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Primary Resistance to Immune Checkpoint Inhibitors in Patients with Metastatic Melanoma: Prognosis, Subsequent Therapies and Survival

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Nothing in life is to be feared,
it is only to be understood.
Now is the time to understand more, so that we may fear less.

Marie Skłodowska Curie

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List of Abbreviations

AJCC	American Joint Committee on Cancer
ALM	Acral lentiginous melanoma
APC	Antigen-presenting cell
BRAF	Human proto-oncogene
Carbotaxol	Carboplatin plus paclitaxel
cKIT	Human proto-oncogene
CMMR	German Central Malignant Melanoma Registry
CR	Complete response
CTLA	Anti-cytotoxic T-lymphocyte-associated antigen
CVD	Chemotherapy with cisplatin, vinblastine and dacarbazine
DC	Disease control
DDG	German Society of Dermatology
DTIC	Dacarbazine
EMA	European Medicines Agency
ESMO	European Society of Medical Oncology
FDA	Food and Drug Administration
FUP	Follow-up time
ICC	Investigator's choice of chemotherapy
IQR	Interquartile range
Ipi	Ipilimumab
IT	Immunotherapy
JAK	Janus-activated kinase
LDH	Lactate dehydrogenase
LMM	Lentigo-maligna melanoma
MHC-I	Major histocompatibility complex one
Nivo	Nivolumab
NM	Nodular melanoma
NRAS	Human proto-oncogene
ORR	Objective response rate
(m)OS	(median) overall survival
PD-1	Programmed cell death protein 1
PD	Progressive disease
Pembro	Pembrolizumab
(m)PFS	(median) progression-free survival
PR	Partial response
RECIST	Response evaluation criteria in solid tumours
SD	Stable disease
SPSS	Statistical Package for Social Sciences

SSM	Superficial spreading melanoma
Tregs	Regulatory T-cells
Y	Year(s)

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1 Introduction

1.1 Malignant melanoma and its classification

Melanoma is a highly aggressive type of skin cancer which can metastasize via lymphatic or haematogenous ways early on. It develops in the melanocytes, the pigment producing cells which are mainly found in the skin, but also occur in the eye, meninges and mucous membranes.

Depending on the stage of the disease at the time of diagnosis, the survival rates vary. In melanoma staging, both clinical and pathological classifications are used. The TNM classification of the "American Joint Committee on Cancer" (AJCC) (Gershenwald et al., 2017) has been the most common and widely accepted staging system for many years. It is used in many countries worldwide and also provides the standard for histopathological diagnosis in the German guidelines (S3-Leitlinie zur Diagnostik, Therapie und Nachsorge des Melanoms) of how to treat malignant melanoma. As can be seen in Table 1, it gives information about the anatomical extent of the melanoma by including thickness and ulceration of the primary tumour (T), the spread to regional lymph nodes (N) and the presence or absence of distant metastases (M). In total it comprises 4 stages of the disease with a various number of substages. The AJCC Melanoma Staging System is regularly reviewed and updated, the eighth edition from December 2017 being the latest and most current version. It is very valuable in clinical decision making concerning treatment and the prognosis of the disease.

The basis of the latest edition is the new analysis of data from more than 46,000 melanoma patients stage I-III and a database from the seventh edition AJCC which consists of approximately 10,000 stage IV patients. (Gershenwald et al., 2017) The patient data was analysed in regard to 5- and 10-years (y) survival according to disease stage and it is not surprising that the rates showed a variety. While stage I patients had a 98% chance of surviving the next five years and a 95% probability for the next ten years, the percentage dropped by approximately 10% for stage II patients, being 90% and 84%. With progress of the disease, the survival decreased to 77% and 69% in stage III patients. On a positive note, these "*survival outcomes for equivalent stage groupings were substantially higher than those for similar stage groups of patients in prior editions, including the seventh edition*". (Gershenwald et al., 2017)

The AJCC did not perform any calculations on survival outcomes for stage IV melanoma patients since the management of patients with stage IV melanoma has undergone rapid change in recent years and is still evolving. Historically, the median overall survival (mOS) of stage IV patients was around 8 months and the 5-y survival rate was approximately 10%. (Garbe et al., 2011)

Table 1

TNM Classification of melanoma

Stage	Primary tumour (T)	Regional lymph node metastases (N)	Distant metastases (M)
0	Not available	None	None
IA	≤ 1.0mm with or without ulceration	None	None
IB	>1.0-2.0 mm without ulceration	None	None
IIA	>1.0-2.0 mm with ulceration or >2.0-4.0 mm without ulceration	None	None
IIB	>2.0-4.0 mm with ulceration or >4.0 mm without ulceration	None	None
IIC	>4.0 mm with ulceration	None	None
IIIA	<0.8-1.0 ± ulceration or >1.0-2.0mm without ulceration	1-3 clinically occult nodes	None
IIIB	Up to 2.0 mm ± ulceration or up to 4.0 without ulceration	1 clinically detected or no regional lymph node disease and presence of in-transit/ satellite metastases or 2-3 from which at least 1 which was clinically detected	None
IIIC	Any tumour thickness ± ulceration	2-3 from which at least 1 which was clinically detected or 1 clinically occult/detected and presence of in-transit/ satellite metastases or ≥4 clinically occult nodes or ≥2 clinically occult nodes and presence of intransit/ satellite metastases	None
IIID	>4.0 mm with ulceration	or ≥4 clinically occult nodes or ≥2 clinically occult nodes and presence of intransit/ satellite metastases	None
IV	Any tumour thickness	Any	Distant metastases

Note: adapted from: Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma of the skin. In: Amin MB, Edge SB, Greene FL, et al, *eds. AJCC Cancer Staging Manual. 8th ed. New York: Springer International Publishing; 2017:563-585.(Amin et al., 2017)*

1.2 Systemic therapies

In recent years the long-term prognosis for patients with stage IV metastatic melanoma has significantly improved. New management options in the form of immunotherapies and targeted agents have evolved and are changing the therapeutic landscape for advanced melanoma. Especially patients with advanced unresectable disease benefit from these two new classes of systemic agents. (Garbe et al., 2016)

1.2.1 Chemotherapy

Before checkpoint inhibitors and targeted therapies were approved and introduced into clinical use the treatment options for metastatic melanoma were limited. In the past, the first-line treatment was the chemotherapeutic agent dacarbazine. Studies showed a response rate of 5% - 12% and a mOS of up to 9.1 months. (Chapman et al., 2011)

An overview of trials evaluating the most commonly used chemotherapeutic agents can be found in Table 2. Fotemustine, a chemotherapeutic agent that is able of crossing the blood-brain-barrier, was compared to dacarbazine in a phase III trial (Avril et al., 2004) showing an improved response rate (15.2% vs. 6.8%) and a slight increase in OS (7.3 months vs. 5.6 months). (Garbe, 2012)

Other chemotherapeutic treatment options, namely combination chemotherapy and biochemotherapy (chemotherapy plus interferon-α or interleukin-2) demonstrated improved response rates but no improved OS. (Ives et al., 2007)

Also, polychemotherapies (combination of cisplatin, vinblastine, dacarbazine, tamoxifen) did not show a survival benefit compared to monotherapy with dacarbazine. (Atkins et al., 2008)

Table 2
Overview of trials evaluating the most commonly used chemotherapeutic agents

Study	Stage	Endpoint	Outcome	Agent
(Avril et al., 2004)	IV	mOS ORR	7.3m 5.6m 15.2% 6.8%	fotemustine vs. dacarbazine
(Bedikian et al., 2006)	III or IV	mOS	7.8m	dacarbazine

Study	Stage	Endpoint	Outcome	Agent
(Schadendorf et al., 2006)	IV	mOS ORR	11.6m 5.5%	dacarbazine
(Atkins et al., 2008)	IV	RR mOS	19.5% 13.8% 9m 8.7m	biochemotherapy CVD
(Flaherty et al., 2013)	IV	mOS	11.3m 11.1m	carboplatin+paclitaxel vs. carboplatin+paclitaxel+sorafenib

1.2.2 Immune checkpoint inhibitors

Several immunotherapies, namely the anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4 checkpoint inhibitor ipilimumab and the programmed cell death protein (PD)-1 checkpoint inhibitors pembrolizumab and nivolumab have been approved recently. These monoclonal antibodies target specific immune checkpoint receptors which leads to an increased activity of T-lymphocytes and an enhanced immune response against cancer cells. Their mechanism of action is presented in detail in 1.3. Table 3 gives an overview of clinical trials evaluating CTLA-4 checkpoint inhibitor ipilimumab, PD-1 checkpoint inhibitors pembrolizumab and nivolumab and combined immunotherapy of ipilimumab plus nivolumab.

Table 3
Summary of trials evaluating ipilimumab, PD-1 inhibitors and combined immunotherapies

Clinical trial	Stage	Therapy groups	Outcome
NCT00094653 (Hodi et al., 2010)	unresectable stage III or IV melanoma after previous chemotherapy or IL-2	ipi + gp100 vaccine ipi + placebo gp100 + placebo	mOS: 10m,10.1m, 6.4m 1-y OS: 43.6%, 45.6%, 25.3% 2-y OS: 21.6%, 23.5%, 13.7% mPFS: 2.76m, 2.86m, 2,76m
NCT00324155 (Robert et al., 2011)	previously untreated III or IV	ipilimumab + DTIC DTIC + placebo	mOS: 11.2m, 9.1m 1-y OS: 47.3%, 36.3% 2-y OS: 28.5%, 17.9% 3-y OS: 20.8%, 12.2%
(Schadendorf et al., 2015) Pooled Analysis		ipilimumab	mOS: 11.4m 3-y OS: 22%
Check Mate-066 (Robert et al., 2015a)	unresectable stage III/IV BRAF wt melanoma, treatment naïve	nivolumab DTIC	ORR: 40%, 13.9% mPFS: 5.1m, 2.2m 1-y OS: 72.9%vs. 42.1%

Clinical trial	Stage	Therapy groups	Outcome
Check Mate-037(Weber et al., 2015)	unresectable stage III/IV after ipi or after ipi and BRAF inhibitor (for pats with BRAFV600 mutated melanoma)	nivolumab ICC (DTIC or carbotaxol)	ORR: 31.7%, 10.6% mOS 16m, 14m
Check Mate-069 (Hodi et al., 2016, Postow et al., 2015)	unresectable stage III/IV melanoma, treatment naïve	nivo + ipilimumab ipilimumab	ORR: 59%, 11% mPFS: 2-y PFS: 51.3%, 12% 2-y OS: 63.8%, 53.6%
Check Mate-067 (Hodi et al., 2018, Larkin et al., 2015)	unresectable stage III/IV melanoma, treatment naïve	nivo + ipilimumab nivolumab ipilimumab	mOS: not reached, 36.9m, 19.9m mPFS: 11.5m, 6.9m, 2.9m 4-y OS: 53%, 46%, 30% 4-y PFS: 37%, 31%, 9%
Keynote 002 (Ribas et al., 2015)	ipilimumab refractory melanoma	pembro 10mg/kg pembro 2mg/kg ICC	6-m PFS: 38%, 35%, 16%
Keynote 006(Robert et al., 2015b)	unresectable stage III/IV melanoma with ≤ 1 prior systemic therapy	pembro 10mg/kg (every 2 weeks) pembro 10mg/kg (every 3 weeks) ipilimumab	At 33.9m of mFUP mOS: 32.3%, 15.9% mPFS: 8.3%, 3.3% ORR: 42%, 16%

Note: adapted from: Amaral et a., 2017 Immunotherapy in managing metastatic melanoma: Which treatment when? (Amaral et al., 2017)

1.2.2.1 CTLA-4 monotherapy

The first immune checkpoint inhibitor approved in the EU for treatment of melanoma was ipilimumab in July 2011. Ipilimumab is a human monoclonal antibody that binds to the checkpoint molecule cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). By blocking it an antitumour immune response is induced. (Wolchok et al., 2013a)

A dose of 3 mg/kg body weight was approved for the treatment of patients with non-resectable or metastatic melanoma who had received prior treatment. The approval in the EU followed the one by the Food and Drug Administration (FDA) a few months earlier (also 3 mg/kg body weight of ipilimumab was approved as monotherapy for patients with advanced melanoma with the minor difference that it could be prescribed regardless of pre-treatment). The recommended induction regime for ipilimumab by the European Medicines Agency (EMA) is four intravenous infusions at a dose of 3 mg/kg body weight, administered intravenously over a period of 90 minutes, every 3 weeks. (Hanaizi et al., 2012)

At that time the estimated mOS for metastatic melanoma was 8-10 months and the rate for 5-y survival 10%. (Balch et al., 2009, Garbe et al., 2011)

The introduction of ipilimumab resulted in a significant improvement of long-term survival for the patients with advanced melanoma. Studies where ipilimumab was administered as monotherapy demonstrated a mediocre mOS (10.1 months) but an increased OS with a 1-y survival rate of 45.6%-39.3% and 2-y survival rate of 23.5%-24.2%. (Hodi et al., 2010, Wolchok et al., 2010)

A pooled analysis of over 1800 patients showed a mOS of 11.4 months and a 3-y survival rate of 22%, even higher for treatment naïve patients with mOS of 13.5 months and a 3-y survival rate of 26%. (Schadendorf et al., 2015) A plateau in the OS was perceived around year three, meaning that the patients alive at that time experienced a long-term survival benefit. (Schadendorf et al., 2015)

During the studies it was observed that some patients experienced side effects from ipilimumab. These side effects can be mostly explained by the immunostimulatory effect of ipilimumab. Side effects of ipilimumab mostly affect the gastrointestinal tract (colitis), the liver (hepatitis), the endocrine system (thyroiditis, hypophysitis), the skin and other organs. Often, the side effects are reversible or can be well controlled by a prompt treatment.

1.2.2.2 Anti-PD-1 monotherapy

In 2015, two inhibitors against the checkpoint programmed cell death protein 1 (PD1) were approved for the treatment of metastatic melanoma: nivolumab and pembrolizumab. The approval was based on a study (Robert et al., 2015a) that presented prolonged OS and PFS for patients treated with nivolumab in comparison to DTIC. After one year 72.9% treated with nivolumab were still alive, in the DTIC group only 42.1%. (Robert et al., 2015a) Also, the objective response rate depicted a strong effect of nivolumab (40% vs. 13.9%). (Robert et al., 2015a)

Both anti–PD-1 agents nivolumab and pembrolizumab have shown superior OS, PFS, and objective response rate (ORR), with a better safety profile, than ipilimumab alone. (Larkin et al., 2015, Robert et al., 2015b) In the phase 3 KEYNOTE-006 trial involving patients with advanced melanoma, the OS rate at 33 months was 50% in the pembrolizumab group, as compared with 39% in the ipilimumab group.

In the CheckMate 067 trial, the mOS in the nivolumab group was 37.6 months, 52% of the patients were alive in the nivolumab group alive after three years, as compared with 34% of those in the ipilimumab group. In this trial, patients whose disease had progressed during ipilimumab therapy could have received subsequent anti–PD-1 agents. These results indicated similar survival outcomes with regard to the use of anti–PD-1 agents as monotherapy. The survival rate at three years was 58% among patients in the nivolumab-plus-ipilimumab group. The 4-y update of the CheckMate 067 study reported a 4-y OS of 46% (95% CI: 41-52) for patients treated with nivolumab.

1.2.2.3 Combined immunotherapy with CTLA-4 plus PD-1

Ipilimumab, being the first approved checkpoint inhibitor in the EU and US, has demonstrated an improved outcome in patients treated for advanced melanoma. To increase the number of patients who benefit from immunotherapy, complementary combinations have increasingly been investigated and introduced into clinical use.

The results of two phase II studies and two phase III study indicated that the combination of ipilimumab with nivolumab leads to a significantly improved outcome. (Hodi et al., 2016, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

A 4-y update of one of the studies (CheckMate 067) confirmed this and reported a 4-y OS of 53% (95% CI: 47-58) for patients treated with combined immunotherapy. (Hodi et al., 2018)

All four studies included patients who did not receive treatment previously. They compared the combination of ipilimumab plus nivolumab with ipilimumab monotherapy, the phase III trials included a group for nivolumab monotherapy.

Around 60% of the patients responded to the combined immunotherapy (objective response rates of 61%, 59%, 58%, 57.6%) with complete response (CR) between 10% and 20% (22%, 22%, 19%, 11.5%). (Hodi et al., 2016, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

Two studies reached the median PFS (mPFS), 11.5 months for the combination therapy. (Larkin et al., 2015, Wolchok et al., 2017)

The incidence of adverse events was very similar in all three studies. Over 90% of the patients receiving the combination of CTLA-4 and PD-1 checkpoint inhibitors

experienced side effects with around 50% being grade 3 or 4. (Hodi et al., 2016, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

The use of a combination therapy with ipilimumab plus nivolumab is beneficial for patients in terms of response rates, PFS and long-term survival. However, the high incidence of severe adverse events has to be taken into consideration when choosing a treatment.

1.2.2.4 CTLA-4 plus PD-1 vs. CTLA-4 vs. PD-1

The combination of the CTLA-4 blocker ipilimumab with anti-PD1 checkpoint blockers has shown an improvement in the treatment response of melanoma patients. These two classes of checkpoint inhibitors increase antitumour immune response through complementary and nonredundant mechanisms which act synergistically. (Postow et al., 2015)

Several studies proved the advantage of combined inhibition of T-cell checkpoint pathways by ipilimumab plus nivolumab over monotherapy. (D'Angelo et al., 2017, Hodi et al., 2016, Hodi et al., 2018, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

Throughout these studies the combined therapy resulted in high response rates around 60% with CR in one-fifth of the patients in comparison to ipilimumab monotherapy where only a maximum of 19% of the patients responded objectively (and CR in 0-5%). (Hodi et al., 2016, Hodi et al., 2018, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

Trial groups treated with nivolumab only, showed a higher response rate than ipilimumab, but still lower than the combination therapy (43.7% and 52% and CR in 8.9% and 16%). (Larkin et al., 2015, Wolchok et al., 2017)

The numbers for OS for the combination therapy are promising with 2-y survival rates around 64%, 3-y OS of 58% and a 4-y OS of 53%. (Hodi et al., 2018, Larkin et al., 2015, Wolchok et al., 2017)

Bringing monotherapies of ipilimumab and nivolumab into comparison the rates for 2-y survival were lower than for the combined immunotherapy (45% for ipilimumab, 59% for nivolumab). (Wolchok et al., 2017)

While in other studies the median for PFS was not yet reached, the CheckMate 067 study did and showed a clinically meaningful improvement with mPFS of 11.5 months, while the number for nivolumab was almost half of it (6.9 months) and ipilimumab on the bottom (2.9 months). (Wolchok et al., 2017)

The superiority of the combination of CTLA-4 antibody ipilimumab plus PD-1 inhibitor nivolumab over the monotherapy of each agent can be seen in the high rates of response, CR, survival and mPFS. In contrast, unfortunately the severe and life-threatening adverse events of combination therapy were high as well. In general, more than 90% of patients experienced side effects while being treated with the combination immunotherapies, with a similar or only slightly lower number with either agent alone. Also, side effects of a potential immunological cause occurred with a similar frequency. (Wolchok et al., 2017)

However, the number of patients presenting with grade 3 or 4 adverse events is quite different. While nivolumab monotherapy lead to severe adverse events in up to 21% of patients (Wolchok et al., 2017) and ipilimumab in up to 27,3% (Larkin et al., 2015), when receiving both agents more than 50% were affected (with numbers ranging from 51%-59%). (Hodi et al., 2016, Larkin et al., 2015, Postow et al., 2015, Wolchok et al., 2017)

Adverse events from immune checkpoint inhibitors require quick recognition and treatment. Management recommendations for a wide range of immune related adverse events of all grades were developed and approved by the European Society of Medical Oncology (ESMO) in the form of Clinical Practice Guidelines. (Haanen et al., 2017)

1.3 Mechanisms of action of checkpoint inhibitors

CTLA-4 and PD-1 checkpoint inhibitors are both monoclonal antibodies targeting specific checkpoint receptors with the effect of an increased activity of the immune system via T-lymphocytes.

