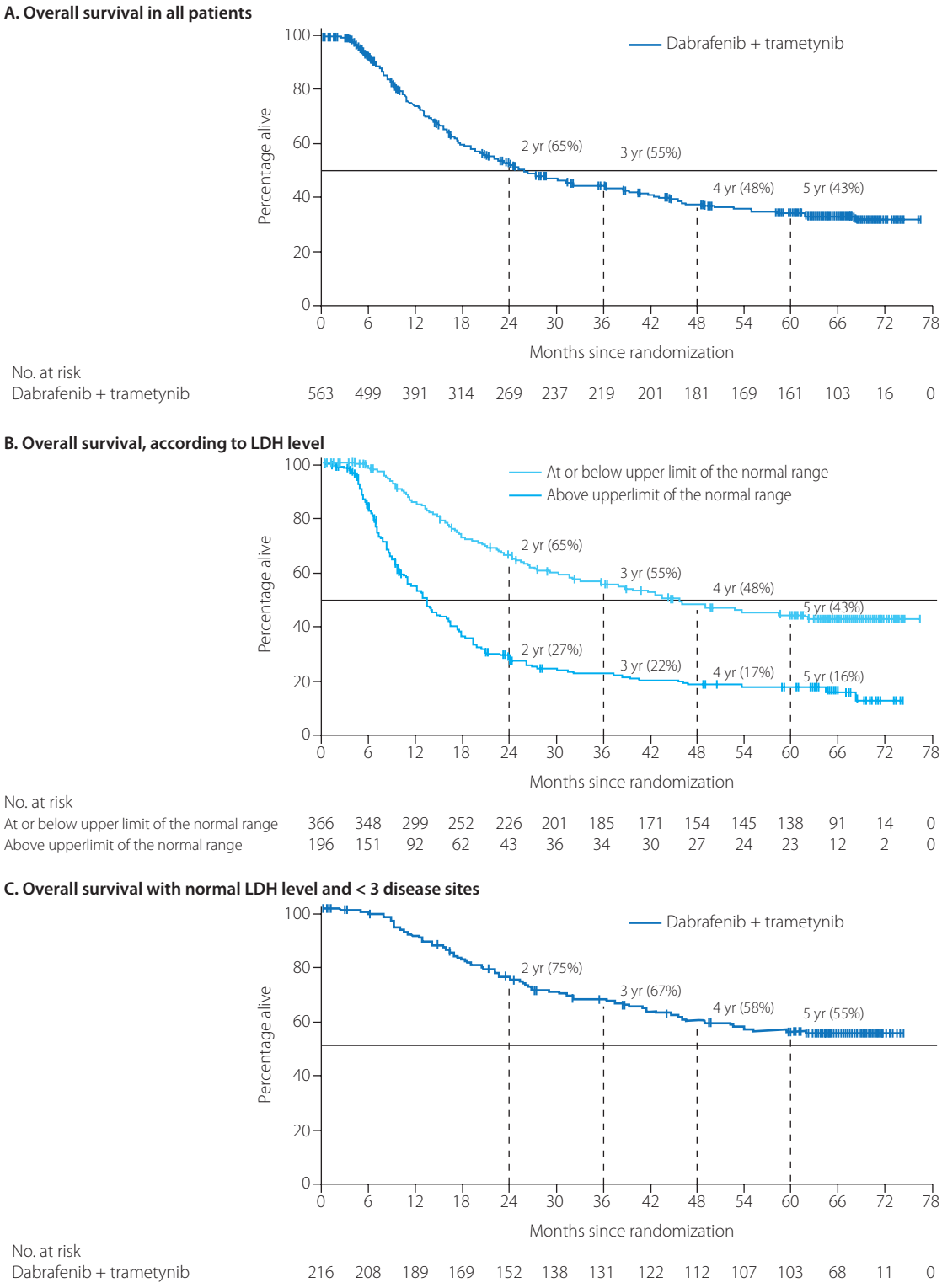

Figure 5. OS in the general population (A), depending on LDH (B) concentration, and in the group of patients with normal LDH concentration and with fewer than 3 locations of disease (C) [23]

3 occupied areas, the percentage of 5-year OS was estimated at 55 % (95% CI: 48–61) (fig. 5).

Of the 161 patients who lived at the time of analysis, 69 (43%) received dabrafenib, trametinib or both. Further anticancer therapy was administered to 72 patients (45%). Most often it was immunotherapy – 56 patients (78%), 42 (67%) received anti-PD-1 and 30 (42%) anti-CTLA-4 antibodies. In the remaining 89 patients (55%) no anticancer treatment of the next line was administered.

In the whole analysed population, further treatment lines were administered in 299 out of 563 patients (53%). Immunotherapy was the most frequent choice – 196 of 299 patients (66%) received it, including 151 (51%) treated with anti-PD-1 and 102 (34%) with anti-CTLA-4 antibodies.

Objective responses were recorded in 68% of patients, including total responses at 19% (109 patients). The percentage of 5-year OS in the group of patients with CR was 71%; in patients with PR this percentage was 32%, and in patients with disease stabilization – 16% (fig. 1). 6). The median of the CR period was 36.7 months. Table I shows the factors influencing survival free from disease progression and overall survival.

Summary

The use of anti-PD-1 antibodies and BRAF/MEK inhibitors in the group of patients with positive *BRAF* mutation allows to achieve long-term overall survival in about 1/3 of patients with advanced melanoma. Long-term responses to treatment are most often observed in patients with low disease severity and normal lactate dehydrogenase concentration before systemic treatment. In patients with melanoma with more aggressive course, a combination of anti-CTLA-4 and anti-PD-1 antibodies seems to be more effective in immunotherapy. However,

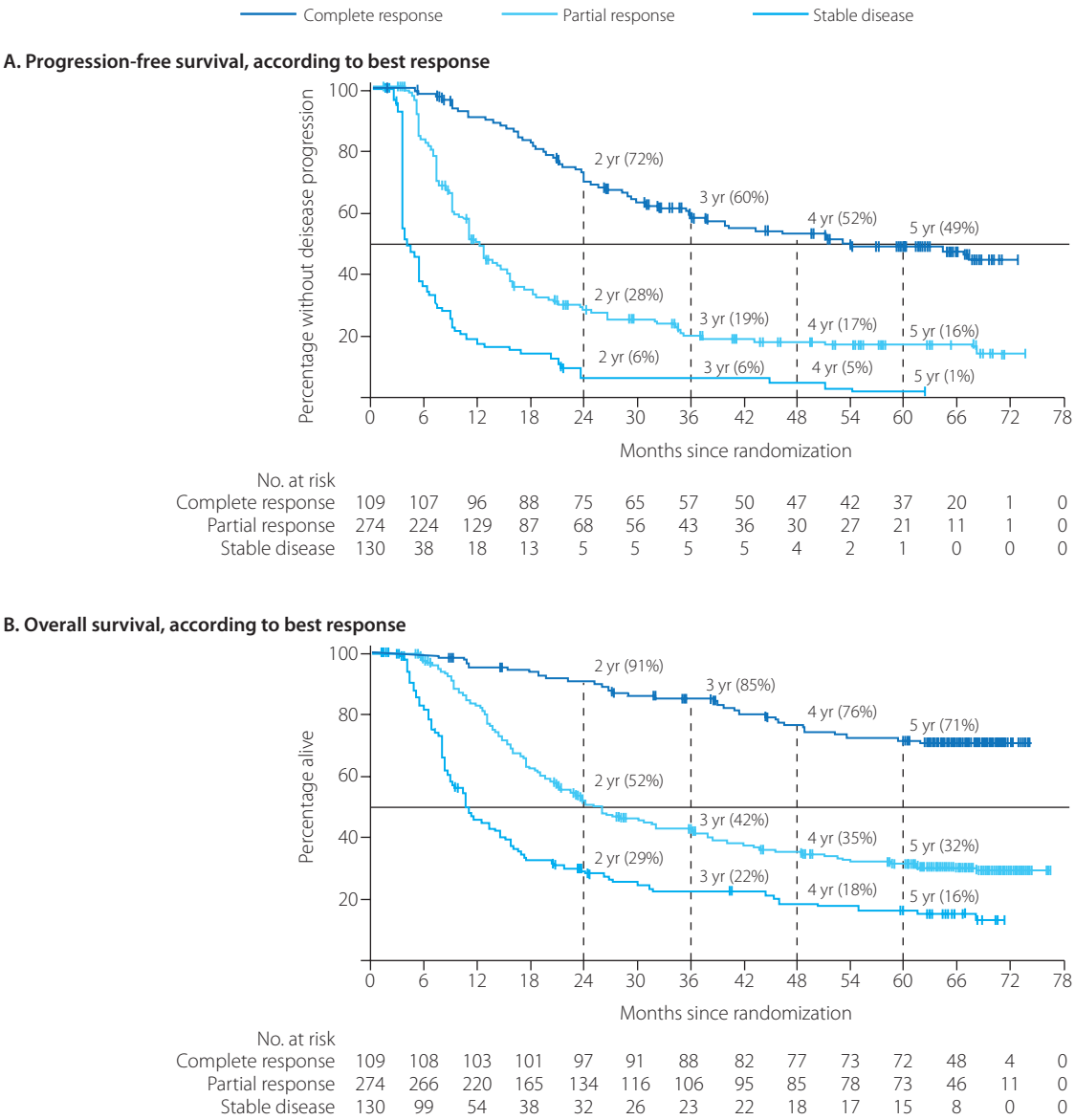

Figure 6. PFS depending on the response to treatment (A), OS depending on the response to treatment (B) [23]

Table I. Analysis of factors influencing survival free from disease progression and overall survival

Variable	Result tested (n)	PFS		OS	
		HR (CI 95%)	p	HR	p
Sex	Women (238) *vs.* men (313)	0.74 (0.61–0.90)	0.003	0.68 (0.55–0.84)	<0.001
BRAF mutation	V600E (482) *vs.* V600E or V600K plus V600K (69)	0.65 (0.49–0.87)	0.004	0.77 (0.55–1.06)	0.11
General condition	ECOG 0 (398) *vs.* ECOG ≥1 (153)	0.68 (0.55–0.85)	<0.001	0.49 (0.39-0.62)	<0.001
LDH concentration	Normal (359) *vs.* elevated (192)	0.50 (0.40–0.64)	<0.001	0.47 (0.37–0.61)	<0.001
Number of locations with disease	<3 locations (282) *vs.* ≥3 locations (269)	0.72 (0.58–0.91)	0.005	0.58 (0.46–0.74)	<0.001

this group of patients still requires new therapeutic options. Several ongoing clinical trials are aimed at answering the question about the correct sequence of treatment and the appropriateness of using immunotherapy in combination with targeted treatment.

Conflict of interest: none declared

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Review article

Akademia Czerniaka

Combined or sequential treatment of advanced melanoma?

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Melanoma is a malignant neoplasm with a very high rate of growth in the number of cases. In Poland, in the years 1980–2010, the number of cases of melanoma increased threefold. Although the incidence rates of melanoma are rising, the mortality rate due to melanoma is falling. In recent years, the treatment of patients with melanoma has changed to a great extent. Thanks to the development of molecular research, the presence of specific mutations in melanoma cells was discovered. The progress in understanding the molecular mechanisms occurring in this neoplastic cells and the interaction between the immune system cells and melanoma cells contributed to the development of new classes of drugs: immunotherapy and targeted therapy.

With the use of checkpoint inhibitors, long-term remission of the disease can be achieved, which has been confirmed in many clinical trials that have shown improvements in overall survival (OS) and progression free survival (PSF). However, the predominant problem is the low response rate to checkpoint inhibitors and the time between the initiation of therapy and the response to treatment. This is not the case with targeted therapies, where the response rate is high and the response time is very short. Therefore, a promising treatment strategy can be a combination of these two classes of drugs, so that one can try to achieve a quick and long-term response to the treatment. The paper discusses the current treatment options for melanoma patients in the spreading phase of the disease and analyzes the benefits of combined and sequential treatment.

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Key words: melanoma, immunotherapy, targeted therapy, sequential treatment

Introduction

Melanoma is a malignant neoplasm with a very high rate of growth in the number of cases. In Poland, in the years 1980–2010, the number of cases of melanoma increased threefold [1, 2]. Although the incidence rates of melanoma are rising, the mortality rate due to melanoma is falling. Low grade melanoma has a very good prognosis and is usually completely curable with surgical methods, while 5-years survival rate reaches as much as 99%. However, melanomas with metastases to regional lymph nodes or distant metastases are characterized by much worse prognosis: 5-year survival rates are 63% and 20% respectively [3]. Despite significant progress, the prognosis of patients with melanoma in the spreading phase of the disease is still unsatisfactory and new treatment strategies are constantly being sought.

In recent years, the treatment of patients with melanoma has changed to a great extent. Thanks to the development of molecular research, the presence of specific mutations in melanoma cells was discovered. It is estimated that melanoma cells show *BRAF* V600 mutation in about 50% of patients with disseminated melanoma [4]. The progress in understanding the molecular mechanisms occurring in melanoma cells and the interaction between the immune system cells and melanoma cells contributed to the development of new classes of drugs: immunotherapy and targeted therapy. Immunotherapy is based on immune checkpoint inhibitors (ICIs), which inc-

lude anti-cytotoxic T-lymphocyte antigen 4 antibodies (anti--CTLA-4) and anti-programmed death receptor-1/ligand-1 (anti-PD-1/anti-PD-L1) antibodies. The first registered ICI drug was ipilimumab (anti-CTLA-4), followed by nivolumab and pembrolizumab (anti-PD-1). Another group of drugs is targeted therapy, which includes BRAF inhibitors (BRAFi; vemurafenib, dabrafenib and encorafenib) and MEK inhibitors (MEKi; cobimetinib, trametinib, binimetinib). It is also worth mentioning the introduction of oncolytic talimogene laherparepvec virus (T-VEC) to the treatment of patients with melanoma.

With the use of checkpoint inhibitors, long-term remission of the disease can be achieved, which has been confirmed in many clinical trials that have shown improvements in overall survival (OS) and progression free survival (PSF). However, the predominant problem is the low response rate to checkpoint inhibitors and the time between the initiation of therapy and the response to treatment. This is not the case with targeted therapies, where the response rate is high and the response time is very short. Therefore, a promising treatment strategy can be a combination of these two classes of drugs, so that one can try to achieve a quick and long-term response to the treatment. The paper discusses the current treatment options for melanoma patients in the spreading phase of the disease and analyzes the benefits of combined and sequential treatment.

Immunotherapy

Anti-CTLA-4 or anti-PD-1 monotherapy

Ipilimumab

Ipilimumab is a recombinant human monoclonal antibody, IgG1 subclass, with a half-life of 12–14 days, binding to the CTLA-4 (CD152) molecule [14]. By blocking CTLA-4, the anti-neoplastic immune response is activated. Three phase II studies – CA184-022, CA184-008 and CA184-007 [5–7] – using ipilimumab in monotherapy in patients with advanced melanoma showed a median survival rate of 8.6–11.0 months and a percentage of 1-year survival rate of 39–48%. Subsequent analyses showed a 2-year survival rate of 30% [8] and a 3-year survival rate of 25% at the dose of 10 mg/kg of body weight. [9]. However, there is no data available to indicate that this drug leads to permanent cures in melanoma patients. In 2010, the results of the phase III study (MDX010-20) using ipilimumab in patients with advanced melanoma were presented [10]. The median survival time of patients treated with ipilimumab was 10.0 months, of patients treated with ipilimumab and gp100 – 10.1 months and in both cases it was significantly higher than in the control group (median survival time: 6.4 months). In 2011, in a study evaluating the efficacy of ipilimumab at the dose of 10 mg/kg of body weight (in combination with dacarbazine) in the first-line therapy, long-term responses were demonstrated in some patients and an improvement in total survival rate (the percentage of 2-year OS was 28.5%) [11, 12]. The analysis of 12 clinical trials confirmed the potential long--term effect of ipilimumab on the survival rate, and the 10-year survival curve reached a plateau at about 20% [13]. However, ipilimumab is not currently used in monotherapy as first-line option treatment.

