Diploma Thesis

Adjuvant Melanoma therapy with Nivolumab, Pembrolizumab, or Dabrafenib plus Trametinib – a real life study

submitted by

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Graz, 2nd of August 2022

Affidavit

I hereby declare that the following diploma thesis has been written entirely by myself and without any help from third parties. I further assure that no sources other than those cited in this thesis have been used, and that all material from external sources, either literally or by content, has been explicitly indicated.

Graz, 2nd of August 2022 Nina Kainbacher eh

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List of Abbreviations

AE	Adverse event
AJCC	American Joint Committee on Cancer
BMI	Body mass index
BRAF	V-Raf murine sarcoma viral oncogene homolog B1
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CI	Confidence interval
CK	Creatine kinase
CLND	Complete lymph node dissection
CMNS	Congenital melanocytic nevus syndrome
CNS	Central nervous system
CRP	C-reactive protein
CTLA4	Cytotoxic T-lymphocyte-associated antigen 4
DNA	Deoxyribonucleid acid
D+T	Dabrafenib + Trametinib
ECOG	Eastern Cooperative Oncology Group
ERK	Extracellular signal-regulated kinase
FAMMM	Familial atypical multiple mole melanoma syndrome
GDP	Guanosine diphosphate
GGT	Gamma-glutamyltransferase
GTP	Guanosine triphosphate
HIV	Human immunodeficiency virus
HR	Hazard ratio
HRAS	Harvey rat sarcoma virus
IPI	Ipilimumab
irAE	Immune related adverse event
LDH	Lactate dehydrogenase
MAP2K	Mitogen-activated protein kinase kinase
MEK	Mitogen-activated protein kinase kinase
NIV	Nivolumab
NLR	Neutrophil to lymphocyte ratio

NRAS	Neuroblastoma rat sarcoma virus
OS	Overall survival
PD-1	Programmed cell death protein 1
PEM	Pembrolizumab
PET	Positron emission tomography
PFS	Progressive-free survival
PTEN	Phosphatase and tensin homolog
Ras	Rat sarcoma virus
RFS	Recurrence-free survival
ROS	Reactive oxygen species
RTX	Radiation therapy
SLND	Sentinel lymph node dissection
SOS	Son of sevenless
S100B	S100 calcium-binding protein B
UVR	Ultraviolet radiation
WT	Wild-type

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Zusammenfassung

Einleitung

Mit dem Einsatz von Immuntherapien und zielgerichteten Therapien gelang in den letzten Jahren ein Durchbruch in der onkologischen Medizin. Diese Studie soll nun erste Ergebnisse zur Wirksamkeit und Sicherheit in der Praxis liefern.

Methoden

Für diese Studie rekrutierten wir 51 Melanompatienten im Stadium III oder IV der Erkrankung nach R0-Resektion, die danach eine adjuvante Behandlung mit den PD-1-Antikörpern Nivolumab oder Pembrolizumab oder eine Kombination aus den BRAF/MEK-Inhibitoren Dabrafenib und Trametinib erhielten. Primärer Endpunkt der Studie war das rezidiv-freie Überleben (RFS), sekundäre Endpunkte inkludierten das Gesamtüberleben (OS), sowie eine Subgruppen- und Nebenwirkungsanalyse.

Ergebnisse

Die Überlebensanalyse ergab ein 1-Jahres RFS von 70 % in der D+T, 59 % in der NIV und 50 % in der PEM Gruppe. Nach zwei Jahren zeigte sich ein RFS von 44 % in der D+T+, 43 % in der NIV und 50 % in der PEM Gruppe. Kombinierte Ergebnisse beider PD-1-Antikörper ergaben ein 1-Jahres RFS von 58 % und ein 2-Jahres RFS von 43 %. Das Gesamtüberleben war mit 82 % in der PD-1-Gruppe und 78 % in der BRAF/MEK-Gruppe sehr ähnlich. Faktoren, die das RFS in der PD-1-Gruppe signifikant erhöhten, waren ein Alter unter 65 Jahre (HR für Rezidiv 0,43 vs. 1,96; p = 0,045; 95 % CI), nur ein positiver Lymphknoten (HR für Rezidiv 0,5 vs. 1,5; p=0,02; 95 % CI) und Zeit zum Therapiestart unter drei Monaten (HR für Rezidiv 0,4 vs. 2,2; p=0,028; 95 % CI). Immun-vermittelte Nebenwirkungen hatten keinen signifikanten Einfluss auf das RFS. In der BRAF/MEK-Gruppe konnte kein Faktor mit signifikantem Einfluss auf das RFS identifiziert werden. Schwere Nebenwirkungen traten in 25,8 % in der PD-1-Gruppe und in 25 % in der BRAF/MEK-Gruppe auf. Insgesamt konnten keine neuen toxischen Risiken beobachtet werden.

Conclusio

Immuntherapien und zielgerichtete Therapien bleiben weiterhin sehr effektive Methoden, um Melanome im fortgeschrittenen Stadium zu behandeln. Dennoch bleibt vor allem das frühe Rezidiv des Melanoms auch heute noch eine Herausforderung in der Medizin, da ein Teil der Patienten nicht auf die Behandlung anspricht und es zu schwerwiegenden Nebenwirkungen kommen kann. Somit ist weiterhin intensive Forschung zur Verbesserung der Therapie erforderlich.

Abstract

Introduction

With the use of immunotherapy and targeted therapy, a breakthrough in oncological medicine was achieved in the last few years. This study should now bring first results of efficacy and safety in real-life practice.

Methods

For this study, we retrospectively enrolled 51 stage III or IV melanoma patients after R0-resection, who received adjuvant treatment with PD-1-antibodies nivolumab or pembrolizumab or a combination of BRAF/MEK-inhibitors dabrafenib and trametinib. Primary endpoint was recurrence-free survival (RFS), secondary endpoints included overall survival (OS) and subgroup and safety analyses.

Results

Survival analysis revealed a one-year RFS of 70 % in the D+T, 59 % in the NIV, and 50 % in the PEM treated group. After two years, subjects showed a RFS of 44 % in the D+T, 43 % in the NIV, and 50 % in the PEM treated group. If we combine the two PD-1-antibodies, 58 % of patients were relapse free after one year, and 43 % after two years. OS was very similar in PD-1 (82 %) and BRAF/MEK treated patients (78 %). In the PD-1 treated group, factors that significantly improved RFS were age under 65 (HR for relapse 0.43 vs. 1.96; p=0.045; 95 % CI), only one positive lymph node (HR for relapse 0.5 vs. 1.5; p=0.02; 95 % CI), and a time to start of therapy that is less than three months (HR for relapse 0.4 vs. 2.2; p=0.028; 95 % CI). Immune-related adverse events due to immunotherapy did not indicate a reduced recurrence risk in our study. In the BRAF/MEK group, no factor leading to an improvement in RFS could be observed. Serious adverse events were similar in the PD-1 (25.8 %) and BRAF/MEK treated group (25 %) and no new toxicity signs were identified.

Conclusion

Immunotherapy and targeted therapy agents continue to be very effective treatments for advanced melanoma. Nevertheless, there are multiple difficulties, since a number of patients do not respond to the treatment or experience severe adverse events. Therefore, early recurrence of advanced melanoma remains a challenge in medicine even to this date and intensive research is still needed for therapy improvement.

1 Introduction

1.1 Melanoma

Melanoma, while not the most common, is the most serious form of all skin cancers. Due to its tendency to spread, it is accountable for about 80-90 % of skin cancer deaths (1,2). The tumor originates from melanocytes, the type of cells which produce the skin pigment melanin. It typically occurs in the skin but because melanocytes are derived from neural crest cells, it can also develop anywhere in the body, in places such as the mucosa, intestines, or the uvea. The most common areas are sun exposed areas such as upper and lower extremities, the head, the neck, or the torso. In women, the lower extremity appears to be the most common localization, while in men, it is the torso, especially the back. The older the patients, the more frequently a melanoma is located on the head or neck (3). In these patients, the type of melanoma is more commonly lentigo maligna melanoma, which accounts for 10 % of melanoma cases, but has an increased incidence and occurs on chronic sun exposed skin areas in the elderly. With 70 % of all cases, the most common type of melanoma is the superficial spreading melanoma. It is associated with the presence of many moles, sun exposure, and has a slow growth process (4). Then again, there is nodular melanoma, which is not associated with the mentioned factors. It accounts for 20 % of all melanoma cases and shows little pigmentation. This sometimes leads to misdiagnosis and thus to a higher mortality rate. At 5 %, acral lentiginous melanoma is the rarest of the four main melanoma types. It occurs on palms, soles, and subungual and is also not linked to sun exposure and moles. It is the most common type in Asian and African people and tends to have the worst prognosis due to surgical difficulties and late diagnosis (5,6).

1.1.1 Epidemiological facts

In Austria, 1521 people are recorded with a diagnosis of melanoma (2019), although it is assumed that not all cases are included in the statistics. Based on the number of inhabitants in Austria in 2019 and the available statistics, an incidence of 17.3 per 100,000 inhabitants can be calculated. While there is an increase in incidence, the

five-year median survival rate has also increased in recent years. At 88 %, women show a slightly higher five-year survival rate than men at 83 %. Accounting for 4 % of all malignancies, melanoma is the seventh most common malignancy in women and the sixth most common in men (7). These numbers are also consistent with melanoma statistics from the USA, where an incidence of 18 per 100,000 citizens can be observed (8).

Globally, data shows similar results. In general, melanoma is more common in women before the age of 40 than in men. Above the age of 70, the incidence reaches higher levels in men than in women (9). The lifetime risk for developing melanoma is more than 25 times higher in people with white skin than in people with darker pigmented skin (2.6 % vs. 0.1 %), meaning that in Western countries, approximately 1 out of 50 will develop the malignant disease (10). This is also why there is a north-south divide in Europe, with the Scandinavian countries having the highest incidence and lightest skin color, and the Mediterranean countries having the and lowest incidence with 5-7 per 100,000 citizens per year and the darkest skin type (8,11). Australia shows the world's highest incidence with 54 cases per 100,000 citizens per year, New Zealand shows similar trends. In these countries, melanoma is the third most common type of cancer. It is assumed that light skin color paired with high UV exposure causes this condition (12,13). As we will see in section 1.1.2, the risk can be very individual and increases with several risk factors.

1.1.2 Etiological risk factors

1.1.2.1 Ultraviolet radiation

There are several risk factors for developing melanoma. It is well known that UV radiation is a major risk factor for developing melanoma due to inflammatory, immunosuppressive, and DNA damaging processes (14). The UV spectrum consists of UVA (wavelength 315-400 nm), UVB (280-315 m) and UVC (100-280 nm) waves. Evidence has shown that UVA and UVB radiation can cause DNA damage, which can lead to the development of skin cancers including melanoma (15-17). Particularly UVA radiation causes direct DNA damage through methylation and indirect alteration through oxidation and the generation of reactive oxygen species (ROS), leading to mutations and thus to uncontrolled cell growth and

differentiation (14). It is evident that the sun is an important risk factor, and it does not matter whether UVR comes directly from the sun or from artificial tanning beds. Another component of the suns danger is the fact that it suppresses the immune system by expelling CD4+ lymphocytes and dendritic cells. Dendritic cells play an important role in the presentation of antigens. These cells make antigens visible for other cells such as CD4+ lymphocytes, whose function is to kill infected host-cells, recruit other cells of the immune system, and secrete cytokines. Furthermore, cytokines like interferon and interleukin are important signal proteins, which activate other immune cells such as natural killer cells and macrophages to fight off infected or cancerous cells. They also increase the occurrence of major histocompatibility complex (MHC), which facilitates the recognition of diseased cells or tissues even more (18). Sunburns have been shown to cause more damage than chronic intermittent sun exposure, with the latter being more associated with actinic keratosis as a precursor to squamous cell skin cancer (11).

Taking all these factors into account, sunburns are a major risk factor for the development of a cancerous process. If they occur in childhood, the risk is even doubled, so prevention in childhood and adolescence is even more important (14,19). However, different melanoma types show a different association to sun exposure. While the superficial spreading type and lentigo maligna melanoma are associated with intermittent sun exposure, UVR plays no role in the development of the nodular type and the acral lentiginous melanoma. All these mechanisms of sun damage typically affect fair skin types since they have less melanin as a protective factor against UV radiation (20).

Overall, sun protection is a major contributor to prevention of some types of melanocytic malignancies. Studies show that regular use of sunscreen can significantly reduce the individual risk of developing skin cancer (21). In addition, physical protection in the form of clothing can also help.

1.1.2.2 Immune system

Melanoma is an immunogenic tumor, and its relationship with immune cells in the tumor microenvironment significantly influences cancer cell proliferation, progression, and metastasis. It is suspected of hiding from immune cells, making it easier to pass by our cells' defense mechanisms. This circumstance enables a

therapeutic intervention, these cancer immunotherapies will be discussed later (22). As mentioned above, UV radiation suppresses and modulates the immune system in several ways. These mechanisms also occur in immunocompromised people. In addition, immunocompromised conditions, such as HIV or people treated with immunosuppressive medication due to organ transplantation or autoimmune diseases may be accompanied by a higher risk of developing melanoma (23).

1.1.2.3 Melanocytic nevi and genetics

Since approximately one third of all melanomas, usually of the superficial spreading type, are associated with preexisting moles, the total count of moles is a major risk factor for the occurrence of melanoma (24,25). Other types like lentigo maligna and nodular melanoma are not associated with the presence of nevi. A dysplastic nevus, meaning that the nevus is atypical in appearance regarding its size, color, border, or surface, as well as its histological appearance, has a higher risk of evolving into melanoma than common moles (26,27).

People with a genetic condition called dysplastic nevus syndrome, also known as familial atypical multiple mole melanoma (FAMMM), are at particular high risk for developing melanoma. It is believed to be due to mutations in the CDKN2A gene on chromosome 9p21, which encodes for production of tumor suppressor protein p14ARF. This protein also has a major impact on the expression of the tumor suppressor protein p53, the most frequently mutated protein in all types of cancers (28,29). Tumor suppressor genes are important because they can disrupt the cell cycle of dysregulated cells to allow enough time for DNA repair mechanisms and induction of apoptosis, if needed (29). Criteria for FAMMM are a high total count of nevi, often more than 50, the presence of dysplastic nevi as described above, and a family history of melanoma in more than one first- or second-degree relative (30). Although inherited in an autosomal dominant manner, penetrance can vary. The estimated risk for developing melanoma by the age of 80 is between 58 % and 92 %. Affected individuals also suffer from a higher risk of developing other malignant diseases, especially pancreatic cancer (30). A second genetic disorder to mention is the congenital melanocytic nevus syndrome (CMNS). Unlike FAMMM, the melanotic lesions in the skin are already present at birth. Due to atypical proliferation of melanocytes in the nervous system, CMNS is also associated with neurological

symptoms. Genetically, it is mostly based on mutations in the NRAS spectrum of neural-derived crest cells such as melanocytes are (31,32). Large or giant congenital nevi, which can occupy the entire body and have a high risk of transforming into melanoma, are rare and occur in less than 1 of 20,000 newborns worldwide (33,34).

Another rare genetic disease to mention is Xeroderma pigmentosum. It is an autosomal recessive hereditary disease associated with UVR hypersensitivity caused by a malfunction in DNA repair mechanisms. The hypersensitivity results in multiple sunburns, many freckles, and changes in skin pigmentation. People with this genetic condition show a higher risk of developing several types of skin cancers due to their reduced ability to repair DNA damage (35).

Conclusively, there are many genes that can predispose people to develop melanoma. About 10 % of melanoma patients report one or more family member also affected with the disease, meaning that a positive family history can be taken as a precursor to individual risk. Although not all genetic connections are fully understood, genetics may play an important role in understanding the evolution of melanoma (36).

1.1.2.4 Skin type

Melanin absorbs UV rays and has antioxidant properties. Hence, melanin may be a protective factor against melanomagenesis (37). As a result, fair skin types are affected by melanoma much more frequently than darker skin types, which is also reflected in the incidence rates (10). Especially people with red or blonde hair, blue or green eyes, freckles, and a preference for sunburn rather than tan are particularly at risk (11). Figure 1 graphically represents the different skin types on the Fitzpatrick scale. It shows that the lighter the skin, the higher the risk of sunburns and therefore the risk of developing some form of melanoma.