The immune response is regulated by both inhibitory and stimulatory pathways which in turn are controlled by immune checkpoints. These checkpoints are certain inhibitory signal molecules which are upregulated following T-cell activation. They serve the function of protecting healthy tissue from damage by regulating the extent and duration

of the inflammatory response. Malignant tumours utilize these checkpoints to reduce the inherent immune response. (Pardoll, 2012)

The checkpoint CTLA-4 can be found on activated T-cells and regulatory T-cells (Tregs). T-cells' activation or priming involves two signals to be complete. The first one is binding of the major histocompatibility complex one (MHC-I complex) presented on the surface of an antigen-presenting cell (APC) to a T-cell receptor on a naive T-cell. The MHC-I displays the tumour-associated peptide antigen. The second signal is the interaction of CD80/86 on the APC with CD28 on the T-cell. After this activation of the T-cell, CTLA-4 is expressed on the surface of it. CTLA-4 competes with CD28 to ligate CD80/86 and since CTLA- 4 has a higher affinity to CD80/86, it replaces CD28. By disrupting the initial binding T-cell activation is downregulated and consequently the T-cell mediated immune response as well. (Garbe et al., 2011, Liu et al., 2018)

When ipilimumab, a CTLA-4 antibody binds to it the interaction with CD80/86 is prevented. That way the ligation of CD28 on the T-cell to CD80/86 on the APC is kept intact and the immune response follows and is not dampened. Ipilimumab can also deplete Tregs which express CTLA-4. Tregs are a subpopulation of T-cells. Their task is to suppress the activation of the immune system in certain situations in order not to recognise the body's own cells as foreign. (Melero et al., 2015, Pardoll, 2012) This reduces the risk of developing autoimmune diseases, allergies and rejection following transplantation. (Pardoll, 2012)

An accumulation of Tregs in the tumour was observed in different tumour entities. (Shitara and Nishikawa, 2018) It is assumed that in this case the regulatory T-cells inhibit the activation of effector T-cells in order to induce the immune response to the tumour. (Melero et al., 2015)

CTLA-4 inhibits T-cells during an early phase of immune response in the lymphatic organs, the priming phase. PD-1 however, downregulates T-cells at a later stage in peripheral tissues, the effector stage. The mechanism by which T-cells are inhibited is different from CTLA-4 not only regarding the timing in the immune response. (Seidel et al., 2018)

Like CTLA-4 the expression of PD-1 is present only on T-cells which have been activated. Moreover, PD-1 is also expressed on B-, natural killer cells, monocytes and dendritic cells. (Amaral et al., 2017)

PD-1 has two ligands, PD-L1 and PD-L2. PD-L1 is expressed on many hematopoietic cells and also on tumour cells and its expression is regulated by IFN-γ. (Brown et al., 2003) On binding of PD-1 with its ligands, some parts phosphorylate and dephosphorylate resulting in inhibition of T-cell activation which over time leads to T-cell exhaustion. (Saeidi et al., 2018)

Inhibitors of the PD-1 pathway like nivolumab and pembrolizumab prevent and reverse T-cell exhaustion, increase the activation of T-cells and thereby enhance the immune response against tumours. (Luke and Ott, 2015)

1.4 Resistance mechanisms

Despite the increasing number of patients that benefit from the treatment of immune checkpoint inhibitors, there is still a part of patients that does not respond at all. Primary (or innate) resistance can be seen in around half of patients treated with anti-PD-1 inhibitors (40%-65%). (Larkin et al., 2015, Robert et al., 2015a, Robert et al., 2015b) The number is even higher in patients receiving anti-CTLA-4 antibodies. (Hodi et al., 2010). For the combination of PD-1 antibodies and CTLA-4 antibodies, about 23% of patients present with primary resistance. (Hodi et al., 2018, Larkin et al., 2015)

Mechanisms of resistance are closely linked to the steps of immune response. Due to its complexity not all mechanisms of resistance to immune checkpoint inhibitors are fully understood.

The interaction between the immune system and tumour cells is called cancer immunoediting. The process of tumour progression under immunosurveillance is divided into three phases: the elimination phase, the equilibrium phase and the immunological escape phase. (Schreiber et al., 2011)

If cancer cells manage to survive the first two phases and escape a competent immune system, they grow and establish an immunosuppressive tumour microenvironment in the escape phase. (Mittal et al., 2014)

1.4.1 Tumour mutational burden and neoantigens

Tumour mutational burden has been identified as a factor in the response to immune checkpoint blockers. It is a feature of the tumour itself, tumour-intrinsic. Tumours like melanoma with high levels of non-synonymous mutations show a high response to immune checkpoint inhibitors in comparison to tumours with lower mutational burden. (Colli et al., 2016, Hugo et al., 2016, Snyder et al., 2014, Van Allen et al., 2015) The only exception seems to be renal cell carcinoma which shows susceptibility to immune checkpoint inhibitors despite a low mutation load.

Moreover, a higher burden of non-synonymous mutations leads to an increase in the generation of neoantigens. (Riaz et al., 2016) Neoantigens play an important role in the identification of cancer cells and the subsequent immune response against the tumour. T-cells can recognize cells as foreign when they express antigens that are not present in the normal cells and tissues in the body. Tumour cells express tumour-associated antigens in a high level, but since these antigens can also be found in some normal tissues tolerance can develop. (Lu and Robbins, 2016)

Somatic mutations that lead to the expression of neoantigens are not present in normal tissues hence neoantigens are ideal to prime T-cells and be targeted by immunotherapy. (Lu and Robbins, 2016) Lack of sufficient or suitable neoantigens diminishes the formation of tumour-reactive T-cells and presents one mechanism of resistance to immunotherapy. (O'Donnell et al., 2017)

1.4.2. Microsatellite instability

Closely linked to neoantigens and a high tumour mutational burden is the deficiency of DNA-mismatch repair proteins which leads to microsatellite instability. DNA-mismatch repair proteins maintain genomic stability by recognizing and repairing DNA replication errors.

Impaired DNA-mismatch repair results in an accumulation of errors in the DNA, microsatellite instability. Microsatellites are repetitive DNA sequences distributed across the human genome. They serve the function of maintaining the chromosome structure and are found in centromere regions and at the end of chromosomes. Due to their repetitive structure and the tandem arrangement of the bases, changes of the microsatellite DNA regarding mutations and length can occur easily. (Kawakami et al., 2015)

Cancers expressing microsatellite instability are associated with an increased response to immune checkpoint inhibitors. (Le et al., 2017, Le et al., 2015)

In a small trial, 40% of patients with mismatch-repair deficiency responded to the anti-PD-1 agent, (Le et al., 2015) whereas the mismatch-repair proficient patients did not show any response. (Le et al., 2017)

1.4.3 PD-L1 expression

Relating to a high tumour mutational burden, a study reported that an increased mutational load is linked to elevated PD-L1 expression in melanoma. (Madore et al., 2016)

The PD-1/PD-L1 pathway is a major mechanisms of tumour immune escape. In the case of malignant melanoma PD-L1 can be expressed on cancer cells. When the ligand PD-L1 interacts with its receptor PD-1 on T-cells, the recognition and destruction of cancer cells by the host immune response is inhibited. (Botti et al., 2017)

Concerning immune checkpoint inhibitors, an increased expression of PD-L1 affects the response to immunotherapy in a positive way. PD-L1-positive melanoma patients with ≥5% PD-L1 tumour expression receiving combined immunotherapy of ipilimumab plus nivolumab as well as patients only receiving nivolumab monotherapy had an increased PFS compared to PD-L1-negative patients. (Larkin et al., 2015, Long G. V. et al., 2016)

1.4.4 Lymphocyte exhaustion

Closely related to the expression of PD-L1 is T-cell exhaustion which is induced by PD-1 and its ligand in case of prolonged antigen exposure. This occurs in chronic viral infections and cancer. (Schietinger and Greenberg, 2014, Wherry, 2011) The negative regulatory signal that is triggered by the binding of PD-1 to its ligand leads the T-cell into a dormant state and thereby to lose effector functions. The expression level of PD-1 receptors and the ligand abundance defines the degree of T-cell exhaustion. (Pauken and Wherry, 2015) The exhausted state in T-cells with a low-to-intermediate PD-1 expression can be reversed, one mechanism of anti-PD-1 inhibitors. (Sznol and Chen, 2013)

1.4.5 Other mechanisms of resistance

Mechanism of antigen presentation can also result in resistance to immune checkpoint blockade.
Tregs, which express CTLA-4 can have a negative effect on dendritic cells. Tregs produce IL-10 which reduces the expression of class II MHC. (Commeren et al., 2003) MHC I can be defective when mutations in ß2-microglobulin occur, a protein necessary for the folding and transport of MHC class I to the cell surface. (Zhao et al., 2016)

MHC expression is enhanced by IFN-γ. Mutations in genes involved in the IFN-γ pathway also result in resistance to immune checkpoint blockade. IFN-γ signalling is important for the T-cell mediated immune response since MCH play a crucial role in tumour antigen presentation. Involved in the signalling pathway are also Janus-activated kinase 1 (JAK1) and Janus-activated kinase 2 (JAK2). IFN-γ binds to IFN-γ receptor 1 and IFN-γ receptor 2 and signals through JAK1 and JAK2 pathway. Mutations in both IFN receptors and JAK2 receptors were found in melanoma tumours that were refractory to PD-1 inhibitors. (Zaretsky et al., 2016) Also, mutations in JAK1 and JAK2 were shown in patients not responding to anti-PD-1 treatment, despite a high tumour load. (Shin et al., 2017)

1.5 Aims and objectives

1.5.1 Aims

This study aims to identify and characterize stage IV melanoma patients who are primary resistant to checkpoint immunotherapy. Primary resistance to therapy was defined as progressive disease in the first staging that took place approximately three months after starting the therapy.

We aim to assess patients' characteristics, best response to immunotherapy, mOS and survival rates as well as mPFS and PFS rates. Moreover, our aim is to evaluate prognostic and/or predictive factors of primary resistant patients.

1.5.2 Objectives

1. Is primary resistance associated with a worse outcome?
2. What was the best response to first-line immunotherapy?
3. Do primary resistant patients respond to further immunotherapy?
4 Is there a difference in response considering BRAF status?

2 Patients and Methods

2.1 Data collection

For this study an approval of the local ethics committee is available for this analysis (approval number 676/2016BO2).

Data from the German Central Malignant Melanoma Registry (CMMR) of the German Society of Dermatology (DDG) were used. The CMMR was established in 1983 by the German Society of Dermatology in cooperation with the Federal Health Office and ensures the systematic collection of data. Several hospitals at national level as well as in Switzerland and Austria are involved.

A total of 607 patients newly diagnosed with stage IV melanoma from the Department of Dermatology at the University Hospital in Tübingen were identified in the CMMR for the period January 2015-August 2018. From these, data from 530 patients was available for this analysis and we focused on the 347 melanoma patients that were considered primary resistant according to the definition previously mentioned in 1.5.1. Originally, the patient population included 355 melanoma patients treated with immunotherapy. Due to missing data on best response, 8 patients were excluded from this retrospective analysis. The cut-off data analysis was March 2019.

The relevant data for this analysis were obtained by research in the patient record database of the University Hospital Tübingen (SAP ISH GUI for Windows, Copyright 1993-2004) and the database of the CMMR of the DDG.

The data was collected with Epi Info™ an open-source statistical software.

Information from the CMMR was compiled in a Statistical Package for Social Sciences (SPSS) table and evaluated according to the following criteria: gender, date of birth, date of stage IV diagnosis, stage at initial diagnosis (TNM stage), localization of primary tumour and histopathological characteristics of the primary tumour. Further information was obtained from electronic patient records, including BRAF, NRAS and cKit mutation status, S100 tumour marker value, lactate dehydrogenase (LDH) value, type, start and end dates of systemic and local therapies for metastatic stage IV, radiological evaluations since stage IV diagnosis including number of organ metastases, best response to therapy

and time of last follow-up or death from any cause. These data were entered into Epi Info™ and exported as an excel table into SPSS using appropriate variables. The data from the CMMR and the data from Epi Info™ were merged into a final SPSS file which was used for the statistical analysis.

2.2 Patients' population and variable definition

A first analysis was performed with all 530 stage IV melanoma patients. However, the focus of the analysis was put on patients with metastatic melanoma receiving immunotherapy as first-line treatment. All the analysis refers to patients treated with immunotherapy as first-line therapy unless otherwise stated.

Some of our analysed patients had also received local therapies like surgery or radiotherapy but these were not considered in the analysis.

The following variables were included in the analysis: gender, age, localization and histological subtype of primary melanoma, stage at initial diagnosis, number of organs with metastases, presence of brain and liver metastases, BRAF mutation, LDH and S100 level at stage IV diagnosis and date of death or last observation were considered. Follow-up time was defined as the time between the date of diagnosis of stage IV disease and the date of the last follow-up or death.

The total collective of 347 patients was divided into two subgroups according to response to the first-line immunotherapy at the time of first staging – primary resistant and disease control (DC). DC included the patients that were evaluated having a best response of CR, partial response (PR) or stable disease (SD). Progressive disease (PD) meant primary resistance to immunotherapy at the first staging, usually three months after initiation of immunotherapy administration. The response was evaluated according to response evaluation criteria in solid tumours (RECIST) 1.1.(Eisenhauer et al., 2009)

2.3 Statistical analysis

The entire patients' population was characterized using descriptive statistical analysis and frequency tables and cross tables. For certain variables, mean, median and standard deviation were calculated. Kaplan-Meier curves were used to determine survival curves

and median survival time using log-rank tests for statistical significance testing. Differences with a *p*-value of 0.05 were evaluated as significant.

In addition, the 1-, 2- and 3-y survival was calculated with a 95 % confidence interval. Follow-up time was defined as the time between the date of diagnosis of stage IV melanoma and the date of last follow-up or death. Survival probabilities were calculated based on the date of diagnosis of stage IV disease. In the OS analyses all causes of death were considered.

For the statistical evaluation of this work, the statistics program IBM SPSS Statistics Version 24.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) and the PRISM program (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA) were used.

3 Results

3.1 Description of the patients' collective

A total of 347 patients were included in this retrospective analysis. Originally, the patient population included 355 melanoma patients treated with immunotherapy. Due to missing data on best response, 8 patients were excluded from this retrospective analysis. The median follow-up time was 23 months (95% CI: 20.5-25.5).

Table 4
Patients' Characteristics

Characteristics	**All** n=530	**IT collective** n=347*	**IT** n=347*		χ^2 **test**♣
			Primary resistant n=144 (41.5%)	**DC** (CR, PR, SD) n=203 (58.5%)	
Age distribution					0.383
median		68(54.0-74.0)			
<60	197 (37.2%)	108 (31.1%)	39 (27.1%)	69 (34%)	
60-75	180 (34%)	127 (36.6%)	55 (38.2%)	72 (35.5%)	
>75	153 (28.8%)	112 (32.3%)	50 (34.7%)	62 (30.5%)	
Gender					0.079
Male	301 (56.8%)	207 (59.7%)	78 (54.2%)	129 (63.5%)	
Female	229 (43.2%)	140 (40.3%)	66 (45.8%)	74 (36.5%)	
Tumour localization♦					0.007
Head and neck	85 (20.6%)	59 (21.5%)	16 (15.1%)	43(25.4%)	
Trunk	144 (34.9%)	81 (29.5%)	24 (22.6%)	57 (33.7%)	
Extremity	166 (40.2%)	118 (42.9%)	57 (53.8%)	61 (36.1%)	
Other	18 (4.3%)	17 (6.1%)	9 (8.5%)	8 (4.8%)	
Histological subtype♦					0.013
SSM	134 (35.6%)	80 (31.1%)	33 (34%)	47 (29.4%)	
NM	118 (31.4%)	80 (31.1%)	22 (22.7%)	58 (36.3%)	
LMM	17 (4.5%)	14 (5.4%)	1 (1%)	13 (8.1%)	
ALM	37 (9.8%)	32 (12.5%)	16 (16.5%)	16 (10%)	
Mucosal	18 (4.8%)	17 (6.6%)	9 (9.3%)	8 (5.0%)	
Other	52 (13.9%)	34 (13.3%)	16 (16.5%)	18 (11.2%)	
Stage at initial diagnosis♦					0.130
I	89 19.7%)	52 (17.4%)	21 (17.5%)	31 (17.4%)	
II	129 (28.5%)	91 (30.5%)	29 (24.3%)	62 (34.8%)	
III	159 (35.2%)	105 (35.3%)	44 (36.7%)	61 (34.3%)	
IV	75 (16.6%)	50 (16.8%)	26 (21.7%)	24 (13.5%)	
Number of organs with metastases					0.03
1-3	462 (87.2%)	309 (89%)	122 (84.7%)	187 (92.1%)	
>3	68 (12.8%)	38 (11%)	22 (15.3%)	16 (7.9%)	

Characteristics	**All** n=530	**IT collective** n=347*	**IT** n=347*		χ^2 **test**♣
			Primary resistant n=144 (41.5%)	**DC** (CR, PR, SD) n=203 (58.5%)	
Brain metastases					0.901
No Brain metastases	404 (76.2%)	283 (81.6%)	117 (81.2%)	166 (81.8)	
Brain metastases	126 (23.8%)	64 (18.4%)	27 (18.8%)	37 (18.2%)	
Liver metastases					0.065
No liver metastases	338 (63.8%)	222 (64%)	84 (58.3%)	138 (68%)	
Liver metastases	192 (36.2%)	125 (36%)	60 (41.7%)	65 (32%)	
BRAF mutation♦					0.529
BRAF mutation	216 (59.7%)	96 (44.9%)	35 (42.2%)	61 (46.6%)	
BRAF wild type	146 (40.3%)	118 (55.1%)	48 (57.8%)	70 (53.4%)	
LDH level♦					0.016
Normal	279 (62.1%)	200 (66.2%)	73 (58.4%)	127 (71.8%)	
Elevated	170 (37.9%)	102 (33.8%)	52 (41.6%)	50 (28.2%)	
S100 level♦					0.000
Normal	230 (51.5%)	168 (54.7%)	51 (40.8%)	117 (64.3%)	
Elevated	217 (48.5%)	139 (45.3%)	74 (59.2%)	65 (35.7%)	

* 8 patients excluded due to lack of information on best response
♣ χ^2 test performed between primary resistant group and DC group
♦ Patients for which the information was unknown were excluded

3.2 Prognostic factors and survival probabilities

3.2.1 Age and Sex

For the total collective (n=347), the median age at diagnosis of metastatic melanoma was 68 years (IQR (interquartile range) [54.0-74.0]). The patients' age ranged between 19 years (minimum) and 91 years (maximum) and the standard deviation was 14.135 years. Table 4 shows the division of patients into three age groups. The majority (36.6%) of our patient population (127/347 patients) were between 60 and 75 years old at the time of stage IV diagnosis of melanoma, 32.3% of the patients (112/347) were over 75 years old and 31.1 % (108/347 patients) were below 60 years of age.