Nivolumab and pembrolizumab

Nivolumab has a structure of a human monoclonal IgG4 antibody with a half-life of approximately 26 days and is specific to the PD-1 receptor. The mechanism of action of both nivolumab and pembrolizumab is to bind the drug to the PD-1 receptor and block the interaction with PD-L1 and PD-L2 ligands, which in turn activates T-lymphocytes for an immune response against neoplastic cells [14]. Two large phase III studies, CheckMate-066 [15] and CheckMate-037 [16], confirmed the efficacy of nivolumab in the treatment of melanoma patients. In the CheckMate-066 study, nivolumab was used as the first-line treatment in patients without *BRAF* mutations. The comparator in the study was dacarbazine. The percentage of 2-year-old OS for nivolumab was 57.7% and PSF 39.2% [17], the percentage of 3-year-old OS was 51.2% and PFS 21.6%. The number of objective responses to nivolumab was 40% and to dacarbazine it was 13.9% [18]. The OS median for nivolumab was 37.5 months and 11.2 months for dacarbazine. In turn the median PFS was 5.1 and 2.2 months respectively [15, 18]. In the CheckMate-037 study, nivolumab was used as a follow-up treatment in patients after ipilimumab and BRAFi if a mutation was found in the *BRAF* gene. Dacarbazine or paclitaxel with carboplatin were used as comparators. The median OS in this study was 16 months for nivolumab and 14 months for chemotherapy and the median PFS: 3.1 and 3.7 months, respectively; objective responses were obtained in 27% and 10% patients, respectively [19].

Pembrolizumab is a humanized monoclonal IgG4 antibody with a half-life of about 27 days [20]. Its efficacy was confirmed in phase III KEYNOTE-006 study, with the participation of patients with advanced melanoma, who had not been previously taken part in systemic treatment. Patients were assigned to 3 groups of patients receiving pembrolizumab every 2 or 3 weeks or ipilimumab [21]. The median OS was 32.7 months in groups treated with pembrolizumab and 15.9 months in groups treated with ipilimumab. The median PFS was 8.4 and 3.4 months respectively [22, 23]. Objective responses were obtained in the group receiving pembrolizumab every 2 or 3 weeks in 33.7% and 32.9% of patients respectively, and in the group treated with ipilimumab in 11.9% [21].

Combination therapy using anti-CTLA-4 with anti-PD-1

The first attempts to combine CTLA-4 and PD-1 inhibitors were made in phase I, where the combination of nivolumab at a dose of 1 mg/kg of body weight with ipilimumab at a dose of 3 mg/kg of body weight was tested [24]. The basic study that evaluated the efficacy of combination therapy: nivolumab with ipilimumab was the phase III study CheckMate-067 [25]. The

median OS was not obtained in the group receiving nivolumab with ipilimumab, in the group with nivolumab it was 36.9 months, and in the group with ipilimumab 19.9 months. The median PFS reached 11.5 months, 6.9 months and 2.9 months respectively, with objective responses of 57.6%, 43.7% and 19% respectively. It should be noted that the combination of nivolumab with ipilimumab gave better results in patients with low expression of PD-L1 in melanoma tissue (<5%). In case of high expression of PD-L1 in melanoma tissue, the treatment results for monotherapy with nivolumab and combined therapy with PFS and OS were similar [25]. Adverse events related to treatment in grade 3 and 4 have been reported:

- in the group receiving nivolumab with ipilimumab – in 185 (59%) out of 313 patients,
- in the group receiving nivolumab – in 70 (22%) out of 313 patients,
- in the group receiving ipilimumab – in 86 (28%) out of 311 patients [26, 27].

Another study that evaluated the association of anti-CTLA-4 with anti-PD-1 was the III/IV phase of CheckMate-511 study [28]. It compared 2 different doses of both drugs, i.e. nivolumab at a dose of 1 mg/kg of body weight with ipilimumab at a dose of 3 mg/kg of body weight (NIVO1+IPI3) and nivolumab at a dose of 3 mg/kg of body weight with ipilimumabat a dose of 1 mg/kg of body weight. (NIVO3+IPI1). Objective response to treatment was obtained in 45.6% of cases in the NIVO3+IPI1 group and 50.6% in the NIVO1+IPI3 group, and the percentage of complete remissions – (CR) was 15.0% and 13.5% respectively. The median OS was not obtained in any of the groups and the median PFS was 9.9 months in the NIVO3+IPI1 group and 8.9 months in the NIVO1+IPI3 group. Annual PFS was 47.2% and 46.4% respectively and annual OS was 79.7% and 81% respectively.

The association of pembrolizumab at a dose of 2 mg/kg of body weight with ipilimumab at a low dose of 1 mg/kg of body weight at the phase Ib of KEYNOTE-029 study was also evaluated. The number of objective responses to treatment was 61%, including 15% of CR. In 27% of treated patients adverse effects were reported in grades 3 and 4 [29, 30].

Targeted therapy

Monotherapy with BRAF orMEK inhibitors

Vemurafenib

Vemurafenib is a low molecular weight BRAF serine threonine kinase inhibitor used in melanoma patients with mutations in the *BRAF* gene [31]. In phase III of the BRIM-3 study, vemurafenib and dacarbazine were compared in patients with advanced melanoma, who had not previously undergone systemic treatment, with the presence of mutations in the *BRAF V600E* gene [32]. The median OS in the group receiving vemurafenib was 13.6 months and in the group with dacarbazine – 9.7 months in the analysis before cross-over to vemurafenib (in the analysis after cross-over the median OS was 10.3 months). The median PFS was 6.9 and 1.6 months respectively [32, 33]. The 1-, 2-, 3- and 4-year survival rate constituted 56% and 46%, 30% and 24%, 21% and 19%, as well as 17% and 16%, respectively. In the group receiving vemurafenib, 48% of responses to treatment were reported, and in the group treated with dacarbazine – 5% [32].

Dabrafenib

Dabrafenib is a reversible BRAF V600 kinase inhibitor used in melanoma patients with mutations in the *BRAF* gene. In the BREAK-3 study, dabrafenib was compared with dacarbazine in patients with advanced melanoma with the present *BRAF* V600E mutation who had not received previous systemic treatment. Because of the planned cross-over to dabrafenib after disease progression, the primary endpoint was PFS, which in the group with dabrafenib was 5.1 months and in the group with dacarbazine – 2.7 months [34]. According to data presented at the ASCO conference in 2013, the median OS in the dabrafenib-treated group was 18.2 months and in the dacarbazine-treated group 15.6 months, while the number of responses to dabrafenib treatment was 59% [17].

Encorafenib

Encorafenib, like dabrafenib and vemurafenib, it is an inhibitor of BRAF V600 kinase. However, it differs from them by 10 times longer half-life of dissociation (>30 h). This probably results in higher antineoplastic activity and at the same time less activation of the MAPK pathway in healthy tissues, which is responsible for the development of adverse effects [37]. The combination of encorafenib with binimetinib compared to encorafenib or vemurafenib was evaluated in the COLUMBUS study [43, 44].

Trametinib

Trametinib is an oral, low-molecular, selective inhibitor of MEK1 and MEK2 kinase. Trametinib was evaluated in phase III of the METRIC study and compared with chemotherapy (dacarbazine or paclitaxel) in patients with advanced melanoma with *BRAF* V600E/K mutation [35]. In this study, the median PFS was 4.9 months for trametinib and 1.5 months for chemotherapy, and 1-, 2-, 5-year total survival for trametinib and chemotherapy was 60.9% and 49.6%, 32.0% and 29.4%, 13.3% and 17.0% respectively. In the vast majority of patients at the early stage of treatment, a cross-over (n = 70, 65%) to trametinib was used [36].

Binimetinib

Binimetinib, just as trametinib, is an oral, low-molecular, selective inhibitor of MEK1 and MEK2 kinase. Its efficacy was assessed in a phase III study with NEMO randomization, where it was compared with dacarbazine in patients with advanced melanoma with *NRAS* mutation. The median OS in the group receiving binimetinib was 11 months, and in the group receiving dacarbazine – 10.1 months. The median PFS was 2.8 and 1.5 months respectively, and the rate of responses was 15% and 7% respectively [38].

Therapy combined with BRAF and MEK inhibitors

Vemurafenib with cobimetinib

The efficacy of the combination of vemurafenib and cobimetinib was confirmed by phase III of coBRIM study [39]. It showed the advantage of combination of vemurafenib and cobimetinib over monotherapy with vemurafenib. The median OS and PFS for the combination was 22.3 and 12.3 months for the combination and 12.3 and 7.3 months for the vemurafenib respectively. Similarly, the number of objective responses for vemurafenib with comimetinib was 70% and 50% respectively. One of the advantages of using the combination in comparison with monotherapy with BRAF inhibitor was significantly lower number of skin complications.

Dabrafenib with trametinib

The combination of dabrafenib and trametinib was evaluated in two studies of the third phase of COMBI-d [40] and COMBI-v [41]. In the first one, dabrafenib with trametinib was compared to dabrafenib and in the second one to vemurafenib. The median OS in COMBI-d was 25.1 months for the combination and 18.7 months for the trametinib, the median PFS was 11 and 8.8 months respectively, while the number of objective responses to treatment ranged from 69% to 53%. In the COMBI-v study, the median OS for the combination was 25.6 months and for vemurafenib 18 months, while the median PFS was 11.4 and 7.3 months, respectively, and the number of responses to treatment was 64% and 51%. The updated 5-year follow-ups in COMBI-d and COMBI-v showed a 4-year PFS of 21% and a 5-year PFS of 19%. Four-year OS was 37% and five-year OS was 34%. Total remission was observed in 19% of patients with 5-year-old OS at 71% [42].

Encorafenib with binimetinib

The efficacy of the combination of encorafenib and binimetinib was evaluated in the COLUMBUS study [43, 44]. Encorafenib and binimetinib were compared with encorafenib or vemurafenib. The OS median for the combination was 33.6 months and 16.9 months for vemurafenib. The median PFS for encorafenib with binimetinib was 14.9 months, for encorafenib 9.6 months and for vemurafenib 7.3 months. However, the number of objective responses to treatment was 64%, 52% and 41% respectively.

Immunotherapy with targeted therapy

Combinations of immunotherapy and targeted therapy are currently being tested in many clinical trials. This treatment is used in patients with melanoma with the current mutation in the *BRAF* gene. This combination of therapies seems very promising. It enables a significant number of responses to be obtained in a short time using BRAFi/MEKi and a long-term maintenance of these responses during treatment with checkpoint inhibitors. Another justification for this management strategy is based on different mechanisms of action of individual therapies, which, due to their complementary action, may improve the effects of treatment [45].

The first combinations of targeted therapies with immunotherapy for melanoma referred to ipilimumab and vemurafenib. However, the study was discontinued due to significant hepatotoxicity of combined therapy [46]. Subsequent studies concerned the combination of ipilimumab or nivolumab or pembrolizumab with dabrafenib and trametinib [47]. The first results of treatment with pembrolizumab, dabrafenib and trametinib are promising, with PFS of 16 months and 59.8% response rates with a median response time of 18.7 months and acceptable treatment toxicity [47]. The study evaluating the toxicity of the combination of atezolizumab, vemurafenib and cobimetinib resulted in 71.8% objective responses and 39.3% with a median response duration of 17.4 months [48]. Currently, patients with advanced melanoma are undergoing a number of clinical trials to combine targeted therapy with immunotherapy, but most often these are phase I and phase II studies [49]. However, the results of treatment of patients with advanced melanoma are still yet to come, as they require confirmation in subsequent clinical trials.

Strategy of management of patients with advanced melanoma

At present, the following therapies are registered for the treatment of patients with melanoma in inoperable grade III and grade IV:

Combined treatment

a) patients with a present mutation in the *BRAF* gene:
- dabrafenib with trametinib
- encorafenib with binimetinib
- vemurafenib with cobimetinib
- nivolumab with ipilimumab

b) patients with no mutation in the *BRAF* gene:
- nivolumab with ipilimumab (currently not refundable in Poland).

Monotherapy

a) patients with a mutation in the *BRAF* gene:
- dabrafenib
- trametinib
- vemurafenib
- nivolumab
- pembrolizumab
- iplimumab

b) patients with no mutation in the *BRAF* gene:
- nivolumab
- pembrolizumab
- iplimumab.

In the light of current studies, the use of targeted therapies as monotherapy may be justified only in case of significant complications during BRAFi-MEKi combination therapy [39–

44]. However, if complications occur during combined therapy, the combination of BRAFi and MEKi with a different toxicity profile should be considered first. In the case of checkpoint inhibitors, the use of immunotherapy as a monotherapy is preferred in most patients due to its low toxicity. The combination of nivolumab and ipilimumab should be recommended especially in patients with asymptomatic CNS metastases [50–52]. However, the sequence of therapies, especially in patients with the present mutation in the *BRAF* gene, remains a problem.

A very practical solution was proposed in the work by Schvartsman et al. [50]. He distinguished two prognostic groups among patients with melanoma in inoperable grade III and in grade IV: low and high risk (table I). Then, depending on the risk group and the presence or absence of metastases in the CNS, he presented 4 possible scenarios of management (fig. 1–4):

- low-risk patients without metastases in CNS;
- low-risk patients with metastases in CNS;
- high-risk patients without metastases in CNS;
- high-risk patients with metastases in CNS.

Such a division can be very useful in everyday medical practice. It seems that if the patient is in good general condition, the tumor mass is small and occurs in 1–2 locations and the LDH level is normal or slightly elevated, the best option is to start therapy with checkpoint inhibitors. Of course, attention should be paid to possible contraindications to immunotherapy and a discussion with the patient about other available therapeutic options.

In situations where the disease is rapidly progressing and a mutation is present in the *BRAF* gene, BRAFi and MEKi are the therapies of choice (in some cases checkpoint inhibitors may also be considered). One can also optionally start treatment with BRAFi and MEKi (for a short period of time) to stop the neoplastic process and then move on to immunotherapy. However, new therapeutic options and drug combinations are being sought to achieve long-term survival in this group of patients.