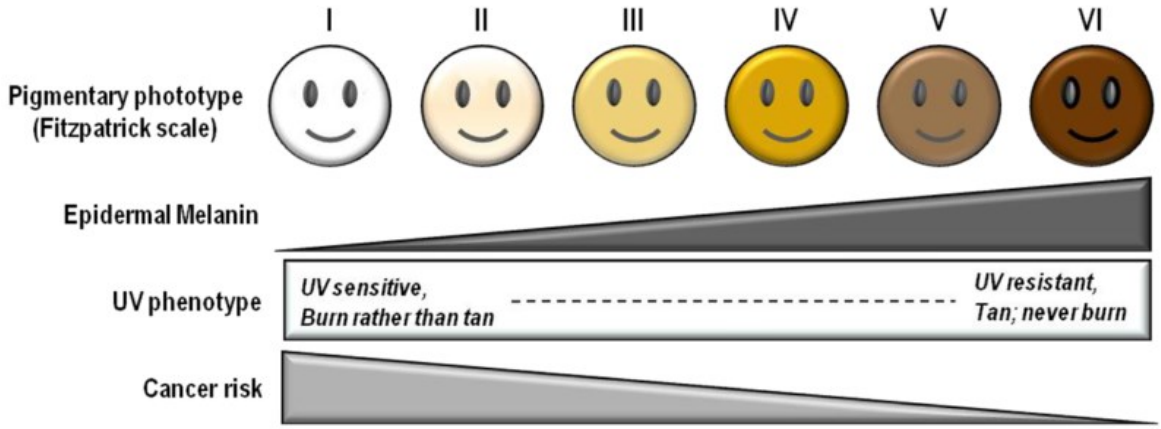


Figure 1: Skin types on risk: *Fair skin types tend to burn rather than tan and have a higher risk of getting skin cancer, while darker skin types with higher pigmentation are more resistant to UVR and less susceptible for getting sunburns. Thus, DNA damage happens less often, and skin cancer risk decreases with an increase in pigmentation.*

Reference: D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci 2013 -06-07;14(6):12222-12248.

1.1.3 Diagnosis

Although not all melanomas show distinctive features, dermoscopy, also known as epiluminoscopy, is generally an effective tool for early melanoma diagnosis. It involves an examination of the skin using surface microscopy. There are malignancy signs and algorithms to follow, e.g. the ABCDE assessment and the sign of the “ugly duckling”.

Table 1: ABCDE Assessment

A	***Asymmetry***
B	***Border***
C	***Color***
D	***Diameter***
E	***Evolution***

If the lesion looks asymmetrical, this can be a sign for malignancy. The border of the lesion should be defined and clear, with no uneven or irregular borders. In a benign lesion, the color is usually uniform and medium brown. Black, blue, or red pigments should not be visible. Next, a diameter greater than 5 mm may be suspicious. Evolution, which includes changes in color, size, border, surface, or shape of the lesion as well as additional sensations like itching or bleeding, is one of the most significant indicators for a malignant process.

The “ugly duckling sign” means, that the mole looks very different from the others on the person’s body. Considering that most moles of a body look alike, the “ugly duckling” is a warning sign for melanoma. ABCDE and the ugly duckling sign are easy to evaluate for the overall population and a good rule to follow when doing health screenings on one’s own body.

With epiluminoscopy, the structure of skin can be analyzed more precisely. The main three things to consider are symmetry, structure, and color. Pigment network can be accurately assessed and plays an important role in differentiation between benign and malign. Malignant lesions often have a more structureless appearance. Moreover, variability in color can also be evaluated better. Four or more colors can indicate malignancy, especially white or blueish areas suspect. If two or three of them are positive, a lesion is classified as suspicious for melanoma. With a sensitivity of 92 % and a specificity of 95 %, dermoscopy is a great tool to allow early melanoma detection (38).

Another technique which is in use for early melanoma discovery is the FotoFinder® (39). It is a digital dermoscopy for monitoring suspicious lesions over time. This allows for analysis of the entire body over time in order to detect new nevi or the evolution of preexisting ones. Skin cancers other than melanoma can also be detected with the additional use of fluorescence applications. In summary, the best prevention of melanoma is to regularly examine one’s body to check if any mole looks different or bigger than normal, and to see a dermatologist once a year for a close examination with dermoscopy.

If a lesion shows signs of malignancy, a histological biopsy must be taken for a definitive diagnosis. The recommended practice is to remove the whole efflorescence, so the thickness can be used for staging. Punch biopsy or shaving only are not recommended (40). A nodular melanoma, which often lacks pigmentation, can easily be misdiagnosed when using these rules.

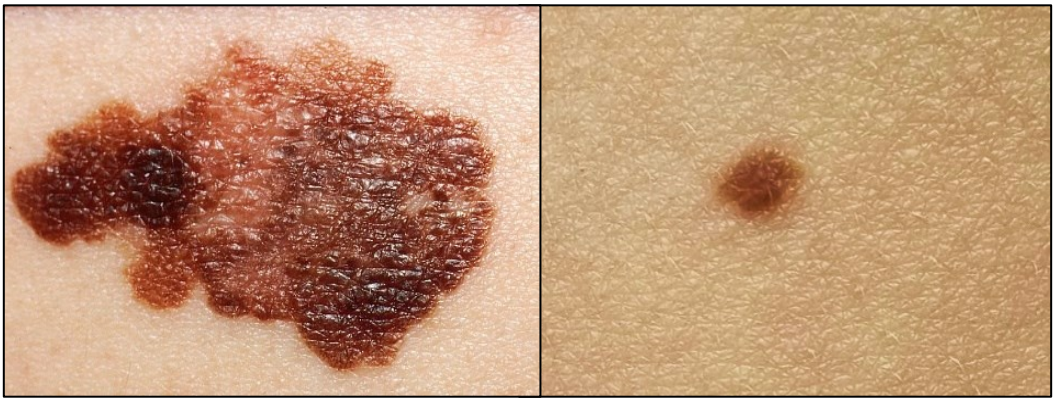


Figure 2: ABCDE Assessment: *On the left side, a highly suspicious lesion is shown. It is asymmetric, bigger than normal and many shades of brown with darker and lighter areas can be identified. For comparison, the right side shows a normal mole in a monotonous brown color with small diameter, also no irregular shape or border can be detected.*

Reference: National Cancer Institute, AV Number: AV-8500-3850

1.1.4 Staging

Melanoma is staged by the American Joint Committee on Cancer 8th edition 2017 (AJCC), which is based on the TNM system. T stands for tumor, N for lymph node metastases and M for distant metastases. The Breslow's depth, which divided the tumor depth into five stages, was mostly replaced by the eighth edition of AJCC staging manual, which is our focus. The classification allows to select the right therapy, prognosis and survival can be assessed more precisely. Tables 2-5 describe the TMN categories (41).

Penetration depth is determined with a biopsy of the whole lesion. Deep skin penetration and positive ulceration status are negative prognostic factors. After the biopsy of the primary tumor, the spread of metastases is evaluated using a radiographic examination such as a PET scan, if required. MRI can be very sensitive when brain metastases are suspected, but PET/CT, especially with 18F-FDG as a tracer, is preferred when it comes to the detection of soft-tissue, nodal or visceral metastases (42).

Table 2: TNM Classification – T

T Category	*Thickness*	*Ulceration*
TX	Tumor cannot be assessed	-
T0	No primary tumor	-
Tis (Melanoma in situ)	Melanoma cells only at the very top layer of skin	No
T1	≤1.0 mm	Unknown
T1a	<0.8 mm	No
T1b	<0.8 mm	Yes
	0.8-1 mm	Yes or no
T2	>1.0-2.0 mm	Unknown
T2a	>1.0-2.0 mm	No
T2b	>1.0-2.0 mm	Yes
T3	>2.0-4.0 mm	Unknown
T3a	>2.0-4.0 mm	No
T3b	>2.0-4.0 mm	Yes
T4	>4 mm	Unknown
T4a	>4 mm	No
T4b	>4 mm	Yes

To further classify the N stages, there are designations for special types of metastases that lie between the tumor and the nearest lymph node:
Microsatellite metastases: The tumor has spread to an area less than 2 cm away with microscopic micrometastases.
Satellite metastases: The tumor has visibly spread to an area less than 2 cm away.
In-transit metastases: Tumor cells more than 2 cm away from the primary tumor, on their way to the next lymph node station.

Any patient in any category with an ulcerated or larger than 0.8 mm tumor is now offered a sentinel lymph node biopsy. With a sentinel lymph node biopsy, clinically occult tumor involved lymph nodes can be detected. Clinically occult refers to normal lymph nodes in PET scan or other radiographic examinations and no clinical signs of metastasis (41).

Table 3: TNM Classification – N

N Category		***Number of affected regional lymph nodes or microsatellite, satellite, or in-transit metastases or matted nodes***
NX		Regional lymph nodes not assessed
N0		No regional lymph node metastases
N1		1 tumor-affected regional lymph node **or** the presence of microsatellite, satellite, or in-transit
	N1a	1 clinically occult
	N1b	1 clinically detected
	N1c	Microsatellite, satellite, or in-transit
N2		2 or 3 tumor-affected regional lymph nodes **or** 1 + microsatellite, satellite, or in-transit
	N2a	2 or 3 clinically occult
	N2b	2 or 3 lymph node metastases, at least one of them detected
	N2c	1 clinically occult or detected + microsatellite, satellite, or in-transit
N3		≥4 tumor-affected regional lymph nodes **or** 2 or 3 + microsatellite, satellite, or in-transit
	N3a	≥4 clinically occult
	N3b	≥4, at least 1 clinically detected or the presence of any number of matted nodes
	N3c	≥2 clinically occult or detected and/or any number of matted nodes + microsatellite, satellite, or in-transit

Table 4: TNM Classification – M

M Category		**Anatomical site**
M0		No distant metastases detected
M1		Distant metastases detected
	M1a	Skin, soft tissue, muscle, or non-regional lymph nodes
	M1b	Lung (+-M1a sites)
	M1c	Visceral organs (non-CNS, +- M1a or M1b sites)
	M1d	CNS (+- M1a, M1b or M1c sites)

A sentinel lymph node biopsy is a surgical procedure with the use of a radioactive tracer in combination with methylene blue for locating the first lymph node in the lymphatic system near the primary tumor. This is the lymph node where cancer cells would be most likely to spread. Sometimes there can be more than one. The tracer and the blue dye are injected intradermally near the tumor before the procedure, which makes it possible to detect which lymph node is the one that accumulates the substances first. Next, suspected lymph nodes are surgically removed and sent to the pathology laboratory to be examined for the presence of tumor cells. The procedure is usually performed in patients with melanoma or breast cancer because these tumors are more likely to spread to lymph nodes before spreading to distant organs. If the biopsy is negative, the tumor is unlikely to have spread. Compared to a complete lymph node dissection, the procedure is less invasive and spares patients the side effects of potentially unnecessary, more invasive surgeries. In addition, studies have shown that there is no benefit from complete lymph node dissection in patients with a positive SLND (43,44). If only small metastases are present, process observation should be considered instead of complete dissection (44). CLND is required in case of pronounced lymph node involvement. Although melanoma is a cancer that has an affinity to spread in lymph nodes first, distant metastases are common and can occur in any organ including the brain, bones, liver, and lung. Even with the primary tumor being very small, melanoma can spread throughout the body due to its phenomenal ability to evade the host's immune system (45).

The classification in table 5 presents the stages into which melanoma can be divided into. No metastasizing processes in lymph nodes can be detected up to stage IIC. Stages IIIA, IIIB, IIIC, and IIID are defined by the presence of lymph node metastases. If distant metastases are present, the tumor is assigned to stage IV, with the penetration depth and the lymph node metastatic spread being irrelevant (41).

Table 5: TNM Classification – Stages

Stage	***T***	***N***	***M***
0	Tis	N0	M0
IA	T1a/T1b	N0	M0
IB	T2a	N0	M0
IIA	T2b/T3a	N0	M0
IIB	T3b/T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1a/b or T2a	N1a or N2a	M0
IIIB	T0, T1a/b, T2a/b, or T3a	Any N1 or N2	M0
IIIC	T0, T1a/b, T2a/b, T3a/b, or T4a/b	Any N1 or N2	M0
IIID	T4b	N3	M0
IV	Any T	Any N	M1

1.1.5 Pathogenesis and signaling cascades in melanoma

The genesis of melanoma is a complex process. It all starts with melanocytes starting to grow and organize abnormally. This process is influenced by the risk factors mentioned, such as DNA damage from UVR or a weakened immune system that overlooks diseased cells. In addition, DNA alterations lead to other mutations that drive tumor growth. Once the cells get out of control, it begins with an initial radial growth phase, followed by a vertical phase and an invasive radial phase. In the initial radial phase, the formation has not yet reached the basal lamina and spreads only superficially at epidermis. In the vertical growth phase, it begins to penetrate deeper skin layers such as papillary and reticular dermis, where it can reach structures like blood and lymph vessels. Now melanoma can progress to the invasive radial growth phase and spread further throughout the body. To achieve successful spreading, the tumor uses various techniques to evade the immunological reaction of the host. A major factor of these processes is the expression of the vascular endothelial growth factor. It is known for its proangiogenic functions, but studies show that it suppresses cells of the immune system in many ways (46). It does not only stimulate the tumor growth, but it also helps evading the immune response of the host and leave the tumor proliferation unrecognized. Additionally, in patients with advanced melanoma, high serum levels of VEGF are observed, indicating that there may also be a connection between high serum levels and a bad prognosis (47). To trick the immune system, melanomas also stimulate immune checkpoints, which are important regulators of the immune system that prevent immune cells from self-attacking the host. If the immune checkpoint pathway is upregulated, the immune reaction is suppressed, and melanoma can spread undetected.

1.1.5.1 Immune checkpoint pathways

A key immune checkpoint is the programmed cell death protein 1 (PD-1). During its clonal expansion, the PD-1 protein is particularly expressed by regulatory T cells, but also by other types of T cells, B cells, and natural killer cells. It is an inhibitory protein of the immune system that can induce apoptosis in antigen-specific T cells. When it connects with its binding protein programmed death ligand 1 (PD-L1), which is mainly expressed by dendritic cells and macrophages, the process is initiated

(48). Normally, PD-L1 expression protects against autoimmune reactions. Disorders of PD-L1 expression have shown to be strongly associated with development of autoimmune diseases (49). Because melanoma can upregulate PD-1 and some types can even express PD-L1, it can downregulate anti-tumor activity (50). This is where immunotherapies like the monoclonal antibodies nivolumab and pembrolizumab come into play. As immune checkpoint inhibitors, they inhibit PD-1, which by upregulating the immune response helps identify and destroy tumor cells. Another immune checkpoint that melanoma affects is the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4). Like PD-1, it is primarily expressed in all regulatory T cells, but also in other immune cells after activation. After connecting to its binding proteins B7-1 and B7-2 on antigen-presenting cells, it downregulates the T-cell proliferation, especially in lymph nodes, and thus also the immune response (51). Ipilimumab, acting as a checkpoint inhibitor by inhibiting CTLA4, is used to treat unresectable or advanced melanoma, usually in combination with nivolumab. Furthermore, ongoing trials are showing good results when using it as monotherapy for neoadjuvant treatment in resectable stage III melanoma, and currently there is a focus on using it in earlier stages (52).

1.1.5.2 Tumor mutations

About 50 % of melanomas show mutations in the BRAF gene on chromosome 7 which codes for the protein B-Raf. It is a proto-oncogene, which, when mutated, causes cells to become malignant and stimulates uncontrolled cell proliferation. In particular, the most frequently observed V600E mutation of the BRAF gene is an important driver of disease development. In this mutation, the amino acid 600, usually valine (V), is replaced with glutaminic acid (E). This is commonly seen not only in melanoma, but also in other tumors such as glioma, non-small-cell lung cancer, papillary thyroid carcinoma, and hairy cell leukemia, so therapies targeting the B-Raf protein are being addressed in all these malignancies in various studies (53-56). Since therapy resistance occurs after a treatment interval of 6 or 7 months, B-Raf inhibitors are combined with inhibitors of the mitogen-activated protein kinase kinase (MEK1 and 2/MAP2K). For a combination therapy, dabrafenib as a BRAF-inhibitor and trametinib as a MEK-inhibitor are approved.