The whole collective comprises of almost 60% male and 40% female patients. The proportions are similar in the primary resistant group with 54.2% men and 45.8% women and in the DC group where 63.5% are male and 36.5% are female.

3.2.2 Localization of the primary tumour

The localization of the primary tumour was unknown for 20.7% so these were excluded from the analysis. Most patients had their primary tumour on an extremity (42.9%), followed by the trunk (29.5%) and head and neck (21.5%). There is a significant difference between the primary resistant and DC group considering tumour localization. More than 50% of the primary resistant patients (53.8%) were diagnosed with their primary tumour on an extremity while it was only 36.1% of the DC patients. Slightly more than one-fifth of the patients in the primary resistant group had their melanoma on the trunk whereas it was one-third in the DC group. Head and neck as an origin made up only 15.1% in the primary resistant group while the number was 10% higher in the patients with DC (25.4%).

3.2.3 Histological subtype

Histological subtype also presented as a factor with significant difference between the primary resistant patients and DC patients. One-fourth of the patients (25.9%) had an unknown histological subtype so they were excluded from the analysis. In the whole collective the majority of patients were diagnosed with Nodular melanoma (NM) and Superficial spreading melanoma (SSM). Each made up 31.1%. Acral lentiginous melanoma (ALM) was diagnosed in 12.5%, 17 patients (6.6%) had a mucosal melanoma and 14 patients (5.4%) Lentigo-maligna melanoma (LMM). Other histological subtype represented 13.3% of the collective.

The primary resistant patients mostly received the diagnosis of SSM (34%) while it was only second place in the DC group with 29.4%. In the DC group, the majority of the patients had a NM (36.3%) while in the primary resistant group this diagnosis represented only one-fifth of the patients. Mucosal melanoma was distributed with almost 10% in the primary resistant group and half of it (5%) in the DC group. The less frequent histological subtype in the primary resistant group was LMM (1%). In the DC control group, this subtype represented 8.1% of the patients. The least frequent histological subtype in the DC was mucosal melanoma (5%), as previously mentioned.

3.2.4 Stage at initial diagnosis

Most patients, more than one-third of the whole collective, were initially diagnosed with stage III melanoma. A little less (30.5%) were diagnosed in stage II. Almost one-fifth of the patients (17.4%) were diagnosed with stage I melanoma and a similar proportion (16.8%) were in stage IV at the time of diagnosis. When comparing the distribution in the primary resistant and DC group, it can be seen that most of the patients in the primary resistant group also were diagnosed in stage III. In contrast, the DC group patients were diagnosed in equal proportions in stage II and III (approximately 34% in both stages). One-fifth of the primary resistant patients were diagnosed in stage IV, while the number was only 13.5% in the DC group. In both groups, primary resistant patients and patients with DC, the patients who were initially diagnosed in stage I made up 17%.

3.2.5 Number of organs with metastases

When analysing the number of organs with metastases the whole collective presented with almost 90% of patients with one to three metastases. The numbers were similar in the two subgroups, although there was a slightly higher number of patients with more than three metastases in the primary resistant group (15.3% vs. 7.9% in the DC group). The difference between the two groups was significant (χ^2=0.03, see Table 4).

3.2.6 Brain and liver metastases

Regarding the number of brain metastases, 81% of the patients did not have any brain metastases, while 18% did have metastases in the brain. This percentage was similar in the primary resistant patients and DC patients and the difference was not significant.

Regarding the presence of liver metastases, more than 60% of the whole collective did not have liver metastases. When assessing the numbers in the subgroups the number of patients with liver metastases was higher in the primary resistant patients (41.7%) compared to one-third in the DC group.

3.2.7 BRAF status

38.3% of the patients in the whole collective did not have any information on their BRAF status, so they were excluded in the analysis. In the whole collective, as well as the

primary resistant and DC group a little more than half of the patients were BRAF wild type and more than 40% expressed a BRAF mutation. The difference was not significant between both groups.

3.2.8 LDH and S100

When assessing the level of LDH at stage IV diagnosis, 13% of the patients in the whole collective had to be excluded due to lack of information. Two thirds presented with an LDH level in a normal range while one third had an elevated level. There was a significant difference between primary resistant and DC patients. In the primary resistant group 40% of patients demonstrated elevated LDH levels whereas it was only 28% in the DC group.

Regarding the level of S100, 11.5% of the patients did not have any information on the tumour marker S100 and were excluded. More than half of the patients in the whole collective presented with normal levels while 45% had elevated S100 at the time of diagnosis. In the primary resistant group two-thirds of the patients demonstrated elevated levels while two-thirds had normal levels in the DC group and this difference was significant.

3.3 Overall survival according to patients' characteristics (primary resistance (PD) and DC (CR, PR, SD))

Kaplan-Meier curves were applied to assess the effect of several factors on survival of the patients in the two subgroups primary resistance and DC. These factors were: age at time of diagnosis, gender, localization of the tumour, histological subtype, stage at time of initial diagnosis, number of organs with metastases at time of stage IV diagnosis, presence of brain metastases at time of stage IV diagnosis, presence of liver metastases at time of stage IV diagnosis, BRAF mutation, level of LDH and level of tumour marker S100.

Moreover, 1-, 2- and 3-y OS with a 95% confidence interval were assessed.

The primary resistant group comprised 144 patients which is 40.5% of the whole immunotherapy treated collective. The group DC included 203 patients.

The OS, *p*-value of the log-rank-test, mOS and 1-, 2- and 3-y OS for primary resistant patients are displayed in Table 5 and for DC in Table 6.

Four factors were shown to be prognostic (*p*-value of ≤0.05) in the primary resistant group (number of organs with metastases, presence of liver metastases, LDH level at baseline and S100 level at baseline) and two in the DC group (presence of brain metastases and level of S100 at baseline).

Table 5
Overall survival of primary resistant patients according to patients' characteristics

Primary resistant n=144 (41.5%)	**p value**	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
			1-y OS	**2-y OS**	**3-y OS**
Age distribution	*p*=0.068				
<60		13 (8.31-17.69)	52.8 (35.4-70.2)	18.9 (4.2-33.6)	-
60-75		12 (10.43-13.57)	46.7 (32.8-60.6)	21.7 (9.7-33.7)	16.3 (3.4-29.2)
>75		7 (4.26-9.75)	32.2 (18.3-46.1)	10.7 (0.9-20.5)	7.2 (0-15.8)
Gender	*p*=0.863				
Male		10 (7.5-12.5)	38.5 (26.9-50.1)	17 (7.6-26.4)	11(6.8-19.2)
Female		12 (8.46-15.54)	48.8 (35.9-61.7)	17 (6.4-27.6)	-
Tumour localization ♦	*p*=0.766				
Head and neck		10 (8.06-11.95)	43.8 (19.5-68.1)	18.8 (0-38)	12.5 (0-29.4)
Trunk		13 (10.39-15.60)	55.3 (34.1-76.5)	-	-
Extremity		9 (5.93-12.07)	36.6 (22.5-50.7)	14.3 (3.1-25.5)	-
Other		9 (6.08-11.92)	44.4 (11.9-76.9)	11.1 (0-31.7)	-
Histological subtype ♦	*p*=0.187				
SSM		9 (2.03-15.97)	46.2 (8.1-63.8)	19.3 (4.4-34.2)	-
NM		9 (2.6-15.4)	29.5 (8.5-50.5)	-	8.9 (0-24)
LMM					
ALM		19 (8.1-29.9)	60.6 (35.7-85.5)	26 (1.3-50.7)	-
Mucosal		9 (6.08-11.92)	44.4 (11.9-77)	11.1 (0-31.7)	-
Other		8 (4.62-11.38)	15.3 (0-34.7)	-	-
Stage at initial diagnosis ♦	*p*=0.78				
I		7 (4.14-9.86)	42 (19.1-65)	21 (0.6-41.4)	-
II		13 (6.29-19.71)	51 (32.6-69.4)	27.5 (10.4-44.6)	15.7 (0-32.4)
III		12 (8.85-15.16)	42.4 (26.5-58.3)	13.4 (1.4-25.4)	-
IV		8 (5.05-10.95)	36.9 (15.5-58.3)	12.3 (0-28)	-
Number of organs with metastases	***p*=0.024**				
1-3		12 (9.83-14.18)	46.5 (36.9-56.1)	19.5 (11.5-27.5)	11.8 (4.4-19.3)
>3		7 (3.49-10.51)	25.3 (6.5-44.1)	-	-

Primary resistant n=144 (41.5%)	**p value**	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
			1-y OS	**2-y OS**	**3-y OS**
Brain metastases	*p*=0.205				
No Brain metastases		12 (9.69-14.31)	46.7 (36.9-56.5)	18.9 (10.9-26.9)	10.6 (3-18.2)
Brain metastases		7 (4.28-9.72)	27.7 (9.5-45.9)	9.2 (0-21.4)	-
Liver metastases	***p*=0.012**				
No liver metastases		13 (10.65-15.36)	51.4 (39.8-63)	24.2 (13.6-34.8)	14.3 (4.1-24.5)
Liver metastases		9 (6.93-11.07)	32.6 (20.1-45.1)	6.1 (0-12.8)	-
BRAF mutation♦	*p*=0.570				
BRAF mutation		13 (7.91-18.09)	54.9 (37.7-72.1)	17.2 (3.7-30.7)	8.6 (0-19.4)
BRAF wild type		9 (6.76-11.25)	33.3 (20-46.6)	14.6 (4.6-24.6)	-
LDH level♦	***p*=0.007**				
Normal		13 (10.12-15.89)	50.2 (37.9-62.5)	22.3 (11.3-33.3)	11.8 (1.8-21.8)
Elevated		7 (2.81-11.19)	28.7 (15.2-42.2)	6.3 (0-14.3)	-
S100 level♦	***p*=0.003**				
Normal		13 (10.10-15.90)	51.7 (36.8-66.6)	24.9 (11.2-38.6)	8.7 (0-22.4)
Elevated		7 (3.4-10.6)	33.1 (21.5-44.7)	7.4 (0.5-14.3)	-

♦ Patients for which the information was unknown were excluded

Table 6

Overall survival of patients with disease control according to patients' characteristics

DC (CR, PR, SD) n=203 (58.5%)	**p value**	**Median OS *** (months; 95% CI)	**OS** (%; 95% CI)		
			1-y OS	**2-y OS**	**3-y OS**
Age distribution	*p*=0.180				
<60			95.6 (90.7-100)	85.8 (74.2-97.4)	68.4 (50.6-86.2)
60-75			92.3 (85.8-98.8)	87.6 (78.6-96.6)	66 (48-84)
>75			87 (78-96)	66.7 (51.6-81.8)	58.3 (38.1-78.5)
Gender	*p*=0.458				
Male			90.1 (84.4-95.8)	77.5 (67.9-87.1)	63.1 (49.8-76.4)
Female			94.3 (88.8-99.8)	85.6 (76-95.2)	66.2 (47.6-84.9)
Tumour localization♦	*p*=0.047				
Head and neck			96.4 (89.5-100)	79.9 (61.7-98.1)	-
Trunk			82.5 (71.9-93.1)	73.3 (59.6-87)	52.6 (32.6-72.6)
Extremity			96.7 (92.2-100)	86.4 (76-96.8)	71 (52.6-89.4)
Other					
Histological subtype♦	*p*=0.618				
SSM			87.8 (77.6-98)	72.2 (55.7-88.7)	56.2 (32.7-79.7)
NM			90.7 (82.9-98.6)	82.3 (70.7-93.8)	66.2 (49.1-83.3)
LMM					
ALM			-	71.4 (42.8-100)	47.6 (5.1-90.1)
Mucosal					
Other			88.9 (74.4-100)	61 (27.1-94.9)	-

DC (CR, PR, SD) n=203 (58.5%)	**p value**	**Median OS *** **(**months; 95% CI)	**OS** (%; 95% CI)		
			1-y OS	**2-y OS**	**3-y OS**
Stage at initial diagnosis♦	*p*=0.565				
I		32	92.4 (82-100)	-	48.5 (16.9-80.1)
II			93.1 (86.4-99.8)	72.2 (56.3-88.1)	58.4 (36.8-80)
III			87.4 (78.6-96.2)	79.3 (67.5-91.1)	71.5 (56.4-86.6)
IV			95.7 (91.4-100)	-	
Number of organs with metastases	*p*=0.832				
1-3			92.8 (88.9-96.7)	80.6 (73.2-88)	63.4 (52-74.8)
>3			80.2 (60-100)	-	-
Brain metastases	***p*=0.028**				
No Brain metastases			93.4 (89.5-97.3)	82.4 (75-89.8)	71.2 (60.6-81.8)
Brain metastases		31 (24.34-37.66)	84.4 (71.6-97.1)	72.2 (53-91.4)	32.1 (4.1-60.1)
Liver metastases	*p*=0.560				
No liver metastases			93.3 (88.8-97.8)	80.7 (72.1-89.3)	64.3 (51.2-77.4)
Liver metastases			88.6 (80.6-96.6)	80.9 (69.5-92.3)	64.7 (45.5-83.9)
BRAF mutation♦	*p*=0.704				
BRAF mutation			93.1 (86.6-99.6)	80.6 (67.7-93.5)	65.9 (47.1-84.7)
BRAF wild type			89.6 (82.3-96.9)	76.2 (65-87.4)	65.1 (50-80.2)
LDH level♦	*p*=0.185				
Normal			91.9 (86.8-97)	75.7 (65.5-85.9)	62.3 (48.6-76)
Elevated			94 (87.3-100)	-	74 (52.2-95.8)
S100 level♦	***p*=0.006**				
Normal			89.8 (84.1-95.5)	73.7 (63.3-84.1)	54.4 (39.7-69.1)
Elevated			98.5 (95.6-100)	-	80.3 (63.1-97.5)

* mOS was not reached for the majority of DC patients except for patients in stage I at initial diagnosis and patients with brain metastases

♦ Patients for which the information was unknown were excluded

For the primary resistant patients, the number of organs with metastases had a significant influence on survival (*p*=0.024). Patients with one to three metastases at the time of stage IV diagnosis had a mOS of 12 months (95% CI: 9.83-14.18). 46.5% of them were alive after one year (95% CI: 36.9-56.1). In the group with more than three metastases the mOS was 7 months (95% CI: 3.45-10.51). 25.3% of these patients, which is almost 50% less than the number of the patients with one to three metastases, survived one year (95% CI: 6.5-44.1).

Besides the number of metastases, also the presence or absence of liver metastases at time of stage IV diagnosis presented as a prognostic factor in primary resistant patients (*p*=0.012). The mOS for patients without liver metastases at time of stage IV diagnosis was 13 months (95% CI: 10.65-15.36). The 1-y OS was 51.4% (95% CI: 39.8-63) and decreased in a proportional pattern to 24.2% for 2-y survival and 14.3% 3-y survival (95% CI: 13.6-34.8 and 4.1-24.5, respectively). In contrast, patients which presented with liver

metastases at time of stage IV diagnosis had a mOS of 9 months (95% CI: 6.93-11.07), 32.6% survived one year (95% CI: 20.1-45.1). This number declined to 6.1% after 2 years (95% CI: 0-12.8).

The baseline LDH showed to be a prognostic factor in primary resistant patients (p= 0.007). Patients in the primary resistant group with normal LDH level had a mOS of 13 months (95% CI: 10.12-15.89), whereas the number for patients with elevated LDH level was almost half of it (7 months; 95% CI: 2.81-11.19). The 1-y OS for primary resistant patients with a normal LDH level was 50.2% (95% CI: 37.9-62.5). In comparison, only 28.7% of the patients with elevated levels survived one year (95% CI: 15.2-42.2). The difference was even wider for 2-y OS. While more than one-fifth of primary resistant patients with normal LDH level were alive at that mark, it was only 6.3% (95% CI: 0-14.3) of primary resistant patients with elevated LDH levels.

The level of the tumour marker S100 proved to be prognostic in both primary resistant and DC patients (p=0.003 and p=0.006, respectively). The mOS was not reached in the DC group. In the primary resistant group, the mOS was 13 months for patients with a normal S100 level and 7 months for patients with an elevated S100 (95% CI: 10.10-15.9 and 3.4-10.6, respectively). The primary resistant group with a normal S100 level displayed a 1-y survival of 51.7% (95% CI: 36.8-66.6), this was cut in half by time of the second year (24.9%; 95% CI: 11.2-38.6) and the 3-y OS was 8.7% (95% CI: 0-22.4). Primary resistant patients with elevated S100 showed a 1-y OS of 33.1% (95% CI: 21.5-44.7) and a 2-y OS of 7.4% (95% CI: 0.5-14.3). In the DC group with normal S100 levels, the 1-y OS was 89.8% (95% CI: 84.1-95.5) and decreased to 54.4% (95% CI: 39.7-69.1) at the mark of 3-y OS. Unexpectedly, the DC patients with elevated S100 displayed a slightly higher percentage of surviving patients. 1-y survival was 98.5% (95% CI: 95.6-100) and 3-y survival 80.3% (95% CI: 63.1-97.5). This might be due to fact that values for baseline S100 were missing for 11.5% of patients of the collective.

The presence or absence of brain metastases also had a significant influence on survival in the DC group. Median OS for patients without brain metastases at time of entering stage IV was not reached and was 31 months (95% CI: 24.34-37.66) in patients with brain metastases. While patients without brain metastases had a 1-y OS of 93.4% (95% CI: 89.5-97.3), the percentage was about 10 points less in patients with brain metastases (84.4%; 95% CI: 71.6-97.1). The difference in 2-y OS was similar (82.4% vs.72.2%; 95% CI: 75-89.8 and 53-91.4, respectively). Almost three-fourths of patients without

brain metastases were alive after three years, while it was only one-third of the patients with brain metastases at time of diagnosis of stage IV.

3.4 Overall survival according to response

3.4.1 Primary resistance (PD) and disease control (CR; PR; SD)

Figure 1 displays the OS of the whole collective treated by immunotherapy. The corresponding numbers can be found in Table 7.

Almost 60% of the patients were categorized under DC and the best response was distributed as follows: CR (15.6%), PR (24.7%) or SD (18.2%). Two-fifths of the patients were primary resistant and progressed under immunotherapy. Median OS was not reached for patients with CR and PR and 28 months (95%CI: 22.93-33.07) for patients with SD. In comparison, primary resistant patients reached a mOS of 11 months (95% CI: 8.83-13.17).

One-sixth of the patients had a CR. The 2-y OS was 96.3% and the 3-y OS was 90.3% (95% CI: 89.2-100 and 77-100, respectively). Patients with PR made up one-fourth of the whole collective. The 1-y OS was 90.2% and the 3-y OS was 69.8% (95% CI: 83.1-97.3 and 54.5-85.1, respectively). For patients with SD the 1-y OS was 86.4 % (95% CI: 77.6-95.2), 2-y OS was 63.2% (95% CI: 46.7-79.7) and 3-y OS was 24.1% (95% CI: 2.5-45.7).

In comparison to the SD group, the 1-y OS was twice as low in the primary resistant patients 43.1% (95% CI: 34.5-51.7), declined to 17% after two years (3.5 times lower) and two times lower (10.8%) after three years (95% CI: 9.9-24.1 and 4.3-17.3, respectively).

