The clinical impact of BRAF and NRAS mutation in melanoma

Doctoral (PhD) thesis

Kata Czirbesz MD

University of Pécs

Clinical Medical Sciences Doctoral School

Head of the Doctoral School: Prof. Lajos Bogár MD, PhD, Dsc

Head of the Program: Prof. István Kiss MD, PhD, Dsc

Supervisor: Prof. Gabriella Liszkay MD, PhD, med.habil.

Pécs, 2022.

lntroduction

The incidence of melanoma shows continuous increase worldwide, during the past few decades the number of new cases has multiplied. Experimental and clinical research of the last decades investigated the molecular and immunological background of melanoma, which successfully established the base of novel innovative therapeutic modalities such as target-based and immunotherapies, and the clinical application of new prognostic and predictive factors.

According to the Cancer Genom Atlas Network there are four major genomic categories of melanoma: BRAF, NRAS, NF-1 mutant and triple-wild type.

Oncogenic mutations in the BRAF and NRAS proved to be the most common genetic alterations of cutaneous melanoma (40-60% and 15-20% respectively), while KIT gene is frequently affected in mucosal melanomas (5-40%), and the majority of uveal melanomas harbors mutations of GNAQ or GNA11 (80%).

Somatic mutations of BRAF and NRAS result in hyperactivation of the mitogen-activated protein kinase (MAPK) signal that drives tumor growth and leads to progression of disease.

ln 90% of cases with BRAF mutation valine to glutamic-acid mutation is present at codon 600 of exon 15 (V600E). The development of specific inhibitors, such as vemurafenib opened a new horizon of melanoma therapy. However, other known rare BRAF mutations also appear (e.g. V600K, V600R, V600D). BRAF mutations more frequently occur at younger ages, on trunk location, and are associated with chronic UV exposure. Although the presence of BRAF mutation is an attractive target of melanoma therapy, its prognostic value is still elusive.

RAS was the first discovered oncogene of melanoma. The incidence of RAS mutation is approximately 20%, with the majority found in NRAS, while mutations of KRAS and HRAS may occur in 1-2% of cases. Mostly glutamin-leucin substitution is detected in exon 3 codon 61 position, while alterations of exon 2 codon 12 and 13 are relatively rare. Similarly, to BRAF, it seems that high UV exposure induces the development of NRAS mutations as well. Unfortunately, specific molecular therapy against NRAS mutated melanoma has not been accepted yet. Previously, based on clinical trials some therapeutic advantages of MEK inhibitory strategy were reported. Furthermore, studies suggested that the immunotherapy was more effective among patients with NRAS mutation, but some studies conclude that NRAS mutation of melanoma has a negative impact of disease outcome.

Until 2011 metastatic melanoma had no accepted effective therapy, the treatment options as chemotherapies displayed a very low level of efficacy. The prognosis of metastatic melanoma patients was poor, with a median survival of 6-10 months, the five-year survival rates were estimated to be less than 5 per cent. ln 2011 the treatment landscape has changed with the approval of two new treatment options involving immunotherapy and targeted agents, which have demonstrated a significantly higher survival benefit for metastatic melanoma patients. Vemurafenib is the first selective small molecule inhibitor of mutated BRAF. Although BRAF inhibitor monotherapy is an effective therapy in BRAF-mutant melanoma, the duration of response is often short lived with developing resistance after approximately 6 months. Re-activation of MAPK signaling occurs the he majority of cases of acquired resistance to BRAF inhibitors. ln an attempt to delay the resistance and enable prolonged inhibition the combination of BRAF inhibitor and MEK inhibitor target therapy was approved by the FDA. Until now 3 different BRAF-MEK inhibitors approved and available, these are vemurafenib/cobimetinib, dabrafenib/trametinib, encorafenib/binimetinib. ln Hungary encorafenib/binimetinib combination is not available, although we could apply for numerous patients in clinical studies.

Although in sentinel lymph node (SLN) positive cases the efficacy of completing lymph node dissection remained questionable, the role of sentinel lymph node biopsy is inevitable in regional staging and designing of therapy, and sentinel lymph node status is one of the most important prognostic factors in melanoma.

The experimental and clinical research revealed the molecular background of melanoma. Nevertheless, the prognostic value of BRAF and NRAS mutation of the primary tumor is questionable.

Aim of the study

The aim of our retrospective study was to determine the progression free survival, overall survival and safety of vemurafenib therapy for BRAF mutated metastatic melanoma and subsequently to prove the clinical benefit for the studied 43 patients, based on real-life data in a single institute analysis. Also, the aim of our study was to compare the combination therapy, retrospectively the efficacy and safety with 38 patients receiving vemurafenib/cobimetinib combination and 80 patients receiving dabrafenib-trametinib therapy based on real-life data. Finally, to assess the correlation between known prognostic factors of melanoma, mutational

occurrence of BRAF and NRAS in the primary tumor, and sentinel lymph node status. Moreover, we investigated the association of these factors with disease outcome.