At almost 30 %, mutations in the NRAS proto-oncogene are the second leading most common genetic alteration in melanoma. Tumors mutated in this gene are considered highly aggressive (57). Other mutations on the RAS spectrum like KRAS and HRAS are also seen in melanoma. Genes on the RAS spectrum encode for the RAS protein, which plays a role in the MAPK/ERK signaling cascades. Therefore, treatment of advanced NRAS-mutated melanoma is based on therapies that affect that pathway, mainly targeting MEK, but immunotherapy is also an approved strategy. BRAF and NRAS mutations are common in cutaneous melanoma, while in acral lentiginous and mucosal melanoma, the most common mutation appears to occur in the KIT proto-oncogene, with 36 % of acral lentiginous and 39 % of mucosal melanomas positive for alteration. Chronical sun-exposed skin also shows an increased frequency of KIT mutations (58).

Other observed mutations that can occur alone or in combination with the above changes are loss-of-function mutations in tumor suppressor genes such as TP53, CDKN2A, PTEN and NF1. TP53 encodes the tumor suppressor protein p53, which regulates cell division and initiates apoptosis in cells with damaged DNA. Therefore, a loss of its function is fatal and supports the development of malignancies. In fact, it is the most common mutated gene in all tumors. CDKN2A is the typical alteration in familial melanoma. Alterations in the NF1 gene, which is responsible for the tumor syndrome neurofibromatosis type 1, are associated with a dysregulation of the MAP/ERK signal pathway. Another alteration in melanoma is the loss of the tumor suppressor gene PTEN. It is also a regulator of the cell cycle and widely distributed in many kinds of tumors. In addition to driver mutations such as BRAF, NRAS or NF1, changes in the MAP2K1, which codes for the kinase MEK1, are also observed in cutaneous melanoma (59).

Melanoma does not always have to carry a mutation. Melanocytic tumors without genetic modifications are referred to as wild-type (WT). Triple-WT means that no changes are shown, neither in BRAF nor in NRAS or NF1 genes.

1.1.5.3 MAPK/ERK signal pathway

The MAPK/ERK signal pathway, also known as RAS-RAF-MEK-ERK pathway, is a cascade of processes in which proteins that provides signals for the regulation of the differentiation and proliferation of cells are activated. The cascade starts with the binding of a mitogen, i. e. a growth factor, to a growth factor receptor such as EGFR (epidermal growth factor receptor). This leads to dimerization and autophosphorylation and thereby to its activation. The activated and now phosphorylated receptor is consequently able to bind and activate further molecules: it ligates to the growth factor receptor bound protein (Grb2), resulting in binding of the molecule SOS, which subsequently connects with RAS. RAS is a proto-oncogene, that can bind to GDP, which is converted in GTP with the help of SOS, resulting activation of RAS. In turn, RAS now launches the activation of B-RAF, which phosphorylates MEK1 and MEK2. Finally, the MEK kinases phosphorylate the extracellular signal-regulated kinases (ERK), which, like the MEKs, belong to MAPK family. As a result, ERK 1 and 2 activate transcription factors and alter the translation of mRNA into proteins that play an important role in cell cycle regulation. Disruption of these processes leads to dysregulation of proliferation, differentiation, apoptosis, and migration, what subsequently promotes malignant transformation. The entire cycle is now a subject of interest in cancer research since a dysregulation of the pathway is found in more than 30 % of all tumors and more than 50 % of all melanomas. Particular attention is paid to the inhibition of B-RAF and MEK (60).

1.1.6 Therapeutical strategies

1.1.6.1 Surgery

Surgery is a key component of cutaneous melanoma therapy and the first option for an attempt to cure. A lesion should always be resected in its whole expansion, punch biopsy only is not recommended. In earlier stages, such as cutaneous melanoma in situ, a curing rate of 98.9 % can be achieved with surgery only, provided that an adequate margin of approximately 1 cm is maintained (61). In addition, depending on the depth of tumor penetration, margins of 1-2 cm are recommended to reduce the risk of recurrence. This often requires a further excision (62).

1.1.6.2 Chemotherapy

Melanoma shows incredible resistance to chemotherapy. Before the era of immunotherapy and targeted treatment, several combinations and chemotherapeutic drugs were tested unsuccessfully for melanoma treatment. Dacarbazine was approved as the only chemotherapeutic drug for advanced melanoma in the 1970s. Its oral equivalent, temozolomide, showed similar results. However, both drugs showed a response rate of only 10-15 % and thus failed to achieve a significant efficacy in terms of life prolongation in clinical studies (63-65). Later, dacarbazine was combined with interferon-alpha or interleukin, called biochemotherapy, which led to better tumor response, but also it did not increase survival rate, only toxicity (66).

1.1.6.3 Radiation therapy

Although cutaneous melanoma is highly resistant to radiation therapy, RTX is part of standard therapy for uveal and choroidal melanoma. Stereotactic radiosurgery and proton beam therapy have proven to be effective for this regard (67,68). Furthermore, adjuvant radiation therapy could be an approach to treat high-risk lymph node metastases in combination with adjuvant systemic therapies. This strategy is currently a subject of research interest (69). In addition, radiation therapy is used palliatively for unresectable or therapy refractive distant metastases.

1.1.6.4 Immunotherapy: T-VEC

Since 2015, Talimogene laherparepvec (T-VEC), a modified herpes simplex 1 virus, is approved for intralesional injection of cutaneous unresectable stage IIIB/C and IV melanoma. It should have an oncolytic effect and simultaneously upregulate the hosts immune response, locally and systemically. Hence, it accounts to immunotherapeutic medication. Currently, there are studies ongoing, in which T-VEC is investigated in several adjuvant and neoadjuvant settings. A recent study showed that patients with resectable stage IIIB/C or IV melanoma, who were treated with T-VEC prior to surgery, had an increased two-year recurrence-free survival at 29.5 % vs. 15.9 % in the group with surgery alone (70).

1.1.6.5 Neoadjuvant immunotherapy

Neoadjuvant immune checkpoint inhibition therapy for melanoma has gained increasing attention in recent years. Various studies are ongoing, particularly on blocking receptors such as CTLA-4 and PD-1 or both. There are indications, that it may be superior to adjuvant therapy alone and there is evidence that the combination of nivolumab and ipilimumab in a neoadjuvant setting leads to improved recurrence-free survival at stage III melanoma, but also to higher toxicity (71). In conclusion, neoadjuvant immunotherapy shows promising data, but there are no results of larger ongoing studies that allow to make a concrete statement yet (72).

1.1.6.6 TILs and CAR-T cell immunotherapy

Currently, research is ongoing to test the efficacy of adoptive cell-transfer (ACT) therapy in metastatic melanoma. In doing so, tumor-infiltrating lymphocytes (TILs) are extracted from cancerous material, examined for anti-tumor activity, and TILs that show activity are expanded in vitro and returned to the host. The aim is to modify patient's immune cells in order to destroy tumor cells more effective. It is the beginning of the production of a chimeric antigen receptor for T cells (CAR-T cells) that modify T cells to present melanoma antigens more effectively to other cells of the immune system (73). Both techniques are successfully used in hematological malignancies. Whether the benefit is similar in melanoma remains to be seen.

1.1.6.7 Immunotherapy: PD-1 inhibitors

Before the development of immunotherapy, prognosis of advanced stage III or IV melanoma was with a five-year survival rate of less than five percent very poor (74). In particular, the PD-1-antibodies pembrolizumab and nivolumab has revolutionized melanoma therapy and improved survival. Several studies show significantly increased RFS and OS in patients with advanced melanoma (75) when treated with these agents in an adjuvant setting.

Immunotherapy is not new. Since melanoma genesis mechanisms are closely related to the host immune system, it has always been a research topic with even more potential in the future. The first tried and tested therapies with interferon and interleukin did not meet expectations and have been superseded. Nivolumab and

pembrolizumab show much better results and less toxicity than interferon, interleukin, and the following used CTLA-4 inhibitor ipilimumab, which is now being tested in neoadjuvant settings. As previously described, PD-1-antibodies block the immune checkpoint protein PD-1 on T cells through binding to the PD-1 receptor. PD-1 itself is a downregulating protein of the immune system and gets upregulated by melanoma cells. Blocking the protein prevents it from interacting with its binding proteins, and disrupting this mechanism results in higher immune system function. On the contrary, the medication can trigger autoimmune adverse reactions that can affect almost every organ in the body. In phase III clinical trials, the number of patients who experienced side effects varies. The most frequently occurring adverse events seem to be unspecific general symptoms like fatigue, nausea, loss of appetite, gastrointestinal disturbances, or skin reactions such as rash and pruritis. Common immune-related side effects affect almost any organ system and can appear as autoimmune inflammations such as colitis, pneumonitis, pancreatitis, vitiligo, thyreoiditis, hypophysitis, elevated liver markers and cardiological and neurological complications (76). Even though grade IV and fatal immune-related adverse events are rare, cases of pneumonitis, multiple organ failure, encephalitis, stroke, neutropenia, and cardiac failure leading to death are reported (77,78). Overall, it can be said that the benefit from PD-1 therapy exceeds the damage of adverse events, since prognosis would be poor without this therapy. Nevertheless, it should be evaluated carefully with involvement and clarification of the patient if treatments with NIV or PEM should be started (77).

The dosage optimum for NIV in an adjuvant setting studied in phase II clinical trials, is intravenous injection of 240 mg every two weeks or 480 mg every four weeks. The treatment should be continued until disease recurrence or occurrence of high-grade adverse events. In contrast to palliative treatment with NIV, for which the duration is unlimited, adjuvant therapy in resected stage III or IV melanoma should last up to one year (79). For PEM, the defined dosage for an intravenous use is 200 mg every three weeks or 400 mg every six weeks for up to one year in an adjuvant treatment unless disease recurrence or unbearable adverse reactions. As in the treatment recommendation with NIV, therapy duration in metastatic or unresectable melanoma is unlimited (80).

1.1.6.8 Targeted therapy: BRAF/MEK-inhibitors

With the evolution of targeted therapy, a breakthrough in advanced melanoma therapy was achieved. The primary targets are the b-RAF protein and the MEK kinases 1 and 2 of the MAPK/ERK signal pathway. Therefore, the active agents dabrafenib as a b-RAF inhibitor and trametinib as an inhibitor of MEK 1 and 2 are used. Through this mechanism, the permanent activation of the MAPK/ERK signal pathway can be stopped, which leads to a reduction in proliferation, and promoted apoptosis. A criterion for the efficacy of the therapy is that melanoma must have a mutation in the BRAF gene, what about half of these tumors do. The aim is to block the uncontrolled cell cycle and thus stop the driver of melanoma growth process. Several phase III clinical trials show an excellent improvement of RFS and OS in patients with metastatic melanoma or resected stage III or IV melanoma treated with adjuvant targeted therapy compared to placebo (81,82). Hereby, the combination of dabrafenib and trametinib showed the best results and longest therapy response. Currently, targeted therapy is part of first-line therapy for advanced BRAF-mutated melanoma. Dosing is determined with 2x75 mg a day of dabrafenib (every 12 hours) plus 2 mg once a day of trametinib. It is recommended to continue the treatment until intolerable toxicity, or a disease progression occurs (83). Reports of adverse events are investigated in phase III clinical studies and range from mild to severe reactions. Some of the most common adverse events seen in approval studies were pyrexia, hyperkeratosis, headache, arthralgia, fatigue, diarrhea, hypertension, skin toxicities and cardiac toxicities (84).

Real-life experience with the efficacy and safety profiles of nivolumab, pembrolizumab, dabrafenib and trametinib has not been evaluated yet but is urgently needed. Therefore, our study shows real-life investigations of adjuvant therapy with these immunotherapy and targeted therapy agents after their approval.

2 Materials and methods

2.1 Study population

Between January 2017 and January 2020, 51 melanoma patients from the Medical University Hospital of Graz were retrospectively enrolled for this study, all of them receiving adjuvant melanoma therapy with the PD-1-antibodies nivolumab (NIV) or pembrolizumab (PEM) or a combination of the BRAF/MEK-inhibitors dabrafenib and trametinib (D+T).

The inclusion criteria were defined as follows:

1. definitive diagnosis of melanoma in a stage III or IV (AJCC 2017)
2. complete resection of the tumor (R0-resection)
3. adjuvant melanoma therapy with nivolumab, pembrolizumab or dabrafenib plus trametinib.

As this is a real-life study, no specific exclusion criteria were defined.

All patients were followed up to April 30th, 2021.

2.1.1 Treatment

Table 6: Adjuvant therapy

	No. (%)
Nivolumab	27 (52.9)
Pembrolizumab	4 (7.8)
Dabrafenib + Trametinib	20 (39.2)
Total	51 (100)

At 52.9 %, the majority of study population received nivolumab, 39.2 % received dabrafenib plus trametinib and only 7.8 % of the patients pembrolizumab. It is assumed that the period till the first application of the adjuvant should be kept short. In most of cases (54.9 %), time until therapy was less than three months. In the D+T

group, 65 % receveid the first dose within less than three months, while 53.8 % in the NIV group received their first dose. An exception was the PEM population, in which all of patients received their first adjuvant therapy more than three months after resection. The mean number of therapy cycles reported was 9.59 in the NIV group and 7.75 in the PEM group, of which 22.2 % in the NIV and 25 % in the PEM group are ongoing at time of the last follow-up. In the D+T group, the mean time of therapy was 6.95 months, 45 % of them ongoing. Overall, 87.1 % in the PD-1 group and 85 % in the BRAF/MEK-group completed the treatment.

2.1.2 Patient characteristics

Table 7: Demography

	Nivolumab N=27 No. (%)	**Pembrolizumab N=4 No. (%)**	**Dabrafenib +Trametinib N=20 No. (%)**
Sex			
Female	14 (51.9)	1 (25)	10 (50)
Male	13 (48.1)	3 (75)	10 (50)
Total	27 (100)	4 (100)	20 (100)
Age			
< 65	14 (51.9)	3 (75)	12 (60)
≥ 65	13 (48.1)	1 (25)	8 (40)
Total	27 (100)	4 (100)	20 (100)
BMI			
< 25	11 (40.7)	0 (0)	6 (30)
≥ 25	15 (55.6)	4 (100)	12 (60)
Not reported	1 (3.7)	0 (0)	2 (10)
Total	27 (100)	4 (100)	20 (100)
ECOG			
0	22 (81.5)	4 (100)	18 (90)
1	4 (14.8)	0 (0)	2 (10)
2	1 (3.7)	0 (0)	0 (0)
3	0 (0)	0 (0)	0 (0)
Total	27 (100)	4 (100)	20 (100)

Demographic data such as age, sex, BMI, and the ECOG performance status were collected. Gender was very evenly distributed in each group and sorted into male and female. Age of patients was sorted into younger than 65 years and ≥ 65 and ranged from 19 to 85 years. 54.8 % in the PD-1 treated group and 60 % in the BRAF/MEK treated group were younger than 65 years. Body mass index was divided into two groups, < 25 and ≥ 25. It is striking that in each group more than 60 % of all patients were overweight with a body mass index above 25. Performance status was very good, with 83.8 % in the PD-1 group and 90 % in the BRAF/MEK group having an ECOG status of 0, meaning that the patient is fully active and able to perform all pre-disease activities without restriction. Only one patient had a performance status of 2, meaning that he is capable of all self-care but unable to perform work activities. No one in the study had an ECOG level greater than 2.

As shown in table 8, we also collected data related to patient laboratory results, including tumor markers LDH and S100B. We further analyzed albumin and neutrophile to lymphocyte ratio (NLR) as well as the C-reactive protein (CRP) as systemic inflammation markers. Normal LDH value should be between 120 and 240 U/l. In the study, the level ranged from 121 up to 564 and samples were also distributed into two different groups: < 240 U/l and ≥ 240 U/l. The median level of LDH was in the reference range in all groups and overall, 25-30 % of patients showed a higher LDH level than 240 U/l. The laboratory reference values for CRP range from 0.6 - 5.0 mg/dl. CRP levels in the study were between 0.5 and 17.4 and most of the patients were within the reference range with median levels of 1.4 in the PD-1 treated group and 2.25 in the BRAF/MEK-inhibitor treated group. S100B was used as most specific tumor marker for melanoma, which should be below 0.105 µg/l. Only 18.5 % in the NIV group, 10 % in the D+T group and nobody in the PEM group was above these levels. For the neutrophile to lymphocyte ratio, we used a cut-off of 3.2, values above this were reported as abnormal, which was the case in 14.8 % of patients in the NIV group, in 20 % of cases in the D+T and in 25 % in the PEM group. Albumin levels in all patients ranged from 2.9 - 5 g/dl. Since laboratory reference values range from 3.5 - 5.4 mg/dl, no values were outside the normal range.