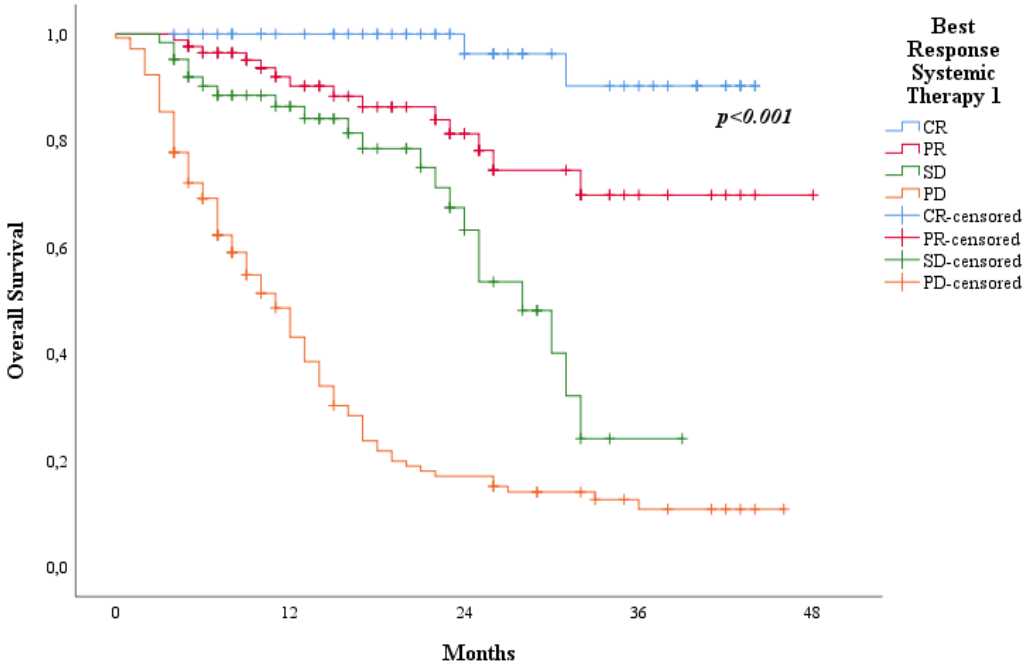

Figure 1. Overall survival according to type of response to the first systemic therapy.

Table 7

Median overall survival and overall survival rates of the collective treated by immunotherapy according type of response

Response	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
CR N=54 (15.6%)	not reached	-	96.3 (89.2-100)	90.3 (77-100)
PR N=86 (24.7%)	not reached	90.2 (83.1-97.3)	81.3 (70.7-91.9)	69.8 (54.5-85.1)
SD N=63 (18.2%)	28 (22.93-33.07)	86.4 (77.6-95.2)	63.2 (46.7-79.7)	24.1 (2.5-45.7)
PD N=144 (41.5%)	11 (8.83-13.17)	43.1 (34.5-51.7)	17 (9.9-24.1)	10.8 (4.3-17.3)
DC (CR, PR, SD) N=203 (58.5%)	not reached	91.8 (87.7-95.9)	80.6 (73.4-87.6)	64.2 (53.4-75)

3.4.2 Objective response and no objective response

Figure 2 shows the collective divided into objective response and no objective response. Objective response included CR and PR. These patient groups made up two-fifths of the whole collective (40.3%). The no objective response group comprised SD and PD and accounted for three-fifths of the collective (59.7%).

As can be seen in Table 8, mOS was reached for the patients with no objective response and it was 14 months (95% CI: 11.6-16.4).

Regarding 1-y OS more than half of patients showing no objective response were alive after that mark (56.3%; 95%CI: 49.1-63.5). This decreased to 30.2% after two years and to 15.9% after three years (95% CI: 22.8-37.6 and 8.1-23.7, respectively).

The mOS was not yet reached for the objective response patients. The 1-y OS was 94.2% (95% CI: 89.9-98.5) declined to 87.5% after two years and was 78.7% after three years (95% CI: 80.6-94.4 and 68.3-89.1, respectively).

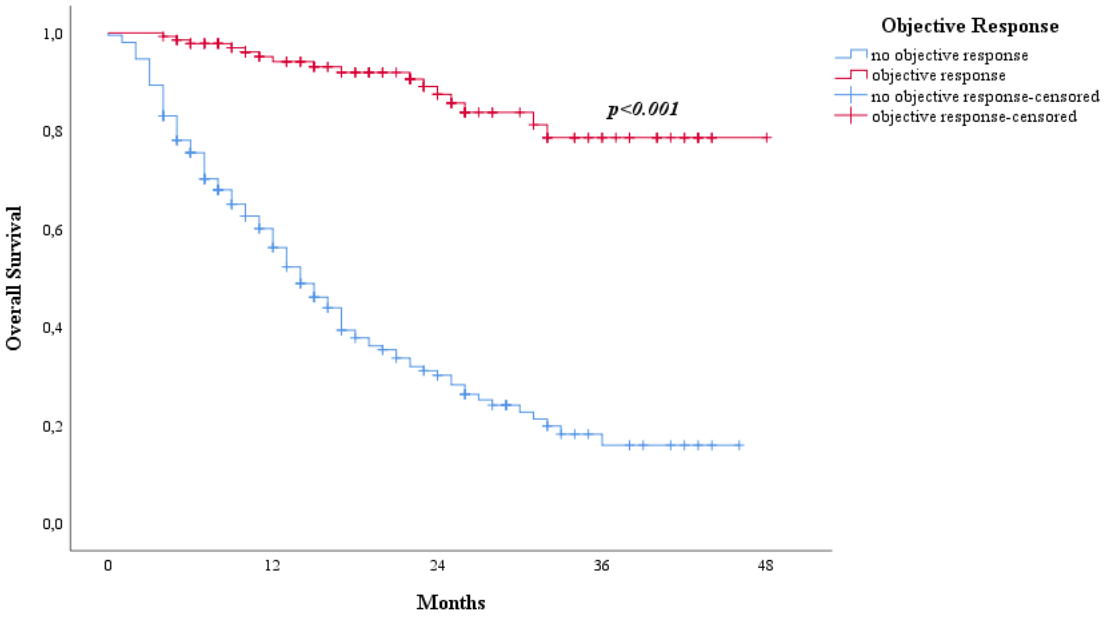

Figure 2. Overall survival according objective response and no objective response.

Table 8

Median overall survival and overall survival rates according objective response and no objective response

Response	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
No objective response	14 (11.57-16.43)	56.3 (49.1-63.5)	30.2 (22.8-37.6)	15.9 (8.1-23.7)
Objective response	Not reached	94.2 (89.9-98.5)	87.5 (80.6-94.4)	78.7 (68.3-89.1)-

3.5 Progression-free survival according to response

3.5.1 Primary resistance (PD) and disease control (CR; PR; SD)

Figure 3 displays PFS of the whole collective according type of response. There was a significant difference in PFS of the four response groups, which can be seen in Table 9. Median PFS for primary resistant patients was 4.0 months (95% CI: 3.62-4.38), while it was three times longer (11 months) for SD patients. PR patients displayed a mPFS of 37 months. 86% of the patients that had a CR were progression free at one year and almost 75% at three years. For the primary resistant patients, the 1-y PFS was below 10% and only 1% were progression free at three years.

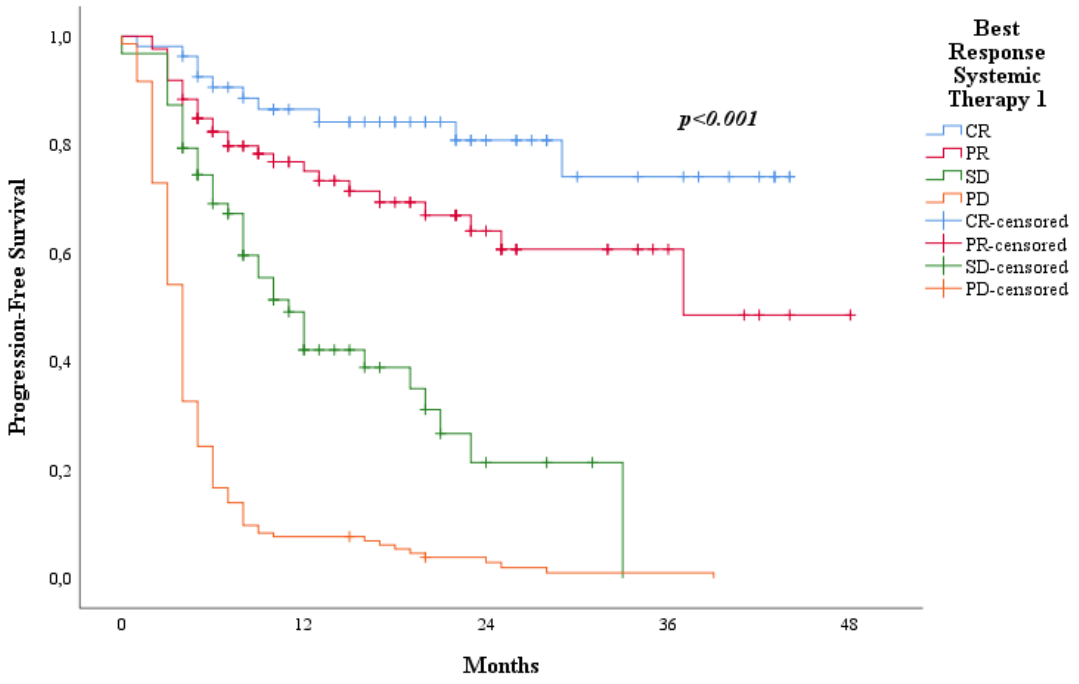

Figure 3. Progression-free survival according to type of response to the first systemic therapy.

Table 9

Median progression-free survival and progression-free survival rates of the collective treated by immunotherapy according type of response

Response	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y PFS	**2-y PFS**	**3-y PFS**
CR	Not reached	86.5 (77.1-95.9)	80.8 (69-92.6)	74.1(57.6-90.6)
PR	37	75.1 (65.5-84.7)	64.1 (51.8-76.4)	-
SD	11 (8-14)	42.1 (28.6-55.6)	21.3 (6-36.6)	-
PD	4 (3.62-4.38)	7.6 (3.3-11.9)	2.9 (0-5.8)	1 (0-2.8)

3.5.2 Objective response and no objective response

We also calculated the PFS according to the groups objective response and no objective response. *Figure 4* displays it and table 10 shows the numbers.

The big difference between the two groups can be seen in figure 4. The PFS for objective response was 79.6% after one year (95% CI: 72.7-86.5). Only one-sixth of patients with no objective response were progression free after the same time period. The decline was very similar in both groups.

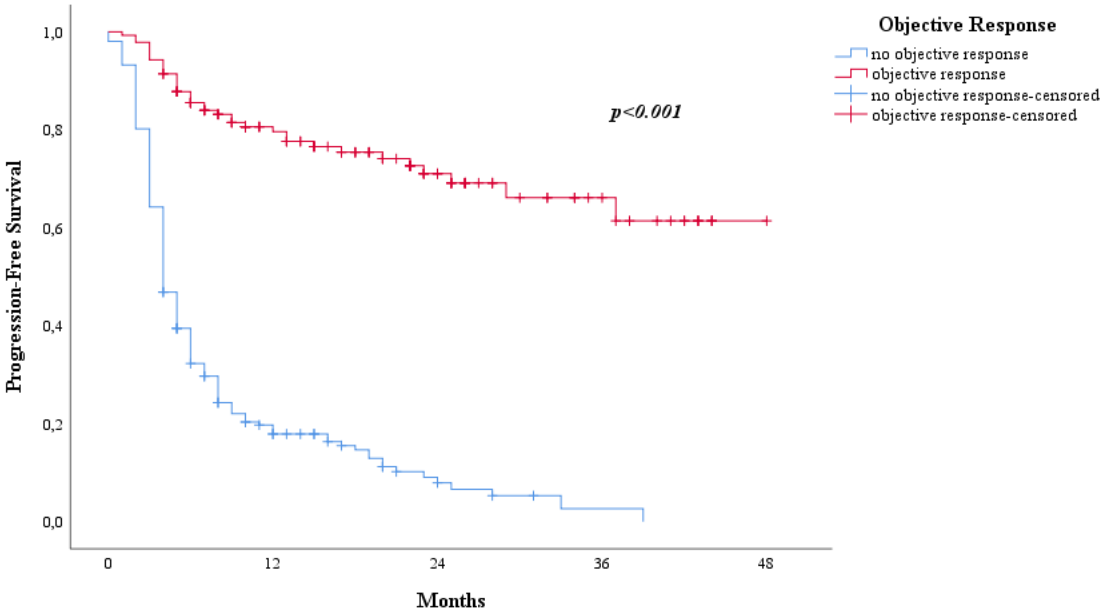

Figure 4. Progression-free survival according to objective response and no objective response.

Table 10
Median progression-free survival and progression-free survival rates according to objective response and no objective response

Response	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
No objective response	4 (3.5-4.5)	17.9 (12.4-23.4)	7.9 (3.2-12.6)	2.6 (0-6.9)
Objective response	Not reached	79.6 (72.7-86.5)	71.1 (62.5-79.7)	66.1 (55.5-76.7)

3.6 Overall survival according to patients' characteristics, considering the type of checkpoint inhibitor

Besides structuring the OS and PFS of all immunotherapy treated patients regarding response, we also analysed it by type of immunotherapy.

The biggest part of the patients (174 patients) received PD-1 monotherapy first-line (nivolumab or pembrolizumab). Two-fifths of the patients (145 patients) received combination therapy of nivolumab plus ipilimumab. The smallest group (26 patients) received CTLA-4 monotherapy.

3.6.1 PD-1 monotherapy

In the PD-1 treated patient group there was a significant difference between the primary resistant group and DC group in terms of tumour localization, histological subtype of the melanoma and level of S100. This is presented in Table 11.

Half of the patients who were primary resistant to PD-1 immunotherapy had their primary tumour on an extremity. For one-fifth of them the tumour was found on the trunk and for one-sixth in the head and neck area. In contrast, the location of the melanoma in patients with SD was equally distributed between extremity (35.2%), trunk (34.1%) and head and neck (26.2%).

Regarding histological subtype, one-third of primary resistant patients had a SSM, followed by 23% with NM and 17% with ALM. Differing from the primary resistant group the SD patients mostly were diagnosed with NM (42.5%), one-fourth of them had SSM and 10% LMM.

As for the level of S100 at time of stage IV diagnosis there was a significant difference between the patients who were primary resistant to PD-1 monotherapy and the ones who had DC (p=0.002). 60% of primary resistant patients showed elevated levels of S100 while it was only one-third in the DC group.

Table 11

Characteristics of patients treated with PD-1 monotherapy divided into primary resistant and disease control

Characteristics	**PD-1 Monotherapy** n= 174		χ^2 test
	Primary resistant n= 75(43.1%)	**DC** (CR, PR, SD) n= 99 (56.9%)	
Age distribution			0.766
<60	17 (22.7%)	27 (27.3%)	
60-75	27 (36%)	35 (35.4%)	
>75	31 (41.3%)	37 (37.3%)	
Gender			0.208
Male	43 (57.3%)	66 (66.7%)	
Female	32 (42.7%)	33 (33.3%)	
Tumour localization♦			0.034
Head and neck	9 (16.1%)	23 (26.2%)	
Trunk	11 (19.6%)	30 (34.1%)	
Extremity	30 (53.6%)	31 (35.2%)	
Other	6 (10.7%)	4 (4.5%)	
Histological subtype♦			0.023
SSM	16 (34%)	20 (25%)	
NM	11 (23.4%)	34 (42.5%)	
LMM	0	8 (10%)	
ALM	8 (17%)	7 (8.8%)	
Mucosal	6 (12.8%)	4 (5%)	
Other	6 (12.8%)	7 (8.7%)	
Stage at initial diagnosis♦			0.580
I	11 (18%)	17 (18.5%)	
II	15 (24.6%)	31 (33.7%)	
III	21 (34.4%)	29 (31.5%)	
IV	14 (23%)	15 (16.3%)	
Number of organs with metastases			0.416
1-3	68 (90.7%)	93 (93.9%)	
>3	7 (9.3%)	6 (6.1%)	
Brain metastases			0.922
No Brain metastases	64 (85.3%)	85 (85.9%)	
Brain metastases	11 (14.7%)	14 (14.1%)	
Liver metastases			0.055
No liver metastases	45 (60%)	73 (73.7%)	
Liver metastases	30 (40%)	26 (26.3%)	
BRAF mutation♦			0.268
BRAF mutation	14 (30.4%)	28 (40.6%)	
BRAF wild type	32 (69.6%)	41 (59.4%)	
LDH level♦			0.124
Normal	40 (63.5%)	61 (75.3%)	
Elevated	23 (36.5%)	20 (24.7%)	
S100 level♦			
Normal	26 (40.6%)	56 (65.9%)	0.002
Elevated	38 (59.4%)	29 (34.1%)	

♦ Patients for which the information was unknown were excluded

3.6.2 Nivolumab plus ipilimumab

Similar to the PD-1 monotherapy, the group of patients treated with combined immunotherapy also displayed a significant difference between primary resistant patients

and DC patients regarding tumour localization and level of S100 tumour marker. This is presented in Table 12.

For almost 70% of patients who progressed under the combined immunotherapy the melanoma was found on an extremity. For one-fifth of the primary resistant patients the tumour was localized on the trunk. In the DC group the localization of the primary tumour is more evenly distributed. One-third of them had the primary tumour on the trunk, one-third on an extremity and 26% in the head and neck area.

Concerning the level of S100, the distribution in the nivolumab plus ipilimumab-treated group is very similar to the PD-1checkpoint inhibitor-treated group. 60% of the primary resistant patients showed elevation in S100. In the DC group only one-third demonstrated an elevation of this tumour marker.

Table 12

Characteristics of patients treated with combination therapy of nivolumab plus ipilimumab divided into primary resistant and disease control

Characteristics	**Nivolumab +ipilimumab** n= 145		χ^2 **test**
	Primary resistant n=52 (35.9%)	**DC** (CR, PR, SD) n= 93 (64.1%)	
Age distribution			0.875
<60	20 (38.5%)	37 (39.8%)	
60-75	20 (38.4%)	32 (34.4%)	
>75	12 (23.1%)	24 (25.8%)	
Gender			0.095
Male	25 (48.1%)	58 (62.4%)	
Female	27 (51.9%)	35 (37.6%)	
Tumour localization♦			0.007
Head and neck	3 (8.6%)	19 (26.4%)	
Trunk	7 (20%)	25 (34.7%)	
Extremity	24 (68.6%)	24 (33.3%)	
Other	1 (2.8%)	4 (5.6%)	
Histological subtype♦			0.345
SSM	15 (39.5%)	25 (34.2%)	
NM	7 (18.4%)	20 (27.4%)	
LMM	0	5 (6.8%)	
ALM	7 (18.4%)	8 (11%)	
Mucosal	1 (2.6%)	4 (5.5%)	
Other	8 (21.1%)	11 (15.1%)	
Stage at initial diagnosis♦			0.204
I	8 (17.8%)	12 (15.8%)	
II	10 (22.2%)	28 (36.8%)	
III	17 (37.8%)	28 (36.8%)	
IV	10 (22.2%)	8 (10.6%)	
Number of organs with metastases			0.088
1-3	41 (78.8%)	83 (89.2%)	
>3	11 (21.2%)	10 (10.8%)	
Brain metastases			0.402
No Brain metastases	37 (71.2%)	72 (77.4%)	
Brain metastases	15 (28.8%)	21 (22.6%)	

Characteristics	Nivolumab +ipilimumab n= 145		χ^2 test
	Primary resistant n=52 (35.9%)	**DC** (CR, PR, SD) n= 93 (64.1%)	
Liver metastases			0.767
No liver metastases	30 (57.7%)	56 (60.2%)	
Liver metastases	22 (42.3%)	37 (39.8%)	
BRAF mutation♦			0.165
BRAF mutation	18 (69.2%)	28 (52.8%)	
BRAF wild type	8 (30.8%)	25 (47.2%)	
LDH level♦			0.096
Normal	27 (56.3%)	62 (70.5%)	
Elevated	21 (43.7%)	26 (29.5%)	
S100 level♦			
Normal	18 (40%)	57 (64.8%)	0.006
Elevated	27 (60%)	31 (35.2%)	

♦ Patients for which the information was unknown were excluded

3.6.3 Ipilimumab monotherapy

The group of patients treated with ipilimumab monotherapy did not display any significant differences between primary resistant patients and patients with DC as can be seen in Table 13. The group consisted of a number of 26 patients, two-thirds of them showed a progression under monotherapy with ipilimumab whereas one-third had DC.