Methods

From November 2012 to October 2015 we have selected a number of 43 BRAF mutated, metastatic melanoma patients, treated with vemurafenib. Patients were at least 18 years of age and all, of them had unresectable stage lllC or stage IV (M1a, M1b or M1c disease) melanoma with measurable lesions according to the Response Evaluation Criteria in Solid Tumors iRECIST) [20]. ln baseline characteristics the following parameters were registered: gender, age, Eastern Cooperative Oncology Group (ECOG) performance status, mean Breslow tumor thickness, ulceration, melanoma metastasis stage (M1a, M1b, M1c), lactate dehydrogenase (LDH) level at enrollment (normal or elevated), treatment lines (first, second, third, fourth), genotype of the BRAF mutation status (V600E, V600K, undefined). Patients with brain metastasis were included in case of stable disease.

Cobas 4800 V600 mutation test, which is for the assessment of the BRAF mutation status from DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue was used to identify BRAF V600 mutation status. The Cobas test was designed to detect the predominant BRAF V600E mutation with high sensitivity and was performed at the Department of Pathology at the National Institute of Oncology. Study patients were stratified based on the American Joint Committee on Cancer stage (lllC, M1a, M1b or M1c), ECOG performance status. All patients received continuous vemurafenib therapy according to the approved prescribing information (at a dose of 960 mg twice daily orally) unless unacceptable side effects or disease progression occurred. Safety evaluations were conducted every 4 weeks, including physical examination, electrocardiography, dermatologic evaluation and laboratory tests, that included complete blood count, chemical testing and urine analyses. The patients were monitored for adverse events at each visit and the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.03 were used for all grading. Dose reductions/interruption were determined for intolerable Grade 2 toxic effects or worse. When toxicity that resulted in a dose reduction improved to Grade 1 or less, the dose was restarted at 720 mg after a second presence of the toxic effects at 480 mg twice daily. Cutaneous squamous-cell carcinoma did not require dose modification. At baseline, patients underwent computed tomography and magnetic resonance imaging of the brain. We conducted tumor assessment

according to RECIST, version 1.1, at baseline, at weeks 9, and every 12 weeks thereafter until tumor progression, death or intolerable toxicities.

ln our second study From November 2015 and December 2018 we have selected a number of 118 BRAF mutated metastatic melanoma patients, treated with vemurafenib+cobimetinib and dabrafenib+trametinib in our Institute. Patients were over 1-8 years of age and they had unresectable Stage lllC or stage lV melanoma. ln baseline characteristics the following parameters were registered: gender, age, Eastern Cooperative Oncology Group (ECOG) performance Status, mean Breslow tumor thickness, ulceration, melanoma metastasis stage (M1a, M1b or M1c), lactate dehydrogenase (LDH) level at enrollment (normal or elevated), treatment lines (first, second). 38 (32%) the patients received vemurafenib+cobimetinib, 960 mg vemurafenib twice a day continuously and 60 mg cobimetinib once a day on a 21 day-on/7 day off schedule for 28 day dosing cycles unless unacceptable side effects or disease progression occurred. 80 (68%) patients received dabrafenib-trametinib, the daily dose of dabrafenib was 2x150 mg, and that of trametinib was 1x2 mg both were administered continuously, scheduled for 28 -day dosing cycle unless disease progression or unacceptable side effects. ln 39 patients from the dabrafenib+trametinib group cerebral metastasis were present (49%). Efficacy and safety results were compared by our retrospective analyses. Contrast enhanced computed tomography (CT) was performed at baseline and every 12 weeks afterwards. Tumor response was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Adverse Events were assessed by the Common Terminology criteria for Adverse Events, version 4.03. Dose modification and interruption were determined for Gr3 or worse following the labeled indications. Treatment was continued until progression, intolerable AE or death. We retrospectively analyzed the collected data and investigate the efficacy and toxicity characteristics: the overall response rate (ORR), the progression free survival (PFS), the overall survival (OS) and the adverse events.

Our third retrospective single center study involved 159 patients who were surgically treated for melanoma in the National Institute of Oncology (Hungary) between October 2011 and July 2015. From the institutional database the following clinico-pathological data were collected: age, gender, location of primary tumor, Breslow thickness, ulceration, histological subtype, mitosis count, lymphovascular and perineural invasion, tumor infiltrating lymphocytes, signs of regression and SLN status. The SLN biopsy was performed in cases with intermediate tumor thickness (1-4 mm); less than 1 mm if the primary tumor was ulcerated, Clark-level more than lll, lymphovascular invasion or high mitosis activity was present; more than 4 mm Breslow

thickness if the tumors were not ulcerated. SLN biopsy was performed by double labeling technique 4-8 weeks after primary surgery. ln cases of positive SLNs complete regional lymph node dissection (RLND) was indicated. SLNs were histologically investigated in serial sections stained with hematoxylin and eosin. ln addition, HMB45, S100 and Melan-A immunohistochemistry were performed to confirm SLN status as we previously reported.