Considering previous therapy alongside R0-resection, only one person in the NIV and one in the D+T group had a systemic therapy with Interferon. Radiation therapy

was more frequently represented with 22.2 % in the NIV, 25 % in the PEM, and 15 % in the D+T group. Regarding to lymph node surgery, a sentinel lymph node biopsy (44.4 % NIV, 75 % PEM, 40 % D+T) was significantly more often performed than a complete lymph node dissection (25.92 % NIV, 25 % PEM, 35 % D+T) in each group.

Table 8: Blood results and treatment before adjuvant therapy

	Nivolumab N= 27	**Pembrolizumab N= 4**	**Dabrafenib + Trametinib N= 20**
Lab before adjuvant – Median (Range)			
LDH	199 (121-436)	186.5 (177-564)	197.5 (121-298)
S100B	54 (26-7250)	36 (19-43)	61 (27-310)
CRP	1.4 (0.5-15.6)	2.1 (0.7-2.6)	2.25 (0.7-17.4)
Albumin	4.4 (2.9-4.9)	4.75 (4.7-4.8)	4.6 (4-5)
Lab before adjuvant – No. (%)			
LDH ≥ 240 U/l	7 (25.9)	1 (25.0)	6 (30.0)
S100B ≥ 0.105 µg/l	5 (18.5)	0 (0.0)	2 (10.0)
NLR ≥ 3.2	4 (14.8)	1 (25.0)	4 (20.0)
Therapy before adjuvant – No. (%)			
Interferon	1 (3.7)	0 (0.0)	1 (5.0)
RTX	6 (22.2)	1 (25.0)	3 (15.0)
Lymph node surgery – No. (%)			
SLND	12 (44.4)	3 (75.0)	8 (40.0)
CLND	7 (25.92)	1 (25.0)	7 (35.0)

2.1.3 Tumor characteristics

An inclusion criterion for this study was the diagnosis of stage III or IV melanoma. The vast majority of patients, 90.4 % in the PD-1 group and 85 % in the BRAF/MEK group, were classified as having tumor stage IIIB or IIIC. Only one patient in the entire study had a stage IV tumor. Ulceration was common at 38.7 % in the PD-1 and 50 % in the BRAF/MEK group, and tumor penetration was mostly less than 4 mm in all groups. Acral lentiginous melanoma as a relatively rare type of melanoma could not be observed frequently. Only two patients in the PD-1 group and one of the BRAF/MEK group were affected by this tumor type. A criterion for tumor stages III and IV is the presence of at least one lymph node metastasis, which can also be occult. At around 75 %, most patients in all groups had only one affected lymph node.

Considering the MAPK/ERK signaling pathway as therapeutic target of the BRAF/MEK-inhibitors dabrafenib and trametinib in patients with a tumor mutation in the BRAF proto-oncogene, almost all tumors in the D+T group had a BRAF V600 E, D or K mutation. These mutations were significantly less common in the PD-1 group. Mutations in the NRAS gene were found in 38.2 % of tumors in the PD-1 group. Tumors mutated in this gene are considered highly aggressive (57). Wild-type tumors were classified as tumors with no mutation in either BRAF, NRAS, or any other gene examined. Other mutations observed were MAP2K1 (P124L), CDKN2A (C172T), BRAF (K601E), BRAF (L597R) (V600R), HRAS (G13V), BRAF (G469K), HRAS (Q61R), CDKN2A (R80X), PTEN (5del:p.T2fs), CDKN2A (E88X) and PTEN (W111R). It should be noted that tumors can show more than one mutation.

Table 9: Tumor characteristics

	PD-1-inhibitors N=31	**BRAF/MEK-inhibitors N=20**
	No. (%)	**No. (%)**
Stage		
IIIA	1 (3.2)	2 (10)
IIIB	10 (32.3)	8 (40)
IIIC	18 (58.1)	9 (45)
IIID	2 (6.5)	0 (0)
IV	0 (0)	1 (5)
Total	31 (100)	20 (100)
Ulceration		
No	16 (51.6)	5 (25)
Yes	12 (38.7)	10 (50)
Not reported	3 (9.7)	5 (25)
Total	31 (100)	20 (100)
Acral lentiginous melanoma		
No	29 (93.5)	19 (95)
Yes	2 (6.5)	1 (5)
Total	31 (100)	20 (100)
Thickness		
< 4 mm	16 (51.6)	14 (70)
≥ 4 mm	7 (22.6)	2 (10)
Not reported	8 (25.8)	4 (20)
Total	31 (100)	20 (100)
Lymph node metastases		
= 1	23 (74.2)	15 (75)
> 1	6 (19.4)	5 (25)
Not reported	2 (6.5)	0 (0)
Total	31 (100)	20 (100)
Mutation		
BRAF V600 E, D, K	8 (23.5)	19 (82.6)
NRAS	13 (38.2)	0 (0)
WT	5 (14.7)	0 (0)
Others	8 (23.5)	4 (17.4)

2.2 Study design

This study is one of the first study to analyze real-life data on the use of the PD-1 antibodies nivolumab and pembrolizumab and the combination of BRAF/MEK-inhibitors dabrafenib plus trametinib in clinical routine. Thus, real-life experience with these active substances can finally be compared to results of existing phase III clinical studies. Between January 2017 and January 2020, patients with the diagnosis of stage III or IV melanoma after complete tumor resection were admitted to the University Clinic for Dermatology and Venereology of the Medical University Hospital of Graz to perform the study.

Medical data includes demographics such as age, gender, weight, BMI, performance status and laboratory values such as LDH, CRP, S100B, albumin, and NLR. We also collected data on previous therapies like Interferon or radiation therapy, or whether the patient had complete lymph node dissection or sentinel lymph node biopsy. Furthermore, tumor-specific characteristics such as tumor stage, histological classification, BRAF mutation status, ulceration status, penetration depth and the status of lymph nodes and metastases were recorded. Each patient was followed up to April 30th, 2021. Data was extracted from openMEDOCS and, after complete pseudonymization, a Microsoft Excel list was created individually for each patient.

Primary endpoint of the study was recurrence-free survival (RFS), which in our setting also functioned as progression-free survival (PFS), defined as the time between R0-resection and the occurrence of the first melanoma recurrence in adjuvant treated patients. Patients without melanoma recurrence during the follow-up period were censored on the date of last patient contact. Secondary endpoints included overall survival (OS), a multivariate subgroup analysis and the documentation of adverse events. OS is defined as the time between R0-resection and the date of death from any reason and was also censored at the last patient contact if the patient was alive at that time. Close monitoring of side effects is required to establish a safety profile of the adjuvant medication. To determine the severity of adverse events, we divided them into clinical severity degrees I-V. In therapy with the checkpoint inhibitors NIV and PEM, immune-related adverse events (irAE) were examined more closely. To identify patients at risk, another secondary endpoint was a detailed subgroup analysis that included all mentioned parameters

of patient and tumor characteristics and the presence of the adverse events. The collected real-world data enables an objective assessment of the efficacy and safety of these drugs and enables a comparison with results of existing pivotal studies. In addition, we generated descriptive statistics of treatment failures, which recorded the frequency of specific melanoma recurrences and median time to recurrence. Further treatment, including a therapy switch of available systemic therapies, was also recorded.

2.3 Analytical methods

For descriptive statistics and logistic regression models, IBM SPSS for Windows version 26.0.0.1 was used. Categorical parameters such as patient characteristics like age, sex, BMI, ECOG and previous therapies and tumor specific characteristics such as tumor stage, mutations, penetration depth, type of tumor and the lymph node status were presented as absolute and relative frequencies, while scale-type variables such as blood results were presented as mean, median and range. To compare the distributions of parameters, a Chi-square test for categorical variables and a Student's T-test for continuous variables were performed.

Kaplan-Meier curves were constructed for survival analysis, visualizing the outcomes and time-to-event connections of different patient groups. The Mantel-Cox log-rank test was used to compare the groups and calculate the significances of the results. Patients who did not have an event such as a recurrence or death during observation time were considered censored. To obtain the most conclusive results, only variables with missing values below 5 % were used for survival analysis.

A Cox proportional hazards regression model was performed to identify predictive cofactors of early melanoma recurrence. The Cox regression models were presented as hazard ratios and 95 % confidence intervals (CI) and tested for significant influence using the log-rank test for calculating the p-value. All p-values were two-sided and values < 0.05 % were considered significant. Potential cofactors affecting outcome were patient and tumor characteristics, laboratory results, previous therapies, and adverse events. To graphically visualize the strength of the correlation between the outcome and the influencing factors, forest plots were created with Microsoft Excel.

3 Results

This chapter presents our findings in detail and demonstrates important correlations. Profession-free survival as our primary endpoint is described in detail in section 3.1. We analyzed the PFS not only in every treatment arm, but also according to lymph node interventions, stages, and adverse events. Every survival function is graphically represented as a Kaplan-Meier curve. The same applies for overall survival, which is presented in section 3.2.

After the survival analysis, a subgroup analysis was performed for both PD-1 treated patients and BRAF/MEK treated patients. It is described in section 3.3 and presents connections between special patients or tumor characteristics and recurrence-free survival rate. To further discuss the safety profile of the investigated active agents, an analysis of adverse events with special interest in immune-related adverse events in immunotherapy treated patients was performed in section 3.4.

Finally, observations from recurrence cases and subsequent initiated therapies are described in section 3.5.

3.1 Progression-free survival

Progression-free survival was the primary endpoint of this study. First, we compared the PFS of nivolumab, pembrolizumab and the combination of dabrafenib plus trametinib. Thereafter, both PD-1-antibodies Nivolumab and Pembrolizumab were pooled together, to get more precise results due to an increased number of cases. In addition, we analyzed if there is a difference of the PFS in patients in stage IIIA and B compared to ≥ stage IIIC, in patients with SLND or a CLND, and in patients who experienced immune-related adverse events during immunotherapy treatment. At one year, the percentage of patients alive without relapse was 59 % in the NIV group, 50 % in the PEM group, and 70 % in the D+T group. After two years of follow-up, the percentage of patients alive without relapse was 43 % in the NIV group, 50 % in the PEM group, and 43 in the D+T group. When merging the two PD-1-antibodies, 58 % of immunotherapy treated patients were relapse free after one year and 43 % after two years. Thus, comparing the different treatment arms, initial differences in RFS level out over time and were similar at the end of the follow-up time. The mean time of recurrence-free survival was 18.8 months in the PD-1 group and 19.1 months in the BRAF/MEK group. Overall, two-year-comparison was very similar among patients receiving NIV, PEM or D+T and no statistically significant difference was identifiable.

Survival analysis according to tumor stages revealed no significant difference between earlier and higher melanoma stages. In the PD-1 treated group, 63.6 % of patients in stage IIIA or B experienced a two-year recurrence-free interval, while only 34.3 % experienced the same in later stages of melanoma. In the BRAF/MEK group, we observed the opposite effect. Patients in later stages showed an improved recurrence-free survival with 48 % after two years in contrast to only 37.3 % in earlier stages. Nevertheless, PFS was very similar in all stages and no significant difference could be observed, neither between all treatment arms, nor between stages IIIA, B and stage IIIC or above. Sections 3.1.1, 3.1.2, and 3.3.3 graphically present survival analyses of the different treatment arms and of different melanoma stages.

3.1.1 PFS: Nivolumab vs. Pembrolizumab vs. Dabrafenib plus Trametinib

Table 10: PFS in NIV, PEM and D+T

Adjuvant therapy	*No. of Patients*	*Recurrences – No. (%)*	*Progression-free Survival (95% CI)* *Mean in months (range)*
Nivolumab	27	14 (51.9)	18.9 (13.5-24.2)
Pembrolizumab	4	2 (50)	13.5 (5.1-21.9)
Dabrafenib + Trametinib	20	10 (50)	19.1 (14.4-23.7)
Overall	51	26 (51)	19.8 (16-23.5)

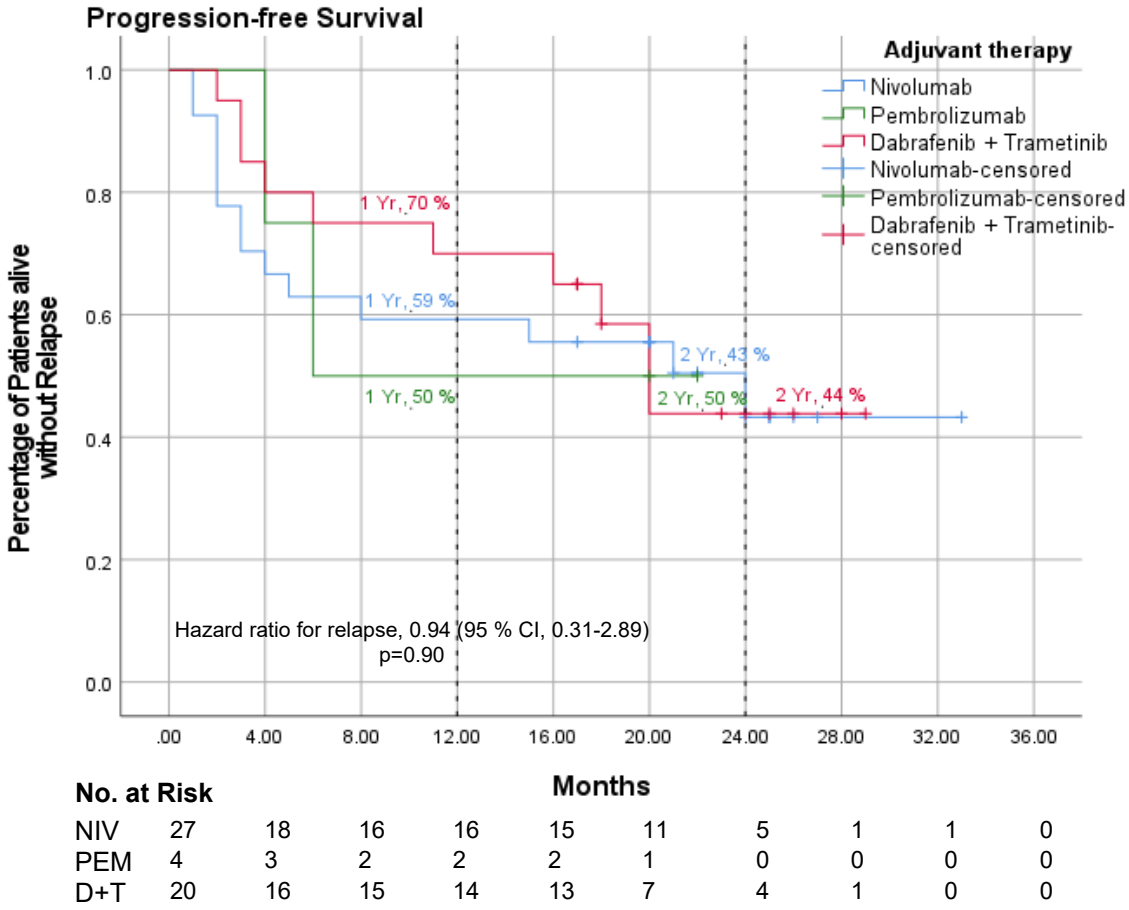
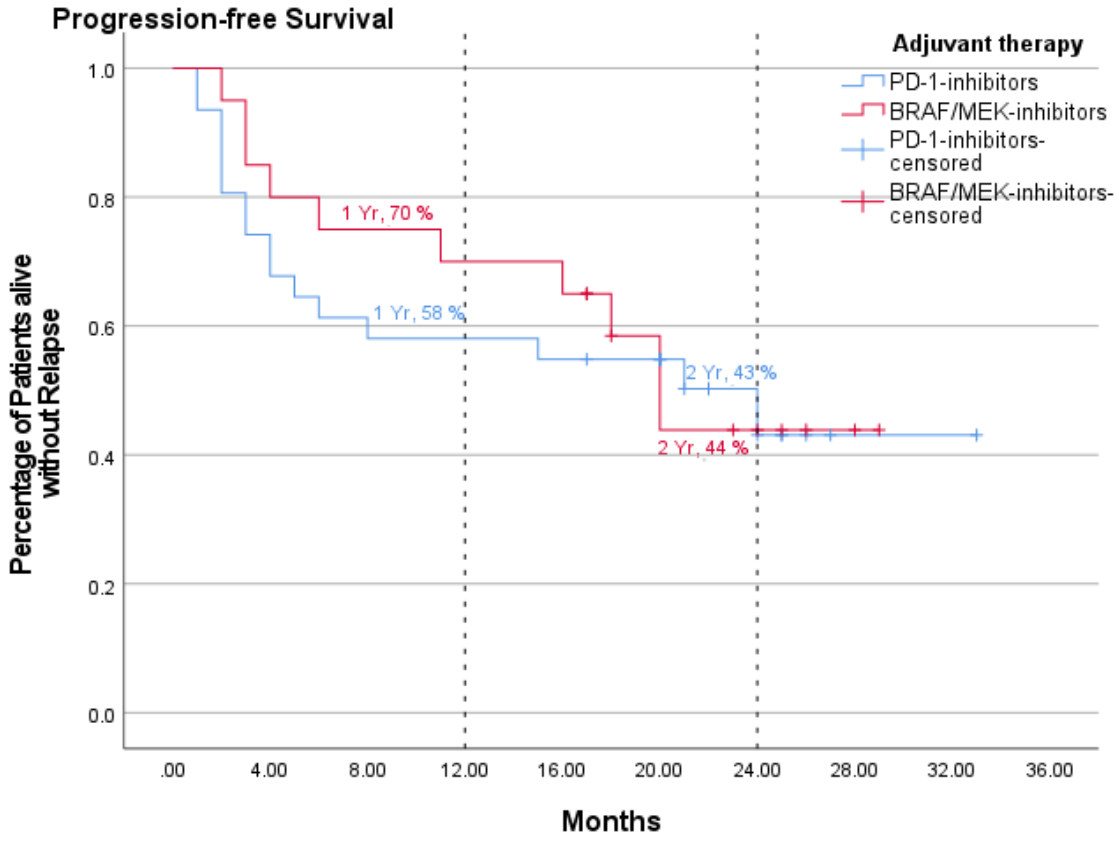