Table I. Prognostic groups of patients according to Schvartsman et al. [50]

Factor/group	Low-risk group	High-risk group
Tumor mass size (total volume)	<10 cm	≥10 cm
Number of locations where metastases were found	<3 locations	≥3 locations
LDH concentration value	Normal	≥2 × upper limit of normal
Performance status	0 or 1	≥2

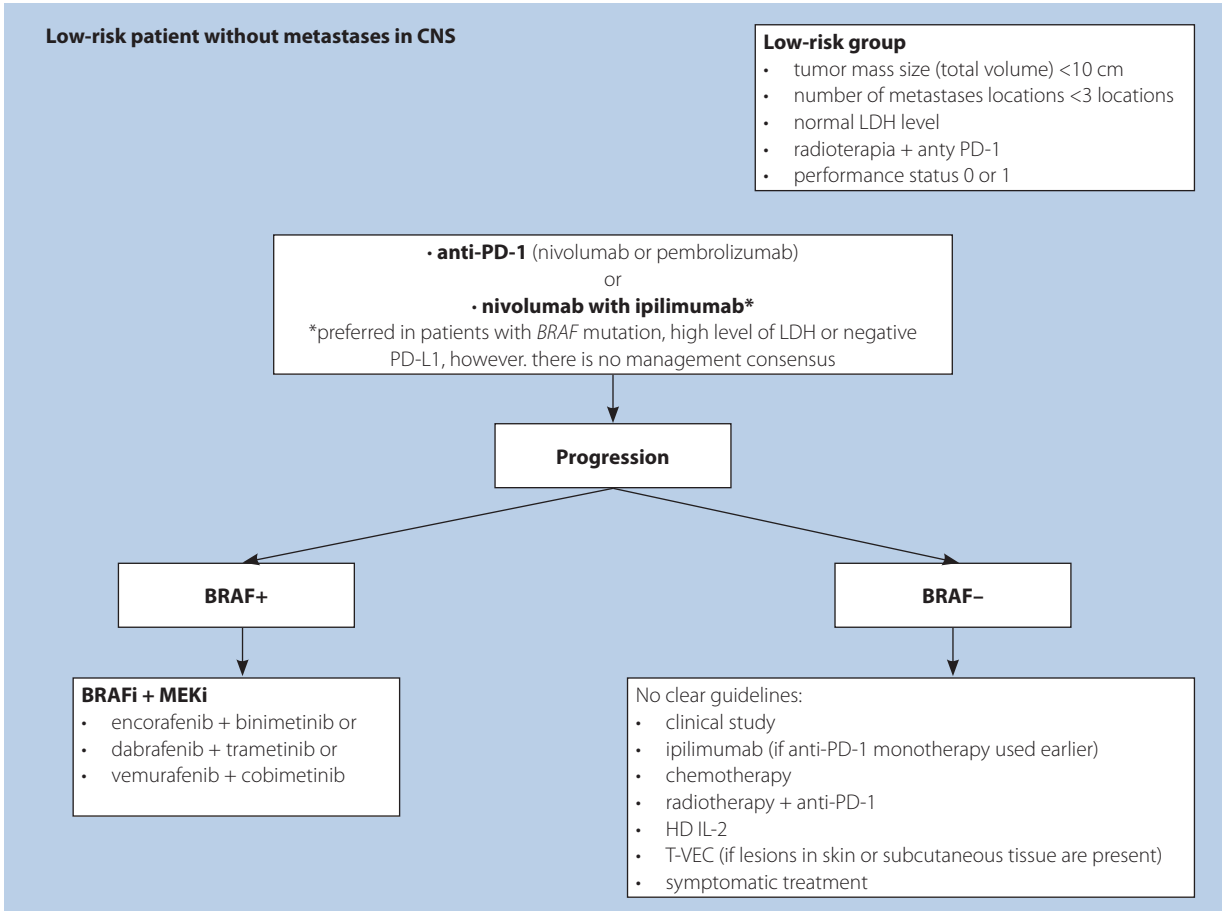

Figure 1. Management algorithm 1. Low-risk patient without metastases in CNS

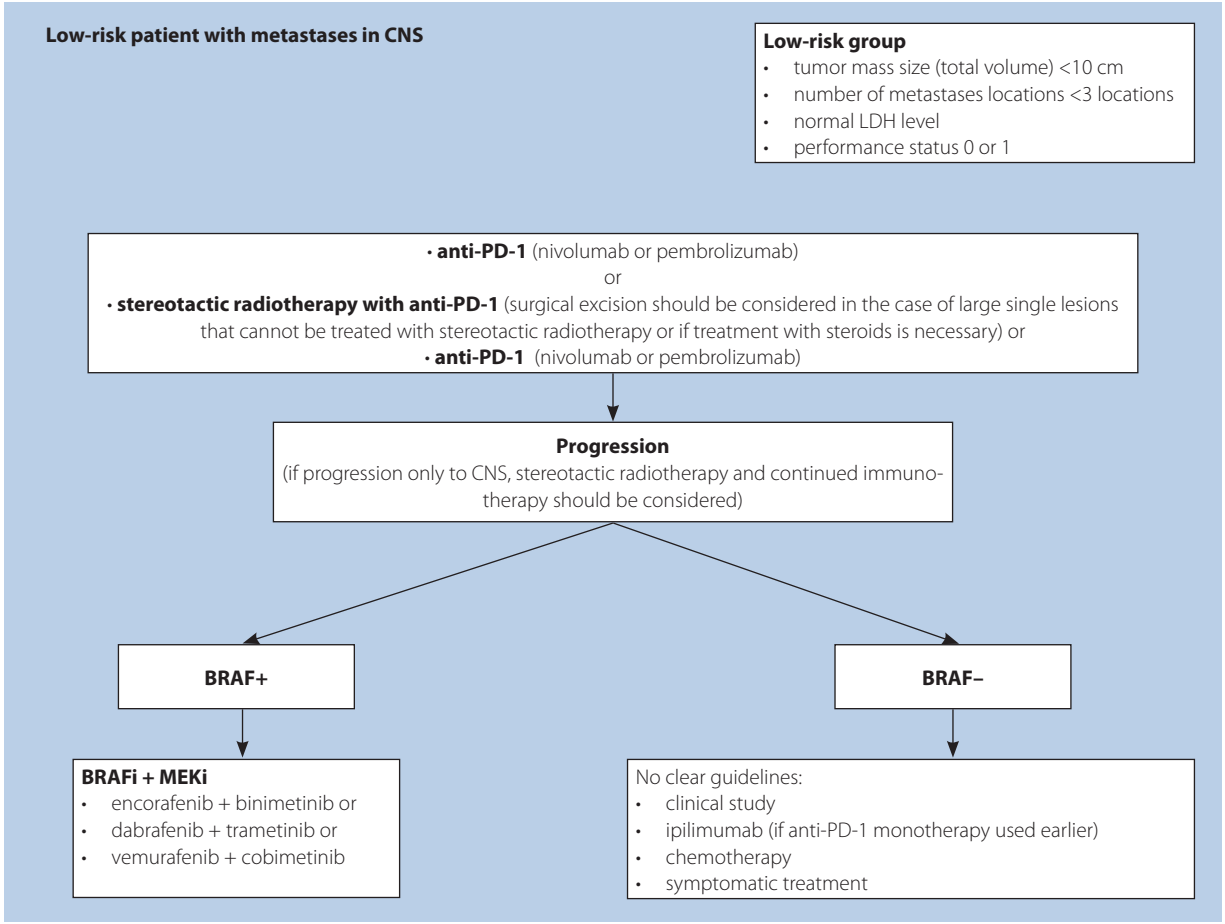

Figure 2. Management algorithm 2. Low-risk patient with metastases in CNS

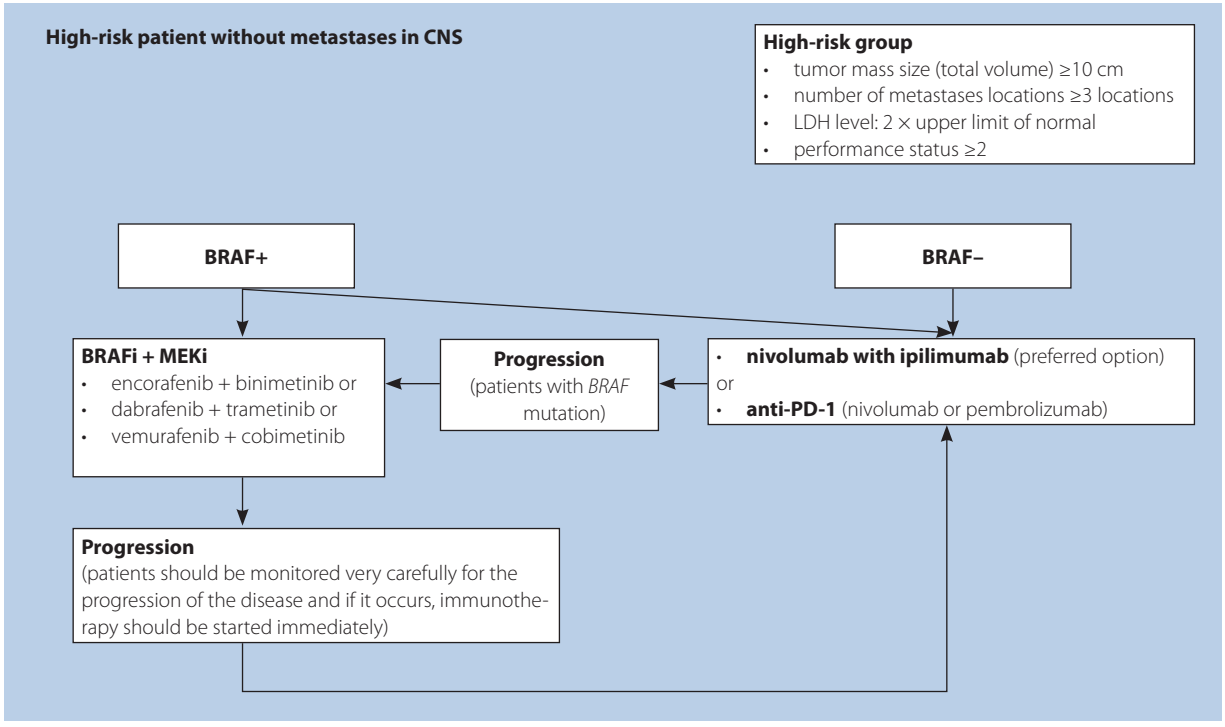

Figure 3. Management algorithm 3. High-risk patient without metastases in CNS

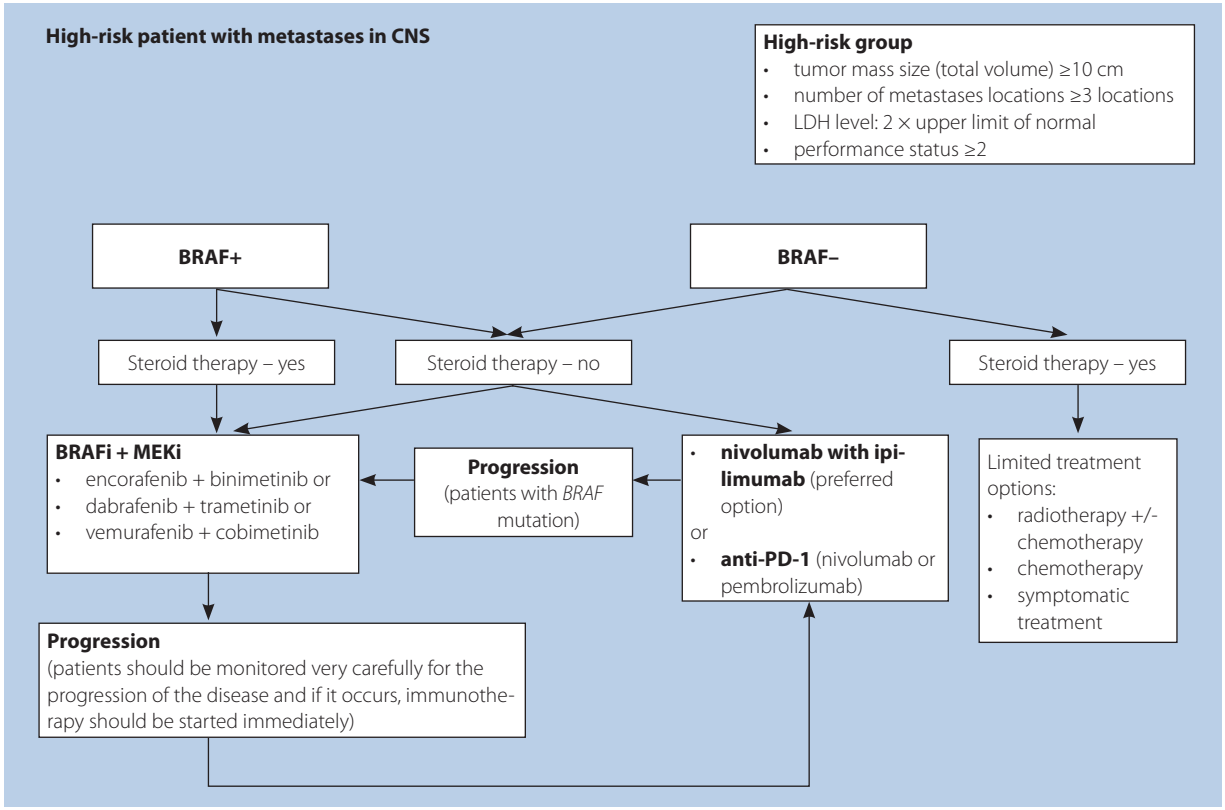

Figure 4. Management algorithm 4. High-risk patient with metastases in CNS

Summary

The emergence of new therapies has certainly improved the survival rates of melanoma patients. However, there is still a group of patients in whom the efficacy of the treatment is unsatisfactory and further research is needed to answer the question: "Why do these patients not respond to the treatment applied?". Other problems have also emerged in relation to the toxicity of new drugs and the sequence and duration of treatment, which require further research.

Conflicts of interest: Bożena Cybulska-Stopa: lectures, analyses, conferences sponsored by BMS, MSD, ROCH, Novartis, Pierre Fabre.

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Review article

Akademia Czerniaka

Mechanisms of melanoma resistance to treatment with BRAF and MEK inhibitors

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Several mechanisms of resistance to inhibition of *BRAF* activity in melanoma cells have been described so far. Genetic studies have shown that mutations in MEK1 kinase (MAP kinase kinase), which result in constitutive activation of ERK kinase, result in resistance to treatment. Another mechanism of the acquired BRAF inhibition resistance is the accumulation of activating mutations in the *NRAS* oncogene, which drives the activation of CRAF. This in turn leads to a permanent activation of the signal transduction to *MEK* and *ERK*. Another important mechanism of resistance is the formation of variants of the *BRAF* V600E gene splicing, including variants that lack exons 4 to 8 containing the *RAS*-binding domain. The presence of the *p61 BRAF* V600E variant leads to the constitutive *ERK* signal, which is resistant to *RAF* inhibition. In addition, treatment resistance is affected by hyperactivation of tyrosine kinase receptors such as platelet-derived factor receptor β (PDFRβ), insulin-like growth factor 1 receptor (IGF-1R) and erythropoietin-producing hepatocellular receptors (EPH) – leading to the induction of the 3-phosphoinositol kinase pathway (PI3K) in patients treated with BRAF or MEK inhibitors. Another interesting path of BRAFi/MEKi resistance is over-expression of the epidermal growth factor receptor (EGFR) through negative feedback in patients treated with BRAF inhibitors (BRAFi) – EGFR is not normally expressed in untreated melanomas.

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Key words: BRAF, MEK, melanoma, MAPK, drug resistance

Introduction

Before the discovery of activating mutations in the *BRAF* gene, melanoma was considered to be the skin cancer with the worst prognosis. Classical dacarbazine-based chemotherapy regimens provided few therapeutic options. In 2002, in a breakthrough study conducted by the Cancer Genome Project at the Sanger Institute, *BRAF* [1] mutations were identified in over 60% of melanoma patients. Currently, melanoma is responsible for over 80% of skin neoplasm deaths, although its cases account for only 1% of all skin neoplasm cases. Until 2011, when the Food and Drug Administration (FDA) approved vemurafenib (the first drug acting selectively on a specific mutant protein in the signalling pathway of BRAF/MEK), there was virtually no effective therapy for patients with melanoma with metastases. The group of drugs that include vemurafenib (as well as dabrafenib and encorafenib) is referred to as BRAF (BRAFi) protein inhibitors [2].