Patients with negative SLNs received low dose interferon-α therapy for 18 months. ln positive SLN-cases intermedier or high dose interferon-α therapy was indicated after regional block dissection. According to the decision of multidisciplinary oncoteam in disseminated cases targeted, immune, and chemotherapy were performed. Regression of primary tumor was classified as lower or higher than75%o, and late or early regression, similarly to the protocol of the College of American Pathologists. Follow-up data were obtained from the institutional database and National Cancer Registry of Hungary. The follow-up period ended in October 2019.

Genetic subtyping

For further analysis molecular categorization was performed. Genomic DNA was isolated from formalin-fixed, paraffin embedded tissue (FFPET) using the cobas® DNA Sample Preparation kit. The target DNAs were amplified and detected on the cobas z 480 analyzer using the amplification and detection reagents provided in the BRAF/NRAS Mutation Test (LSR) kit.

BRAF/NRAS Mutation Test (LSR) uses primers that define specific base-pair sequences for each of the targeted mutations. Amplification occurs only in the regions of the BRAF or NRAS genes between the primers; the entire gene is not amplified. BRAF sequences range from 101 to 120 base pairs. NRAS sequences range from 94 to 121 base pairs.

The test is designed to detect the following mutations (n=36) at a percent mutation of 5% or greater:

- V600E, V600E2, V600D, V600K, V600R, and K601E in BRAF exon 15
- G466A, G466V, G469A, G469R, and G469V in BRAF exon 11
- G12A, G12C, G12D, G12R, G12S, G12V, G13A, G13C, G13D, G13R, G13S, G13V, and A18T in NRAS exon 2
- A59D, A59T, Q61-Ht, Q6l-Hc, Q61K, Q61L, Q61P, and Q61R in NRAS exon 3
- K117Nc, K117Nt, A146T, and A146V in NRAS exon 4

Statistical analysis

Numeric parameters were compared by Mann-Whitney or Kruskal-Wallis test with post hoc analysis. Categorical data were analyzed by Chi-square test or Fisher's exact probability test. Survival periods were determined as the time period from the date of SLN biopsy to the date of last visit or defined complete event (death, progression, distant metastasis). Thus, overall survival (OS), disease-specific survival (DSS), progression-free survival (PFS) and distant metastasis-free survival (DMFS) were calculated. Survival analyses were done using the Kaplan-Meier method and log-rank statistics. Univariate and multivariate analysis of prognostic factors was done using Cox's regression model. Probability of sentinel lymph node positivity was assessed by binary logistic regression model. Differences were considered to be statistically significant when p-value proved be lower than 0.05. All statistical calculations were performed by Statistica 13.4 (TIBCO Software, Palo Alto, CA).

Results

ln our first analyses a total of 43 metastatic melanoma patients received vemurafenib. The median follow-up time was 15.9 months. The study included 22 women (51.2%), 21 men (48.8%), the mean age was 57 years (range: 27-77). We found an ECOG 0-1 performance status in 91% of the patients. According to the AJCC staging system 70% of the patients had stage M1c status, including 6 patients with a history of stable brain metastasis. LDH blood level was 49% <ULN. 72.1% of the patients received vemurafenib therapy in second or third line. Out of these patients 39 had V600E, 2 of them had V600K BRAF mutation genotype, while 2 patients' mutation was undefined. 22 (51.1%) patients had a confirmed objective response. Complete response was achieved in 5 patients (11.6%) and 17 (39.5%) patients achieved a partial response. Stable disease was seen in 27.9 % of patients. We experienced primary resistance in 5 patients (11.6%). Median PFS was 6.48 (95% Cl:4.8-15.0) months, median OS was 11.47 (95% Cl: 8.08-NA) months. We observed a non-significant difference in median PFS between the V600E and the V600K mutation subtype groups (6.7 months versus 1.8 months) and in median OS between the V600E and the V600K mutation groups (12.3 versus 8 months). 46.51% of the patients are still alive, the longest period of administering vemurafenib has been 26 months in this population. Analyzing our subgroup with cerebral metastasis, 50% objective responses were observed; complete response was achieved in 1 patient (16.6%), partial response in 2 patients (33.35) with vemurafenib treatment. 3 patients had progressive disease (50%).

Median PFS was 4.5 months, median OS was 6.8 months in the group of, patients with cerebral metastasis. We found significant association between LDH level at enrolment and OS, as the strongest predictive factor, both in univariate (HR:4.89, Cl:1.97-12.13, p=0.000613) and multivariate analysis (HR:4.65, Cl: 1.32-16.38, p=0.0168).