Figure 3: *PFS in Nivolumab vs. Pembrolizumab vs. Dabrafenib plus Trametinib*

3.1.2 PFS: PD-1-inhibitors vs. BRAF/MEK-inhibitors

Table 11: PFS in PD-1-inhibitors and BRAF/MEK-inhibitors

Adjuvant therapy	*No. of Patients*	*Recurrences – No. (%)*	*Progression-free Survival (95% CI)* *Mean in months (range)*
PD-1-inhibitors	31	16 (51.6)	18.8 (13.8-23.8)
BRAF/MEK-inhibitors	20	10 (50)	19.1 (14.4-23.7)
Overall	51	26 (51)	19.8 (16-23.5)



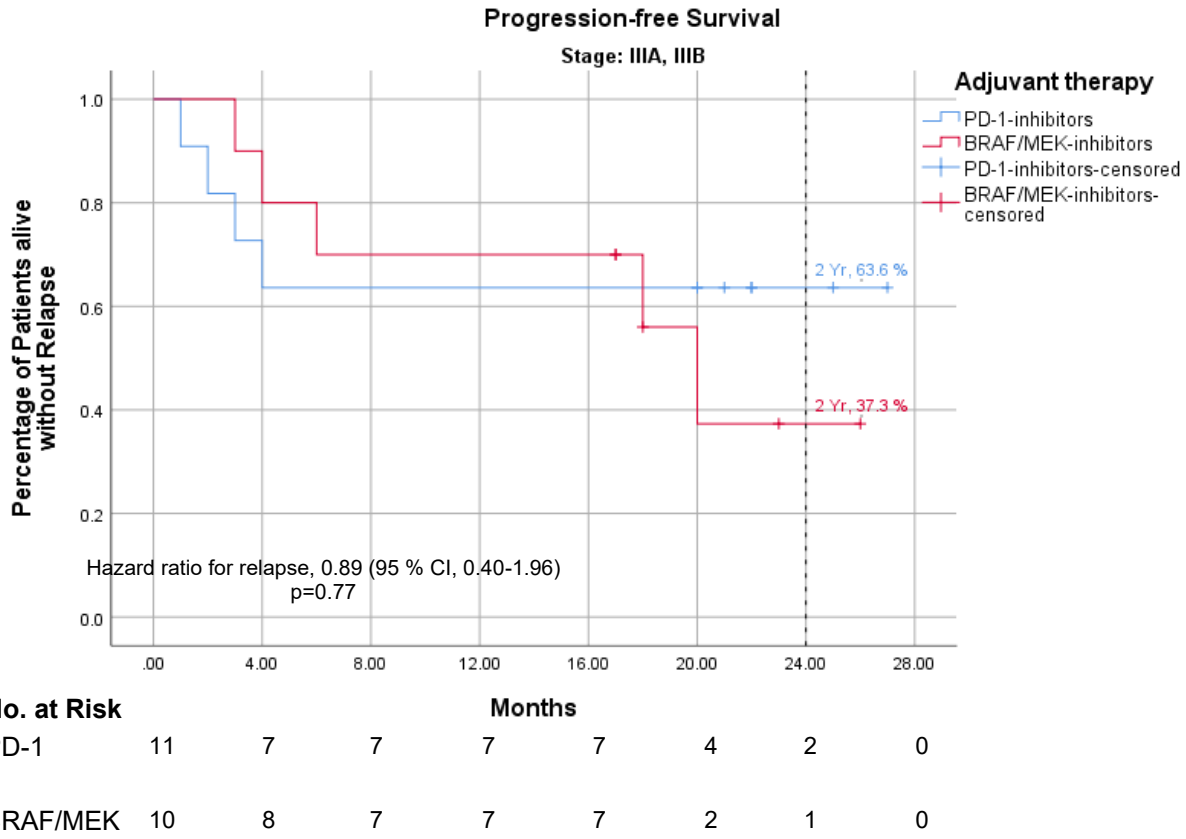
PD-1	31	22	18	18	17	15	6	1	0	0
BRAF/MEK	20	16	15	14	13	7	4	1	0	0

Figure 4: *PFS in PD-1-inhibitors vs. BRAF/MEK-inhibitors*

3.1.3 PFS according to tumor stages

Table 12: PFS in stages IIIA and IIIB

Adjuvant therapy	***No. of Patients***	***Recurrences – No. (%)***	***Progression-free Survival (95% CI)*** *Mean in months (range)*
PD-1-inhibitors	11	4 (36.4)	18 (11.2-25.1)
BRAF/MEK-inhibitors	10	5 (50)	20 (16-24)
Overall	21	9 (42.9)	18.2 (13.5-22.8)

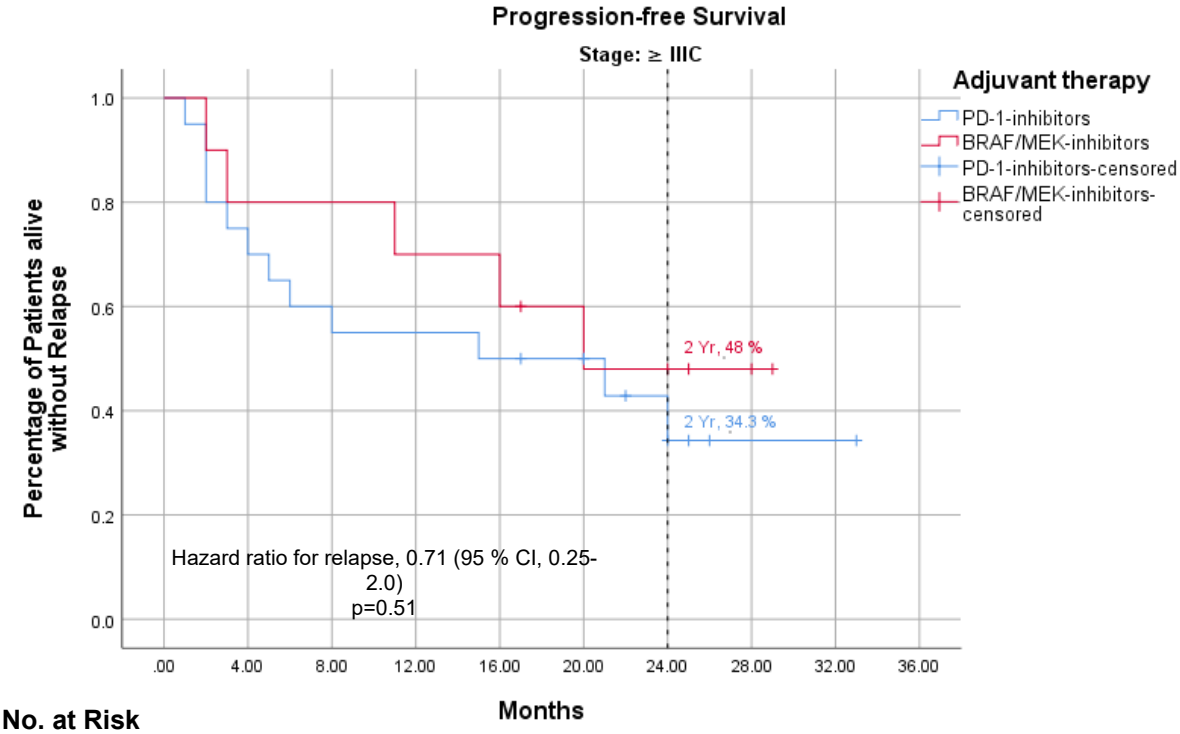


No. at Risk								
PD-1	11	7	7	7	7	4	2	0
BRAF/MEK	10	8	7	7	7	2	1	0

Figure 5: *PFS in stage IIIA and IIIB*

Table 13: PFS in stage ≥ IIIC

Adjuvant therapy	*No. of Patients*	*Recurrences – No. (%)*	*Progression-free Survival (95% CI)* *Mean in months (range)*
PD-1-inhibitors	20	12 (60)	17.3 (11.4-23.2)
BRAF/MEK-inhibitors	10	5 (50)	19.5 (13-26.1)
Overall	30	17 (56.7)	18.8 (14-23.5)



No. at Risk

PD-1	20	14	11	11	10	7	4	1	0	0
BRAF/MEK	10	8	8	7	6	4	3	1	0	0

Figure 6: *PFS in stage ≥ IIIC*

3.1.4 PFS according to lymph node intervention

Table 14: PFS in patients with SLND vs. CLND in the PD-1-antibodies treated population

Therapy	*No. of Patients*	*Recurrences – No. (%)*	*Progression-free Survival (95% CI)* *Mean in months (range)*
SLND	10	6 (60)	16.6 (7.9-25.3)
CLND	8	4 (50)	15.4 (8.8-21.9)
Overall	18	10 (55.6)	17.9 (11.4-24.3)

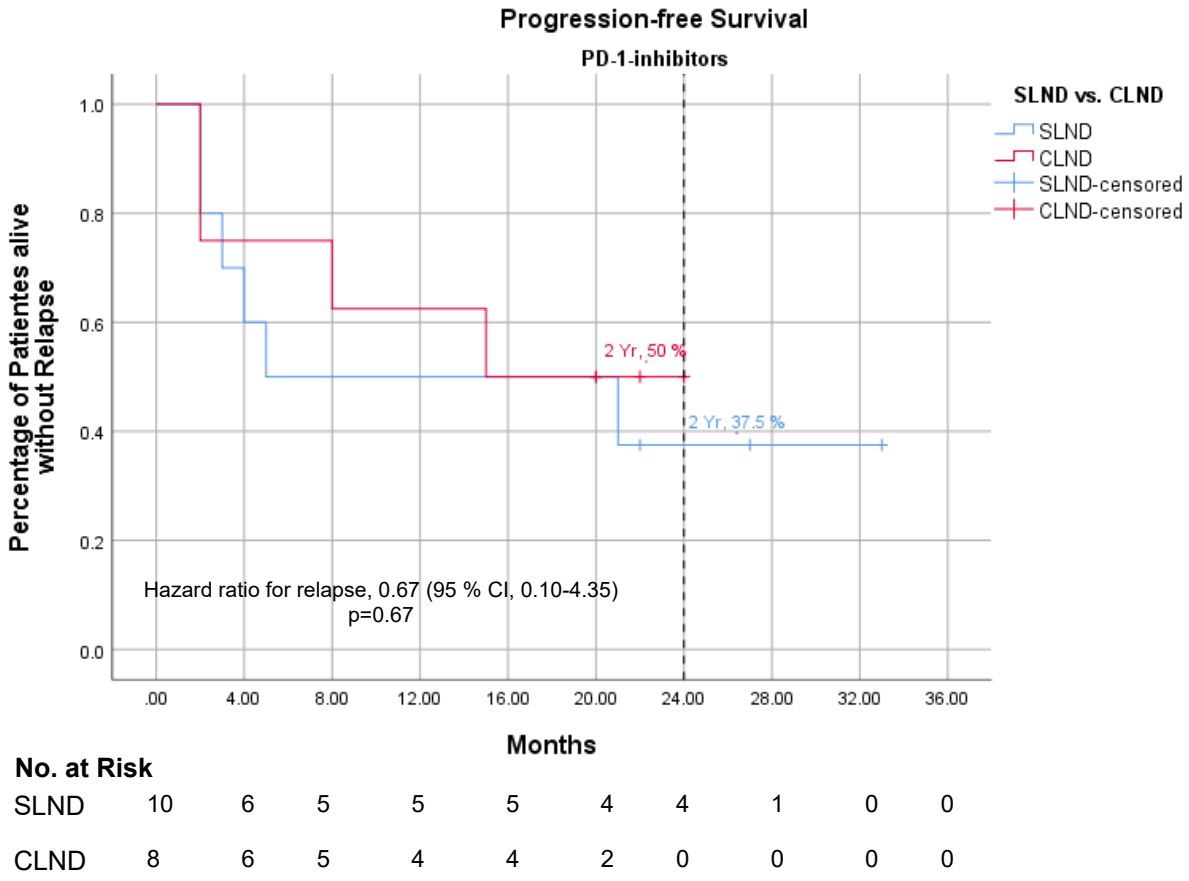


Figure 7: *PFS: SLND vs. CLND in PD-1-antibodies treated patients*

Table 15: PFS in patients with SLND vs. CLND in the BRAF/MEK-inhibitors treated population

Therapy	***No. of Patients***	***Recurrences – No. (%)***	***Progression-free Survival (95% CI)*** *Mean in months (range)*
SLND	8	5 (62.5)	12.5 (5.8-19.2)
CLND	7	4 (50)	20.6 (14.6-26.5)
Overall	15	9 (60)	16.9 (11.5-22.3)

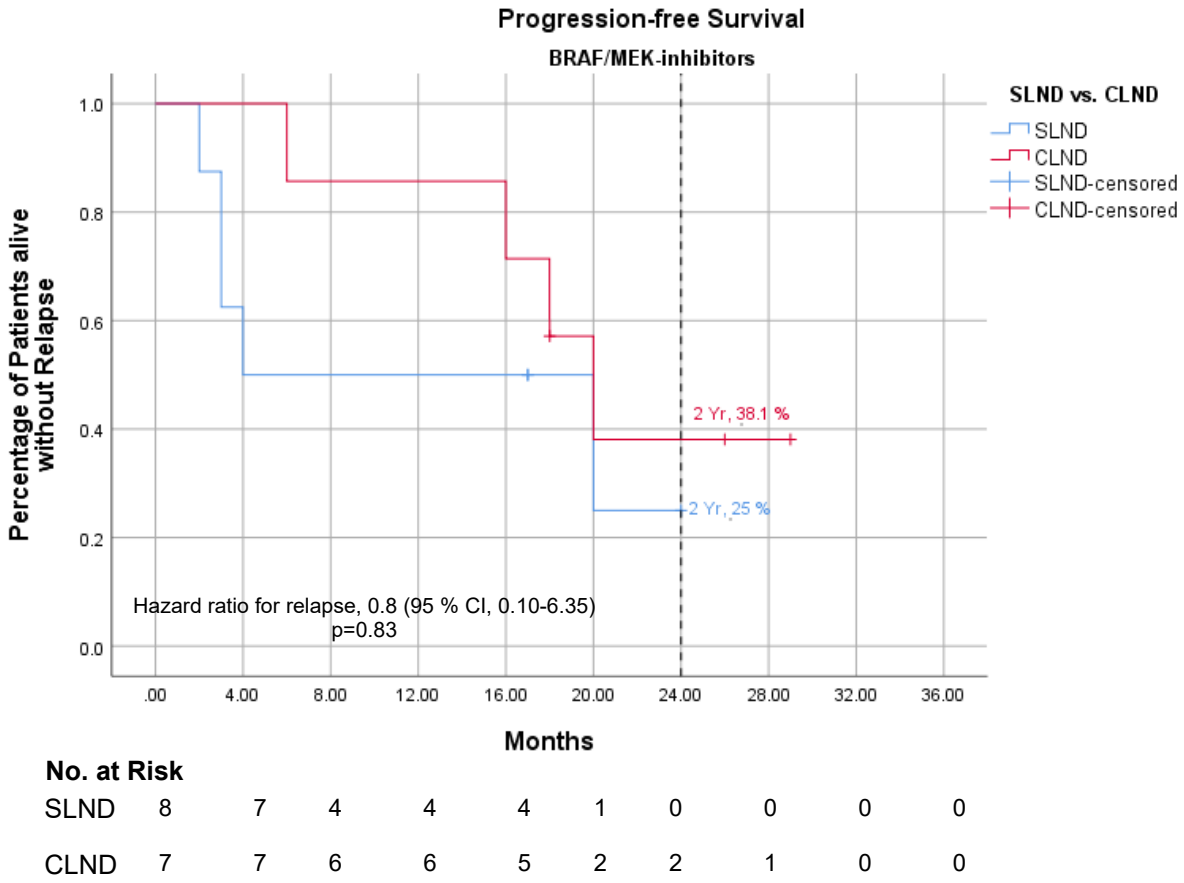


Figure 8: *PFS: SLND vs. CLND in BRAF/MEK-inhibitors treated patients*

In the PD-1 group, 50 % of those who received CLND were progression-free vs. 37.5 % in those who received SLND at two years. In the BRAF/MEK group, there are similar results with a progression-free survival rate of 38.1 % after two years in CLND patients vs. 25 % in SLND patients. However, these findings were consistent and not statistically relevant with p-values of 0.67 (0.10-4.35, 95 % CI) in the PD-1 and 0.83 (0.10-6.35, 95 % CI) in the BRAF/MEK group.