Table 13

Characteristics of patients treated with CTLA-4 antibody monotherapy divided into primary resistant and disease control

Characteristics	Ipilimumab n= 26		χ^2 test
	Primary resistant n= 17 (65.4%)	**DC** (CR, PR, SD) n= 9 (34.6%)	
Age distribution			0.198
<60	2 (11.7%)	3 (33.3%)	
60-75	8 (47.1%)	5 (55.6%)	
>75	7 (41.2%)	1 (11.1%)	
Gender			0.484
Male	10 (58.8%)	4 (44.4%)	
Female	7 (41.2%)	5 (55.6%)	
Tumour localization♦			0.056
Head and neck	4 (26.7%)	0	
Trunk	6 (40%)	2 (25%)	
Extremity	3 (20%)	6 (75%)	
Other	2 (13.3%)	0	
Histological subtype♦			0.555
SSM	2 (16.7%)	2 (28.6%)	
NM	4 (33.3%)	4 (57.1%)	
LMM	1 (8.3%)	0	
ALM	1 (8.3%)	1 (14.3%)	
Mucosal	2 (16.7%)	0	
Other	2 (16.7%)	0	

Characteristics	Ipilimumab n= 26		χ^2 test
	Primary resistant n= 17 (65.4%)	DC (CR, PR, SD) n= 9 (34.6%)	
Stage at initial diagnosis♦			0.676
I	2 (14.3%)	2 (22.3%)	
II	4 (28.6%)	3 (33.3%)	
III	6 (42.9%)	4 (44.4%)	
IV	2 (14.2%)	0	
Number of organs with metastases			0.114
1-3	13 (76.5%)	9 (100%)	
>3	4 (23.5%)	0	
Brain metastases			0.215
No Brain metastases	16 (94.1%)	7 (77.8%)	
Brain metastases	1 (5.9%)	2 (22.2%)	
Liver metastases			0.067
No liver metastases	9 (52.9%)	8 (88.9%)	
Liver metastases	8 (47.1%)	1 (11.1%)	
BRAF mutation♦			0.494
BRAF mutation	3 (27.3%)	3 (42.9%)	
BRAF wild type	8 (72.7%)	4 (57.1%)	
LDH level♦			0.537
Normal	6 (42.9%)	4 (57.1%)	
Elevated	8 (57.1%)	3 (42.9%)	
S100 level♦			
Normal	7 (43.8%)	4 (50%)	0.772
Elevated	9 (56.2%)	4 (50%)	

♦ Patients for which the information was unknown were excluded

3.7 Overall survival according to type of checkpoint inhibitor

Figure 5 shows the OS of the immunotherapy-treated collective divided by type of checkpoint inhibitor. There was no significant difference in OS between the patients treated with PD-1 monotherapy, combined immunotherapy of nivolumab plus ipilimumab and ipilimumab monotherapy. Table 14 shows that the mOS was 15 months for the ipilimumab-treated group (95% CI: 9-21) while it was 26 months - almost twice as high- for the patients treated with PD1-monotherapy or combined immunotherapy of nivolumab plus ipilimumab (95% CI: 19.7-32.3 and 20.5-31.5, respectively). Regarding survival rates, the numbers are very similar between the PD1-monotherapy group and the combination immunotherapy group. 1-y OS was around 70%, dropped gradually to 40% after three years. In the ipilimumab group the 1-y OS was 65.4% (95% CI: 47.2-83.6) and decreased by half after three years.

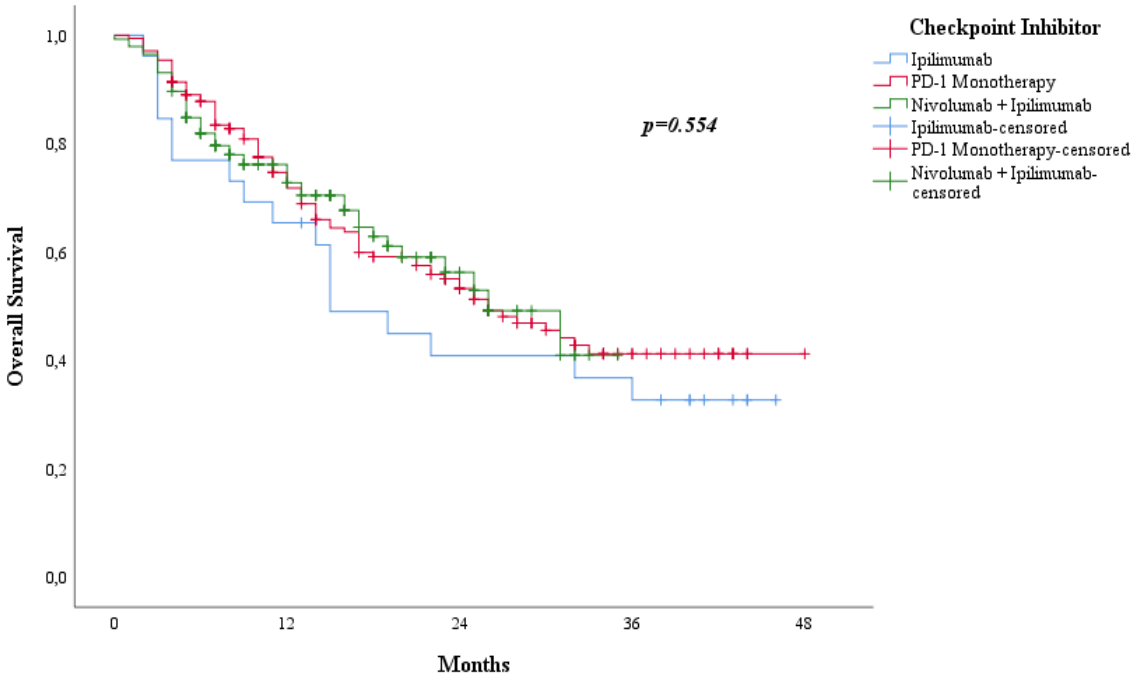

Figure 5. Overall survival according to type of checkpoint inhibitor.

Table 14

Median overall survival and overall survival rates according to type of checkpoint inhibitor

Type of checkpoint inhibitor	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
Ipilimumab N=26	15 (9-21)	65.4 (47.2-83.6)	40.9 (21.7-60.1)	32.7 (14.3-51.1)
PD1-Mono N=174	26 (19.7-32.3)	71.8 (64.7-78.9)	53.2 (45-61.4)	41.2 (32-50.4)
Nivo+Ipi N=145	26 (20.5-31.5)	72.8 (65-80.6)	56.2 (44.8-67.6)	41 (22.6-59.4)

3.8 Progression-free survival according to type of checkpoint inhibitor

In terms of PFS there is no significant difference between the different types of therapies as can be seen in *Figure 6*. The mPFS ranged from 4.0 months for ipilimumab monotherapy (95% CI: 3.34-4.66) over 8.0 months for PD1-monotherapy (95% CI: 5.52-10.48) and was 9.0 months for the combined immunotherapy (95% CI: 1.77-16.23). This is displayed in Table 15. The 1-y PFS was 27% in the ipilimumab group, 40% in the PD-1 monotherapy group and 50% in the combination immunotherapy group.

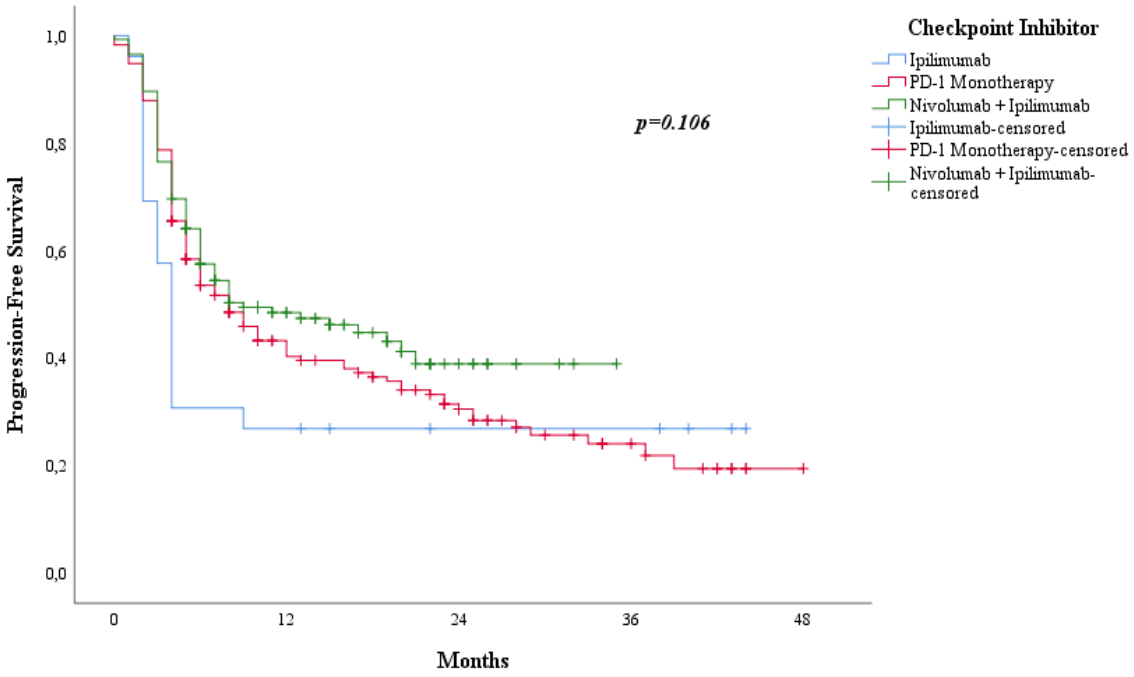

Figure 6. Progression-free survival according to type of checkpoint inhibitor.

Table 15

Median progression-free survival and progression-free survival rates according to type of checkpoint inhibitor

Type of checkpoint inhibitor	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
Ipilimumab	4.0 (3.34-4.66)	26.9 (9.8-44)	-	-
PD1-Mono	8.0 (5.52-10.48)	40.8 (33.2-48.4)	31.1 (23.7-38.5)	24.7 (16.9-32.5)
Nivo+Ipi	9.0 (1.77-16.23)	49.5 (41.1-57.9)	40 (30-50)	-

3.9 Comparison between PD-1 monotherapy and nivolumab plus ipilimumab, considering patients' characteristics

When comparing patients' characteristics between the two large therapy groups – PD-1 monotherapy and combined immunotherapy – significant differences were observed (see Table 16).

There was a significant difference between the PD-1 monotherapy group and the combined immunotherapy group in terms of age groups, BRAF mutation, number of organs with metastases, presence of brain metastases and level of LDH at time of stage IV diagnosis.

The proportion of patients in the age group 60-75 was similar in both therapy groups. However, there were 40% of the patients above 75 years of age in the anti-PD-1 monotherapy subgroup compared to 25% in the combination therapy group. The combination therapy group had a higher proportion of younger patients (40% vs. 25%).

Concerning BRAF mutation the PD-1 therapy group had a higher proportion (42%) of BRAF wildtype patients than the combined immunotherapy group (22.8%).

Regarding the number of organs with metastases, the percentage was twice as high in the combined immunotherapy-treated group than in the PD-1 monotherapy group. Additionally, the number of patients with brain metastases was also higher in the nivolumab plus ipilimumab group. One-fourth of the patients had brain metastases while it was only 14.4% in the PD-1 group.

There was no significant difference between the presence or absence of liver metastases between the two groups.

As for the LDH level at time of stage IV diagnosis, the information was missing for a bigger part of PD-1 treated patients (14.4%) than for the combined immunotherapy treated ones (8.4%).

These results show that the groups were not homogenous.

Table 16

Patient characteristics' and crosstabs of PD-1 mono- and combination therapy showing heterogeneity between the groups

Characteristics	**PD-1 Mono- or Combination therapy** n= 319		χ^2 test
	PD-1 Monotherapy	**Nivolumab + ipilimumab**	
Age			0.007
<60	44 (25.3%)	57 (39.3%)	
60-75	62 (35.6%)	52 (35.9%)	
>75	68 (39.1%)	36 (24.8%)	
BRAF♦			0.003
BRAF mutation	42 (36.5%)	46 (58.2%)	
BRAF wild type	73 (63.5%)	33 (41.8%)	
Number of organs with metastases			0.033
1-3	161 (92.5%)	124 (85.5%)	
>3	13 (7.5%)	21 (14.5%)	
Brain metastases			0.013
No Brain metastases	149 (85.6%)	109 (75.2%)	
Brain metastases	25 (14.4%)	36 (24.8%)	

Characteristics	PD-1 Mono- or Combination therapy n= 319		χ^2 test
	PD-1 Monotherapy	Nivolumab + ipilimumab	
Liver metastases			0.072
No liver metastases	118 (67.8%)	86 (59.3%)	
Liver metastases	56 (32.2%)	59 (40.7%)	
LDH level♦			0.4
Normal	101 (70.1%)	89 (65.4%)	
Elevated	43 (29.9%)	47 (34.6%)	
S100 level♦			
Normal	82 (55%)	75 (56.4%)	0.819
Elevated	67 (45%)	58 (43.6%)	

♦ patients for which the information was unknown were excluded

3.10 Overall survival according to type of checkpoint inhibitor considering best response

3.10.1 PD-1 monotherapy

Figure 7 depicts the OS of patients treated with PD-1 monotherapy according to response to therapy. As can be seen in Table 17, the proportion of patients with CR, PR and SD was very similar, around 20%. Primary resistant patients made up 43% of the PD-1 monotherapy-treated group. The mOS was not reached for the patients with CR and PR at the time of the data cut-off analysis. SD patients showed a mOS of 25 months (95% CI: 20.61-29.39) and in the primary resistant patients the mOS was 12 months (95% CI: 9.58-14.42). In terms of survival rates, the 1-y OS for the primary resistant patients was 46.4% which dropped to 18.9% after two years and 12.2% after three years (95% CI: 34.4-58.4, 9.1-28.7 and 3.2-21.2, respectively).

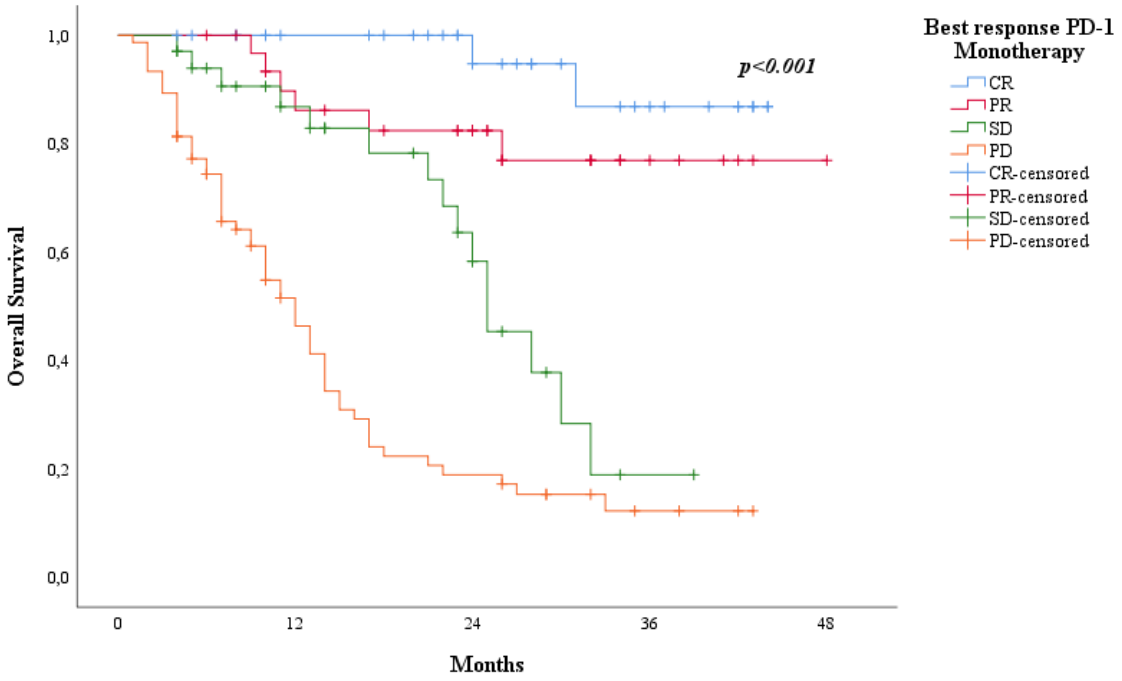

Figure 7. Overall survival of PD-1 monotherapy treated patients according to best response.

Table 17

Median overall survival and overall survival rates of PD-1 monotherapy treated patients according to best response

Best response to PD-1 Monotherapy N=174	**Median OS** (months; 95% CI)	**OS** (%; 95% CI) **1-y OS**	**2-y OS**	**3-y OS**
CR N=33 (19%)	not reached	-	94.7 (84.7-100)	86.8 (69.4-100)
PR N=32 (18.4%)	not reached	86.2 (73.7-98.7)	82.4 (68.3-96.5)	76.9 (60.2-93.6)
SD N=34 (19.5%)	25 (20.61-29.39)	86.8 (74.6-99)	58.3 (37.7-78.9)	18.9 (0-40.7)
PD N=75 (43.1%)	12 (9.58-14.42)	46.4 (34.4-58.4)	18.9 (9.1-28.7)	12.2 (3.2-21.2)

3.10.2 Nivolumab plus ipilimumab

Figure 8 depicts the OS of ipilimumab plus nivolumab-treated patients according response to therapy. As can be seen in Table 18, the largest proportion of patients are the ones primary resistant (36%), closely followed by PR (33%). One-fifth had SD under the combined immunotherapy and 11% CR. Similarly to the PD-1 therapy group, the mOS was not reached at the time of the data cut-off analysis. SD patients had a mOS of 31 months which is six months longer than in the PD-1 monotherapy

group (95% CI: 9.95-52.05). The primary resistant patients had a mOS of 9 months, three months less than in the PD-1 group (95% CI: 5.31-12.69). In terms of survival rates, the 1-y OS for the primary resistant patients treated with combination therapy was 36.4% and dropped to 10.1% after two years (95% CI: 0-20.7 and 9.1-28.7, respectively).