The RAS/MAPK pathway (fig. 1) is one of the best known pathways of signal transduction from the cell environment to the cell nucleus in which, as a result of the action of these signals, the transcription of genes associated with the processes of cell growth, division and differentiation is initiated. It can be said that it is a cascade of phosphorylation of subsequent proteins (Ras-Raf-MEK-ERK), where an activated protein phosphorylates a subsequent protein and thus the signal is transmitted up to the transcription factors (among others

c-Myc and CREB). Activated Ras stimulates RAF kinase protein kinase activity, RAF kinase phosphorylates and activates MEK (MEK1 and MEK2), and MEK phosphorylates and activates mitogen-activated protein kinase (MAPK). RAF and ERK (also known as MAPK) are serine/threonine protein kinases and MEK is a serine/tyrosine/threonine kinase. This signalling pathway is activated by growth factors, hormones and cytokines that interact with the membrane receptor with tyrosine kinase activity (RTK). As a result of these interactions, the membrane receptor is phosphorylated and binds several different proteins, which in turn transmit a signal to the RAS (*rat sarcoma*) protein. RAS protein then binds guanosine triphosphate, which activates it and in turn transmits the signal to the whole next cascade of protein kinases activated by mitogens (mitogen activated protein kinases – MAPK). The first protein in this pathway is RAF (rapidly accelerated fibrosarcoma protein): BRAF or CRAF. RAF proteins have serine/threonine kinase activity and phosphorylate and activate MAPK kinase, also known as MEK – MAPK/ERK, which in turn phosphorylates another ERK kinase (extracellular signal regulated kinase), which after phosphorylation moves to the cell nucleus, where it activates target transcription factors through phosphorylation. Disturbances in this pathway can lead to neoplasms on the one hand, and several human malformations on the other [3].

Disturbances in this pathway, including oncogenic ones, may occur at different levels as a result of gene mutations – the *RAS* gene is one of the most frequently mutated oncogenes in human cancers [4]. Disturbances in the whole RAS/MAPK pathway occur in 98% of melanomas [5]. About 50% of these cancers (but for comparison, only 7–10% of all cancers in general) have a mutation in the *BRAF* gene. On the other hand, 80–90% of these mutations are of the V600E missense type, where amino acid number 600 is glutamic acid and not valine found in wild-type protein. This mutation changes the

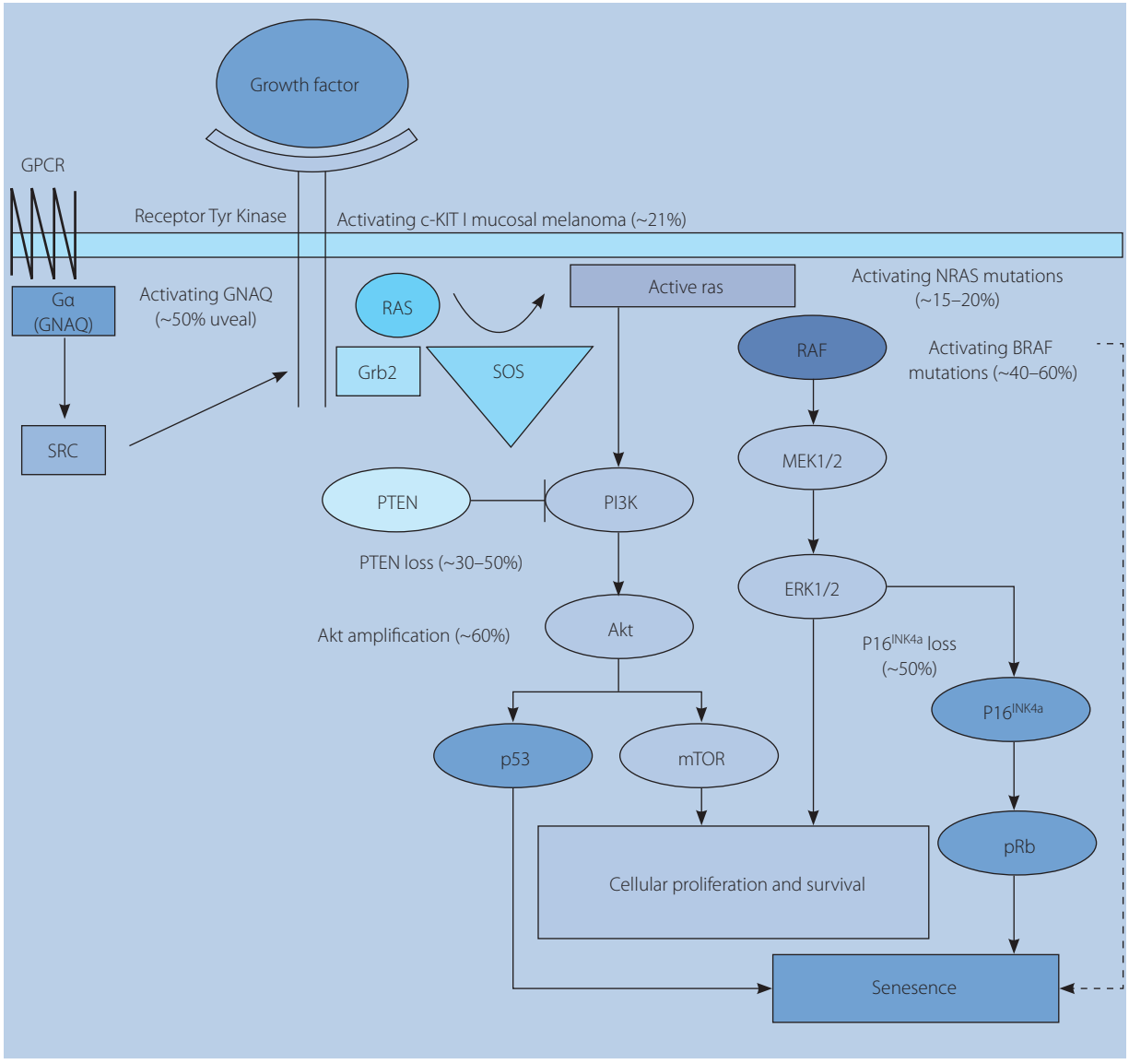

Figure 1. RAS pathway in melanoma cells

conformation of BRAF protein, increasing its kinase activity, resulting in constitutive activation of the MAPK/ERK pathway. In the same place, but much less frequently, there are also other substitutions, e.g. V600K (7.8%), V600R (1%) (lysine and arginine substitution, respectively). In melanoma cells mutations also occur in other MAPK/ERK pathway genes, most often in the *NRAS* gene (13–25%) [6, 7].

It is worth mentioning that mutations in the *BRAF* gene do not depend on UV radiation and are not sufficient to cause melanoma. The oncogenesis process is the result of many mutations in different genes. There are also other mutations in melanoma, e.g. in tumor suppressor genes *TP53*, *PTEN* and *CDKN2A*, as well as in the telomerase gene promoter [5]. Also important for melanoma development are mutations induced by UV radiation, which are characterized by transitions of C->T nucleotides at the 3' end of pyrimidine dimers [8], such mutations occur in the above mentioned cancer suppressor genes, the telomerase gene and in *p16*. However, the issue of mutations induced by UV is complicated, they are different in different melanomas and molecular classification is based more on mutations that are not caused by UV rays [5]. Moreover, it is not easy to determine whether a given mutation was caused by UV, and it is not always certain [9].

In molecular terms, melanomas can be classified into 4 groups based on the mutations that occur in them: in *RAS*, in *BRAF*, in *NF1*, and the fourth group consists of so-called triple--negative melanomas [5, 10, 11]. It is interesting that in primary melanomas mutations in *RAS* and *BRAF* genes never occur simultaneously. As for the *MEK* gene mutations (MAPK), there is no such good data as for mutations in many other genes, but since MEK inhibitors are also used in melanoma therapy together with BRAF inhibitors, *MAP2K1* mutations occur in 5.38% of patients with melanoma while *MAPK1* mutations occur in 1.77% of patients with melanoma. Mutations in specific *MAPK* genes are often criteria for inclusion in clinical trials with MEK inhibitors (MEKi) [12].

BRAF protein normally acts as a dimer, but the *V600E* mutation causes it to act as a monomer. Inhibitors used in melanoma therapy, such as vemurafenib, have an effect on this monomer. Approximately 20% of patients with the *BRAF V600E* mutation do not respond to this drug [6]. The reasons for this are complex and may be due, among other things, to the heterogeneity of the cancer – e.g. not all of its cells have a target mutation, or to the loss of certain cancer suppressor genes, such as *PTEN* and *NF1*, which cause primary resistance to BRAFi/MEKi [6].

Similarly, after a few months of using BRAFi, melanoma cells become resistant to therapy, which may have different causes. In general, mutations that cause resistance act by increasing the frequency of *RAF* dimerization, although the most common cause of such resistance is reactivation of BRAF/ /MEK or another pro-proliferative signal transduction pathway. This reactivation may pass through ERK or other proteins. The reactivation can also be done by activating other RAFs, e.g. ARAF and CRAF. Another signal transduction pathway, such as PI3K-AKT-mTOR, can also be activated in the process of creating BRAFi/MEKi resistance. In inhibitor-resistant melanoma cells with *BRAF* V600E mutation there is a type-β platelet-derived growth factor receptor (PDGFRβ) over-expression, but other changes in these cells are also present. There is also an increased activation of the *IGFR1* receptor (a receptor of insulin – like growth factor 1), which leads to activation of the PI3K-AKT--mTOR pathway. Finally, in some resistant cells, elevated levels of epidermal growth factor receptors are also found [2]. From the physiological point of view, in general, it is always about reactivation of the signalling pathway transmitting information about proliferation. It is not possible to determine the cause of the drug resistance in all cases.

Some causes of BRAFi/MEKi drug resistance are related to the *BRAF* gene itself. However, these are not typical mutations in the gene itself that change its structure, but those that cause changes in the splicing of the gene transcript. The result is a protein product again capable of forming dimers (which removes the activity of inhibitors that act on the monomer) or over-expression of this gene, resulting in more protein product that may result in the presence of dimers. Indirectly, *RAS* gene mutations can also influence dimerization [6].

Mechanisms of primary resistance of melanoma to treatment with BRAF inhibitors

Malignant melanomas are genetically very heterogenous. Moreover, they gain new mutations in the course of the metastasis process [13]. A significant proportion of melanoma tumor cells carry the *BRAF* V600E mutation and about 20% of patients with melanoma with this *BRAF* V600E mutation do not respond to treatment with BRAF inhibitors [14]. In addition, different cancer cells in the same patient may carry different mutations responsible for resistance to BRAF inhibitors [6]. The main proposed mechanisms of primary resistance to BRAF inhibitors, such as *PTEN* loss, *RAC1P292S* mutation, MAP3K8 over-expression, hepatocyte growth factor (HGF) secretion by stromal cells, *NF1* suppressor gene loss and *CCND1* amplification, are summarized in table I.

Future studies should provide a more detailed explanation of the mechanisms underlying the BRAF inhibitor resistance and the discovery of common mechanisms of resistance to different groups of chemotherapeutic agents. An important objective of the studies is to identify biomarkers of primary resistance to BRAF inhibitors so that the therapeutic response can be predicted before the onset of the therapy.

Loss of the *PTEN* gene

The *PTEN (*phosphatase and tensin homolog) gene is a suppressor gene – the protein encoded by this gene (phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase – PTEN, MMAC1) is involved in cell cycle regulation. PTEN catalyzes PIP3 pho-

Table I. The most important mechanisms of primary resistance to BRAF inhibitors

Mutation/ other cause of resistance	Proposed mechanism	Bibliography
CCND1 amplification	The *CCND1* gene codes cyclin protein D1 – the key regulator of the cell cycle. Amplification of the *CCND1* gene and increase of cyclin D1 protein level maintain cell proliferation in the presence of BRAF inhibitors.	[22, 23]
$RAC1_{P29S}$ mutation	$RAC1_{P29S}$ mutation maintains MAPK signalling pathway transmission in the presence of BRAF inhibitors, proliferation persists despite BRAF inhibition.	[16, 51]
MAP3K8 over-expression	The *MAP3K8* gene encodes the COT protein that activates the MAPK/ERK signalling pathway. Increased level of COT protein results in maintenance of proliferation despite BRAF inhibition.	[18, 19]
Loss of the *NF1* suppressor gene	*NF1* is the negative regulator for the RAS signalling pathway. Mutation of *NF1* resulting in loss of functional neurofibromin 1 protein (NF1) causes an increase in RAS level, activation of CRAF and consequently activation of MAPK pathway, which results in maintenance of proliferation in the presence of BRAF inhibitors.	[21, 52]
Loss of the *PTEN* gene	*PTEN* is the PI3K/AKT pathway suppressor. Loss of *PTEN* results in constitutive activation of this signalling pathway and enables cell proliferation even in the case of BRAF inhibition.	[15]
Secretion of hepatocyte growth factor (HGF) by stromal cells	HGF secretion leads to activation of the HGF receptor – MET – and activation of the MAPK/ERK and PI3K/AKT.	[25, 26]

sphorylation in the 3'position of the inositol ring, which results in inhibition of the PI3K/AKT signalling pathway and finally inhibition of cell proliferation. The loss of the functional *PTEN* gene occurs in over 10% of melanoma cases and is one of the most common mutations responsible for BRAF inhibitor resistance [15]. Loss of PTEN expression leads to constitutive activation of the PI3K/AKT signalling pathway, which results in cell proliferation, cell growth and inhibition of apoptosis. The mechanism of resistance to BRAF inhibitors is inhibition of apoptosis induced by BIM protein (BCL2L11) [15].

*RAC1*P29S gene mutation

RAC1 protein (rac family small GTPase 1 cell migration-inducing gene 5 protein) is a GTPase, a regulator of the cell cycle, cellular adhesion, cell mobility (by interacting with the cytoskeleton) and cell differentiation. The P29S mutation in the *RAC1*gene, according to Watson and Li [16] study results, occurs in 3.3% of cases of melanoma, so not very often. The presence of this mutation positively correlates with the mitotic index, the size of the lesion, as well as the occurrence of metastases [16]. However, the P29S mutation occurs in up to 20% of patients who do not respond to treatment with BRAF inhibitors [17]. Moreover, the presence of this mutation in melanoma cell lines causes resistance to BRAF inhibitors [16].

MAP3K8 gene over-expression

The *MAP3K8* gene codes for the MAP3K8 protein (mitogen--activated protein kinase kinase kinase 8, otherwise COT, EST, ESTF, MEKK8, TPL2, Tpl-2, c-COT, AURA2). The MAP3K8 protein can activate the MAPK/ERK signalling pathway. Increased level of COT protein results in maintenance of proliferation despite BRAF inhibition. In the case of primary over-expression of *MAP3K8*, administration of BRAF inhibitors leads to even higher COT production and, as a result, to further intensification of proliferation [18, 19]. *MAP3K8* gene modifications occur in about 1.5% of all patients with melanoma, often (in about 33% of cases) they are present in Spitz nevi [20].

Loss of NF1 protein function

The *NF1* gene encodes the neurofibromin protein (neurofibromin or neurofibromatosis-related protein – NF-1), which belongs to the group of proteins that activate GTPases. Neurofibromin is a negative regulator of RAS, the first protein of the MAPK signalling pathway. The loss of the functional *NF1* gene product means an increase in RAS protein level and activation of the MAPK pathway, also in the presence of BRAF inhibitors. The loss of *NF1* causes resistance to RAF, MAPK and BRAF inhibitors through constitutive activation of the MAPK kinase signalling pathway [21].