The most common adverse events (AEs) included follicular hyperkeratosis, maculo-papular rash, arthralgia and photosensitivity. Grade 3 AEs, such as cutaneous squamous-cell carcinoma, QTcB interval prolongation, rash and arthralgia were reported in 7 patients (17%). We found no Grade 4 side effects and, in our population, there were no patients with assigned to vemurafenib developed second primary melanoma. Most of the toxic effects were related to the skin (52%) as vemurafenib associated skin manifestations: follicular hyperkeratosis, maculo-papular rash, photosensitivity, alopecia, verruca vulgaris, keratoacanthoma, pruritus, palmo-plantar hyperkeratosis, squamous cell carcinoma. 1 patient had serum bilirubin level elevation. Development of keratoacanthoma or cutaneous squamous cell carcinoma was detected in 5 patients (12%). Median development of the first keratoacanthoma or squamous cell carcinoma was 8 weeks (range: 7 to 32 weeks). Out of the 5 patients 1 patient had cutaneous squamous cell carcinoma KA mixed type. 2 patients (5%) experienced herpes zoster in this population and in case of both patients the infection developed after 6 months of treatment. After 1 month of vemurafenib treatment 1 patient developed 1 to 2 cm, subcutaneous, painful nodules, located dominantly in the under extremities. After the excision of one of these nodules the histopathology showed vemurafenib associated neutrophilic panniculitis. ln this population we had no cases of permanent treatment discontinuation, Dose-reduction was required in 13 patients (30%). 13 patients' treatment was discontinued because of adverse events and after their resolution these patients were able to continue vemurafenib. ln 8 patients a level 1 (75%) dose modification, in 5 patients a level 2 (50%) dose modification was required. The most common reasons for treatment discontinuation were maculopapular rash and arthralgia.

Our second study included 118 patients, 60 men (51%), 58 women (49%). The mean age was with dabrafenib-trametinib 58.8 years, with vemurafenib-cobimetinib 60.7 years. Mean Breslow tumor thickness was 6.3 mm in vemurafenib-cobimetinib group and 4.5 mm in dabrafenib-trametinib group. LDH blood level was on vemurafenib-cobimetinib arm 45%> ULN, and on dabrafenib-trametinib arm36%>ULN. According to the AJCC staging system 77% of the patients had M1c status, including 39 (49%) patients in dabrafenib-trametinib group with cerebral metastasis at the beginning of the therapy. On the vemurafenib-cobimetinib arm

there were no patient with brain metastasis. The median follow-up time was 12 months (3-43) with dabrafenib-trametinib and 18 months (3-43) with vemurafenib-cobimetinib therapy. With dabrafenib-trametinib therapy objective response rate (ORR) was turned to be 82% (7% complete remission, 76% partial remission, 7% stabile disease, 10% progressive disease) and we observed a median PFS of 8.5 months. The median OS was 12 months. With vemurafenib+cobimetinib therapy we found 76% objective response rate (15% complete remission, 61% partial remission, 14% stabile disease, 10% progression disease). The median PFS was 8 months, the median OS proved to be 18 months.

We have been selected a subgroup - which included dabrafenib-trametinib patients with cerebral metastasis at the beginning of the therapy where we found significant association between progression free survival and the presence of brain metastasis ($p=0.0002$) and also between overall survival and brain metastasis ($p<0.0001$). Analyzing our subgroup with cerebral metastasis we observed a median overall survival of 11 months (3-26) and a progression free survival of 7 months (1-26) in contrast of the patients without brain metastasis, whom we reached significantly better survival rates with a median overall survival of 18.5 months (5-43), and a median progression survival of 14 months (3-43).With vemurafenib-cobimetinib therapy objective response rate (ORR) was turned to be 76% (15% complete remission, 60% partial remission, 13% stabile disease, 10% progressive disease) and we observed a median PFS of 8 months. The median OS was18 months. With both combination therapies we reached 90% disease control rate. We found significant association between LDH level at enrollment and PFS with vemurafenib-cobimetinib ($p=0.0184$) and dabrafenib-trametinib ($p=0.0042$) therapy, however between LDH level and OS was only significant with dabrafenib-trametinib ($p=0.0056$). With vemurafenib-cobimetinib therapy elevated liver function was detected most frequently (50%) as treatment-related adverse event. Rash (34%), diarrhea (39%), increased creatinine-kinase (CK) (32%), photosensitivity (29%), blurred vision (24%) occurred in remarkable proportion of patients. Serous retinopathy was diagnosed in 13% of patients. The incidence of any Gr3 or Gr4 adverse event was 34%. Neutrophil panniculitis was diagnosed in 3 patients (8%). Development of second primary melanoma was detected in 1 patient, keratoacanthoma or cutaneous squamous cell carcinoma was detected in 1-1 patients (5%). ln 9 patients (24%) a level 1 (75%) dose modification with vemurafenib and cobimetinib, in 2 patients a level 2 (50%) dose modification with vemurafenib was required. Temporary treatment interruption was required in 16 patients with vemurafenib (42%), and 14 patients (37%) with cobimetinib. ln this population we had 6 patients (16%) of permanent cobimetinib