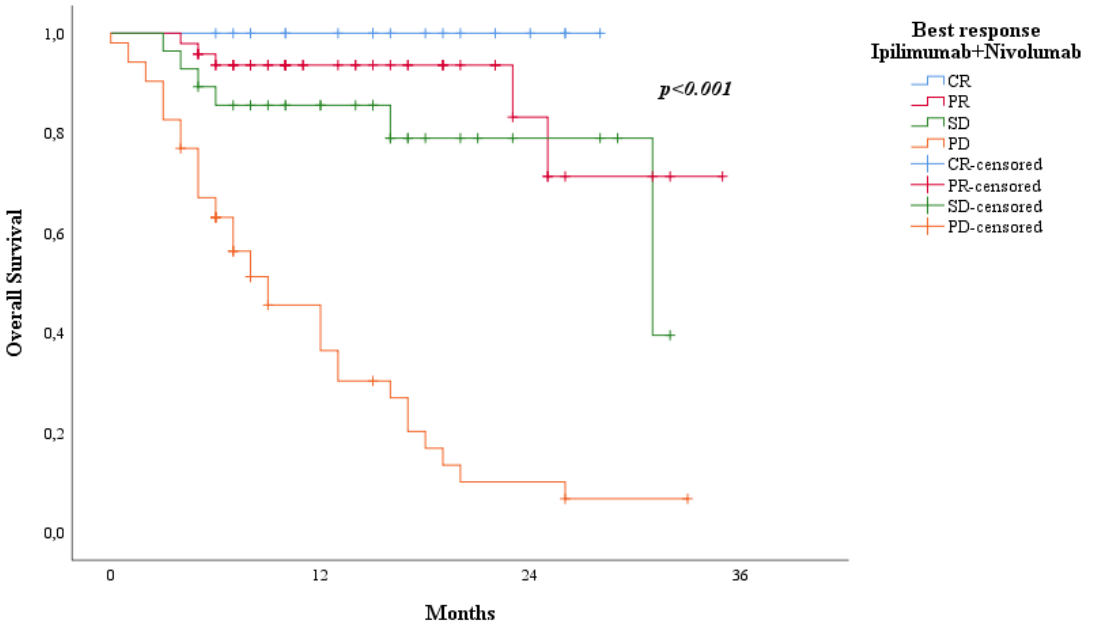

Figure 8. Overall survival of patients treated with combination therapy of ipilimumab plus nivolumab according to best response.

Table 18

Median overall survival and overall survival rates of combination therapy treated patients with ipilimumab plus nivolumab according to best response

Best response to Nivolumab + Ipilimumab N=145	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
CR N=17 (11.7%)	not reached	-	-	-
PR N=48 (33.1%)	not reached	93.6 (86.5-100)	83.2 (72.9-100)	-
SD N=28 (19.3%)	31 (9.95-52.05)	85.6 (72.5-98.7)	79 (61.8-96.2)	39.5 (0-95)
PD N=52 (35.9%)	9 (5.31-12.69)	36.4 (21.5-51.3)	10.1 (0-20.7)	-

3.11 Progression-free survival according to type of checkpoint inhibitor considering best response

3.11.1 PD-1 monotherapy

Regarding PFS of the patients treated with PD-1 monotherapy a significant difference can be detected depending on the type of response (p<0.001). *Figure 9* demonstrates the different graphs. Median PFS as well as 1-, 2- and 3-y PFS can be understood from Table 19. At the time of the data cut-off analysis the mPFS was not reached in the CR group. For PR patients mPFS was 37 months (95% CI: 13.45-60.55), for the SD patients it was 10 months (95% CI: 7.65-12.35) and primary resistant patients it was 4 months (95% CI: 3.48-4.52).

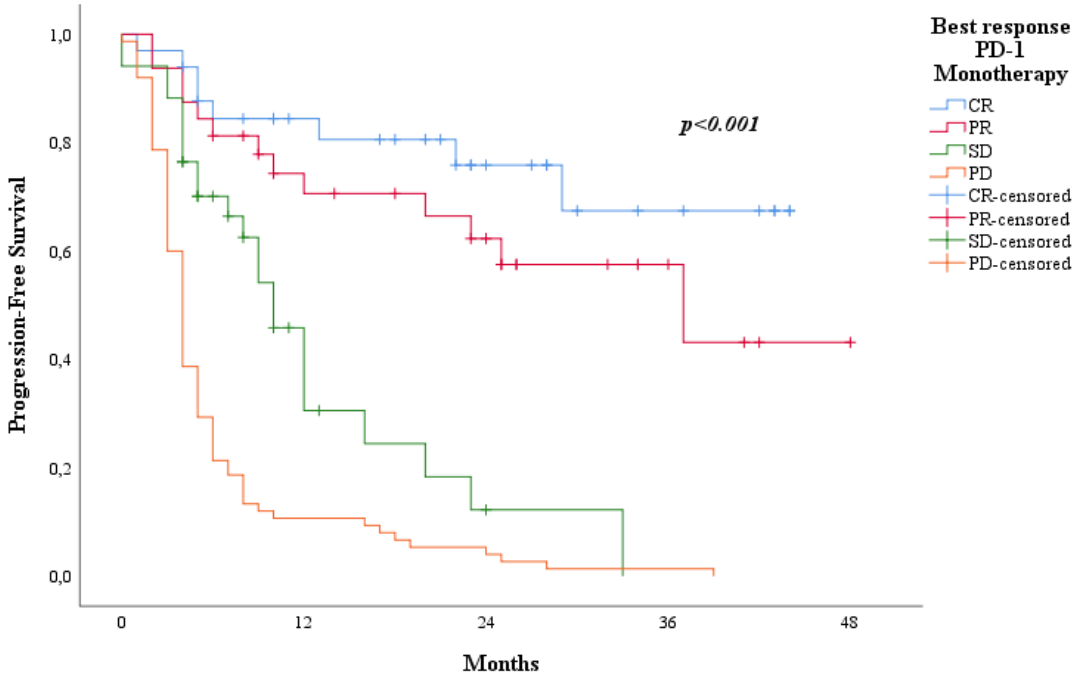

Figure 9. Progression-free survival of PD-1 monotherapy treated patients according to best response.

Table 19

Median progression-free survival and progression-free survival rates of PD-1 monotherapy treated patients according to best response

Best response	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
CR N=33	Not reached	80.6 (66.5-94.7)	75.9 (59.8-92)	67.4 (46.2-88.6)
PR N=32	37 (13.45-60.55)	70.6 (54.3-86.9)	62.3 (44.3-80.3)	-
SD N=34	10 (7.65-12.35)	30.6 (11.8-49.4)	12.2 (0-27.3)	-
PD N=75	4 (3.48-4.52)	10.7 (3.6-17.8)	4 (0-8.5)	1.3 (0-3.8)

3.11.1 Nivolumab plus ipilimumab

Concerning PFS of the patients treated with combined immunotherapy of ipilimumab plus nivolumab a significant difference can be detected depending on the type of response (p<0.001). *Figure 10* demonstrates the different graphs. Median PFS as well as 1-, 2- and 3-y PFS can be understood from Table 20. The mPFS was not reached in the CR and PR group at the time of data cut-off analysis. For SD patients the mPFS was 19 months and for the primary resistant patients it was 3 months (95% CI: 7.17-30.84 and 2.26-3.74, respectively).

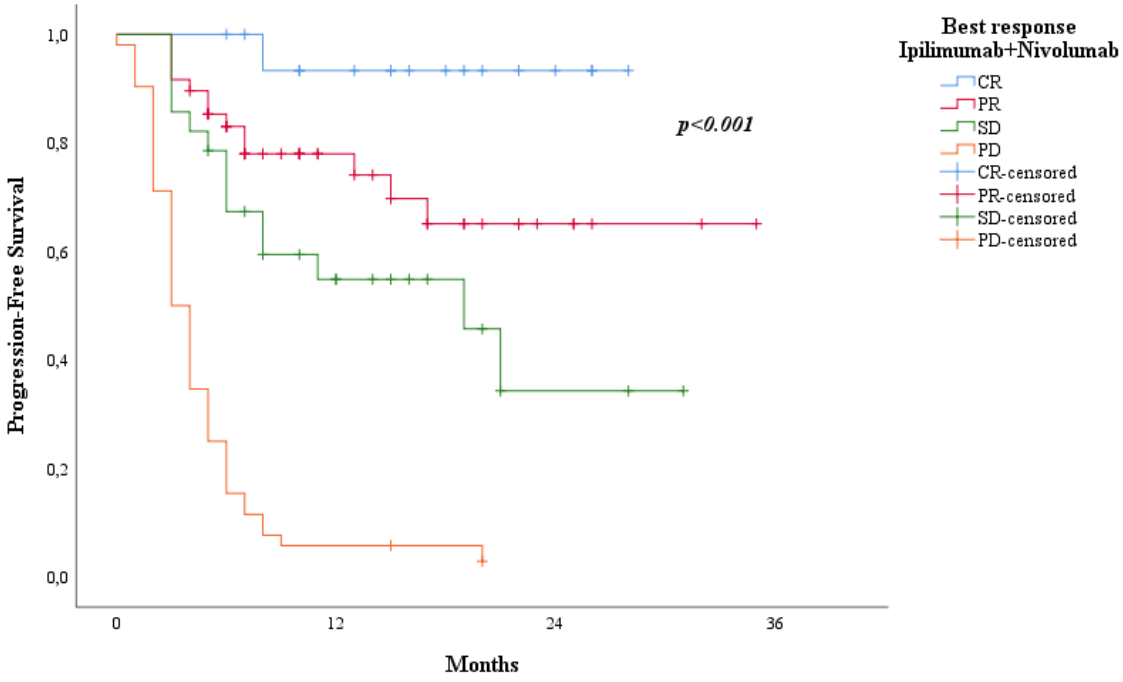

Figure 10. Progression-free survival of patients treated with combination therapy of ipilimumab plus nivolumab according to best response.

Table 20

Median progression-free survival and progression-free survival of combination therapy treated patients with ipilimumab plus nivolumab according to best response

Best response to **Ipi +Nivo**	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
CR N=17	Not reached	93.3 (80.8-100)	-	-
PR N=48	Not reached	78 (65.8-90.2)	65.1 (48.2-82)	-
SD N=28	19 (7.17-30.84)	54.9 (35.7-74.1)	34.3 (8.4-60.2)	-
PD N=52	3 (2.26-3.74)	5.8 (0-12.1)	2.9 (0-8)	-

3.12 Overall survival according type of checkpoint inhibitor considering objective response

3.12.1 PD-1 monotherapy

When dividing the OS of patients treated with PD-1 inhibitor according to objective response and no objective response a significant difference can be noticed (p<0.001). The data is presented in *Figure 11* and Table 21.

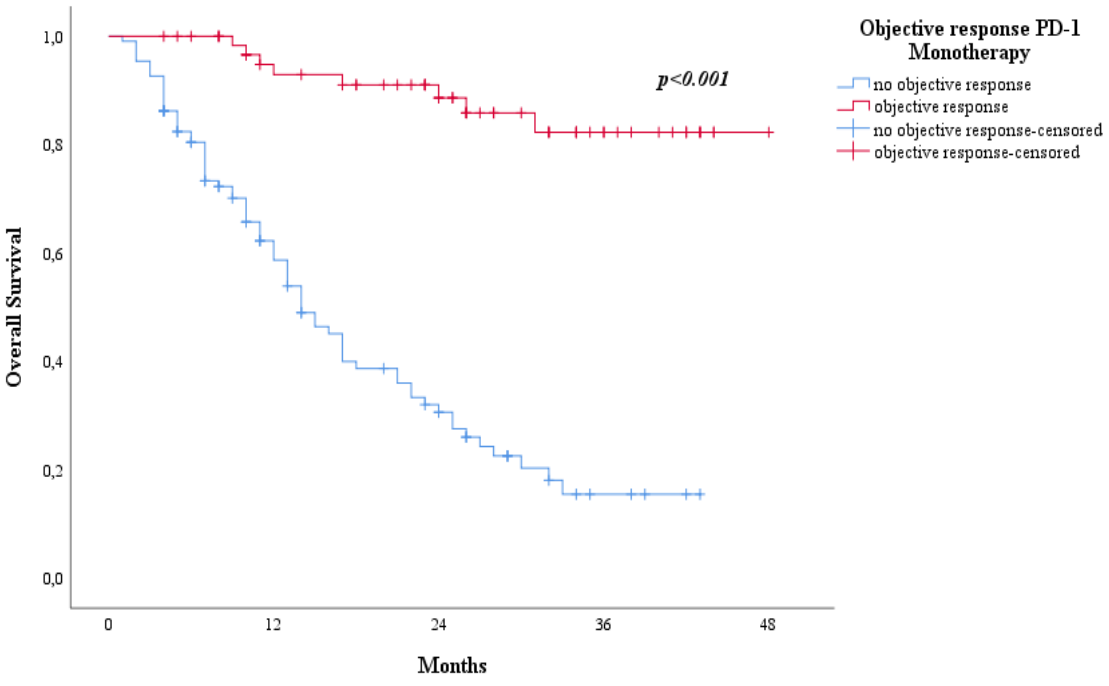

Figure 11. Overall survival of PD-1 treated patients according to objective and no objective response.

Table 21

Median overall survival and overall survival rates of PD-1 treated patients according to objective and no objective response

Response to **PD-1 Monotherapy**	**Median OS** (months; 95% CI)	**OS** (%; 95% CI) **1-y OS**	**2-y OS**	**3-y OS**
No objective response	14 (11.26-16.75)	58.7 (48.9-68.5)	30.6 (20.6-40.6)	15.5 (6.1-24.9)
Objective response	Not reached	92.9 (86.2-99.6)	88.6 (80-97.2)	82.3 (70.5-94.1)

3.12.2 Nivolumab plus ipilimumab

Equally to the PD-1 group, a significant difference regarding OS (p<0.001) is evident between the no objective response and objective response group treated with combined immunotherapy and the data are displayed in *Figure 12* and Table 22.

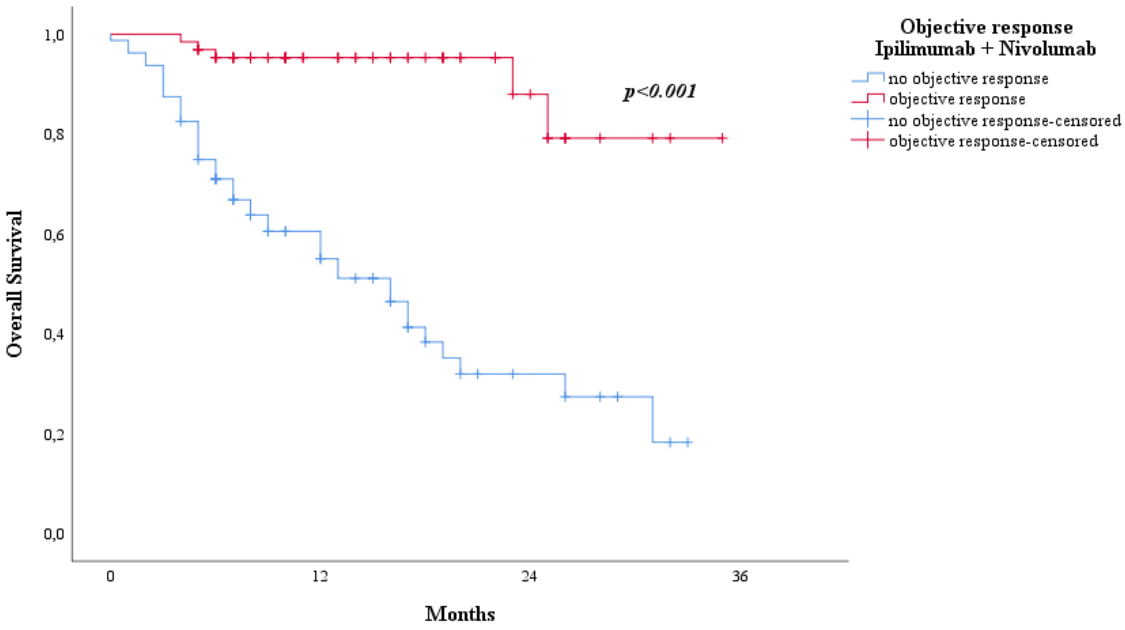

Figure 12. Overall survival of combination therapy treated patients with ipilimumab plus nivolumab according to objective and no objective response.

Table 22

Median overall survival and overall survival rates of combination therapy treated patients with ipilimumab plus nivolumab according to objective and no objective response

Response to **Ipi +Nivo**	**Median OS** (months; 95% CI)	**OS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
No objective response	16 (11.41-20.59)	55 (43.2-66.8)	31.9 (18.2-45.6)	18.3(1-35.7)
Objective response	Not reached	95.3 (90.2-100)	88(73.3-100)	-

3.13 Progression-free survival according to type of checkpoint inhibitor considering objective response

3.13.1 PD-1 monotherapy

The PFS of patients treated with PD-1 monotherapy according to objective and no objective response is demonstrated in *Figure 13*. The mPFS was not reached for objective response and was 5 months for no objective response (95% CI: 4.31-5.7) (see Table 23).

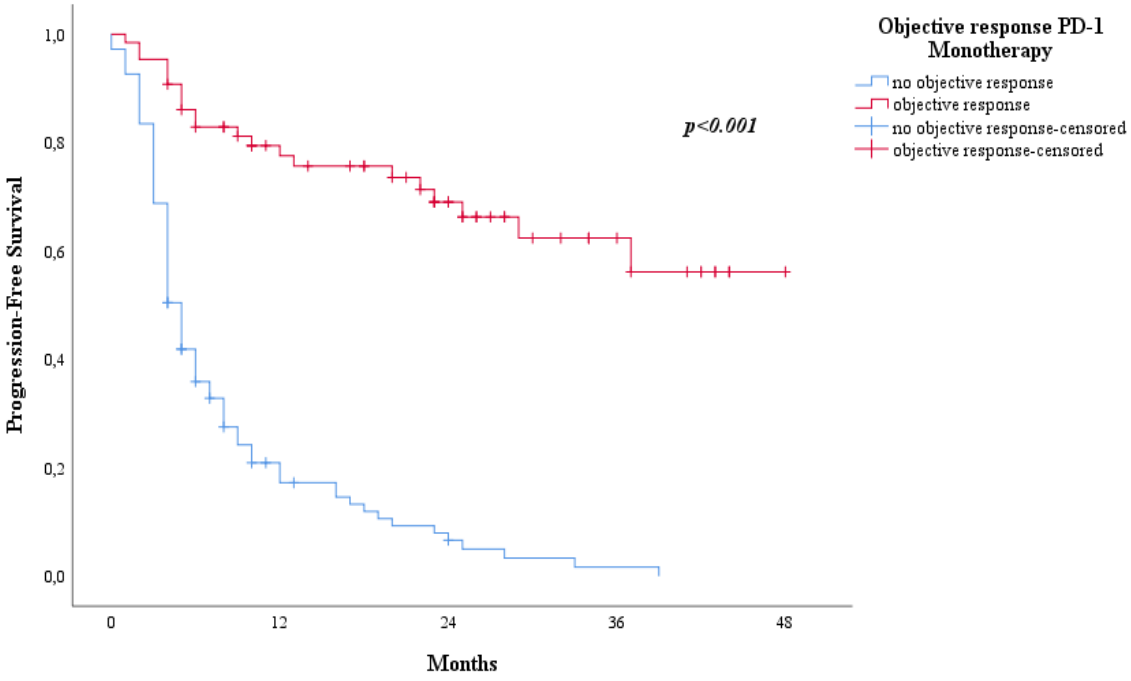

Figure 13. Progression-free survival of PD-1 treated patients according to objective and no objective response.