CCND1 gene amplification

The *CCND1* gene *(BCL1*) codes for cyclin D1(cyclin D1 or otherwise B-cell lymphoma 1 protein), the key protein responsible for cell cycle regulation (G1/S phase transition). BRAFi resistance due to *CCND1* amplification is an example of BRAF inhibitor resistance associated with gene copy number change. In cells where the *CCND1* gene was amplified, increased production of cyclin D1 occurs and as a result BRAF inhibition is not sufficient to inhibit proliferation [22]. Amplification of *CCND1* is observed in more than 38% of melanoma samples, which indicates that a large group of patients is potentially resistant to BRAFi and could benefit from CDK4/6 inhibitor therapy [23, 24].

Secretion of the hepatocyte growth factor by the stromal cells

The hepatocyte growth factor (HGF) is a factor responsible for cell growth, mobility and morphogenesis. Pleiotropic activity of HGF occurs through its receptor, a transmembrane tyrosine kinase, encoded by the cMet proto-oncogene. An example of a mechanism of primary resistance to BRAF inhibitors, which

is associated with the interaction of the tumor microenvironment, is HGF secretion by the stromal cells (including fibroblasts). HGF secretion leads to activation of the HGF receptor – MET protein – and activation of the MAPK/ERK and PI3K/AKT pathways (fig. 2). Their activation results in maintenance of proliferation in the presence of BRAF inhibitors [25, 26].

Mechanisms of secondary resistance of melanoma to treatment with BRAF inhibitors

During treatment, most patients develop secondary resistance to BRAFi/MEKi. The most frequent mechanism of secondary resistance to BRAFi/MEKi treatment is connected with reactivation of signal transduction through the MAPK/ERK pathway. Activation of this pathway may result from both the action of BRAF activating proteins activated by BRAF protein and the secondary activation of *BRAF* itself.

Reactivation of signal transduction by MAPK/ERK

Reactivation of signal transduction from the cell membrane to MAPK/ERK kinases (*upstream reactivation*) is a result of over-expression of receptors tyrosine kinases, which leads to cell division by activating ARAF and CRAF kinases in place of BRAF. Melanoma cells with the *BRAF* mutation V600E during treatment with BRAFi/MEKi can develop treatment resistance by switching the signal to different RAF isoforms (ARAF, BRAF or CRAF) and the resulting reactivation of transmission within the ERK pathway [14]. In melanoma cells there may be over-expression of ARAF or CRAF proteins while BRAF is blocked. ERK protein is a negative regulator of RAS protein. The BRAF inhibitor inhibits the growth of neoplastic cells by inhibiting the ERK pathway. The signal transduction blockage through the ERK pathway stops RAS regulation, inducing partial *RAS* activity. Activation of RAS leads to the creation of *BRAF* V600E dimers. BRAF inhibitors bind to one of the monomers, leading to the transactivation of the second monomer, not bound by the drug. Such activation of BRAF results in partial activation of signal transduction and contributes to the reduction of treatment efficacy [27, 28].

The MAPK/ERK signalling pathway may also be activated by the accumulation of activating mutations in the *RAS* gene as *RAS* promotes cell division by phosphorylating the ARAF and CRAF proteins to compensate for BRAF inhibition. After binding GTP, mutant RAS protein does not dissociate to an inactive form associated with GDP and is constantly activated. The PGD-related mutant RAS protein associated with PGD also promotes *BRAF* V600E dimerisation, reactivation of the ERK signalling pathway and ultimately influences the formation of resistance to BRAF inhibitors, as these drugs are exclusively associated with *BRAF* V600E monomers [29–31].

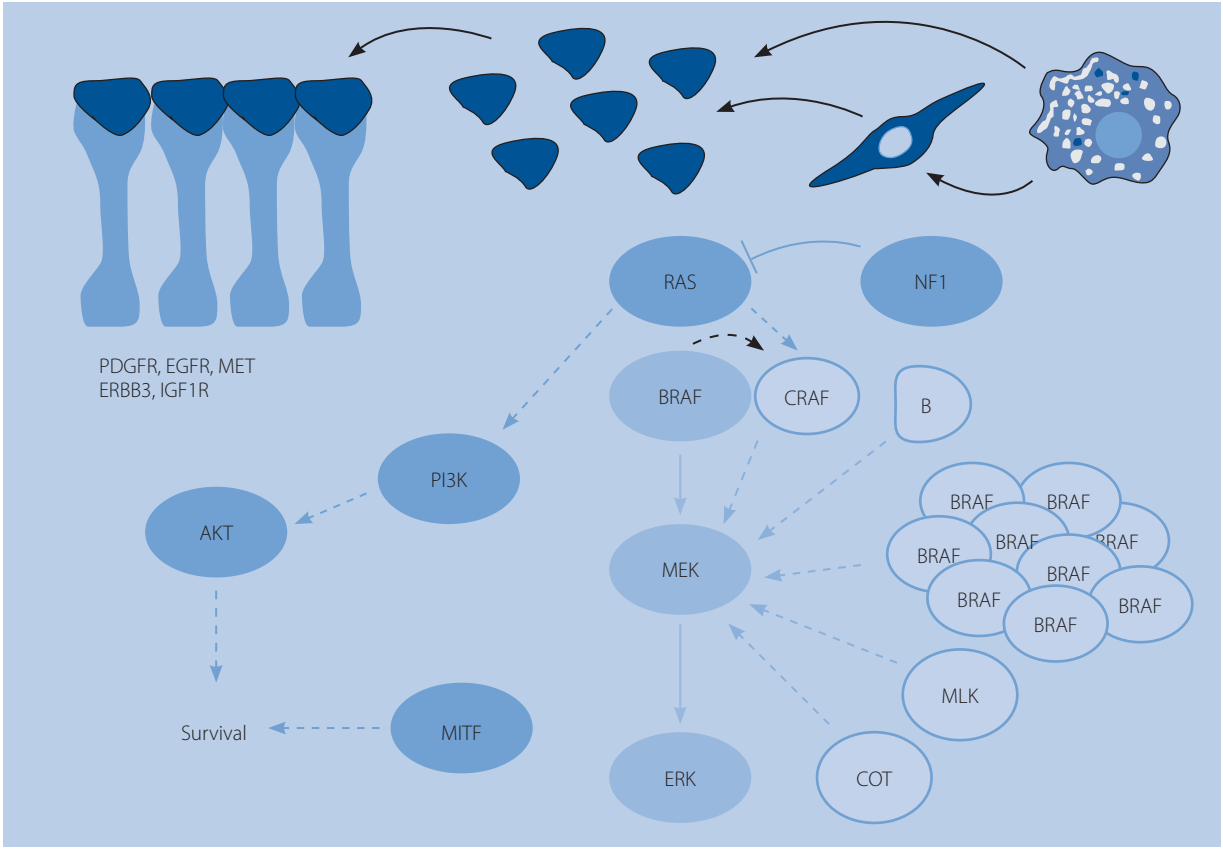

Figure 2. Activation of signalling transduction pathways in the development of drug resistance to BRAFi/MEKi

Activation of signal transduction below MAPK/ /ERK proteins

The activation of signal transduction to target genes by the RAS-RAF-MEK-ERK signalling pathway can occur due to activating mutations in the genes for MEK1/MEK2 (mitogen-activated protein kinase 1/2) proteins. As a result of the activation of MEK proteins (downstream reactivation), the initiation of the signal transfer at the *BRAF* level is no longer necessary for the activation of target genes, thus eliminating the effect of BRAF inhibition [6].

BRAF protein reactivation

Reactivation of BRAF protein function may occur by many mechanisms, among which amplification of the mutant *BRAF* allele is frequent. The BRAF protein is over-expressed due to an increase in the number of gene copies. As a result of the presence of very many copies of BRAF protein in the cell, the inhibitor dose (the number of inhibitor molecules in the cell) is no longer sufficient (in proportion) to inhibit their activity. Increasing the amount of mutant BRAF V600E protein results from increasing the number of gene copies and may lead to spontaneous dimerization of this protein, reactivation of the ERK signalling pathway and become a cause of drug resistance. This type of resistance has been defined as dose-dependent. In *in vitro* studies, higher doses of vemurafenib lead to overcoming drug resistance [32].

In addition, the *BRAF* V600E variant, which is produced by alternative splicing of p61BRAF V600E, has been described in patients with secondary resistance to vemurafenib. This variant forms dimers independently of activation by RAS kinase, which makes BRAF inhibition ineffective, due to the above-mentioned BRAFi activity only towards *BRAF* V600E monomers. Alternative BRAF splicing isoforms appear to be formed as a result of mutations or epigenetic changes [33, 34].

PI3K/AKT signalling pathway activation

The PI3K/AKT signalling pathway communicates with the ERK pathway, and inhibiting one pathway can cause the other to become more active (figure 2). Blocking ERK signaling can lead to an adaptive PI3K/AKT overactivity that compensates for BRAF inhibition and drives resistance. Abnormal PI3K/AKT signaling is a common feature of melanoma and causes resistance by stimulating alternative pathways that reduce dependence on ERK signaling. Mutations leading to increased activity of the PI3K/AKT pathway were identified in 22% of melanomas with acquired BRAF inhibition resistance. It was shown that within a few days after the administration of BRAF inhibitors the expression of AKT protein was increased [35–37].

In preclinical studies it was initially hypothesized that during BRAFi/MEKi treatment there was a strong selection pressure on cells with gain of function mutations leading to increased PI3K/AKT pathway activity in the presence of MAPK pathway inhibition. It was assumed that melanoma cells with such mutations will divide, because they have an advantage in terms of survival and proliferation when their metabolism is not affected by *BRAF* inhibition. In clinical observations, it is this intensive proliferation of cells with an activated AKT pathway that may explain the presence of high tumor mass and rapid progression in patients who responded to *BRAF* inhibition and then developed secondary resistance by this mechanism. Additionally, the PI3K/AKT pathway is activated by growth factors that are associated with RTK, such as PDGFR-β and IGF-1R. When *BRAF* is blocked by an inhibitor, tumor cells may increase the expression of PDGFR-β and IGF-1R in a compensatory manner. This in turn leads to a persistent PI3K/AKT signaling that prevents apoptosis and promotes cell survival. Activated AKT phosphorylates as many as 9000 substrate proteins, thus regulating various processes such as cell survival, proliferation and migration, and affecting drug resistance.

The AKT substrate proteins include molecules such as: ASK1 (apoptotic signal kinase 1), Bim (B cell leukemia/lymphoma-2 interacting mediator of cell death), Bad (B cell leukemia/lymphoma-2 associated death agonist), MDM-2 (murine double minute-2), p21(p21 cyclin dependent kinase protein inhibitors, Cip1), XIAP (X-linked inhibitor of apoptosis), Foxo3a (forkhead box O3) and many others [38]. High expression of the above mentioned RTK receptors on the surface of melanoma cells is associated with acquired resistance to vemurafenib both *in vitro* and *in vivo*. In addition, PI3K and AKT activating mutations can increase the signal strength of the AKT signalling pathway. This in turn intensifies the anti-apoptotic signals and the regulation of expression of the most important proliferative genes described above. These changes allow melanoma cells to survive and replicate independently of BRAF inhibition, which clinically causes the acquired resistance [6, 37, 39].

Increased activation of the EGFR signalling pathway

Increased expression and activation of the epidermal growth factor receptor (EGFR) may also be associated with BRAF or MEK inhibition resistance. Once activated, the EGRF complex formed by Grb2 and Sos proteins binds directly or by combining Shc adaptor protein with specific tyrosine residues on the receptor. This leads to conformational changes in the Sos protein, which can recruit and activate Ras-GDP. Then ERK-activated MAPK kinases eventually move to the nucleus to induce cell proliferation by phosphorylating specific transcription factors such as Elk1 and C-myc, [40]. Reduced *SOX10* (sex determining region Y-box 10) activity described in some melanomas may lead to signaling via TGF-β and consequently to increased expression of EGFR receptor gene and platelet-derived growth factor receptor (PDGFRB) [6, 41].

Tumor microenvironmental activation

The research on drug resistance focuses mainly on the mechanisms of drug resistance, which are a result of changes in the properties of cancer cells. It is now known, however,

that disease progression and resistance to targeted BRAFi/MEKi therapies are not exclusive derivatives of genomic and epigenetic cancer cell modifications.

The microenvironment of the tumor promoting MAPK inhibition resistance is important and this relationship is complex and includes interactions between the tumor and stromal cells, among others [42]. Recent studies have shown that macrophages and factors derived from fibroblasts clearly contribute to the development of resistance to MAPK pathway inhibitors. In the presence of fibroblasts, the adjacent melanoma cells acquire a differentiated, aggressive mesenchymal phenotype. After BRAFi treatment, these melanoma cells maintain a high level of phosphorylated ribosomal protein S6 (pS6), and thus active signaling through mTOR, which is suppressed in BRAFi-sensitive cells without contact with the stromal cells [43]. Activation of mTOR leads to phosphorylation and activation of the ribosomal kinase S6 p70 and eukaryotic protein binding factor 4E 1E, thus promoting increased protein translation and cell growth. mTOR is a kinase that links cell stimulation with growth factors and nutrient availability with protein synthesis and cell growth [44].

In recent years it has been estimated that fibroblasts facilitate melanoma progression and two-way communication exists through direct contact between melanoma cells and fibroblasts. Additionally, melanoma cells respond to fibroblast' secretion of growth factors and cytokines promoting cell survival and growth, including TGF-β and VEGF. Fibroblasts in contact with melanoma cells also release extracellular matrix components (e.g. laminin IV), which facilitate secondary migration (metastasis) of melanoma cells [45, 46]. Within the tumor, fibroblasts show hyperactivation of the MAPK pathway, which results in a qualitative change in the extracellular matrix of the tumor by β1 integrin and FAK kinase, which induce ERK, ensuring that melanoma cells can avoid effective treatment [47].

In melanoma, as a result of HGF secretion by stromal cells, resistance to BRAFi/MEKi may develop. HGF may bind RTK on the surface of melanoma cells, which will increase intracellular signaling promoting *RAS* expression, which ultimately leads to activation of the MAPK pathway. Moreover, it is known that HGF contributes to the development of resistance to BRAF inhibitor treatment by reducing the expression of genes encoding pro-apoptotic factors [25, 26].

The influence of fibroblasts on melanoma cells may differ significantly in elderly patients, because aging fibroblasts are more invasive. Recent studies have shown that aging fibroblasts increase the secretion of the sFRP2 factor – β-catenin inhibitor – which reduces the expression of MITF, leading to reduced expression of the APE1 redox regulator and makes melanoma cells more sensitive to oxidative stress, thus promoting secondary resistance to BRAF inhibition [48].

Macrophages of the tumor stroma secrete TNF-α factor, which promotes MITF expression depending on NF-β, which leads to BRAFi/MEKi resistance. Additionally, TNF-α has been proven to block apoptosis in cells in which BRAF is inhibited and to contribute to melanoma invasion and angiogenesis [49, 50].