discontinuation, and 2 patients (5%) had to discontinue vemurafenib therapy. ln the dabrafenib-trametinib group the most frequent treatment-related Adverse event was blood CK elevation (24%), increased liver function (13%), fever (13%), diarrhea (8%), rash (9%) and leukocytopenia (6%). Serous retinopathy was diagnosed in 2% of the patients. There were no secondary non-melanoma skin cancers occurred during dabrafenib-trametinib therapy. The incidence of any Gr3 or Gr4 AEs was 10%. Temporary treatment interruption was required in 19 patients with dabrafenib (24%) and 15 (19%) patients with trametinib. ln 11 patients (14%) with dabrafenib a level 1 (75%) dose modification was required, in 3% level one (75%) and 3% level 2 (50%) dose modification was needed with trametinib. No permanent discontinuation was needed because of any AE. The majority of adverse events occurred in the 2 months of therapy with both combinations.

ln our third study the median follow-up period of the studied 159 patients was 61 months (range: 1-96 months). The median age was 59 years (range: 18-83 years). Out of 159 patients 71 were male (44.7%) and 88 were female (55.3%). The most frequent location of the primary tumor was the trunk (77; 48.4%), followed by the lower extremities (47; 29.6%), then the upper extremities (35; 22%). Median Breslow thickness was 1.8 mm (range: 0.51-20 mm). Forty-nine of the primary tumors were ulcerated (30.8%). The most frequent histological subtype was superficial spreading melanoma (SSM) (124; 78%), then 31 cases, (19.5%) of nodular melanoma (NM), while in 4 cases (2,5%) other histological types were present. The median mitosis count was 4/mm^2 (range: 0-31), in 132 tumors (83%) neither lymphovascular nor perineural invasion was detected. ln 86 cases (54.1%) tumor infiltrating lymphocytes (TIL) were detected. Early regression of the primary tumor occurred in 9 patients (5.7%), late regression in a lower rate than 75%, occurred in 50 tumors (31.4%), while 75% was exceeded in 21 patients (13.2%). The primary tumor of 90 patients harbored BRAF mutation (56.6%), V600E in 87%, V600K in 10% and V600R in3%. NRAS mutation was detected in 29 patients (18.2%) exon 3 codon 61 (97%), exon 2 codon 13 (3%), 28 patients proved to belong to the double wild type group (17.6%), and genotyping was not valid in 12 patients (7.5%). The SLN status was negative in 130 cases (81.8%), while 29 cases showed positivity (18.2%). SLN status showed positive association with Breslow thickness of the primary tumors (p=0.008). ln sentinel negative cases the median Breslow thickness was 1.64 mm (range: 0.51--20 mm), whereas that of positive cases was 2.45 mm (range: 0.79-15 mm). SLN status and ulceration of the primary tumor showed significant association as well (p=0.007), in negative SLN cases 26.2%, in positive cases 51.7% of the studied tumors were ulcerated. Additionally, mitosis

count of the primary tumor differed between SLN negative and positive groups (p=0.009), 3/mm^2 (range: 0-31) and 5/mm^2 (1-30), respectively. Obviously, the presence of lymphovascular and/or perineural invasion of primary tumor was associated with SLN status (p=0.004); it was present in 34.5% of SLN positive, and only 12.3% of negative cases. A slightly higher occurrence of BRAF mutation was detected in the primary tumors of SLN positive than negatives cases (65.5% and 54.6%, respectively), however, the difference was not statistically significant. Interestingly, NRAS mutation of the primary tumor was present only in 6.9% of SLN positive tumors, while 20.8% in negative cases, however it was not statistically significant either. Analyzing the correlation of different prognostic factors and SLN status, multivariate analysis revealed that the only significant parameter was Breslow thickness of the primary tumor (OR: 4.222; 95% CI:1.201-14.873; p=0.025), while the other studied variables did not affect risk of sentinel positivity. Evaluating the parameters of BRAF, NRAS mutant and double wild type primary tumors, in case of age and Breslow thickness of primary tumor significant differences were revealed (p=0.001 and p=0.018, respectively). BRAF mutant patients were younger, NRAS mutant primary tumors were thicker than that of the other two groups. Trunk location was slightly more frequent, 58.6% of NRAS mutant, 51.1% of BRAF mutant and 31.1% of double wild type tumors. 24.1% of NRAS-mutant tumors proved to be nodular melanoma, while 16.7% of BRAF mutant, and 17.9% of double wild type tumors belonged to that histological category. At the last follow-up 130 patients were alive (81.8%), and 29 died (18.2%). A total of 123 patients (77.4%) were tumor free, 7 received innovative therapies due to metastatic disease (4.4%). ln 29 cases (18.2%) progression of the disease was detected, locoregional progression in 7 patients (4.6%) and distant metastasis in 22 cases (13.8%), respectively. Out of the 22 distant metastatic cases 9 had previous locoregional progression.