Table 23

Progression-free survival and median progression-free survival of PD-1 treated patients according to objective and no objective response

Response to **PD-1 Monotherapy**	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI) **1-y OS**	**2-y OS**	**3-y OS**
No objective response	5 (4.31-5.7)	17.2 (9.6-24.8)	6.6 (1.1-12.1)	1.7 (0-4.8)
Objective response	Not reached	77.6 (67.2-88)	6.9 (56.8-81.2)	62.4 (48.3-76.5)

3.13.2 Nivolumab plus ipilimumab

PFS according to objective and no objective response for patients who received combined immunotherapy is illustrated in *Figure 14*. Similarly to the PD-1 group, mPFS for objective response was not yet reached at the time of data cut-off analysis and it is 5 months for ipilimumab plus nivolumab treated patients who did not have an objective response (95% CI: 3.84-6.16) (see Table 24).

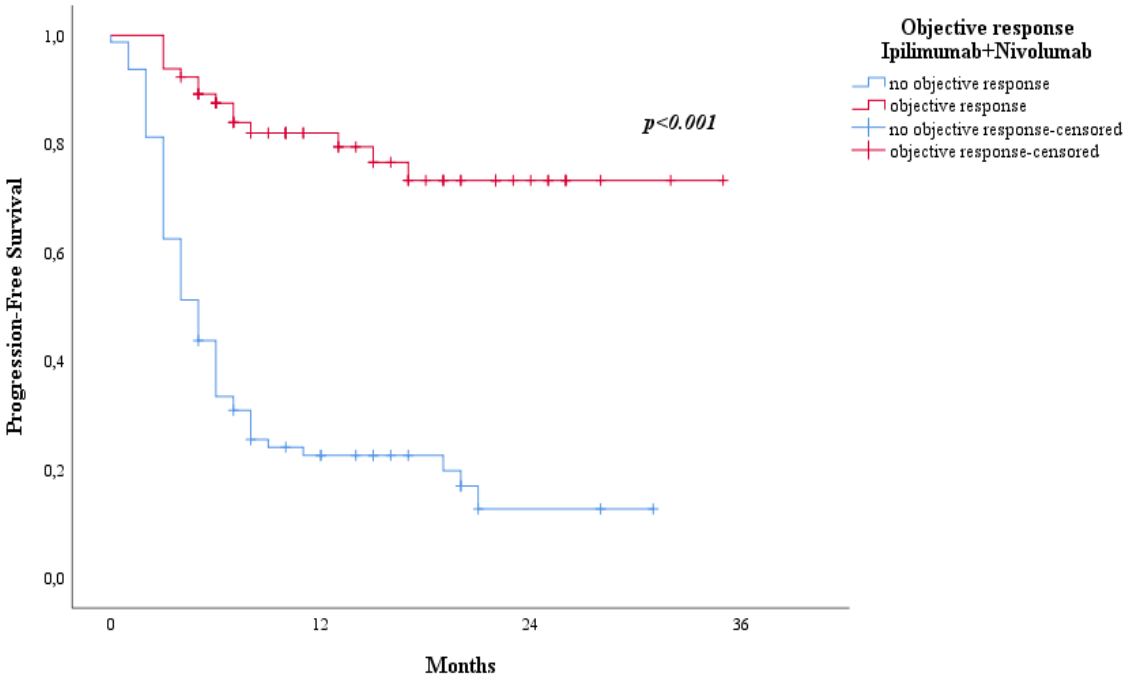

Figure 14. Progression-free survival of combination therapy treated patients with ipilimumab plus nivolumab according to objective and no objective response.

Table 24
Median progression-free survival and progression-free survival of combination therapy treated patients with ipilimumab plus nivolumab according to objective and no objective response

Response to **Ipi +Nivo**	**Median PFS** (months; 95% CI)	**PFS** (%; 95% CI)		
		1-y OS	**2-y OS**	**3-y OS**
No objective response	5 (3.84-6.16)	22.6 (13.2-32)	12.7 (2.5-22.9)	-
Objective response	Not reached	82 (72.2-91.8)	73.3 (60.6-86)	-

3.14 Second-line therapies in primary resistant patients

We also analysed further lines of therapy for the primary resistant patients. Besides the type of systemic therapy, we assessed best response. Moreover, we evaluated the type of therapy in regard to BRAF mutation.

11.1% of the primary resistant patients did not have any available information on a second-line therapy and more than one third did not receive any second-line therapy. Half of the 144 primary resistant patients received a second-line systemic therapy though, compare Table 25 and *Figure 15*. The majority were treated with immunotherapy again (24.3%), while 16.7% received chemotherapy and one-eighth received targeted therapy.

Table 25
Second-line systemic therapy of primary resistant patients

Second-line systemic therapy	**N (%)**
Immunotherapy	35 (24.3%)
Targeted Therapy	18 (12.5%)
Chemotherapy	24 (16.7%)
No systemic therapy	51 (35.4%)
Not available	16 (11.1%)

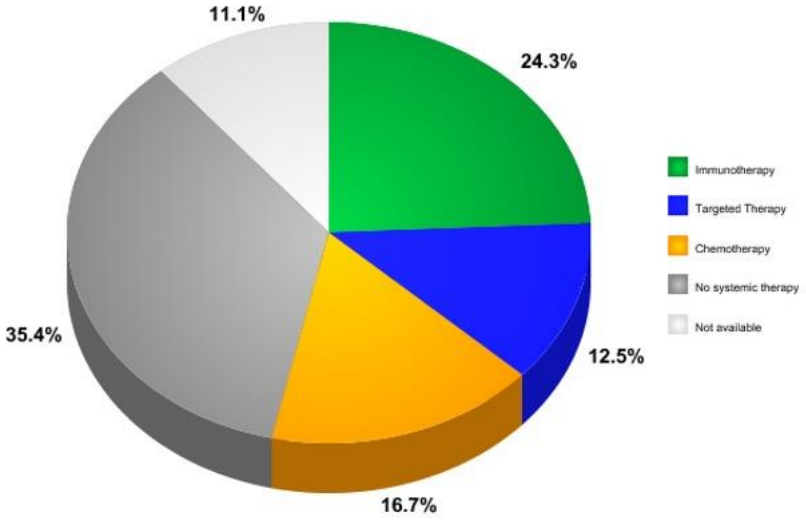

Figure 15. Second-line systemic therapy of primary resistant patients.

The analysis of second-line therapy in terms of BRAF mutation showed that approximately one-fourth of the primary resistant patients harboured a BRAF mutation. The results of this analysis are displayed in table 26 and figure 16. There was no information available on second-line therapy for 8.5% of them. About one-fifth of them did not receive any systemic therapy second-line. Almost half of the primary resistant patient with a positive BRAF status received targeted therapy second-line. More than 10% were treated with second-line immunotherapy and 5% received chemotherapy.

Table 26

Second-line systemic therapy of primary resistant patients with BRAF mutation

Second-line systemic therapy	**Primary resistant patients with BRAF mutation** N=35 (24.3%)
Immunotherapy	5 (14.3%)
Targeted Therapy	17 (48.6%)
Chemotherapy	2 (5.7%)
No systemic therapy	8 (22.9)
Not available	3 (8.5

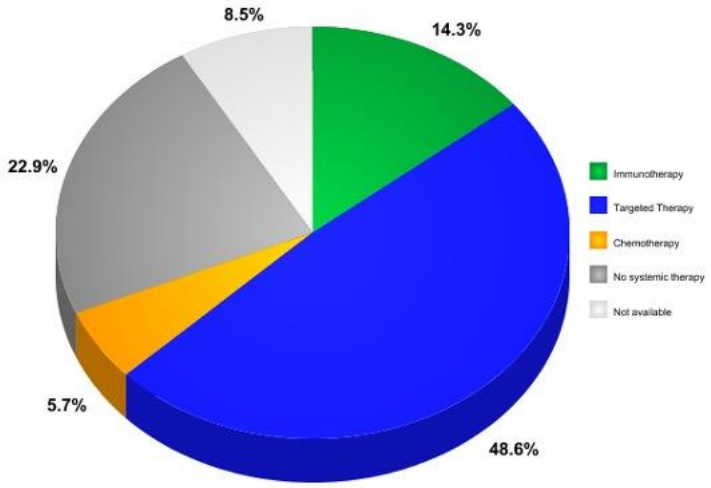

Figure 16. Second-line systemic therapy of primary resistant patients with BRAF mutation.

Since almost half of the primary resistant patients with a positive BRAF status received targeted therapy second-line, we analysed their response. There was no information on the response for one patient. Most of primary resistant patients (52.8%) had a PR to second-line targeted therapy, followed by an equal number of patients with SD and PD (compare Table 27). About 6% had a CR.

Table 27

Response to second-line targeted therapy in primary resistant patients

Response	N (%)
CR	1 (5.9%)
PR	9 (52.8%)
SD	3 (17.6%)
PD	3 (17.6%)
Not available	1 (5.9%)

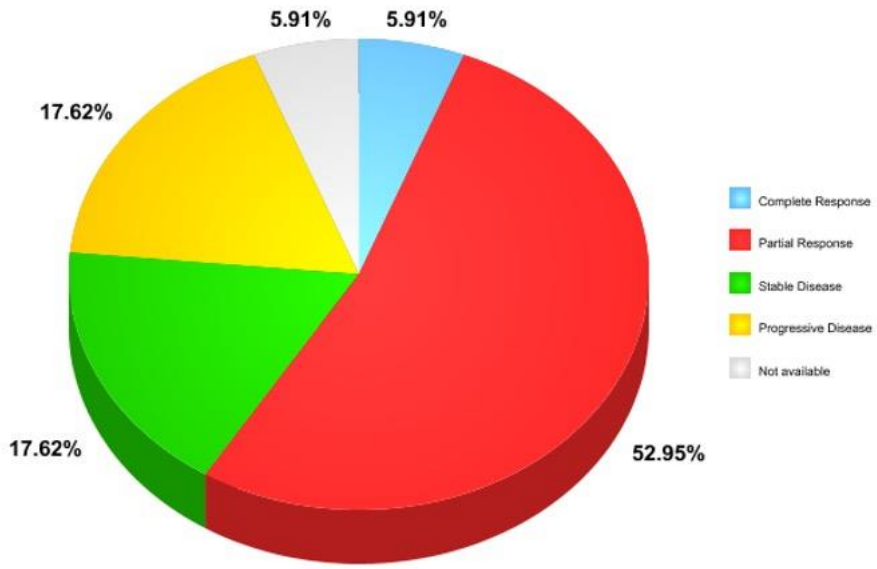

Figure 17. Response to second-line targeted therapy in primary resistant BRAF mutated patients.

Since one-fourth of the primary resistant patients received immunotherapy second-line, we assessed their response to it, compare Table 28 and Figure 18. Data on response was unavailable for 8.6%. The majority of the primary resistant patients showed also resistance to the second-line immunotherapy, 57.1% progressed under it. A similar number of patients – around 15% – had PR and SD and only one patient had a CR.

Table 28

Response to second-line immunotherapy in primary resistant patients

Response	**N (%)**
CR	1 (2.9%)
PR	6 (17.1%)
SD	5 (14.3%)
PD	20 (57.1%)
Not available	3 (8.6%)

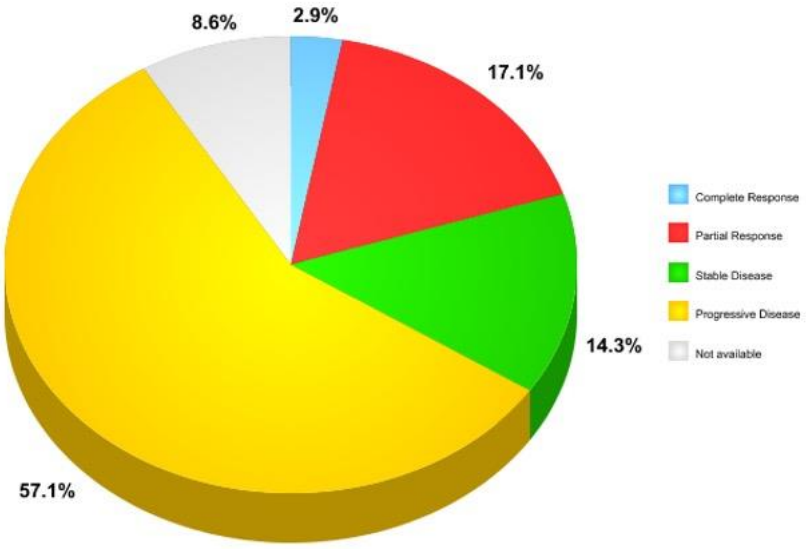

Figure 18. Response to second-line immunotherapy.

The proportion of third-line systemic therapies received by the 35 primary resistant patients that were treated with immunotherapy second-line are shown in Table 29 and *Figure 19*. One-third of the patients had no data about a third-line therapy and one-third did not receive any therapy third-pline. One-fifths received chemotherapy third-line, 14% were treated with immunotherapy and 5% with targeted therapy.

Table 29
Third-line systemic therapy of primary resistant patients receiving second-line immunotherapy

Systemic therapy	**N (%)**
Immunotherapy	5 (14.3%)
Targeted Therapy	2 (5.7%)
Chemotherapy	8 (22.9%)
No systemic therapy	10 (28.55%)
Not available	10 (28.55%)

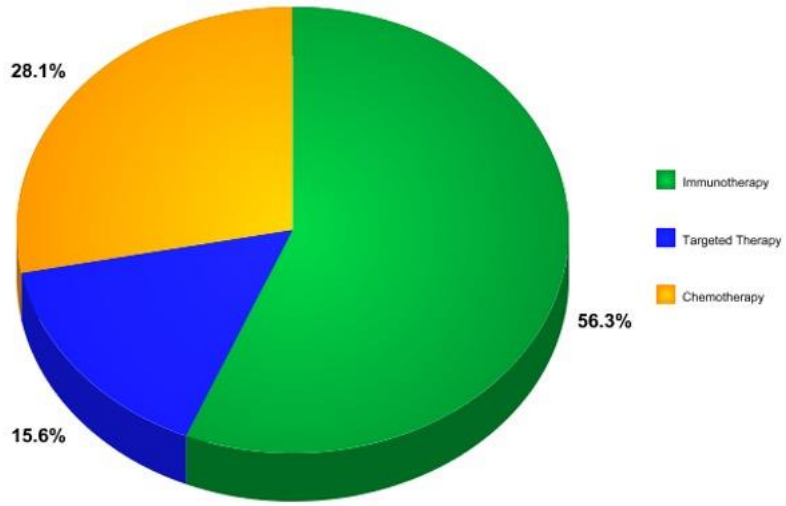

Figure 19. Third-line systemic therapy of patients receiving second-line immunotherapy.

The proportion of third-line systemic therapies received by the primary resistant patients is shown in Table 30 and *Figure 20*. More than half of the primary resistant patients that received a third-line therapy, did receive immunotherapy. Almost one-third received chemotherapy third-line, 15% were treated with targeted therapy.

Table 30

Third-line systemic therapy of primary resistant patients

Third-line systemic therapy	**N (%)**
Immunotherapy	18 (56.3%)
Targeted Therapy	5 (15.6%)
Chemotherapy	9 (28.1%)

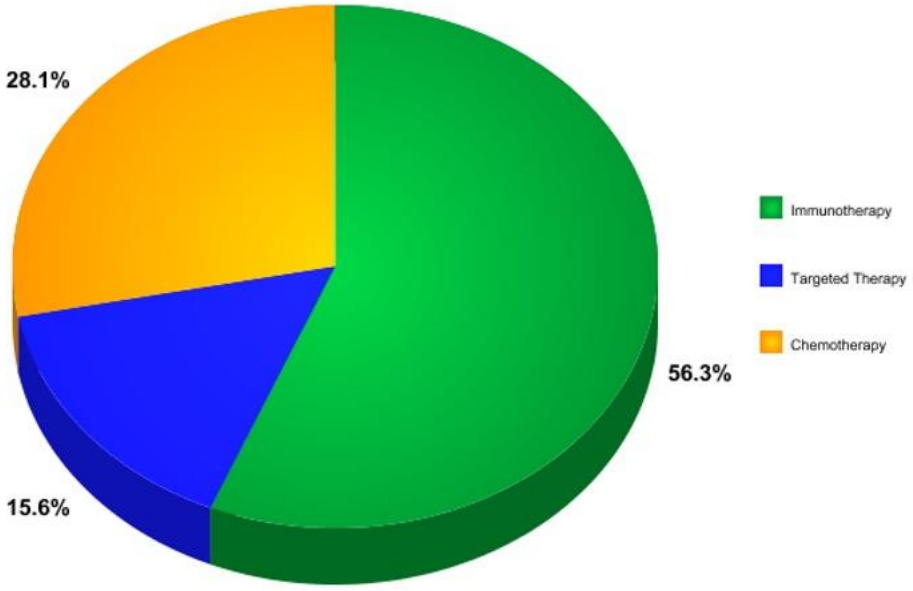

Figure 20. Third-line systemic therapy of primary resistant patients.

4 Discussion of Results

4.1 Most important findings

This work demonstrated that in patients who were primary resistant to immunotherapy the survival was significantly reduced in comparison to responders to immunotherapy.

Patients with primary resistance to immunotherapy presented a worse response to the further therapy lines. In fact, patients that received further therapy lines presented with shorter survival when compared to those that responded to first-line immunotherapy.

Contrary to what we expected, there was no significant difference between mOS and PFS for patients receiving combined immunotherapy and PD-1 monotherapy.

As previously mentioned in Patients and Methods (2.1 Data collection), all the results refer to the collective that includes patients that received first-line immunotherapy, unless otherwise mentioned.

4.2 Patients' collective and prognostic factors

The analysed patient collective consisted of a significant number of patients that translated into a high number of patients treated with immunotherapy (n=347), either in first-line which is the scope of this analysis, as well as in further lines. The size of the collective along with the follow-up time support the statements presented in this discussion when it refers to primary resistant patients. We assessed the OS and survival rates, PFS and evaluated prognostic and predictive factors. For similar retrospective studies in literature a patient collective of 39 to 106 patients receiving first-line immunotherapy can be found. (Cowey et al., 2018, Schilling et al., 2019)

As limitations we need to consider the monocentric and retrospective nature of the study. However, the scope of the documentation with all therapies was very large, and multicentric approach would be difficult to perform in a timely way.

4.2.1 Age and Sex

Comparing epidemiologic data between the collective of patients that received first -line immunotherapy to literature reveals similarities as well as differences.

The majority (36.6%) of our patient population (127/347 patients) were between 60 and 75 years old at the time of stage IV diagnosis of melanoma. 32.3% of the patients (112/347) were over 75 years old at the time of stage IV diagnosis. The median age at diagnosis of metastatic melanoma was 68 years (54.0-74.0) which is slightly higher than Robert et al. (65 years of age) and Postow et al. (65 years of age). (Postow et al., 2015, Robert et al., 2015a) Mean age at the time of stage IV diagnosis of melanoma was 66 years which is also higher than comparable studies from Larkin et al. (60 years of age) and Hodi et al. (56.2 years of age). (Hodi et al., 2010, Larkin et al., 2015). In our collective, the age varied between 19 years (minimum) and 91 years (maximum). The slightly increased median age in our collective can be explained by the fact that we did not consider the exclusion criteria normally present in clinical trials.

The patients' collective comprises approximately 60% males and 40% females. The study from Robert et al. presented a similar ratio between the sexes (58.9% male, 41.1% female) and a higher percentage of male patients was likewise found by Postow et al. (67% males), Hodi et al. (59.3%) and Robert et al. (61%). (Hodi et al., 2010, Postow et al., 2015, Robert et al., 2015a, Robert et al., 2014). When response was considered, i.e. PD and DC, the proportions stayed similar with males being the predominant group (54.2% males and 45.8 females vs.63.5% males and 36.5% females, respectively).