Summary

The main mechanisms of resistance to BRAF inhibitors are:

1. Loss of inhibitory function of ERK kinase – BRAF inhibitor inhibits tumor growth by inhibiting the ERK pathway. This secondarily inhibits ERK negative feedback to RAS, which partially restores RAS activity. It leads to the formation of *BRAF* V600E dimers induced by RAS. BRAF inhibitors bind one and transactivate the other activate the other *BRAF*, reducing the effectiveness of BRAF inhibitor treatment.
2. Mutations activating the *RAS* gene – mutated *RAS-GTP* becomes constitutively active, increases *BRAF* V600E dimerization, reactivates the ERK pathway and secondarily promotes resistance to BRAF inhibitors that block only monomeric *BRAF* V600E.
3. Alternative *splicing* of *BRAF* V600E – the *BRAF* V600E splicing variant due to mutations or epigenetic changes can form dimers independently of RAS. This makes the BRAF inhibitor ineffective because it blocks only the monomeric *BRAF* V600E.
4. Over-expression of mutant protein BRAF V600E – an increased number of *BRAF* V600E copies in a cell (due to gene copy number increase) may also spontaneously favor *BRAF* V600E dimerization, reactivating the ERK pathway and causing treatment failure in some patients.
5. Activation of alternative RAF protein isoforms – *BRAF* V600E melanoma treated with BRAF inhibitors can acquire resistance by flexible switching between different *RAF* isoforms capable of reactivating the ERK pathway, increasing expression of ARAF or CRAF.
6. The over-expression of COT-COT protein, probably due to gene amplification or yet unidentified mechanisms, may reactivate MEK in the presence of *BRAF* inhibition, stimulating ERK signaling and drug resistance to BRAFi.
7. Mutations activating the *MEK* gene – mutations activating the *MEK1/MEK2* gene make blocking the *BRAF* ineffective, because reactivation of MEK means that the MAPK/ERK pathway can still transmit a signal below *BRAF*, regardless of its inhibition.
8. Activation of the PI3K/AKT signalling pathway. Incorrect PI3K/AKT signaling is a frequent feature of melanoma. Blocking ERK signaling can lead to an adaptive PI3K/AKT overactivity that compensates for *BRAF* inhibition and promotes resistance.
9. Receptor tyrosine kinase (RTK) activation – the PI3K/AKT pathway is activated by growth factors that are associated with RTK, such as PDGFR-β and IGF-1R. With the *BRAF* block, cancer cells can over-express RTK, leading to permanent PI3K/AKT signaling.
10. Activating mutations in *PI3K/AKT* genes – activating mutations in *PI3K/AKT* and *AKT* amplify AKT signaling, which in-

creases anti-apoptotic signals and increases the expression of key proliferative genes, providing the cell with survival signals independent of *BRAF*.

11. EGFR signal path activation – EGFR activation induced by SOX10 suppression and increased TGF-β pathway activity that causes cell ageing is reversed by *BRAF/MEK* inhibition.

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HE4 – not only an ovarian cancer biomarker – a brief review

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Human epididymis protein 4 (HE4) was firstly identified in epididymal epithelial cells and described as a protease inhibitor playing a role in spermatogenesis. Regarding numerous studies proving its diverse potential as a prognostic and predictive factor in ovarian cancer, it was incorporated into ROMA algorithm. Nevertheless, recent studies have shown that serum level of HE4 is not exclusive to ovarian cancer. As a result, doctors using ROMA algorithm for stratifying patients with ovarian cancer must be aware of other conditions that may affect serum level of HE4. This review comprises different conditions connected with high level of HE4 that might impact ovarian cancer diagnosing process. Moreover, discovering increased HE4 level in various conditions should open discussion about its applicability in diseases other than ovarian cancer.

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Key words: human epididymis protein 4, ovarian cancer, ROMA algorithm, biomarkers

Introduction

Human epididymis protein 4 (HE4) was firstly identified in epididymal epithelial cells and described as a protease inhibitor playing role in spermatogenesis. It is encoded by WAP 4-disulfide core domain 2 (WFDC2) [1]. Selective overexpression of HE4 has a well-established role in ovarian cancer tumorigenesis, but little is known about its possible role in other conditions [2]. Regarding numerous studies proving its diverse potential as a prognostic and predictive factor in ovarian cancer, it was incorporated into ROMA algorithm, that is a quantitative test based on the serum level of HE4, CA125, and combined with menopausal status [1]. Using this algorithm allows for stratifying patients into two groups – in a high or low ovarian cancer risk and as a result avoiding unnecessary surgeries [3, 4].

On the other hand, recent studies have shown that serum level of HE4 is not exclusive to ovarian cancer [5–8]. Described conditions comprise both cancer and other diseases. The most numerous group of researches about HE4 as a possible new biomarker is connected with lung cancer, especially non-small cell lung cancer (NSCLC) [9, 10]. Some authors even suggest that HE4 secretion might play an extensive role in NSCLC progression, like in ovarian cancer [7]. Another conditions suggested to be connected with HE4 comprise chronic kidney disease [5], renal fibrosis [11], cancers of intestinal tract [6, 12, 13], breast cancer [8] and heart failure [14].

Due to the fact that, as mentioned above, the increased level of HE4 is detectable not only in patients with ovarian cancer, but also in patients with other diseases, doctors using ROMA algorithm for stratifying patients with ovarian cancer must be aware of other conditions that may affect the serum level of HE4. The main aim of this review was to gather and discuss diseases connected with HE4, described in the literature. According to our best knowledge, a similar review encompassing different possible directions in HE4 usage have not been published yet.

Kidney

HE4 was proven to be expressed in the distal convoluted tubules of the kidney [15] and since then scientists try to find out correlation between its expression and the occurrence of chosen conditions. Chronic kidney disease (CKD) is told to become a worldwide public health problem with mean global prevalence at 13.4%. Owing to the fact that detecting CKD at early stages allows its appropriate management there

is an ongoing need for searching new diagnostic indicators [16]. One of the most recently suggested is HE4. In the study conducted by Yuan et al. (2017) [5] serum level of HE4 increased significantly with renal function decline and was proven to achieve better diagnostic ability, sensitivity and specificity than other laboratory indicators, so as a result, it was suggested as the strongest predictor for CKD. What is more, increased levels of HE4 were detectable even at early stages of CKD. HE4 level in blood samples derived from patients with renal disfunction was positively correlated with level of creatinine, urea and cystatin C as far as acute and chronic renal dysfunctions are concerned and has higher diagnostic value with 100% specificity and sensitivity [17]. On the other hand, various studies proved inverse correlation between HE4 and eGFR with statistical significance [11, 17].

The most common final pathological way of CKD is renal fibrosis. HE4 has been recently reported as one of the mediators of this phenomenon because of inhibiting the degradation of type I collagen [18]. In the study conducted by Wan et al. (2016) [11] higher levels of HE4 were detected in patients with more severe renal fibrosis and significant correlation between HE4 and degree of renal fibrosis was observed. ROC curve analysis pointed out HE4 as a suitable biomarker for the diagnosis of renal fibrosis. Moreover, HE4 is suggested as a biomarker for predicting renal fibrosis in kidney transplant recipients due to its increased level and correlation with the severity of the disease [19].

Lupus nephritis (LN) is a common manifestation of *systemic lupus erythematosus* (SLE) and a major cause of morbidity and mortality in these patients. Increased serum HE4 level was proved to be associated with development of LN in SLE patients in two independent studies [20, 21]. However, detailed mechanism leading to development of LN with HE4 contribution remains unclear and needs further research.

Intestinal tract

Gastric cancer (GC), despite its decreasing incidence ratio, has still low 5-year survival rate, thus needs factors indicating its prognosis and improving treatment [22]. The first investigation concerning HE4 expression in GC was performed by Guo et al. (2015) [6]. They discovered upregulation of this glycoprotein in gastric cancer tissues and significant correlation with Lauren classification, TNM stage and tumor size. Silencing HE4 inhibited proliferation, migration and enhanced apoptosis in studied tissues. Regarding these discoveries, HE4 might play an important role in progression of GC and become a new target for treatment. Another aspect of HE4 in GC concerns sensitivity to radiotherapy. Peng et al. (2019) [23] pointed out hypoxia-induced upregulation of HE4 as a reason for resistance to radiotherapy due to the fact that stable knockdown of HE4 sensitized cancer cells and xenograft tumors to radiotherapy. As a conclusion, radiotherapy connected with HE4 knockdown might become a potential therapeutic aim in GC.

Next example of association between resistance to radiotherapy and expression of HE4 is colorectal cancer (CRC). Shi et al. (2018) [12] demonstrated that WFDC2 deficiency improved the radiation resistance in CRC. miR-149 – a small noncoding RNA regulating post-transcriptional gene expression was proved to inhibit HE4 expression in CRC cells and sensitize CRC to radiation both *in vivo* and *in vitro*. As a result, exogenous administration of miR-149 mimic combined with radiotherapy might become a new therapeutic promise in CRC. In 2017, Kemal et al. [24] detected high level of HE4 in CRC samples in comparison to healthy controls. They also proposed a HE4 as a new biomarker for stage III–IV CRC due to its significantly positive expression especially in this group. However, the study group was relatively small and without any follow-up information. What is more, CRC seems to present wide range of tumor markers used for diagnosing and staging thus searching new biomarkers might be questioned.

HE4 expression was suggested as a marker of early stage of pancreatic adenocarcinoma. In the study by Huang et al. (2015) [13] serum HE4 levels reached sensitivity of 45.9% and specificity of 93.6% with cutoff set at 4.59 ng/mL. Interestingly, combination of HE4 with CA19-9 increased sensitivity to 83.3% and the combined HE4 and CA15-3 to sensitivity of 87.5% thus set consisting of HE4, CA19-9 and CA15-3 might become a new powerful biomarker panel for early detection of pancreatic adenocarcinoma and diagnostic improvement. Lu et al. (2016) [25] research determined that treatment of recombinant HE4 on pancreatic cancer Suit-2 cell caused significant cells growth, increased DNA synthesis and cell viability in comparison to control group without HE4 treatment. Moreover, treatment with HE4 upregulated PCNA (key molecule for DNA synthesis) and downregulated p21 (a critical cell cycle regulator). To conclude, HE4 presents an undeniable role in pancreatic cancer development and might be used as a potential biomarker in its early stage detection.

Lung cancer

Owing to the fact that lung cancer is still a leading cause of cancer morbidity and mortality all over the world, accurate and early diagnostic tools encompassing this malignancy are in the area of scientists' interest [22]. Serum HE4 levels were proven to be significantly higher in NSCLC patients than in benign lung diseases and healthy controls. What is more, its higher level was correlated with high TNM stage, positive lymph nodes metastasis and weight loss [7, 26]. Connecting these facts with documented shorter overall survival (OS) in the group with higher level of HE4 allows us to conclude that serum levels of HE4 might predict poor prognosis in NSCLC patients. Moreover, Mo et al. (2018) [26] observed that HE4 was a satisfying discriminator of lung adenocarcinoma. Although Celik et al. (2017) [27] doubted the reliability of HE4 as a lung cancer biomarker, they admitted that it was a promising candidate for adenocarcinoma treatment. On the

other hand, Korkmaz et al. (2018) [28] suggested a panel of three tumor markers including HE4 for discriminating lung cancer from benign lung lesions and subtyping as small cell lung cancer (SCLC).

Survivors of lung cancer are at high risk of disease recurrence, thus we are in a need of sensitive methods for their postoperative monitoring. Current monitoring system is based on clinical examination and imaging methods. However, this combination might in some cases turn out to be insufficient. Muley et al. (2019) [29] suggested algorithm of serial serum measurements consisting of HE4 and another biomarker – CYFRA 21-1 for a recurrence detection. Their suggestion was based on the observation of 31 out of 115 patients suffering from adenocarcinoma recurrence – serum levels of CYFRA 21-2 and HE4 were significantly higher in samples taken from patients with recurrence in comparison to these derived from patients in remission.

Breast cancer

Breast cancer (BC) is the most commonly diagnosed cancer among women all over the world [22]. Regarding to this fact, contemporary science tries to find out new markers for more effective diagnosis and treatment because the sensitivity and specificity of known biomarkers such as CA15-3 and CEA are rather low [30]. Gunduz et al. (2016) [8] identified a significant elevation of serum HE4 in comparison to healthy control group and a correlation between the levels of HE4 and CA15-3 in patients suffering from BC. On the basis of these results they proposed HE4 as a potential biomarker for BC. Nevertheless, the presented study was conducted on a small group of patients and authors did not achieve any significant results as far as many well-known clinicopathological factors in BC are concerned, thus it seems to be too early to call a HE4 a potential biomarker in this condition. In recent years miRNA has become a new target in cancer research due to its correlation with prognosis, clinical staging and metastases [31]. Lu et al. (2017) [32] decided to combine plasma miR-127-3p and HE4 in BC analysis. They concluded that levels of both plasma miR-127-3p and HE4 were increased in BC and combined detection greatly improved methods of early diagnosis of BC with sensitivity of 87.4%

Heart failure

Although new research suggests HE4 as a potential novel biomarker for heart failure (HF), some obstacles may complicate its usage in monitoring of these patients [33]. Most of them are mentioned above – increased level of HE4 is detectable not only in patients with HF, but also in patients with ovarian cancer [2], CKD [5] or pancreatic adenocarcinoma [13]. However, HE4 as a part of multi-marker model, might be a potential aim in the HF stratification [33]. In the study conducted among 567 patients with HF, those with higher HE4 serum level had an unfavorable clinic profile comprising older age, higher NYHA class, greater number of comorbidities such as atrial fibrillation. Plasma HE4 levels were correlated with numerous HF plasma markers (including NT-proBNP, BNP and galectine-3) with the strongest correlation including GDF15 – an emerging prognostic biomarker of cardiovascular diseases [34]. Similar results were achieved by Piek et al. (2017) [14] – strong correlation especially comprising HF severity based on NYHA and NT-proBNP levels was detected. Levels of HE4 was also correlated with risk factors including age, male sex, hypertension and diabetes. What is more, patients with HE4 levels above the median had worse survival rate. Nevertheless, the study was conducted on a relatively small sample size and, as mentioned above, using HE4 as a marker for HF diagnosing and risk stratification might turn out to be impossible due to frequent coincidence between HF and other conditions which makes HE4 non-specific.

Conclusions

HE4 is not exclusive for ovarian cancer. In recent studies it was proven to have a possible role in diagnosing other conditions, including cancers. Nevertheless, the connection between its biology, genetics and pathological condition remains unclear. The most important conclusion from this review suggests carefulness while using HE4 in ovarian cancer diagnosing and remembrance about other conditions that might affect our judgement. Furthermore, owing to developing branch of using HE4 in other diseases we might ask ourselves the question about its applicability in yet unknown syndromes.

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Review article

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Oncogeriatrics (part 2.)
Normal and pathological ageing

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There are two ageing processes, one physiological, also known as normal ageing, and that of pathological ageing, which depends on the occurrence of chronic diseases. The essence of the former is a gradual, progressive, and a very individual restriction of the organs' functional reserve with age. The changes occur in all cells, tissues and systems and do not affect each organ at the same time, and the rate of change can vary between organs. Therefore, the chronological age alone cannot be the factor determining the terapeutic decisions, including surgical treatment. Among the elderly, there is a distinct situation where acute stress response associated with surgery is imposed on the otherwise ageing-related, reduced physiological reserves and the cumulative effect of any accompanying diseases. Standard preparation for surgery and routine perioperative management in such patients can lead to serious complications. In older persons, even minimal injury can exceed the body's capacity to compensate, especially among those with frailty syndrome. It is critical to understand the physiological changes associated with ageing to better understand the differences in the management of these elderly patients.