Univariate Cox proportional hazard model confirmed previously reported findings that Breslow-thickness of the primary tumor, ulceration, mitosis and invasive spreading pattern highly affected risk regarding every survival endpoint, while patients' age affected risk of DMFS and OS. ln addition, sentinel lymph node status was associated with risk of progression (p=0.001) and distant metastasis free survival (p=0.066), but not that of OS and DSS; however, in case of the latter, a tendency close to significance level was observed. NRAS/BRAF-status reversely affected risk, mutant NRAS was associated with a poorer PFS (p=0.048) and OS (p=0.037), while mutant BRAF was associated with significantly more favorable OS (p=0.045). Results of univariate model regarding SLN and mutational status was confirmed by Kaplan-

Meier curves with log-rank tests. According to SLN status comparison of PFS and DMFS revealed significant differences of SLN negative and positive cases (p=0.001 and 0.004, respectively). DSS showed nearly significant difference (p=0.052; data not shown): the 5-year disease specific survival rate of SLN negative cases was 92.7%, while that of positive cases was 77.5%. No significant difference of OS was found between sentinel-positive and sentinel-negative groups (data not shown). Evaluating the disease outcome of BRAF, NRAS mutant and double wild type patients, in case of almost every endpoints NRAS mutant cases were less favorable, while double wild type and BRAF mutant cases showed a very similar survival pattern with more favorable prognosis.

Therefore, comparison of merged BRAF mutant/double wild type and NRAS mutant cases was performed, which showed statistically significant differences of PFS (p=0.047) and OS (p=0.035), while DMFS and DSS did not differ significantly. 5-year PFS of BRAF mutant/double wild patients was 82.9%, whereas in NRAS positive cases it was only 63.3%. ln multivariate analysis excepting the PFS Breslow thickness still proved to be the strongest independent predictor of every endpoint. Compared to univariate model predictive value of ulceration, mitosis and invasion was weaker, mitosis was associated with the risk of DSS, and invasion with DMFS. On the other hand, similarly to univariate test, SLN positivity preserved the role of prediction on PFS (p=0.005) and DMFS (p=0.034). NRAS mutation proved to be negative predictor of PFS (p=0.047) and nearly significant in DMFS (p=0.06). ln addition patients' age was an independent predictor of OS and DMFS both in univariate and multivariate model.

New findings

1. ln our vemurafenib study population 27.9% of the patients received therapy as first line, and 71.9% second-, third-, or fourth-line. Similarly, to the previously published data in phase ll and lll trials, the median overall survival was 11.47 months. Objective responses were noted in 51.1%. DCR was achieved in79% of patients. Complete responses were attained by 5 patients (11.6%), which is a significantly higher rate than was achieved in the BRIM-2 trial (6%). We experienced primary resistance in 5 patients (11.6%), which results are almost similar comparing to the reported data of randomized clinical trials. Approximately 10 to 15% of vemurafenib treated patients do not experience tumor regression or disease stabilization,

demonstrating intrinsic or primary resistance [27,28]. From the analyzed predictive factors LDH level was significantly associated as the strongest predictive factor. ln our study vemurafenib therapy was well tolerated, the AE profile was almost consistent with the previously reported data. However, less squamous cell carcinoma was observed than the literature data (1-9%). ln this population we had no cases of permanent treatment discontinuation. Dose-reduction was required in 13 patients (30%), compared to the 38% in the BRIM 3 study. The most common reasons for treatment discontinuation were maculopapular rash and arthralgia. Development of keratoacanthoma or cutaneous squamous cell carcinoma was detected in 5 patients (12%) which is a better rate than what was published in the BRIM 3 study (18%).

2. ln our retrospective analysis both BRAF-MEK inhibitor combination therapy showed similar efficacy with a slightly different spectrum of toxicity profile. Our efficacy results with dabrafenib-trametinib treatment are slightly lower as the previously published overall and progression free survival datas, but if we analyzed our subgroup without brain metastases our patients reached also significantly better survival rates with a median overall survival of 18.5 months, and a median progression free survival of 14 months. Comparing the toxicity profiles with dabrafenib-trametinib we observed more frequently fever, and less treatment related adverse events, and no permanent discontinuation was needed because of AEs. With vemurafenib-cobimetinib photosensitivity and rash was more often registered. ln conclusion, knowing the diverse spectrum of BRAF-MEK inhibitor therapies side effects and the patient's concomitant diseases, may help the clinicians deciding which target therapy more personalized for their patients.