3.1.5 PFS according to immune-related adverse events

Table 16: PFS in patients with irAE

PD-1-inhibitors N=31	***No. of Patients***	***Recurrences – No. (%)***	***Progression-free Survival (95% CI)*** *Mean in months (range)*
IrAE yes	14	7 (50)	19.1 (11.6-26.6)
IrAE no	17	9 (52.9)	16.3 (10.9-21.6)
Overall	31	16 (51.6)	18.8 (13.8-23.8)

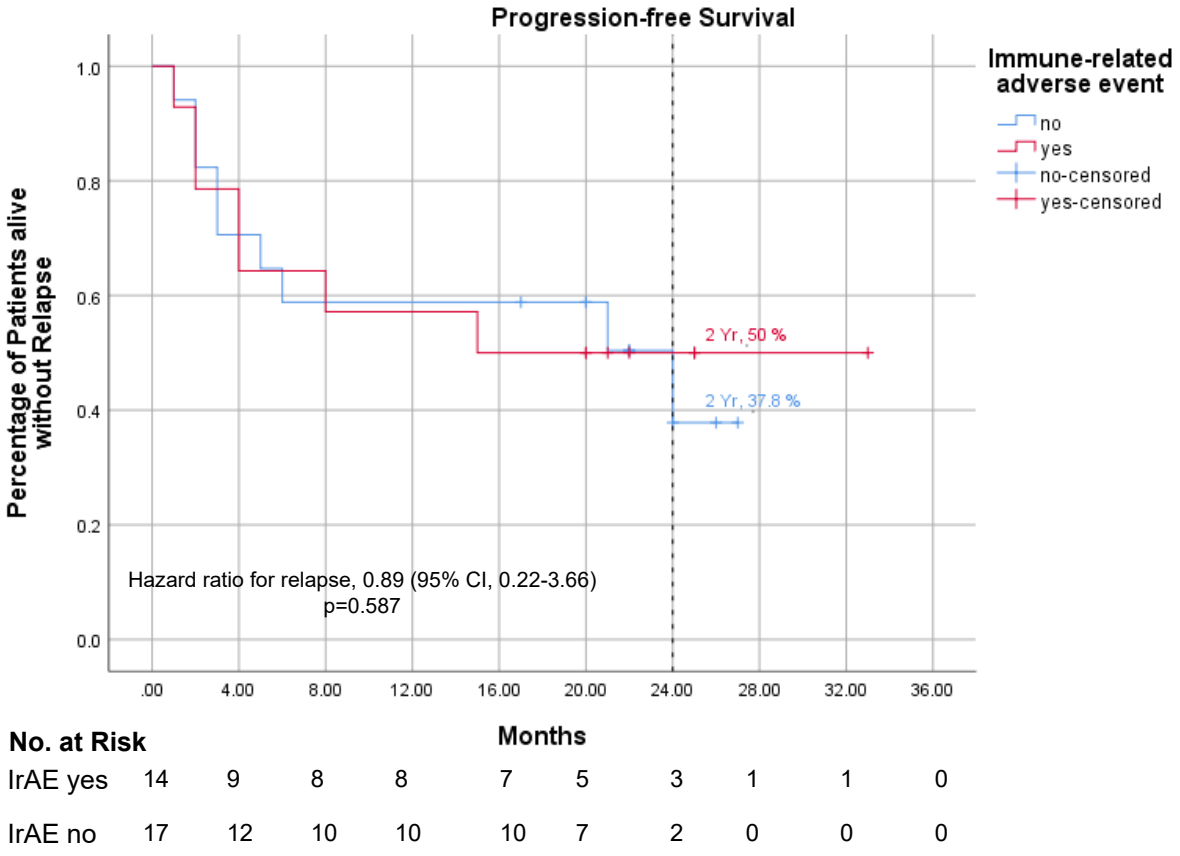


Figure 9: *PFS in PD-1-antibodies treated patients with immune-related adverse events*

Immune-mediated adverse events caused by the mechanism of action of PD-1 inhibitors showed no impact on progression-free survival in our study. 50 % of all patients suffering from immune-related adverse events experienced a two-year recurrence-free survival, while i37.8 % of patients without irAE were recurrence-free at two years. Nevertheless, statistical testing did not show a significant difference (p=0.59, 95 % CI) between both patient groups.

3.2 Overall Survival

Table 17: Overall survival

Adjuvant therapy	*No. of Patients*	*Deaths – No. (%)*	*Survival (95% CI)* *Mean in months (range)*
PD-1-inhibitors	31	4 (12.9)	40.5 (36.3-44.6)
BRAF/MEK-inhibitors	20	4 (20)	26.4 (23.1-29.6)
Overall	51	8 (15.7)	39.7 (36.3-43.1)

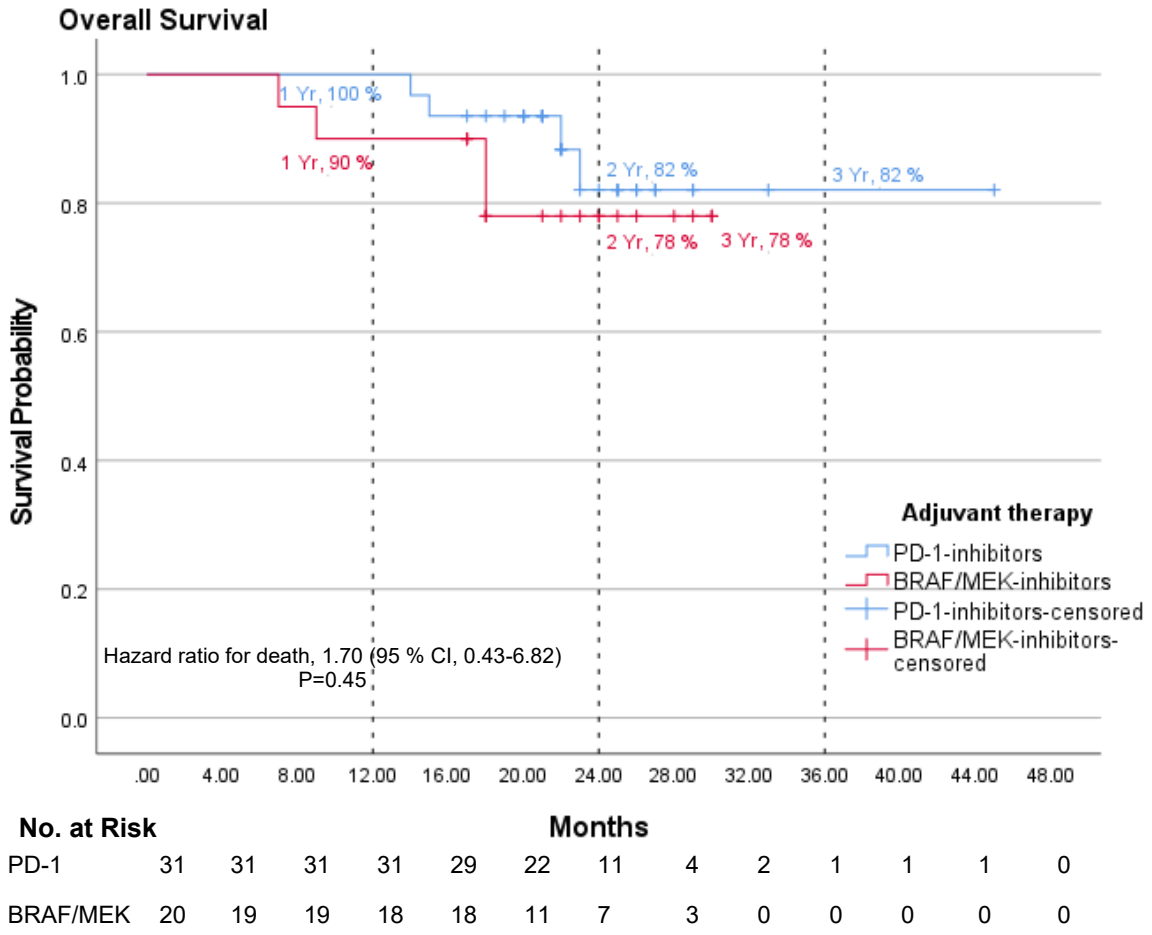


Figure 10: *Overall survival: PD-1-inhibitors vs. BRAF/MEK-inhibitors*

Overall survival functioned as secondary endpoint of this study. To obtain more meaningful results, we combined the two antibodies nivolumab and pembrolizumab in the overall survival analysis. After one year, the percentage of patients alive was 100 % in the PD-1 group and 82 % in the BRAF/MEK group. After three years of follow-up, 90 % of patients in the PD-1 group and 82 % in the BRAF/MEK group were alive.

3.3 Subgroup analysis

Using an unstratified Cox model, we calculated hazard ratios for relapse and examined the influence of a wide range of factors such as age, sex, BMI, $BRAF^{V600E}$ mutation, ulceration and penetration depth of tumor, number and type of positive lymph nodes, as well as previous therapies, lab results and adverse events on recurrence-free survival.

Statistically significant factors in the PD-1 group were patient age, number of positive lymph nodes, and time to start of adjuvant therapy. It has shown that patients younger than 65 years had a significantly lower risk for relapse than patients older than 64 years (HR for < 65 years 0.43 (0.17-1.07) vs. for ≥ 65 1.96 (0.97-3.95) p=0.045, 95 % CI). In the group that received the first therapy less than three months after R0-resection, the risk was significantly lower than in patients that received it later (HR < 3 months 0.4 (0.16-1) vs. ≥ 3 months 2.2 (1.01-4.8) p=0.028, 95 % CI). The number of lymph positive lymph nodes had an impact of the recurrence-free survival in the PD-1 subgroup. Patients with only one positive lymph node had a significant lower risk for recurrence than patients with more than one (HR for only one positive lymph node = 0.5 (0.03-0.82) vs. 1.5 (0.9-0.78) p=0.02, 95 % CI). In addition, we examined the side effects in the PD-1 group with special attention to immune-related adverse events, but no statistically relevant result could be reported regarding irAE. Only in the case of immune-related adverse events ≥ grade III we could see a tendency that people with high grade irAE are on lower risk for relapse than people without irAE ≥ grade III (HR 0.43 in patients with irAE ≥ grade III vs. 1.26 in patients with irAE < grade 3, p=0.394, 95 % CI).

No factor with a significant calculated p-value could be identified in the BRAF/MEK group. In all patients treated with BRAF/MEK inhibitors we could not find any correlation between patient age, number of positive lymph nodes and time of initiation of therapy. Looking at the immune-related adverse events, HR was almost the same in patients with and without the presence of irAE.

Tumor stage had no significant impact on the outcome in either group, while in the PD-1 group we found a trend towards a lower risk of recurrence in patients below stage IIIC compared to patients with tumor stage IIIC or above (HR 0.71 < IIIC vs. 1.87 ≥ IIIC, p=0.273, 95 % CI). Patients with $BRAF^{V600E}$ mutation in the PD-1 group had no RFS disadvantage compared to patients without the mutation.