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Key words: elderly, normal ageing, pathological ageing, frailty syndrome

Surgery itself is aimed at removing the disease and is most often associated with partial or entire organ excision. In addition, it requires a number of invasive or potentially traumatic activities, such as insertion of cannulas into the vascular system, bladder catheterization, intubation, general anesthesia, etc. All of these lead to local tissue damage, disruption of natural barriers and exposure to external factors (physical, chemical and microbiological). This induces many changes in the body affecting the neuroendocrine, cytokine-immune, water-electrolyte and metabolic systems. This, in turn, may be accompanied by oxidative stress and the associated formation of free radicals [1]. Therefore, in the elderly, we have a particularly critical situation in which the acute stress responses associated with surgery are superimposed on the typical ageing-related decrease in physiological reserves (so-called normal ageing) and the cumulative effect of any accompanying diseases (so-called pathological ageing). Standard preparation for surgery and routine perioperative management in such patients is often insufficient and can lead to serious postoperative complications. In older persons, even relatively minor injuries or trauma can exceed the body's ability to compensate, which is especially the case for those with frailty syndrome. In order to better understand the difference in the management of these patients, it is important to be familiar with the physiological changes associated with the ageing process what will be presented in this part of the series.

The essence of so-called **normal ageing** is a gradual, progressive restriction of the functional reserve, which is the difference between the maximum organ function possible to achieve at a given moment of life and the bare minimum necessary to maintain homeostasis [2]. At present, the ageing process is irreversible and increases over time. However, the rate of decline in any given organ's functional reserve varies between individuals and depends on each individual's genetic

conditions and lifestyle. A reflection of the total depletion of functional reserves and adaptive capacity is the so-called "frailty syndrome". It is characterized by organs and systems becoming "ineffective" in the event of a stress factor (surgery, infection, injury, etc.), resulting in an increased risk of adverse outcomes [3]. The details of the frailty syndrome are thoroughly presented in the first part of the series, in the previous issue of *Nowotwory. Journal of Oncology* [4].

In the case of normal ageing, a number of changes occur in all cells, tissues and systems. These changes do not affect each organ at the same time, and the rate of change can vary between organs.

Aging of the **vascular system** can affect both the heart and the vessels. Changes in the sinus node and other parts of the conduction system lead to the development of cardiac arrhythmias that may clinically manifest themselves through syncope and falls. These changes can also lead to a reduction in the maximum heart rate. In turn, the reduction of vagus nerve tension and the reflex activity of the sympathetic nervous system may lead to an increase in resting heart rate. Within the myocardium, an increase in arterial stiffness results in compensatory left ventricular hypertrophy. Progressive myocardial fibrosis reduces ventricular susceptibility and impairs filling during the contraction phase. Left ventricular contractility is also reduced. Together, this leads to a decrease in the left ventricular ejection fraction and a reduction in exercise tolerance. In addition, vascular changes cause an increase in systolic blood pressure and a decrease in diastolic pressure, which have an adverse effect on coronary blood flow. Reduction of the diastolic capacity of precapillary arterioles has a negative effect on peripheral organ perfusion. Decrease in the sensitivity of the baroreceptors increases the risk of orthostatic hypotension [5–7].

In the **respiratory system**, there are changes in the pulmonary parenchyma, as well as the osteoarticular and muscular system of the chest itself. Consequently, this results in a reduction in vital capacity, a reduction of forced expiratory volume in one second and a worsening of gas exchange in the lungs. The residual volume increases by up to 40%. Due to degenerative changes in the joints, ossification of the cartilaginous joints of the ribs and the decrease in the mass and strength of the respiratory muscles with age, chest mobility decreases and, as a result, so does pulmonary ventilation. The clinical manifestation is the acceleration of the breathing rate at rest and effort dyspnea. Reflex reactions in the upper respiratory tract are weakened, which increases the risk of aspiration of gastrointestinal contents and postoperative pneumonia [8, 9].

Ageing of the **digestive system** is associated with reduced secretion of saliva, gastric juice, bile and pancreatic juice. This leads to a worsening of protein and fat digestion, as well as disorders of iron absorption in the small intestine. The reduction in Castle's intrinsic factor production is the reason for poor absorption of vitamin B_{12} and its overall deficiency. More than 70% of elderly people have lactose intolerance associated with the loss of lactase production by the brush border of the small intestine. Due to changes in diet, low fluid intake, use of diuretics and low physical activity, peristalsis slows and constipation occurs as a result. Liver mass and hepatic blood flow are reduced by 30%. The ability of hepatocytes to regenerate after an injury is likewise reduced. The intensity of metabolic processes is smaller, including those responsible for the metabolism of xenobiotics and drugs [9, 10].

From the fourth decade of life, the number of active nephrons decreases in the **renal system**. Despite compensatory overgrowth of other active nephrons, renal blood flow and glomerular filtration rate are reduced by about 10 ml/min/1.73 m^2/decade and, after 65 years of age, 15 ml/min/1.73 m^2/decade. The decrease in glomerular filtration rate is usually not accompanied by a parallel increase in serum creatinine due to a decrease in creatinine production as a result of decreased muscle mass with age. Due to the reduced elasticity of the bladder walls and weakness of the detrusor muscle, the volume of residual urine increases, which likewise increases the risk of urinary tract infection. Decreased activity of the renin-angiotensin system increases the risk of hyponatremia [11].

Ageing of the **endocrine system** is reflected by the extinction of gonadal hormonal function and a decrease in the secretion of growth hormone, dehydroepiandrostenedione, aldosterone, melatonin, thyroxine and insulin. This leads to osteoporosis and lipid and carbohydrate metabolic disorders, and also promotes hyponatremia. Secondary hyperparathyroidism is a commonly diagnosed disorder among elderly patients. It is most often caused by a vitamin D deficiency, which is associated with skin changes, lower exposure to solar radiation and a nutrient-deficient diet [12, 13].

Changes in the **hematopoietic system** lead to disorders in the normal formation of mature blood cells in the bone marrow. This process becomes more critical after the age 60, when the total number of active bone marrow cells decreases by approximately 50% resulting in approximately 30% of the number of cells observed in youth. Erythropoiesis is the most affected, and further contributes to the development of anaemia in the elderly. The chemotactic, phagocytic and bactericidal activity of neutrophils and macrophages decrease, the proliferation of B and T lymphocytes in response to their stimulation decreases, which leads to an overall decrease in immunity, potentially more severe courses of infection, reduced vaccine effectiveness and an increased risk of cancer [14].

With age, there is also a loss of muscle mass and strength, even in physically active people. Between the ages of 45 and 85, an individual's muscle mass is reduced by about 25%. It is estimated that a significant decrease in muscle mass affects 40% of people over 80 years of age. Muscle strength decreases at an even faster rate; after the age of 60, it is possible to lose up to 3% per year. Local accumulation of triglycerides and lipofuscin within muscle fibers and systemic metabolic

and hormonal disorders (insulin resistance and reduction of growth hormone and IGF-1), as well as ageing-related degenerative changes of motor neurons, contribute to a decrease in muscle mass. The overall effect of muscle changes is not only a decrease in physical fitness, but also an increase in the risk of falls [2,15].

Progressive degenerative changes in the joints lead to a decrease in range of motion, which is often accompanied by pain. In the case of degenerative changes in the intervertebral cervical section, in addition to impaired blood flow in the vertebral arteries (cervico-basal syndrome), there may be neurological symptoms accompanying changes in the lumbar section. The effects of these changes include a change in posture and gait and a decrease in general physical fitness [16,17].

Ageing also leads to significant changes in sensory organs. Hardening of the lens nucleus leads to presbyopia, and a decrease in light transmission through the eye lens leads to deterioration of vision resolution, increased visual impairment in the dark and difficulty distinguishing green-blue shades. A reduction in tear production leads to dry eye and an increased risk of conjunctival infection. Impaired reception of high-frequency tones can cause partial or total deafness. A person's sense of smell may also be impaired due to a loss of nerve endings [18–21].

In the nervous system, the atrophy of hippocampal neurons, reduction of neurotransmitter secretion and receptor density for neurotransmitters lead to reduced cognitive ability, increased risk of depression, prolonged reflex reaction time and a related increase in the risk of falls [22].

The aforementioned changes may be compounded by so-called **pathological ageing**, resulting from the impact of associated diseases on the previously described changes, which are characteristic of normal ageing. The result of this is seemingly disproportionate organ ageing. This process is most often caused by the adverse effects of lifestyle and environmental factors, although it can also be caused, in some cases, by genetic background [2]. Vascular changes including macroangiopathy (atherosclerosis) and microangiopathy (diabetic microangiopathy) are of particular importance in cases like these. Impaired perfusion worsens the function of many organs, including the heart, brain, kidneys, intestines and skeletal muscles. It can also be a common cause of death associated with both heart attacks and strokes. Smoking, dust exposure and recurrent lower respiratory tract infections can lead to a faster reduction in lung function and pulmonary reserve. Obesity accelerates the course of degenerative joint processes and reduces the functional reserve of the heart and lungs, in addition to having adverse effects on metabolic

Table I. Review of the organ changes associated with normal aging [2–25]

Vascular system	Reduction in the maximum HR, increase in resting HR, compensatory left ventricular hypertrophy, reduced ventricular ejection fraction, increase in systolic blood pressure, decrease in diastolic pressure, reduction in the coronary blood flow and organ perfusion, decreased sensitivity of the baroreceptors. **Clinical manifestation**: arrhythmias (leading to syncope and falls), reduced exercise tolerance, orthostatic hypotension.
Respiratory system	Reduction in VC, reduction in FEV_1, worsening of gas exchange in the lungs, increased RV, reduced pulmonary ventilation, weakened reflex reactions in the upper respiratory tract. **Clinical manifestation**: acceleration of the breathing rate at rest and effort dyspnoea, increased risk of aspiration and postoperative pneumonia.
Digestive system	Reduced secretion of saliva, gastric juice, bile and pancreatic juice, reduced peristalsis, reduced liver mass and hepatic blood flow, reduced regeneration of the hepatocytes after injury. **Clinical manifestation**: worsened digestion of protein and fat, constipations, vitamin B_{12} deficiency, reduced metabolism of xenobiotics and drugs.
Renal system	Reduction in the number of active nephrons decreases, decreased renal blood flow and glomerular filtration rate, reduced elasticity of the bladder walls and weakness of the detrusor muscle. **Clinical manifestation**: increase in the volume of residual urine increases the risk of infection.
Endocrine system	Decrease in the secretion of growth hormone, dehydroepiandrostenedione, aldosterone, melatonin, thyroxine and insulin, vitamin D deficiency. **Clinical manifestation**: osteoporosis, lipid and carbohydrate metabolic disorders, increased risk of hyponatremia.
Hematopoietic system	Decrease in active bone marrow cells (particularly erythropoiesis), decrease of the chemotactic, phagocytic and bactericidal activity of neutrophils and macrophages, decrease in the proliferation of B and T lymphocytes in response to their stimulation. **Clinical manifestation**: anaemia, overall decrease in immunity, potentially more severe courses of infection, reduced vaccine effectiveness, increased risk of cancer.
Musculoskeletal changes	Increased adiposity, reduced muscle mass, reduced grip strength, reduced body weight. **Clinical manifestation**: decrease in physical fitness, increase in the risk of falls.
CNS and sensory organs	Atrophy of hippocampal neurons, reduction of neurotransmitter secretion and receptor density for neurotransmitters. **Clinical manifestation**: reduced cognitive ability, increased risk of depression, prolonged reflex reaction time, increase in the risk of falls. Presbyopia, deterioration of vision resolution, difficulty in distinguishing green-blue shades, increased risk of conjunctival infection, partial or total deafness, deterioration of the smell.

HR – hearth rate, VC– vital capacity, FEV_1 – forced expiratory volume in one second, RV – residual volume

processes, including those associated with an increase in the risk of type 2 diabetes. Exposure to loud noises accelerates the ageing and degradation of the hearing organ. Exposure to ultraviolet radiation accelerates skin ageing. Exposure to ionizing and infrared radiation accelerates the development of cataracts. Hard physical work and injuries likewise accelerate the degeneration of joints [23, 24].

The effect of postoperative stress is more detrimental to some organs than others. Therefore, the leading causes of postoperative mortality among the elderly are: myocardial infraction, pulmonary and cerebrovascular complications [2, 25]. Therefore, preoperative comprehensive geriatric assessment of organ function and reserves should be obligatory in the older population, particularly undergoing cancer surgery.

Conflict of interests: none declared

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Case report

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A squamous cell carcinoma arising from scrotal epidermal cyst. A case report and review of 94 cases from the world literature

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Epidermal cysts are a common benign skin abnormality, comprising 85–90% of all excised skin cysts. The term epidermal inclusion cyst refers specifically when the cyst resulted from the implantation of epidermal elements in the dermis. Squamous cell carcinomas (SCCs) are common skin lesions; however, a malignant transformation of an epidermal cyst is very rare with incidence of 0.011–0.045%. Few cases of malignant transformation of an epidermal cyst have been reported in the literature so far. This paper presents a case of squamous cell carcinoma arising from a scrotal epidermal cyst.

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Key words: epidermal cyst, squamous cell carcinoma, PD-1 blockade, cemiplimab

Introduction

Epidermal cysts (EC), also known as sebaceous, keratin, follicular infundibular or epidermal inclusion cysts, are extremely common lesions that can occur anywhere in the body. Histologically, they are lined with a thin layer of *squamous epithelium* and develop by buildup of keratin inside the cyst [1].The malignant transformation of an epidermal cyst is very rare clinically. Several neoplasms have been reported to develop in EC including basal cell carcinoma [2], malignant melanoma [3], Merkel cell carcinoma [4], plasmacytoma [5] and squamous cell carcinoma [6].

The development of true squamous cell carcinoma in pre-existing epidermal cysts is a rare event with incidence of 0.011–0.045% [7].

Case report

A 70-year-old male presented with a left scrotal lesion. The lesion was extra-testicular and solid. The initial clinical impression was lymphoma. A CT of chest, abdomen and pelvis was requested, which showed no evidence of lymphadenopathy or any mass lesion.

The patient underwent surgical excision of the scrotal mass. The pre-operative diagnosis and impression was that of a large sebaceous cyst. Intra-operatively, the cystic mass was accidently punctured and revealed a large amount of sebaceous fluid. The entire cystic mass was carefully dissected. The specimen was sent to Pathology.

Gross examination revealed a partially collapsed cyst measuring 3.0 x 2.0 x 1.8 cm with portion of skin attached to it. The inner lining was smooth with the exception of one white raised area measuring 0.8 x 0.8 x 0.5 cm. The entire specimen was serially sectioned and submitted for microscopic examination. The histo-pathological examination revealed infiltrating nests of atypical squamoid cells with surrounding intense desmoplastic stromal reaction, representing an early invasive well-differentiated carcinoma arising from epidermal scrotal cyst. The resection margins were clear (fig.: 1, 2).

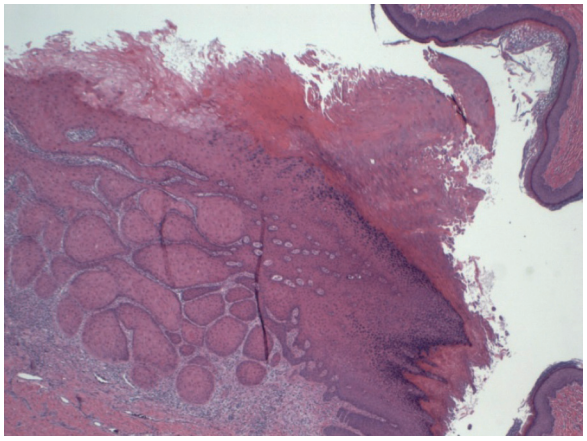

Figure 1. Low power view showing atypical squamoid nests arising from wall of scrotal epidermal cyst

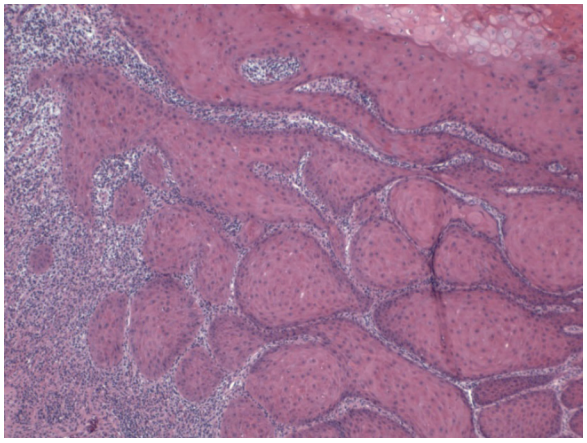

Figure 2. High power view showing the infiltrating nests of atypical squamoid cells with surrounding intense desmoplastic stromal reaction

Discussion

Epidermal cysts are common cutaneous lesions that may occur anywhere on the body. Malignant changes in the epidermal cysts are an uncommon finding.