3. Beside the importance of SLN positivity, Breslow thickness, lymphovascular invasion and NRAS mutation of primary tumor proved to be independent prognostic factors of progression. Therefore, despite the absence of positive SLN, this NRAS positive patient subgroup still requires closer monitoring to recognize progression. ln contrast, examination of our cohort did not confirm any significant association between BRAF mutation, SLN status and survival. ln summary, independently from SLN status, knowledge of mutational status in the primary melanoma lesion supports disease management which manifests additional benefits for the patients.

List of publication

Articles related to this thesis

[1] **Czirbesz K**, Gorka E, Balatoni T, et al. Efficacy of Vemurafenib Treatment in 43 Metastatic Melanoma Patients with BRAF Mutation. Single-Institute Retrospective Analysis, Early Real-Life Survival Data. *Pathol Oncol Res. 2019;25(1):45-50.* **IF: 2.826**

[2] Liszkay G, Mátrai Z, **Czirbesz K**, Jani N, Bencze E, Kenessey I.: Predictive and Prognostic Value of BRAF and NRAS Mutation of 159 Sentinel Lymph Node Cases in Melanoma-A Retrospective Single-Institute Study. *Cancers (Basel). 2021;13(13):3302*. **IF: 6,639**

[3] Gorka E, Fabó D, Gézsi A, **Czirbesz K**, Fedorcsák I, Liszkay G.: Dabrafenib Therapy in 30 Patients with Melanoma Metastatic to the Brain: a Single-centre Controlled Retrospective Study in Hungary. *Pathol Oncol Res. 2018;24(2):401-406.* **IF: 2,433**

Articles not related to this thesis

[1] Balatoni T, Ladányi A, Fröhlich G, **Czirbesz K**, Kovács P, Pánczél G, Bence E, Plótár V, Liszkay G: Biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Pathol Oncol Res. 2020; 26:317-325.* **IF: 3,201**

[2] Kotlan B, Horvath S, Eles K, Plotar V, Naszados Gy, **Czirbesz K**, Blank M, Farkas E, Toth L., Tovari J et al. Tumor-Associated Disialylated Glycosphingolipid Antigen-Revealing Antibodies Found in Melanoma Patients' Immunoglobulin Repertoire Suggest a Two-Direction Regulation Mechanism Between Immune B Cells and the Tumor. *Front Immunol. 2019;10:650*. **IF: 5,085**

[3] Gorka E, Gézsi A, **Czirbesz K**, Liszkay G.: Single Center Retrospective Comparison of the Targeted Combination Therapies in Melanoma Patients. *ELYNS JOURNAL OF CANCER RESEARCH 2017; 2:107-110.*

[4] Gorka E, **Czirbesz K**, Gézsi A, Liszkay G.: Vemurafenib-cobimetinib kombinációs terápia és saját kezdeti tapasztalataink melanoma kezelésében. *Borgyogy Vener Szle 2017; 93:168-171*.

[5] Kovács P, Pánczél G, Melegh K, Balatoni T, Pörneczy E, Lőrincz L, **Czirbesz K**, Gorka E, Liszkay G.: Immunterápiák pszichológiai vonatkozásai a melanoma komplex és multidiszciplináris kezelésében. *Magy Onkol. 2016;60(1):22-27.*

[6] Gorka E, Fabó D, Gézsi A, **Czirbesz K**, Liszkay G. Distance from Primary Tumor Is the Strongest Predictor for Early Onset of Brain Metastases in Melanoma. *Anticancer Res. 2016;36(6):3065-3069.* **IF: 1,937**

[7] Kovács P, Pánczél G, Balatoni T, Pörneczy E, Lőrincz L, **Czirbesz K**, Gorka E, Melegh K, Liszkay G: A melanoma immunterápiájának pszichológiai aspektusai. *Onkológia (az ONCOLOGY magyar kiadása) 2015:5,93-97.*

[8] Kovács P, Pánczél G, Balatoni T, Pörneczy E, Lőrincz L, **Czirbesz K**, Gorka E, Melegh K, Liszkay G.: A melanoma immunterápiájának pszichológiai aspektusai. *ONKOLÓGIA (AZ ONCOLOGY MAGYAR KIADÁSA) 2015; 2:93-97.*

[9] Borbola K, **Czirbesz K**, Liszkay G.: Cutan planocellularis carcinoma Erbitux kezelése. *ONKOLÓGIA (AZ ONCOLOGY MAGYAR KIADÁSA) 2012; 2:165-167.*

Conference publication

[1] **Czirbesz K**, Balatoni T, Baranyai F, Pánczél G, Kispál M, Danyi T, Fröhlich G, Liszkay G.: BRAF-MEK gátló terápiák mellékhatásainak összehasonlítása 118 metasztatikus melanomában szenvedő betegnél az Országos Onkológiai Intézetben. *Borgyogy Vener Szle 2020; 96:286-286.*