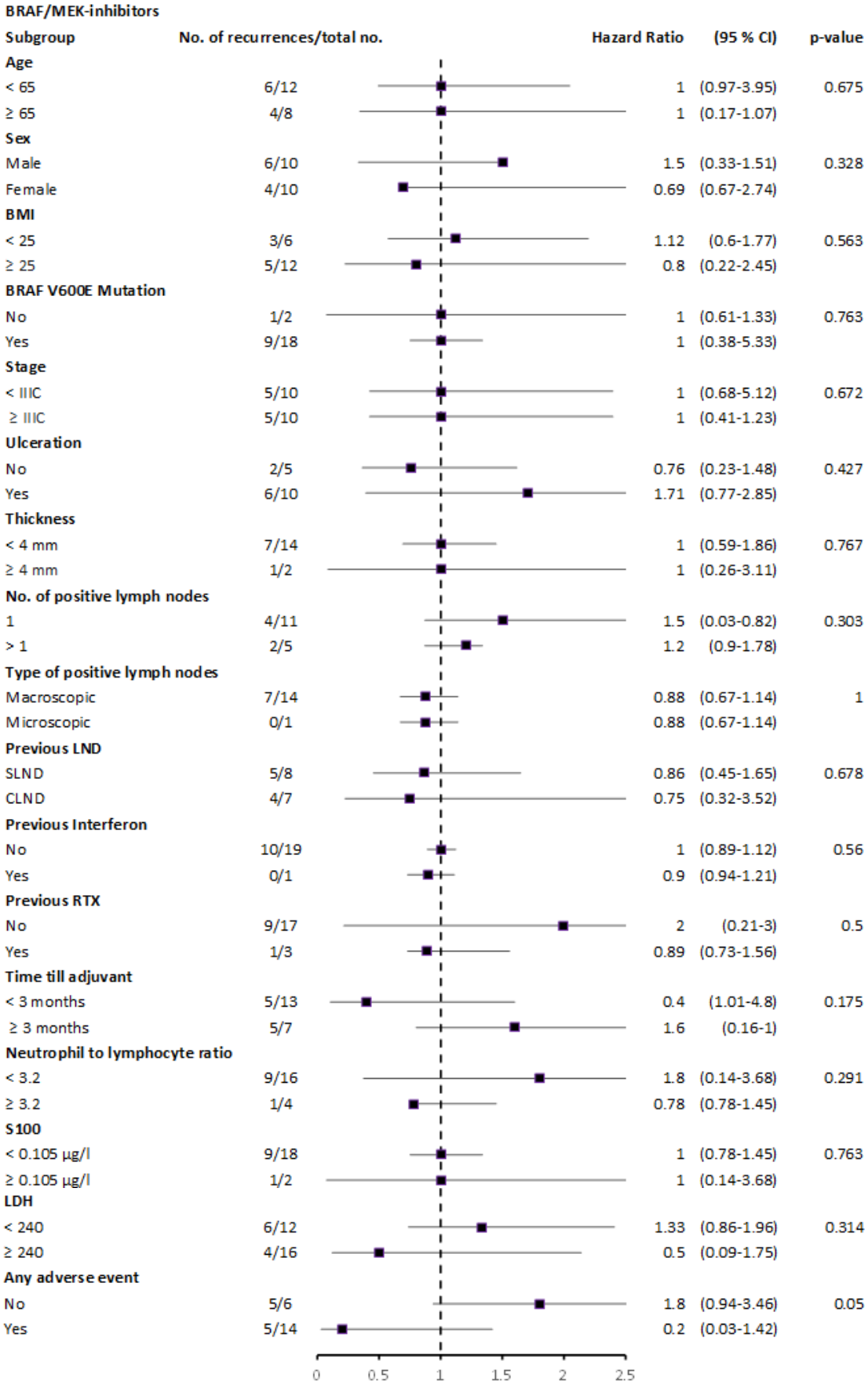


***Figure 11**: Factors affecting outcome in the BRAF/MEK-inhibitors treated group*

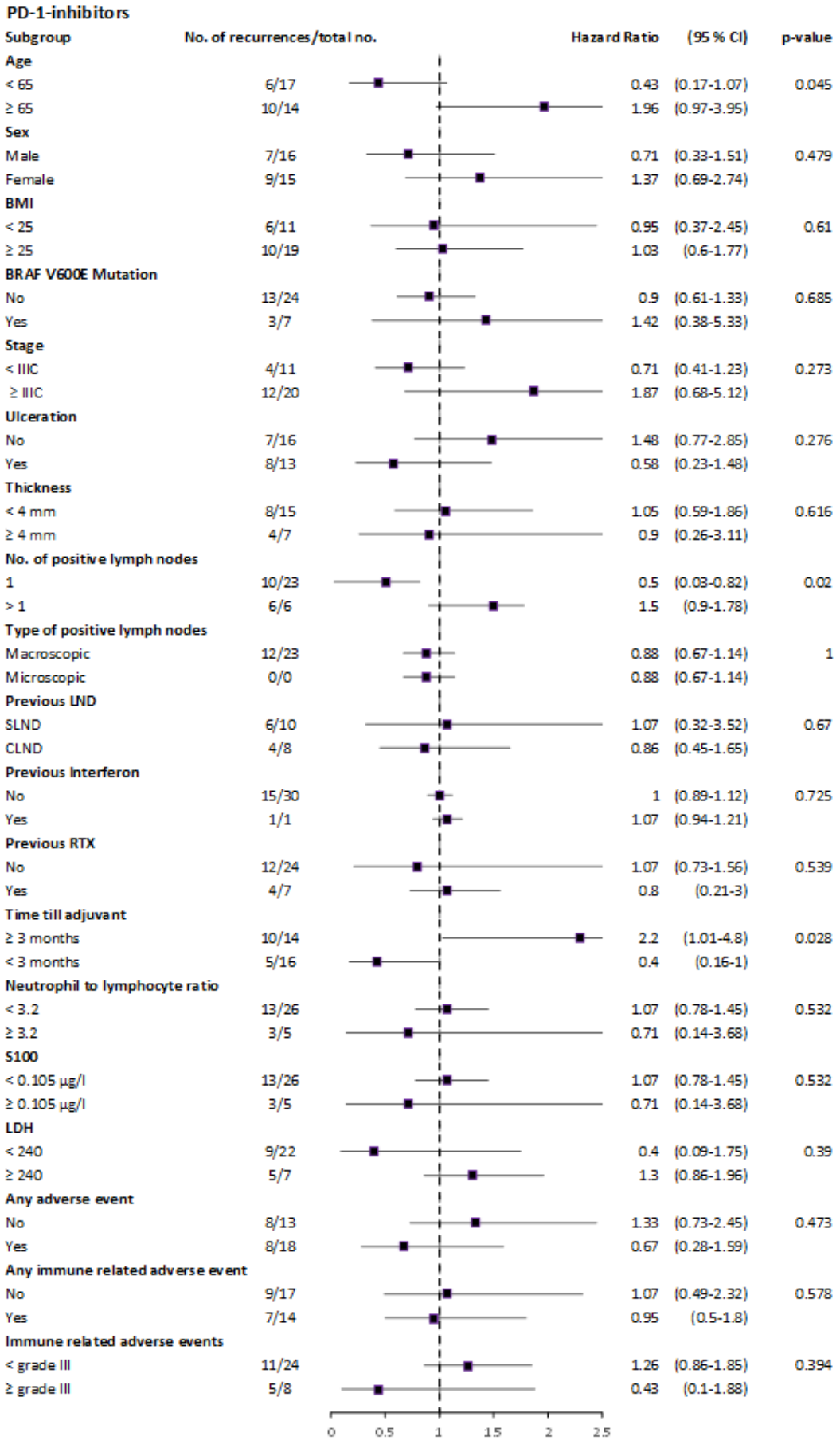


Figure 12: *Factors affecting outcome in the PD-1-antibodies treated group*

Since almost all patients in the BRAF/MEK group had a $BRAF^{V600E}$ mutation, the RFS of patients without the tumor mutation cannot be compared accurately. In both groups, prior therapy, and laboratory results, which include lymph node interventions such as SLND and CLND and prior interferon and radiotherapy, NLR and the value of S100B and LDH, had no significant impact on RFS. Characteristics such as gender and BMI of the patients or penetration depth and ulceration status of the tumor also showed no significant influence on RFS in either group. All results of the subgroup analysis are graphically illustrated in forest plots.

3.4 Adverse events

This chapter aims to provide an overview of adverse events, which occurred during the treatment and the follow-up period with the immunotherapy agents and targeted molecules. Close monitoring of side effects is required to establish a safety profile of the adjuvant medication. We examined which adverse events occurred the most often, which were the most severe and why patients discontinued or ended therapy. In addition, we concentrated on immune-related adverse events, which typically occur in the treatment with immunotherapy drugs. Immune-related adverse events were divided into grade < III and grade ≥ III to achieve a precise analysis of severe immunotherapeutic reactions.

3.4.1 Overview

Adverse events occurred in 58.1 % of patients with PD-1 based treatment and 70 % in the BRAF/MEK-inhibitor treated patients. The most common side effect in the PD-1 group was colitis at 12.9 %. Pneumonitis, hypophysitis, and an increase in CK and lipase levels ranked second at 6.4 %. All adverse events in patients receiving PD-1-inhibitor therapy were assumed to be immune-related. Typical adverse events in the BRAF/MEK group were an increase in CK-levels (25 %), pyrexia (20 %), and neutropenia (15 %). Less commonly observed side effects, affecting only a very small number of patients were vitiligo, oral mucositis, eczema, thyreoiditis, sarcoidosis, myalgia, autoimmune myocarditis, sicca syndrome, papillary edema, rhabdomyolysis, renal colic, polyradiculitis, and an increase in pancreatic and liver enzymes. Median time to onset of side effects was two months in the PD-1 group and one month in the BRAF/MEK group.

Table 18: Adverse events

	PD-1-inhibitors N=31 **No. (%)**	***BRAF/MEK-inhibitors N=20*** **No. (%)**
Any adverse event	18 (58.1)	14 (70)
Mucositis	1 (3.2)	-
Vitiligo	1 (3.2)	-
Eczema	1 (3.2)	-
Colitis	4 (12.9)	1 (5)
Hypophysitis	2 (6.4)	-
Thyreoiditis	1 (3.2)	-
Pneumonitis	2 (6.4)	1 (5)
Sarcoidosis	1 (3.2)	-
Myalgia	1 (3.2)	-
Rhabdomyolysis	-	1 (5)
CK ↑	2 (6.4)	5 (25)
Pyrexia	-	4 (20)
Renal colic	1 (3.2)	-
Toxicity (GGT↑)	-	1 (5)
Polyradiculitis	1 (3.2)	-
Myocarditis	1 (3.2)	-
Sicca syndrome	1 (3.2)	-
Papillary edema	-	1 (5)
Lipase ↑	2 (6.4)	2 (10)
Amylase ↑	1 (3.2)	-
Neutropenia	-	3 (15)

3.4.2 End or break of therapy because of adverse events

Table 19: End or break of therapy because of adverse events

	PD-1-inhibitors N=31 **No. (%)**	***BRAF/MEK-inhibitors N=20*** **No. (%)**
End of therapy because of adverse events	4 (12.9)	3 (15)
Break of therapy because of adverse events	3 (9.7)	6 (30)
Mucositis	1 (3.2)	-
Hypophysitis	2 (6.5)	-
CK ↑	2 (6.5)	3 (15)
Colitis	4 (12.9)	-
Myalgia	1 (3.2)	-
Pneumonitis	1 (3.2)	1 (5)
Myocarditis	1 (3.2)	-
Sarcoidosis	1 (3.2)	-
Lipase ↑	-	1 (5)
Rhabdomyolysis	-	1 (5)
Pyrexia	-	1 (5)
Neutropenia	-	2 (10)

9.7 % in the PD-1-group and 30 % in the BRAF/MEK group discontinued treatment due to adverse events and 12.9 % of the PD-1 and 15 % in the BRAF/MEK group discontinued it permanently. Most common critical events leading to end of therapy or discontinuation were colitis, an increase in CK levels, hypophysitis, myalgia, pneumonitis, sarcoidosis, and myocarditis in PD-1-inhibitor treated patients and an increase in CK and lipase levels, neutropenia, pyrexia, pneumonitis, and rhabdomyolysis in patients treated with BRAF/MEK-inhibitors.

3.4.3 Immune-related adverse events

Immune-related adverse events occurred in 58.1 % of patients in the PD-1 group, 25.8 % experienced grade III or IV adverse events. These severe immune-related adverse events were hypophysitis, colitis, myalgia, an increase in CK and GGT, autoimmune myocarditis, and neutropenia.

Table 20: Immune-related adverse events

	PD-1-inhibitors N=31 **No. (%)**
Immune-related adverse events	18 (58.1)
Immune-related adverse events ≥ grade III	8 (25.8)

3.5 Relapses and subsequent therapy

Overall recurrence rate in both groups was very similar. 51.6 % of patients in the PD-1 group experienced a relapse compared to 50 % in the BRAF/MEK-group. Median time to recurrence was four months in the PD-1 group and 8.5 months in BRAF/MEK-inhibitor treated patients. The median number of detected metastases was very similar at two in the PD-1 vs. 2.5 in the BRAF/MEK group. There are differences in the developed metastases. 25.8 % of the PD-1 based treated patients had local recurrence compared to only 10 % in the BRAF/MEK group. Local recurrence was at 25.8 % the predominant relapse type in the PD-1 treated patients, about 75 % of them having additional lymph node metastases. The predominant type of recurrence in the BRAF/MEK cohort was a combination of distant and lymph node metastases. Distant metastases in general occurred in 22.6 % of PD-1 treated and 30 % of targeted therapy patients. Looking at the distant metastases in detail, the lung (22.6 % PD-1 vs. 20 % BRAF/MEK), skin and soft tissue (12.9 % PD-1 vs. 15 % BRAF/MEK) were most frequently affected. Furthermore, CNS metastases accounted for one third of distant metastases in the BRAF/MEK cohort, compared to only 6.5 % in the PD-1 group. The number of visceral metastases was similar at 9.7 % in the PD-1 and 5 % in the BRAF/MEK cohort.

Four patients of each of both groups died during follow-up time. In the PD-1 group, four of 16 patients could be brought back into complete remission after recurrence. This was achieved with surgery in three of the cases and through combination of surgery and RTX in one case. All these cases were affected by local recurrence in combination with lymph node metastases, one patient experienced local recurrence only. In the BRAF/MEK treated group, one of the 10 recurrences could be brough back into complete remission. This patient had one lymph node metastasis and surgery was performed to achieve remission. In our study, adjuvant therapy switch did not bring an advantage in recurrence cases. Table 21 gives an overview of melanoma recurrence and number of metastases in both groups and types of relapses are presented in detail.

Table 21: Type and number of recurrences plus subsequent therapy

	PD-1-inhibitors (N=31)	*BRAF/MEK-inhibitors (N=20)*
Melanoma recurrence – No. (%)	16 (51.6)	10 (50)
Time to recurrence (months) – Median (range)	4 (1-24)	8.5 (2-20)
No. of metastases – Median (range)	2 (1-7)	2.5 (1-6)
Type of metastases – No. (%)		
Local recurrence	8 (25.8)	2 (10)
Lymph node metastasis only	2 (6.5)	2 (10)
Distant metastasis only	4 (12.9)	3 (15)
Lymph node + distant	3 (9.7)	3 (15)
Lymph node + local	6 (19.4)	1 (5)
Distant metastases		
Skin, soft tissue	4 (12.9)	3 (15)
Lung	7 (22.6)	4 (20)
Visceral	3 (9.7)	1 (5)
CNS	2 (6.5)	4 (20)
Therapy of recurrence – No. (%) of recurrences		
Surgery	6 (19.4)	7 (35)
RTX	5 (16.1)	3 (15)
Systemic therapy	7 (22.6)	6 (30)
- Switch of PD-1 to BRAF/MEK	3 (9.7)	-
- Switch of BRAF/MEK to PD-1	-	5 (25)
- CTLA4 + PD-1	3 (9.7)	2 (10)

4 Discussion

4.1 Background

Immunotherapy and targeted therapy have revolutionized the treatment of advanced melanoma in recent years. With nivolumab, pembrolizumab, dabrafenib and trametinib, game changing adjuvants are available and showed an incredible survival elongation in phase III clinical trials. Before 2011, prognosis of advanced melanoma was poor. Now, a new era of melanoma standard therapy has begun (85,86). Not only do these drugs deliver remarkable results in resected stage III or IV melanoma, even in metastatic melanoma they have proven their effectiveness.

As a PD-1-antibody, nivolumab has shown major advantages over the previous immunotherapy drug ipilimumab, which is a CTLA4-inhibitor. The antibody was approved in august 2018 due to superior results regarding RFS and toxicity over ipilimumab (87). Nivolumab is currently being tested in many different settings and combinations, and it is also approved for the treatment of unresectable or metastatic melanoma. Even in BRAF-mutated melanoma it shows significant survival advantages (88).

The other PD-1-antibody that has shown efficacy in pivotal studies is pembrolizumab. In the KEYNOTE-006 trial, a five-year analysis showed significant superiority in RFS, OS, and toxicity over ipilimumab in patients with advanced melanoma. It shows results similar to studies with nivolumab. Based on this promising data, pembrolizumab was approved in February 2019 for the adjuvant therapy in resected stage III and IV melanoma. For the therapy of unresectable, metastatic melanoma it has been used since 2015. Since 2021, it is also approved in the adjuvant setting of resected stage IIB and IIC melanoma (89). The two antibodies have now established themselves as first-line standard therapy for the treatment of advanced melanoma (90).

Targeted therapy with the BRAF-inhibitor dabrafenib and the MEK-inhibitor trametinib was approved in April 2018 based on the COMBI-AD study. It is a randomized, double-blind trial that involved 870 patients in a resected stage III melanoma with BRAF V600E or V600K mutation. In a five-year analysis, 52 % of patients treated with D+T for one year vs. 36 % in the placebo group were alive without a relapse and no long-term toxicity did occur (91).

All these results are based on pivotal trials and real-life observations lack almost entirely, so now is the time to compare and draw conclusions to see if the treatments can meet expectations in real-world conditions.

4.2 PFS and OS

4.2.1 Nivolumab

First, we discuss the survival analysis of patients treated with nivolumab. Our study showed a one-year recurrence-free survival rate of 59 % and a two-year recurrence-free survival rate of 43 %. The mean RFS in months was 18.9 (13.5-24.2). This does not completely correspond to the results of the largest approval study, CheckMate 238. In this trial, ipilimumab, the standard of care therapy at that time, was compared to nivolumab, which showed a major superiority over ipilimumab. 453 patients with resected stage III or IV melanoma treated with NIV had a four-year RFS of 51.7 % (92). A collaborating preceding study detected a one-year RFS of 70.5 %, so there is a main difference between these figures and our evidence (87).

Overall survival rates were the same in our study compared to the CheckMate 238 nivolumab group. In both, 82 % of patients were alive after three years. Although recurrence-free survival rates do not exactly match treatment expectations, the overall survival outcome remains the same, which is an important finding of this retrospective observation. Finally, the latest follow-up investigation after four years in the landmark study showed a survival of 77.9 % of patients in the NIV group.

Nivolumab not only shows excellent results in a resected stage III or IV melanoma, but it is also effective in an inoperable, previously untreated advanced stage melanoma, as confirmed in the CheckMate-067 study (93).

The dissimilarities in RFS between this study and the landmark study can be explained by the fact that phase III clinical trials collect their patients in a controlled environment, while our data was collected in real-life practice conditions, which means that there are fewer inclusion and exclusion criteria. In addition, a phase III trial is interventional, allowing the setting to be modified, while in a real-world setting, data is collected observational. In contrast to our study, exclusion criteria in the approval study and its collaborators were strict: patients with a higher ECOG level than 1, patients with ocular melanoma or with preexisting conditions such as

autoimmune diseases or non-melanoma cancer without complete remission for more than three years and patients with previous systemic melanoma therapy or the systemic use of glucocorticoids were excluded from the study. All these criteria were not required in our study, what may lead to the different results of RFS.