Among the pre-malignant and malignant neoplasms that have been reported to develop in EC are: basal cell carcinoma [2, 8], malignant melanoma [3], Bowen's disease [9], Paget's disease [10], bowenoid papulosis [11], mycosis fungoides [12], Merkel cell carcinoma [4] and plasmacytoma [5]. All these lesions have a far lesser incidence than squamous cell carcinoma [6]. The development of true squamous cell carcinoma, in a pre-existing cutaneous epidermal cyst, is a rare event with incidence of 0.011–0.045% [7].

SCC also known as epidermoid carcinoma is the second most common skin cancer, after basal cell carcinoma. The rare Merkel cell carcinoma (MCC) is a frequently lethal skin cancer with a higher mortality (33%) than malignant melanoma (MM) (15%) [13]. In contrast, the survival rate for most other non-melanoma skin cancers is excellent. For instance, the 5-year relative survival for basal cell carcinoma (BCC) is 100%, whereas the 5-year relative survival for SCC is slightly less at 95% [14]. Among the above-mentioned skin malignancies, the reports show that the incidence of melanoma has been steadily rising in the recent decades [15].

The literature review revealed that in 1968 McDonald [16] analysed 637 epidermal cysts, but found malignancy in only in 7 (1.1%) cases. Of these, 6 were basal cell carcinomas and only one was a squamous cell carcinoma.

The development of SCC in EC occurs most frequently on the head and neck [17, 18], trunk [19] and thigh. Other reported sites are scrotum [20], perineal regions [7, 21], sublingual gland [22], vulva [23] and breast [24]. After reviewing all reported 94 cases, it was obvious that they are more frequent in males with incidence of 65% (table I). The localization of the lesion was as follows: head and neck (55%), lower limbs (13%), trunk (13%), perineum (8%) and the upper limbs (6%) (figure 3). Malignant transformation of an epidermoid cyst can also occur in the deeper parts of the body other than the skin, such as the intra-cranial region [25] and ovary [26]. It has been reported that the rate of malignant transformation of epidermal cyst into cutaneous squamous cell carcinoma ranges between 0.011% and 0.045% [27, 28]. The documented size of the affected cyst varies between 8 mm and 150 mm. Patients often present with a lesion size between 1 to 4 cm, and the lesion duration ranged from 2 months to 20 years (table I).

The blamed predisposing factors include chronic history, trauma, recurrent infection, chronic sunlight exposure [29, 31], advanced age, skin that is sensitive to ultraviolet radiation, and immunosuppression [32]. Furthermore, chronic inflammation and irritation is classically described to be associated with malignant transformation in lesions behaving similarly to the

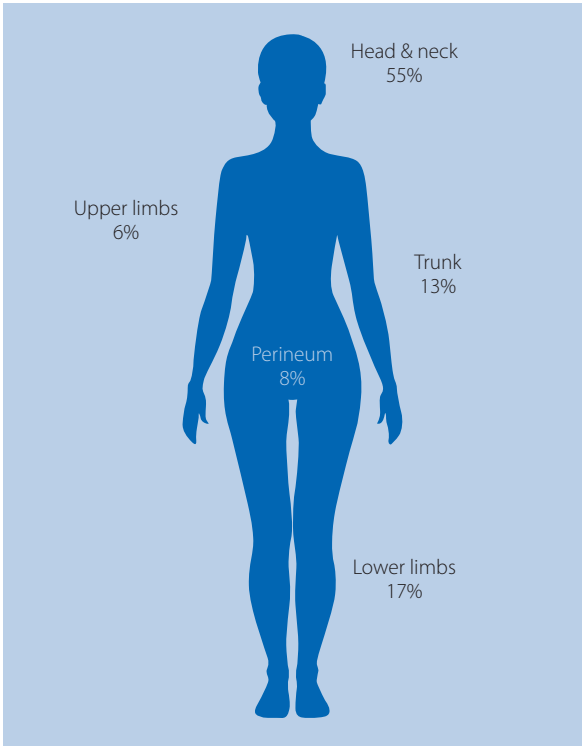

Figure 3. Distribution of 94 cases of squamous cell carcinoma developed in subcutaneous epidermoid cysts

Table I. Malignant transformation of cutaneous epidermal cyst into squamous cell carcinoma: a review of 95 cases reported in literature

Author	Year published	Gender	Age	Site	Size (mm)	Histology	Lesion duration/ /months	Symptoms
Peden: 11 cases [50]	1948	F	43	scalp	–	SCC	180	↑ size
	1948	F	63	scalp	–	SCC	180	–
	1948	M	43	face	–	SCC	1	↑ size
	1948	F	64	forearm	–	SCC	1	↑ size
	1948	M	25	thigh	–	SCC	–	–
	1948	F	48	scalp	–	SCC	300	↑ size
	1948	F	63	shoulder	–	SCC	24	↑ size
	1948	F	75	scalp	–	SCC	24	↑ size
	1948	F	53	scalp	–	SCC	–	–
	1948	M	57	scalp	–	SCC	48	↑ size
	1948	M	79	ear	–	SCC	18	–
Latimer: 2 cases [51]	1949	M	40	face	40	SCC	24	ulcer
	1949	F	5	face	10	SCC	–	↑ size
McDonald [8]	1963	F	43	sternum	–		–	–
Davidson [52]	1976	M	52	ear	–	SCC	2	–
Bauer [53]	1980	M	68	preauricular	30	WD-SCC	–	inflammation
Miler [54]	1981	M	34	scalp	30	WD-SCC	240	↑ size
Yaffe [55]	1982	M	58	ear	25	SCC	132	↑ size
Arianayagam [16]	1987	F	59	thigh	25	WD-SCC	3	↑ size, pain
Sagi [81]	1988	F	60	scalp	–	WD-SCC	120	ulcer
Shah [56]	1989	F	55	buttock	90	WD-SCC	6	–
Davies [57]	1994	M	32	index finger	–	WD-SCC	120	ulcer
Malone [58]	1999	F	92	forehead	35	PD-SCC	–	↑ size
Lopez-Rios: 8 cases [23]	1999	M	68	preauricular	50	SCC	4	–
	1999	M	66	preauricular	15	WD-SCC	2	–
	1999	M	58	ear	25	SCC	132	–
	1999	M	52	ear	20	SCC	132	–
	1999	M	34	retro-auricular	80	SCC	–	–
	1999	M	32	index finger	–	SCC	120	–
	1999	F	59	thigh	50	WD-SCC	3	–
	1999	F	55	buttock	100		6	–
Wong [77]	2000	M	57	buttock	60	WD-SCC	240	↑ size
Morgan: 5 cases [34]	2001	3M	21–80 (mean 56.7)	trunk	–	WD-SCC	–	–
		2F		neck	–	WD-SCC	–	–
				face				
Debaize [78]	2002	F	38	buttock	200	SCC *in-situ*	240	↑ size
Lin [37]	2002	M	68	axilla	65	WD-SCC	2	↑ size
Cameron [59]	2003	M	67	temple	30	PD-SCC	48	↑ size, inflamed
Kume [60]	2004	M	55	sacrum	–	SCC	48	–
Nemoto [61]	2006	F	48	abdominal wall	92	PD-SCC	120	↑ size, pain
Chiu [27]	2007	M	47	thigh	130	WD-SCC	480	↑ size, bleeding
Jehle [48]	2007	M	48	gluteal area	50	WD-SCC	336	↑ size, trauma

Author	Year published	Gender	Age	Site	Size (mm)	Histology	Lesion duration/ /months	Symptoms
Bhatt [22]	2008	F	64	sublingual gland	–	SCC	144	↑ size
Kuvat [31]	2009	M	48	scalp	60	SCC	156	ulcer
Ziadi [28]	2010	M	50	head	15	SCC	3	no change
Antón-Badiola [38]	2010	M	65	retro-auricular	20	MD-SCC	2	ulcer
Pusiol: 2 cases [35]	2010	M	88	face	7	SCC	–	–
	2010	M	96	ear	15	SCC	12	
Kshirsagar [39]	2011	M	72	buttock	100	WD-SCC	120	↑ size, ulcer
Shabbir [62]	2011	M	M	ear	12	SCC	–	–
Moritt: 4 cases [33]	2011	M	48	leg	–	SCC	–	↑ size
	2011	M	68	back	–	SCC	72	↑ size, inflamed
	2011	F	72	scalp	–	SCC	240	↑ size, ulcer
	2011	F	60	face	–	SCC	3	↑ size
Anastasios [63]	2012	F	69	face	9	MD-SCC	18	↑ size
Terada [42]	2012	F	76	nasal		SCC	–	cosmetic
Sumi [64]	2012	F	76	labia majora	125	WD-SCC	–	↑ size
Sinha [65]	2012	M	65	scalp		WD-SCC	72	↑ size
Pusiol: 4 cases [35]	2012	M	88	face	–	SCC	–	–
	2012	M	96	ear	15	SCC	–	–
	2012	M	67	hallux	8	SCC	–	ulcer
	2012	F	86	perineum	15	SCC	–	–
Tokunaga [30]	2013	M	65	neck	90	PD-SCC	420	↑ size, bleeding
Yeh [20]	2013	M	86	scrotum	41	WD-SCC	276	discharge
Cappello [47]	2013	M	63	nasal skin	20	WD-SCC	36	pain, discharge
Skroza [66]	2014	M	63	scalp	30	SCC	24	–
Hasegawa [67]	2014	M	75	buttock	60	SCC	–	–
Fujita [68]	2015	M	48	pre-sacral	120	SCC	1	pain
Satoh [69]	2015	M	76	pre-sacral	70	SCC	36	↑ size
Sridevi [21]	2015	M	68	submandibular	60	WD-SCC	12	↑ size
Suhani [24]	2015	F	60	breast	50		6	–
Sakamoto [17]	2015	M	41	thumb	20	SCC	–	ulcer
Veenstra: 3 cases [70]	2016	F	46	thigh	20	WD-SCC	12	–
	2016	F	89	supra-pubic	40	WD-SCC	1	pain, discharge
	2016	M	61	thigh	12	WD-SCC	6	↑ size
Sze [23]	2016	F	65	vulva	50	MD-SCC	240	↑ size
Lee [71]	2016	M	62	face	25	WD-SCC	–	↑ size
McAllister [36]	2017	M	73	ear	–	SCC	2	ulcer
Rathna [72]	2017	M	30	forehead	20	SCC *in-situ*	36	
Sirvastava [73]	2016	M	28	neck	65	SCC	7	↑ size
Suzuki [74]	2017	M	56	perineum	43	SCC	540	↑ size
Frank [18]	2018	F	64	neck	4	WD-SCC	48	↑ size
Zanguoie [29]	2018	F	77	neck	large	SCC	240	↑ size
Park [7]	2018	M	51	perineal	150	SCC	360	↑ size
Bears [75]	2019	M	44	thigh	15	WD-SCC	5	↑ size

Author	Year published	Gender	Age	Site	Size (mm)	Histology	Lesion duration/ /months	Symptoms
Kim [76]	2019	M	46	nasal alar	9	SCC	–	–
Daisley [6]	2019	M	67	abdominal wall	150	WD-SCC	300	↑ size, pain, ulcer
Niimi [46]	2019	F	71	buttock	100	WD-SCC	12	↑ size, pain
Kasahara [79]	2019	M	50	scrotum	48	WD-SCC	24	firm
Lopez [80]	2019	M	83	peri-coccygeal	61	MD-SCC	10	↑ size, pain
Shah [81]	2019	M	37	scalp	70	PD-SCC	4	↑ size, pain
This case	2019	M	70	scrotum	8	WD-SCC	–	↑ size

WD – well differentiated, MD – moderately differentiated, PD – poorly differentiated

epidermal cyst, such as *pilonidal sinus*, *hidradenitis suppurativa* and *chronic osteomyelitis* [33].

HPV-associated malignant transformation of the epidermal cyst in head and neck area, and the perineum has been reported before. Previous studies looked to the aetiological relation of HPV to the malignant transformation of the EC, however the limited number of cases prevents complete exoneration of HPV as an aetiological factor [29, 30, 34–36].

In the malignant transformation of the EC, the squamous cell carcinoma arises from the lining cells of the epidermal inclusion cyst. The malignancy may be associated with a sudden development of suspicious features in a sebaceous cyst, which has been present for a long time. These signs and symptoms may include the cyst changing into a firmer mass, pain, discharge, inflammation, ulceration, bleeding, rapid increase in size, inflammation or infection not responding to conservative treatment. Such findings may alert the clinician to excise the lump and examine it [7, 31, 37, 38].

Histologically, the lumen of the EC is filled with laminated keratin, and the specimen may reveal scattered islands of severely atypical neoplastic squamous epithelium arranged in small nests or sheets with marked nuclear irregularity, nuclear hyperchromasia, pleomorphism, absence of intracellular bridges, increased mitotic figures and an infiltrative growth pattern [39].

The immunohistochemistry may show positivity of the tumor cells for p53 protein, a tumor marker which is positive in malignancies including SCCs [40–42]. CK5/6 is a cytokeratin marker used to identify breast basal/myoepithelial cells [43] and together with p63+ identify squamous origin in poorly differentiated metastatic carcinomas [42, 44]. CAM 5.2 "commonly used antibody to cytokeratins 8 and CK7", is positive in most epithelial cells as in SCC [30, 45]. The suppressor protein p16 marker may also be present in SCCs [23]. Serum markers, such as SCC-related antigen level, helps in diagnosis and detection, and its upper normal is 1.5 ng/dl [26, 46]. Cytokeratin AE1/AE3 "pancytokeratin" marker, which detects most of the epithelial tissue is also found to be positive in a such cases [42, 45].

The treatment of choice in localized disease is radical surgical excision. Disease free margin specimens are recommended to avoid residual disease or recurrences. Fortunately, despite malignant transformation distant metastatic disease is rare [47]. SCC can metastasize to the regional lymph nodes and lungs [48].

Most of the cases are cured with surgery. In a small percentage of patients, the tumor reaches an incurable stage due to metastatic disease or locally advanced progression, and thus is no longer amenable to surgery or radiation therapy. At this stage palliative systemic chemotherapy or immunotherapy with PD-1 blockade using cemiplimab is indicated [32, 49].

Prognostic factors of local recurrence, metastasis, and disease-specific death, include tumor size larger than 2 cm, gender, preceding lesions, rapid tumor growth, degree of the differentiation and tumor location.

Conclusion

The malignant transformation of an epidermal cyst is a rare condition; this case illustrates the importance of patho-morphological examination of the excised epidermal cysts. Moreover, potential malignancy should be suspected in patients with chronic sebaceous cysts, and the cyst exhibits suspicious features. The most frequently affected region is the head and neck.

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