[2] **Czirbesz K**, Baranyai F, Imrédi E, Pánczél G, Balatoni T, Kispál M, Danyi T, Fröhlich G, Liszkay G.: BRAF-MEK gátló terápiával elért eredményeink 118 metasztatikus melanomában szenvedő betegnél az Országos Onkológiai Intézetben: retrospektív analízis. *Borgyogy Vener Szle. 2019; 95: 251-251.*

[3] **Czirbesz K**, Baranyai F, Imrédi E, Kispál M, Pánczél G, Danyi T, Vízkeleti J, Fröhlich G, Balatoni T, Liszkay G.: BRAF-MEK gátló terápiával elért eredményeink 118 metasztatikus melanomában szenvedő betegnél az Országos Onkológiai Intézetben. Retrospektív analízis. *Magy Onkol. 2019; 63: 1.Supp.:18-18.*

[4] **Czirbesz K**, Geszti F, Gorka E, Pánczél G, Balatoni T, Imrédi E, Kovács P, Melegh K, Kenessey I, Liszkay G.: 49 metasztatikus melanomában szenvedő beteg PD-1 gátló terápiája az Országos Onkológiai Intézetben. *Borgyogy Vener Szle 2017, 93:258-258.*

[5] **Czirbesz K**, Gorka E, Pánczél G, Liszkay G.: Zelboraf terápiával elért eredményeink 43 metasztatikus melanomában szenvedő betegnél az Országos Onkológiai Intézetben, hosszabb követési idővel. Retrospektív analízis. *Magy Onkol. 2017;61:Suppl.1. pp. 13-13.*

[6] **Czirbesz K**, Gorka E, Panczel G, Melegh K, Kovacs P, Gezsi A, Liszkay G.:Efficacy of vemurafenib treatment in 43 metastatic melanoma patient with BRAF mutation. Single-institute retrospective analysis, early real-life survival data with 15.9 months follow up time. *J Eur Acad Dermatol Venereol. 2017;31: 61-61.*

[7] **Czirbesz K**, Gorka E, Melegh K, Imrédi E, Pánczél G, Balatoni T, Liszkay G, Gézsi A.: Zelboraf terápiával elért eredményeink 43 metastaticus melanomában szenvedő betegnél az Országos Onkológiai Intézetben, hosszabb követési idővel. Retrospekív analízis. *Borgyogy Vener Szle. 2016;92: 266-266.*

[8] **Czirbesz K**, Gorka E, Melegh K, Pörneczy E, Horváthy-Kovács A, Antal P, Gézsi A, Liszkay G.: Zelboraf terápiával elért eredményeink metastaticus melanoma kezelésében. *Magy Onkol. 2015; 59 1. Suppl.: 11-12.*

[9] **Czirbesz K**, Gorka E, Horváthy-Kovács A, Pörneczy E, Melegh K, Pánczél G, Kovács P, Liszkay G.: Cutan agniosarcoma. Esetbemutatás (poszter). *Borgyogy Vener Szle. 2015; 91:239-239.*

[10] **Czirbesz K**, Gorka E, Kovács F, Pörneczy E, Melegh K, Hetesi K, Antal P, Liszkay G.: Clinical report of Sentinel-Node Biopsy in 850 Melanoma Patient: A 5-Year Follow-up study. *Pigment Cell Melanoma Res. 2014, 27: 1183-1183.*

[11] **Czirbesz K**, Liszkay G, Gorka E, Melegh K, Pörneczy E, Antal P.:Vemurafenib kezelés klinikai tapasztalatai melanoma malignumban. *Borgyogy Vener Szle 2014; 90: 244-244.*

[12] **Czirbesz K**, Gorka E, Kovács F, Antal P, Borbola K, Liszkay G.: Melanoma malignumban végzett sentinel nyirokcsomó-biopszia klinikai tapasztalatai 850 beteg adatai alapján az Országos Onkológiai Intézetben. Követéses vizsgálat. *Magy Onkol. 2013; 57: suppl.1.17-17.*

[13] **Czirbesz K**, Plótár V, Serester O, Liszkay G.: BRAF V600 mutation in malignant melanoma. *J Dtsch Dermatol Ges 2013; 11: suppl.7. 44.*

[14] **Czirbesz K**, Gorka E, Kovács F, Antal P, Borbola K, Liszkay G.:Melanoma malignumban végzett sentinel nyirokcsomó-biopszia klinikai tapasztalatai 850 beteg adatai alapján az Országos Onkológiai Intézetben. Követéses vizsgálat. *Borgyogy Vener Szle 2013; 89:156.*

[15] **Czirbesz K**, Borbola K, Pánczél G, Gorka E, Liszkay G.: Uvealis melanoma malignum, ductalis emlő carcinoma és pulmonalis adenocarcinoma együttes előfordulása. *Borgyogy Vener Szle 2012; 88:189.*

[16] **Czirbesz K**, Balatoni T, Liszkay G.: A psoriasis vulgaris kezelésének késői mellékhatásaként kialakult bőrtumorok. *Borgyogy Vener Szle 2011; 87:198.*