4.2.2 Pembrolizumab

Patients treated with pembrolizumab showed a 1-year RFS of 50 % and a two-year RFS of 50 %. The mean RFS in months was 13.5. Overall, this is slightly worse than observed in the NIV group. It is also not meeting the expectations of the results of the approval study EORTC (European Organization for Research and Treatment of Cancer) 1325-MG, respectively KEYNOTE-054 with 505 participants who received PEM. In this trial, PEM showed significant results with a 3.5-year RFS of 59.8 % in the intention-to-treat population. As in the previous comparison, the KEYNOTE-054 had several inclusion and exclusion criteria that could affect these results. A main criterion was a cutaneous melanoma with additional lymph node metastases plus the performance of a complete lymph node dissection in the 13 weeks prior to administration of the adjuvant. Additionally, patients with previous autoimmune diseases, infections, corticosteroid therapy, a higher ECOG status than 1, or a previous systemic melanoma therapy were again excluded (94). Nevertheless, the patient population in our study treated with PEM was small, making comparisons difficult. In further analyses, we combined the results of the PEM and NIV group to achieve a higher quality basis for discussion.

A phase III collaborative study for the KEYNOTE-006 investigators, analyzing efficacy of pembrolizumab versus ipilimumab in patients with unresectable stage III or IV melanoma demonstrated a survival benefit in patients treated with different doses of pembrolizumab (n=556) over the ipilimumab treated group (n=278). In this study, in particular the influence of PEM on the OS was remarkable. After five years, patients treated with PEM showed a median OS of 32.7 months vs. only 15.9 months in the IPI group. Conclusively, PEM has also proven to be effective in a palliative setting for life prolongation (95). Compared to our study, all patients of the PEM group stayed alive during a three-year follow-up. However, for better comparison we combined the two PD-1-antibodies and detected a three-year OS of 82 % and a median OS of 40.5 (36.3-44.6) months.

4.2.3 Dabrafenib plus Trametinib

With the combination of targeted therapy substances dabrafenib and trametinib, a median recurrence-free survival interval of 19.1 (14.4-23.7) months was achieved. 70 % of patients stayed relapse-free in the first year and 43 % after a two-year follow-up. Looking at the COMBI-AD trial and its collaborating studies, which are placebo-controlled, double-blind, randomized pivotal trials that tested this kind of therapy for resected melanoma in stage III, discrepancies, particularly regarding the RFS, can again be objectivated. In the COMBI-AD trial, the combination therapy showed significant superiority over placebo. Of the 438 patients treated with the combination therapy in phase III trial, 98 % stayed relapse-free in the first year and 58 % of patients after three years (96). The following five-year analysis shows a similar result, with 52 % of patients staying recurrence-free (91). It is interesting that the relapse curve appears to flatten out as the investigation progresses. Again, these results could be explained by the strict limitations for inclusion and exclusion as in the other landmark studies. Only patients with ECOG score of 0 or 1 and with a fully resected stage III melanoma were included. There must be no radiological or clinical evidence of tumor in the last 12 weeks. Since stage III melanoma involves at least one lymph node, each patient had to undergo a complete lymph node dissection to be included in the study (97).

In our study, overall survival of patients was 78 % after three years. Compared to the COMBI-AD collaborators, in which 86 % of patients stayed alive after three years, the results do not differ that much (97). In contrast to therapy with PD-1 inhibitors, in metastatic, unresectable melanoma it is unclear whether targeted therapy is effective. In a study of 563 randomly assigned patients with advanced, unresectable melanoma, who received the targeted agents, only in one third a long-term survival benefit was achieved with the treatment (98).

In summary, adjuvant therapy with BRAF inhibitors plus MEK inhibitors appears to be superior to PD-1-antibody treatment regarding RFS at first glance, since patients showed a higher recurrence-free survival in the first year with 70 % for BRAF/MEK-inhibitor treatment versus 58 % for PD-1 inhibitors. After a two-year follow-up, the results converge and align with 44 % in the BRAF/MEK and 43 % in the PD-1 group. Then again, PD-1-antibody treatment appears to be superior to BRAF/MEK regarding the OS, as 100 % of PD-1 treated patients were alive after one year and

82 % after three years. In the BRAF/MEK-group, 90 % of patients were alive without relapse after one year and 78 % after a three-year follow-up. To explain the differences of RFS between pivotal studies and our study, it can be stated that excessive inclusion and exclusion criteria in approval studies can result in assigning patients who have a lower risk of disease recurrences. Overall survival is very similar in general, not only if we compare the different adjuvant agents, but also when comparing raw data and data available from phase III studies.

In summary, main differences can be seen between real-life data and phase III studies, especially with regard to progression-free survival. The long-term benefits are very similar in both settings, but real-world data show that adjuvant treatment with PD-1-inhibitors comes closer to investigations of the OS and PFS under ideal conditions, as provided in the existing clinical approval studies.

4.3 Subgroup analysis

It is of particular interest to identify factors that may affect prognosis. One aim of this study was to identify factors that can influence the outcome of disease and thereby predict the response to therapy. In existing studies, several factors such as stage, lymph node interventions, PD-L1 expression and the occurrence of adverse events are tested for their impact to influence the outcome positively or negatively. In our multivariate model, no significant effects of stage of disease, prior therapy and laboratory results were observed in any patient. This includes lymph node intervention such as SLND and CLND and previous interferon and radiation therapy, as well as the neutrophile to lymphocyte ratio and the value of S100B and LDH. Patient characteristics such as gender and BMI and tumor characteristics such as penetration depth and ulceration status also showed no significant influence on the RFS in either group.

An important finding is that in both cohorts we did not detect a survival benefit in patients receiving complete lymph node dissection compared to patients receiving a sentinel lymph node biopsy only. We must not forget that every patient included in this study had at least one lymph node metastasis. Although there is a tendency towards increased RFS in patients with CLND, particularly in BRAF/MEK treated patients, we did not find a statistically significant value. This result is consistent with the results of the DeCOG-SLT trial, a multicenter, randomized, controlled study

examining whether patients with clinically occult sentinel lymph node metastases benefit more from CLND than from observation only (99). No benefit of CLND was found in this study either. As shown in our subgroup analysis, laboratory parameters such as LDH and the calcium binding protein S100B do not appear to have a prognostic influence on outcome, but there are studies on this topic in which S100B is still considered a progression parameter, since higher serum levels of the melanoma specific tumor marker indicate an association with the metastatic burden (100).

4.3.1 PD-1-inhibitors

Factors that showed a significant impact on the RFS or OS were age, number of lymph nodes being positive, and time to start of adjuvant therapy. Patients younger than 65 years had a significantly lower risk of relapse than patients ≥ 65 years (HR for < 65 years 0.43 (0.17-1.07) vs. for ≥ 65 1.96 (0.97-3.95), p=0.045, 95 % CI). For the group which received therapy in less than three months the risk was lower than for patients who received it later (HR < 3 months 0.4 (0.16-1) vs. ≥ 3 months 2.2 (1.01-4.8), p=0.028, 95 % CI). In the BRAF/MEK group we could not see this result related to the time to adjuvant therapy. The number of lymph nodes being positive had an impact of the recurrence-free survival. Patients with only one positive lymph node had a significant lower risk for recurrence than patients with more than one (HR for 1 pos. lymph node = 0.5 (0.03-0.82) vs. 1.5 (0.9-0.78), p=0.02, 95 % CI). This is also consistent with the results of the melanoma-specific survival analysis from the AJCC cancer staging manual (101). As described above, we did not find any significant differences in RFS and OS related to melanoma stages, but hazard ratio for the risk of recurrence in patients with stage III A and B was slightly lower at 0.71 than in patients ≥ stage IIIC at 1.87 (p= 0.273, 95 % CI). Another highly discussed topic is the possibility to predict the response to immunotherapy based on the presence of immune related adverse events, and if these predictions can positively affect the outcome (102). In our study, we could not find results indicating that patients who experience immune-related adverse events show improved survival rates.Furthermore, BRAF mutation status did not affect RFS. Hence, the treatment with nivolumab and pembrolizumab can be assumed as equally effective for tumors with BRAF-mutations and BRAF wild-type tumors. This has also been observed in other studies and meta-analyses (103).

4.3.2 BRAF/MEK-inhibitors

We did not observe a significant influence of any factor in BRAF/MEK-inhibitor receiving patients. There was a tendency, that the presence of adverse events may lead to improved RFS with a hazard ratio for relapse of 0.2 (0.03-1.42), while in patients without any adverse event there was a hazard ratio for relapse of 1.8 (0.94-3.46) ($p = 0.05$, 95 % CI). However, this was not significant. Maybe the effect can be relevant in a greater population size. Unlike in the group receiving the PD-1 based therapy, we could not find any interaction between the age of patients, number of tumor-affected lymph nodes, or time until first adjuvant therapy application. The efficacy for melanoma with NRAS mutations or wild-type melanoma could not be evaluated, since almost all our patients did show a BRAF V600 E, D or K mutation. With the data that we have, targeted BRAF/MEK therapy can be assumed to be equally effective under the different types of BRAF-mutations.

4.4 Safety

Overall, it is known that immunotherapy and targeted agents can trigger severe adverse reactions. In general, they are more tolerable and show less toxicity than chemotherapeutics. Nevertheless, especially immunotherapy drugs can lead to serious side effects since they interfere with the immune system. This includes gastrointestinal, dermatological, rheumatological, cardiovascular, renal, hematological, endocrinological, pulmonological and neurological toxicities.
It is well known that small molecules in targeted therapies also have their specific side-effect profile, as they commonly affect the skin, the musculoskeletal system or lead to pyrexia (104). All these difficulties often require special treatment and are not always easy to manage.

4.4.1 PD-1-inhibitors

In our study, 58.1 % of patients treated with PD-1-antibodies experienced some form of adverse event, 45.2 % of these events were considered immune-related. 25.8 % showed severe immune-related adverse reactions ≥ grade 3. 12.9 % of all patients

had to stop the therapy completely because of uncontrollable reactions, and in 29 % of cases, the treatment had to be temporary discontinued due to adverse events. In the Checkmate 238 trial, almost all patients experienced side effects (96.9 %), but only 14.4 % were considered severe ≥ grade 3. Of the 452 patients treated with nivolumab, 9.7 % had to end or temporary interrupt the treatment due to side effects. The most common adverse event in the Checkmate trial was fatigue (34.5 %). As we did not consider fatigue to be treatment associated, the total number of the presence of any adverse event is not comparable to the number in our study. High grade immune-related adverse events presented in pivotal trial were colitis, rash, and liver toxicity signs with increases in AST and ALT levels (92). A 6.5-year outcome analysis of the Checkmate 067 trial showed 24 % grade 3 or 4 immune-related adverse events in all patients (105), which is close to our results.

In our study, severe immune-related adverse events ≥ grade III, resulting in treatment ending or temporary discontinuation, included colitis and signs of liver toxicity as seen in the landmark trial. In addition, an increase in CK and neutropenia were also accountable reasons in our study. Severe autoimmune myocarditis was acquired in one case of the PEM group. A patient with a history of ulcerative colitis had been in complete remission for the last ten years until the adjuvant therapy with nivolumab, which probably triggered a renewed flare-up and led to the end of therapy. It is striking that all patients with ulcerative colitis in the study showed a flare-up of their underlying disease. A patient with type 1 diabetes developed hypophysitis and thyreoiditis as further endocrinological disorders as a result of therapy with immune checkpoint inhibitors. Patients with the third autoimmune disease in this study, Hashimoto’s thyreoiditis, did not experience any adverse events related to their disease. The risk of autoimmune disease flare-ups has been described before and should be considered when starting a treatment with immune checkpoint inhibitors (106). Overall, the safety results, when compared to the safety analyses of phase III trials, point out many challenges to face in the treatment with immune checkpoint inhibitors. Nevertheless, no new toxic effects or previously unknown adverse events in the treatment with immune checkpoint inhibitors could be identified in our study.

4.4.2 BRAF/MEK-inhibitors

In the side effect analysis of the targeted agents used in our study, 70 % of all patients experienced any form of adverse event, 30 % had to interrupt treatment, and in 15 % of cases adverse events were severe enough to lead to permanent discontinuation. Overall, 25 % of patients experienced serious adverse events including pneumonitis, pyrexia, rhabdomyolysis, hepatotoxicity, and neutropenia. In general, the most common problems reported were pyrexia, neutropenia, and an increase in CK-levels. In COMBI-AD investigator studies, 36 % of the 438 patients experienced severe adverse events, 26 % had to end the treatment due to adverse events, and 66 % resulted in temporary discontinuation. Among the serious events, fever, fatigue, hepatotoxicity, and hypertension occurred. A case of fatal pneumonia was also described. It is interesting that no interactions with blood pressure could be observed in our study. In conclusion, the side-effect profile in real-life is consistent with the existing data, no new toxicities could be identified in the trial, and less severe cases of adverse reactions were observed compared to other trials (97).

4.5 Melanoma recurrence

Overall, melanoma relapse occurred in 51.5 % of patients in the PD-1 treated group and 50 % of patients in the BRAF/MEK treated group. The most common type of recurrence in the PD-1 group was local recurrence, respectively a combination of local recurrence and lymph node metastases. Distant metastases or a combination of distant and lymph node metastases were more represented in BRAF/MEK treated patients than others. Among distant metastases, the lung was the most affected organ in both groups, and CNS metastases were also common in BRAF/MEK treated patients. The only data available regarding recurrence types are from the KEYNOTE-054 trial, in which distant metastases were the most frequently reported type of recurrence in the treatment with pembrolizumab, most of them involving the lungs (88).

In our study, eight relapsed patients underwent a systemic therapy change, either from immunotherapy to targeted therapy or vice versa. In none of these cases a survival benefit could be achieved. In all cases that experienced complete remission again after a recurrence event, surgery was the therapy to achieve these successful

results. Relapses in these cases affected either locoregional areas or lymph nodes. In patients treated with immune checkpoint inhibitors, median time to first recurrence event was four months, which is very early. Patients treated with D+T experienced recurrence events a few months later with a median period to first relapse of 8.5 months. This is reflected in initially increased RFS rates of BRAF/MEK-treated patients, as discussed in section 4.2.3. In conclusion, our study points out, that early melanoma recurrence remains a main problem in the management of advanced melanoma.

4.6 Strengths and limitations

Our single center study provides first real-word data from the use of small-molecule targeted agents and immunotherapy agents, of which we have not yet had any evaluation of survival and safety apart from pivotal studies. Certainly, the small population size limits the interpretation and power of the results, but it allows us to assess therapy response, survival, and safety more accurately. Other limitations of the study might include the facts that specific melanoma types were underrepresented and that patients were only enrolled from one hospital, which can lead to selection bias. Then again, the great advantage of real-world data is that it is completely raw and unselected. This allows different types of bias to be ruled out and to draw conclusions that may be missed in controlled studies. In addition, medical data in our study is collected from a database where it is well documented and can be assumed to be mostly complete and correct.

5 Conclusion and outlook

Immunotherapy and targeted therapy agents remain a very effective treatment for advanced melanoma. Through the conduction of our study, we could achieve a progress in advanced melanoma treatment by analyzing real-world data and identifying key treatment outcome parameters. Although adjuvant treatments do not exactly meet expectations of phase III trials, they prolong recurrence-free and overall survival. Nevertheless, there are multiple difficulties, since not every patient responds to the treatment or severe adverse events occur that negatively affect quality of life. Furthermore, we concluded that the performance of CLND should be evaluated carefully, as it shows no benefit, neither in RFS nor in OS. Nevertheless, we could not find stable parameters that can precisely predict the outcome, so early melanoma recurrence remains a clinical challenge and specific biomarkers are urgently needed to improve treatment and survival.

Currently, there are many studies ongoing, trying to figure out suitable parameters to improve melanoma treatment. For targeted therapy with the BRAF/MEK-inhibitors dabrafenib and trametinib, BRAF-mutation is a suitable indicator for evaluation, whether patients will benefit from treatment or not. For immunotherapy, no specific markers are known to this date. The ability of PD-L1 expression on tumors to predict treatment response to PD-1-inhibitors has been investigated, but studies have shown that the efficacy of PD-1-inhibitors is independent of expression status (94). A new approach to outcome prediction is the analysis of immune-related genes. Clinical trials have shown that the implementation of a classifier of eight immune-related genes has significant value to predict response rate in immunotherapy treated melanoma patients and hence outcome (107). Another potential indicator could be the detailed examination of tumor metabolism with the help of PET/CT. It is reported that many biomarkers detected with PET/CT are valuable in predicting therapy response to immune-checkpoint-inhibitors. In the future, these approaches may help to overcome clinical challenges with adjuvant melanoma therapy (108). In summary, early melanoma recurrence remains a challenge in medicine today, and intensive research is still needed for improvement.

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