4.2.2 Tumour characteristics

In the analysed collective most patients had their primary tumour on an extremity (42.9%), followed by the trunk (29.5%) and head and neck (21.5%). This distribuation is similar to studies of von Schuckmann et al. and de Vries et al. who reported that 42.6% and 50.3% of patients had a primary tumour on an extremity, 35.3% and 35.4% on the trunk and 19.1% and 14.3% in the head and neck area. (de Vries et al., 2008, von Schuckmann et al., 2019)

The localization of the primary tumour was unknown for 20.7% in our collective so these were excluded from the analysis. There is a significant difference between the primary resistant and DC group considering tumour localization (χ^2 test: p=0.007, see Table 4).

More than 50% of the primary resistant patients (53.8%) were diagnosed with their primary tumour on an extremity while it was only 36.1% of the DC patients. Slightly more than one-fifth of the patients in the primary resistant group had their melanoma on the trunk whereas it was one-third in the DC group. Head and neck as an origin made up only 15.1% in the primary resistant group while the number was 10% higher in the patients with DC (25.4%).

One-fourth of the patients (25.9%) had an unknown histological subtype so they were excluded from the analysis. The majority of patients were diagnosed with NM and SSM - 31.1%. each subgroup. This is similar to other studies where SSM were found in the majority of patients, followed by NM. (de Vries et al., 2008, Lidekaite et al., 2017) The next most frequent histological subtype in our collective was ALM (12.5%), followed by mucosal melanoma (6.6%) and LMM (5.4%). In 13.2% of the patients the histological subtype was referred as other and therefore not included in the previous groups.

In the primary resistant group, the majority of the patients were diagnosed with SSM (34%), followed by NM (22,7%), ALM (16.5%), mucosal melanoma (9.3%) and LMM represented only 1% of the primary resistant patients. In 16.5 % of the patients the histological subtype was classified as other.

4.2.3 Metastases

In our collective almost 90% of patients presented with one to three **organs with metastases** and 10% of patients with more than three organs with metastases. In the CheckMate 067 study a slightly higher number, almost 20% of patients, had more than three metastases (Hodi et al., 2018)

The numbers were similar in the subgroup analysis, although there is a slightly higher number of patients with more than three metastases in the primary resistant group when compared to the DC group (15.3% vs.7.9%, respectively). When looking into the patients that received combined immunotherapy, this difference is even more pronounced. 21.2% of the patients in the primary resistant group had more than three organs with metastases compared to 10.8% of the patients in the DC group.

The number of organs with metastases was shown to be a significant prognostic factor for survival in the primary resistant group (p=0.024), see Table 5. The results are similar to those published in the pooled analysis of Schadendorf et al. where the number of organs

with metastases was also a significant prognostic factor for survival ($p<0.0001$ in the univariate analysis, $p=0.0006$ in the multivariate analysis. (Schadendorf et al., 2017).

As for **brain metastases** there was no significant difference between primary resistant patients and DC patients in our collective. 81% did not have any brain metastases, while 18% did present metastases in the brain. It was a significant factor for survival in the DC group ($p=0.028$). In other studies, the presence of brain metastases was usually an exclusion criterion or underrepresented and the numbers therefore quite low: Postow et al. 3%, Hodi et al. 3.5%, Robert et al. 9%, Hodi et al. 12.1%. (Hodi et al., 2018, Hodi et al., 2010, Postow et al., 2015, Robert et al., 2014)

Regarding the presence of **liver metastases**, more than 60% of the whole collective did not have any metastases in the liver. When assessing the numbers in the subgroups the number of patients with liver metastases was higher in the subgroup of primary resistant patients (41.7%) while only one-third had liver metastases in the DC group. Most trials did not assess liver metastases as a baseline patient characteristic. Wolchok et al. did include it as a characteristic and the number of patients with liver metastases was very similar to ours, one-third of the collective. (Wolchok et al., 2013b) Long et al. assessed the site of metastases regarding visceral and non-visceral. Both categories were shown to be significant in the univariate analysis and therefore significant factors for survival. (Long Georgina V. et al., 2016) In our study the prognostic significance of liver metastases was confirmed in the primary resistant group ($p=0.012$).

4.2.4 LDH and S100

Due to lack of information on the LDH level 13% of the patients in the whole collective had to be excluded. Two-thirds presented an LDH level in a normal range while one-third had an elevated level. This distribution is similar to the one presented in the study from Weide et al. (Weide et al., 2012) There was a significant difference in terms of baseline LDH between primary resistant and DC subgroups. In the primary resistant group 40% of patients had elevated LDH compared to 28% in the DC group. LDH was a significant prognostic factor for survival in the primary resistant group ($p=0.007$).

The prognostic value of LDH in melanoma patients has already been demonstrated in several studies. Diem et al. performed an analysis which included 51.5% of patients with elevated serum LDH at baseline that showed a significant shorter survival for these

patients. (Diem et al., 2016). Weide et al. also found LDH to be a prognostic factor for OS in melanoma patients with distant metastasis. (Weide et al., 2012) Recent pooled analyses by Long et al. and Schadendorf et al. confirmed LDH as a prognostic factor for PFS and/or OS. (Long Georgina V. et al., 2016, Schadendorf et al., 2017)

Regarding the **level of S100** at baseline 11.5% of the patients did not have any information on the tumour marker S100 so they were excluded. 45% presented elevated levels of S100 at the time of diagnosis and there was a significant difference between the two groups of our collective. In the primary resistant group two-thirds of the patients had elevated S100. S100 was found to be a significant marker for survival in both subgroups, the primary resistant group and DC group (p=0.003 and p=0.006, respectively). Several studies have shown the prognostic value of S100 in stage IV melanoma patients. Weide et al. assessed a collective with 40% of patients with elevated S100 level and they were shown to have significantly reduced survival in comparison to the ones with normal LDH level at baseline. (Weide et al., 2012)

4.2.5 BRAF mutation

Unfortunately, 38.3% of the patients in the whole collective did not have any information on their **BRAF status**, so they were excluded from this analysis. In the whole collective, as well as the primary resistant and DC group more than 40% of the patients harboured a BRAF mutation. These findings were similar to the Keynote 006 study where 36% of the patients had a BRAF mutation. (Schachter et al., 2017)

It did not prove to be a significant prognostic factor in any of our subgroup analysis. This is in line with the results of Meckbach et al. who did not find any significant survival differences in stage IV patients according mutational status. (Meckbach et al., 2014)

4.3 Survival

Follow-up time was defined from the date of diagnosis of stage IV disease to the date of last follow-up or death.

The median follow-up in our collective treated by immunotherapy was 23 months (95% CI: 20.5-25.5) which we consider to be informative enough to draw conclusions. The Keynote-006 study and CheckMate 069 study had comparable median follow-up of 22.9

months and 24.5 months. (Hodi et al., 2016, Schachter et al., 2017) The CheckMate 066 on the other hand, reported a median follow-up of 8.9 months for nivolumab treated patients (Robert, 2015), much shorter than our study.

The median overall survival for the whole collective was 26 months (95% CI; 20.52-31.48), which is almost 10 months longer that the one reported by our working group. The mOS for stage IV melanoma patients diagnosed between 2013-2014 was 17 months. (Forschner et al., 2017)

4.3.1 Overall survival of primary resistant patients

In our collective 41.5% (n=144) of the patients were defined as **primary resistant patients** while 58.5% (n=203) were defined as having DC. The category DC included patients with a best response of CR, PR and SD. Patients with primary resistance to immunotherapy had a mOS of 11 months (CI 95%: 8.83-13.17), while the mOS for the DC group was not reached.

The clear difference in terms of OS became apparent when comparing 1-y, 2-y and 3-y OS between the two groups. The 1-y survival in the DC group was two times higher compared to the primary resistance patients' group (91.8% vs. 43.1%, respectively). After two years the number decreased to 17% in primary resistant patients (80.6% 2-y OS of DC group) and reached only 10% (64% DC group) after three years.

4.3.2 Overall survival in regard to objective response

We also assessed the **mOS** in terms of **objective response** and **no objective response**. Objective response included patients with CR and PR which made up two-fifths of the whole collective (40.3%). No objective response comprises SD and PD and accounts for three-fifths of the collective (59.7%).

The mOS has not been reached for the patients presenting with objective response and was 14 months (95% CI: 11.57-16.43) for patients with no objective response.

The 1-y survival was 94.2% for patients with objective response (CR not reached, PR 90.2%) compared to 56.3 % of the patients with no objective response (PD 43.1%, SD 86.4%). The 2-y OS was 87.5% (CR 96.3%, PR 81.3%) and 30.2% (PD 17%,

SD 63.2%), respectively. Finally, the 3-y OS was 78.7% (CR 90.3, PR 69.8%) and 15.9% (PD 10.8%, SD 24.1%), respectively.

The previous results show that, patients achieving a response present with better prognosis compared to those who do not. It is important to mention that in this analysis we considered SD as no objective response, which is not always the case in clinical trials.

4.3.3 Progression-free survival

Regarding **PFS** of the immunotherapy collective, we performed the analysis by categorizing into **best response** and also **objective/no objective response**. The differences between the groups were significant in both types of analyses ($p<0.001$).

Median PFS for the primary resistant patients was 4 months (95%CI; 3.62-4.38), which is the same as the mPFS in the no objective response group (SD+PD; 95%; CI: 3.45-4.55). However, there were differences in the 1-, 2- and 3-y PFS between primary resistant patients and patients with no objective response. The 1-y PFS was two times higher in the no objective response group compared to the primary resistant patients' group. The proportions naturally decreased in both groups in a similar manner, reaching 1% in the primary resistant patients and 2.6% in the no objective response patients after three years.

Available literature focuses their analyses on the type of immunotherapy. Due to this fact there were no comparable numbers for mOS and PFS for our immunotherapy collective.

4.3.4 Overall survival and progression-free survival according to type of immunotherapy

We also categorized our collective according to the type of immunotherapy received. There were three groups. The first group consisted of patients who received PD-1 monotherapy as first-line immunotherapy, they made up 50% of the collective. The second group (42%) were patients treated with combined immunotherapy - ipilimumab plus nivolumab and the third group with the smallest number of patients received CTLA-4 monotherapy (7.5%). Due to the small number of patients treated with CTLA-4 monotherapy, they were omitted in the subsequent survival analyses.

The **median overall survival** according to **type of immunotherapy** the patients received did not show any significant difference between the patients treated with

PD-1 monotherapy or combined immunotherapy of nivolumab plus ipilimumab. Median OS was 26 months in both groups (95% CI: 19.7-32.3 vs.20.5-31.5, respectively). The CheckMate 067 study showed a mOS of 36.9 months for nivolumab treated patients and the mOS for combination therapy patients had not been reached (95% CI: 28.3-not reached). (Hodi et al., 2018)

1-y OS was 71.8% for the patients treated with PD-1 monotherapy, decreased to 53.2% at the year 2 mark and finally to 41.2% after 3 years (95% CI: 64.7-78.9, 45-61.4 and 32-50.4, respectively). The CheckMate 067 study reported a slightly higher 3-y OS of 59% and a 3-y OS rate that was around 10% higher than in our subgroup (52%). (Wolchok et al., 2017)

Very similar to the PD-1 monotherapy treated group, 72.8% of patients receiving combined immunotherapy were alive after one year, 56.2% after two years and 41 % after three years (95% CI: 65-80.6, 44.8-67.6 and 22.6-59.4, respectively). Compared to the CheckMate 067 study which reported a 2-y OS of 64% and a 3-y OS of 58%, the survival rates of our combined immunotherapy group are lower. (Wolchok et al., 2017)

In a similar manner there was no significant difference between PD-1 monotherapy and combined immunotherapy regarding **PFS**. While the patients treated with PD-1 monotherapy had a mPFS of 8 months (95% CI: 5.52-10.48), the group that received combined immunotherapy had a mPFS of 9 months (95% CI: 1.77-16.23). In literature a slightly lower number of mPFS is reported for nivolumab, 5.1 months and 6.9 months (95% CI: 3.5-10.8 and 5.1-10.2, respectively). (Hodi et al., 2018, Robert et al., 2015a) whereas the mPFS for combined immunotherapy was slightly higher than ours in the CheckMate 067 study (11.5 months; 95% CI: 8.7-19.3). (Hodi et al., 2018)

The lack of any significant difference between the treatment groups in terms of OS and PFS can be explained by taking a closer look at the groups. When analysing the patient characteristics using the χ^2 test it became evident that they significantly differ in many points (compare Table 16). More patients in the combination therapy group had more than three organs with metastases compared to the group treated with PD-1 monotherapy (14.5% vs. 7.5%, respectively; p=0.033). Regarding the presence of brain metastases, 25% of the patients receiving combination therapy presented with brain metastases compared to 15% in the group receiving PD-1 monotherapy (p=0.013). Finally, the number of patients with elevated LDH was also higher in the combination group (32.5% vs. 24.7%; respectively; p=0.008).

All in all, these significant differences between the therapy groups show that the groups were not homogeneous. In a retrospective study such as this one, these differences cannot be mitigated and there might have been a selection bias regarding choice of treatment. Patients with worse prognostic factors were more frequently treated with the combined immunotherapy.

4.3.5 Survival according to type of immunotherapy and best response

We performed a survival analysis for each type of immunotherapy with regard to best response.

Primary resistance was the largest proportion in the PD-1 monotherapy-treated group with 43%. The proportions for CR, PR and SD were very similar, around 20%. Our PD-1 collective had rather a high percentage of primary resistant patients. Other studies reported numbers of 33%, 38%, 42%. (Hodi et al., 2018, Larkin, 2017, Robert et al., 2015a)

The percentage of primary resistant patients in the combination immunotherapy group was lower than in the PD-1 group. 36% were primary resistant to ipilimumab plus nivolumab therapy, which is also higher than what was reported in other studies - 16% and 24%. (Hodi et al., 2016, Hodi et al., 2018)

Interestingly, our collective showed higher CR numbers for the PD-1 patients compared to what has been reported in the literature. In the group treated with PD-1 monotherapy 19% presented with CR. The CheckMate studies reported 6%, 7.6% and 18% CR. (Hodi et al., 2018, Larkin, 2017, Robert et al., 2014) CR in the combined immunotherapy group was 12% which is around 10% less than previously reported. (Hodi et al., 2016, Hodi et al., 2018)

Moreover, we divided the two types of immunotherapy groups into objective response and no objective response. There was no significant difference in terms of survival for no objective response between the group receiving PD-1 monotherapy and combined immunotherapy. The mOS was 14 months (95% CI: 11.26-16.75) and 16 months (95% CI: 11.41-20.59) and the mPFS was 5 months for both (95%CI: 4.31-5.7 and 3.84-6.16, respectively).

4.4 Strengths and limitations

The current study presents several positive aspects.

First, the high number of patients documented (n=607) and included (n=530), as well as the ones receiving immunotherapy in first-line (n=347).

Second, the follow-up time (FUP=23 months) is long which can support our conclusions.

Third, the primary resistant patient subgroup contains a similar number of patients treated with PD-1 monotherapy as well as combined immunotherapy with ipilimumab plus nivolumab, representing the real-world population of patients that are treated with immunotherapy in stage IV melanoma.

Fourth, the long period of time documented. The patients' collective consists of patients diagnosed with stage IV melanoma between 2015-2018. At that time, immunotherapies were reimbursed so the collective includes not only patients that were initially included in clinical trials but also patients that received immunotherapy as part of standard therapy supporting the fact that our conclusions might be applied to other real-world collectives.

Fifth, the CMMR database is a prospective database that is continuously updated.

Sixth, all therapeutic decisions were validated in a multi-disciplinary setting, since all patients were discussed in the tumour board where the individually best suited therapy options were chosen.

The study presents also some limitations.

First, this is a monocentric, retrospective study and therefore a selection bias cannot be completely excluded. This can be confirmed by the fact that the characteristics of the subgroups receiving PD-1 monotherapy and combined immunotherapy were non-homogeneous, indicating that more patients with worse prognostic factors received combined immunotherapy.

Second, some patients were treated not only in our clinic but also in other institutions, and in a small number of cases, due to the new laws related to the exchange of clinical information, it was seemingly difficult to have a complete overview of the therapies received.

Third, our analysis included only outcomes defined by the systemic therapies received. Local therapies as surgery or radiotherapy that definitely play a role in this setting were not taken into account.

5 Summary

This study aimed to identify and characterize stage IV melanoma patients who are primary resistant to checkpoint immunotherapy. Primary resistance to therapy was defined as progressive disease in the first staging that took place approximately three months after starting the therapy.

Further aims included assessing patients' characteristics, best response to immunotherapy, mOS and survival rates as well as mPFS and PFS rates. Finally, we wanted to evaluate prognostic and/or predictive factors of primary resistance to immunotherapy.

We analysed 530 stage IV melanoma patients that were diagnosed with metastatic melanoma between 2015 and 2018. 347 patients were treated with immunotherapy in first-line of which 144 were considered primary resistant, i.e. the best response was progressive disease that was observed at the time of first staging.

The relevant data for this analysis were obtained by research in the patient record database of the University Hospital Tübingen (SAP ISH GUI for Windows, Copyright 1993-2004) and the database of the CMMR of the DDG. The data were collected with Epi Info™, an open-source statistical software. The data from the CMMR and the data from Epi Info™ were merged into a final SPSS file.

The entire patients' population was characterized using descriptive statistical analysis, frequency tables and cross tables. Kaplan-Meier curves were used to determine survival probabilities and median survival time using log-rank tests for statistical significance. Differences with a *p*-value of 0.05 were considered significant. In addition, the 1-, 2- and 3-year survival rates were calculated with a 95% confidence interval.

Follow-up time was defined as the time between the date of diagnosis of stage IV melanoma and the date of last contact or death. In the OS analyses all causes of death were considered.

The prognostic factors in patients with primary resistance were found to be baseline level of S100, baseline level of LDH, number of organs with metastases and presence of liver metastases.

Patients with primary resistance had a significantly worse prognosis compared to those that did not have primary resistance to immunotherapy (2-y OS: 17% vs.80.6%; 95% CI: 9.9-24.1 and 73.4-87.6, respectively).

Regarding the type of immunotherapy received, we have found no difference in terms of survival between combination immunotherapy and monotherapy with PD-1 (the mOS was 26 months in both groups). This is probably due to the fact that a selection bias was present when choosing the type of immunotherapy received. In fact, when we analysed both groups, patients with worse prognostic factors were treated mostly with combined immunotherapy compared to the monotherapy with PD-1 antibodies.

Our analysis showed that survival has been significantly improved with the new therapies available, namely immunotherapy. In fact, when we compare with other publications that analysed populations with a small number of patients treated with PD-1 based immunotherapy, we can clearly see that our collective performed better. (Forschner et al., 2017)

In conclusion, PD-1 based immunotherapy should be considered upfront in stage IV melanoma patients. First-line therapy should take into consideration the prognostic factors that were previously mentioned. The presence of BRAF mutation should also be considered, although it was not a prognostic factor in our analysis. Decisions should always be taken in a multidisciplinary setting. Future trials and pre-clinical investigations should focus on earlier identification of patients that will most likely be primary resistant to immunotherapy. These are the ones that derive the least benefit from these therapies and probably other therapeutic options should be considered in first-line.

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6 Deutsche Zusammenfassung

Diese Studie hatte das Ziel der Identifikation und Charakterisierung von Stadium IV Melanompatienten, die primär resistent gegen Immuntherapie mit Immuncheckpoint-Inhibitoren sind. Primäre Resistenz gegen die Therapie wurde als Progression im ersten Staging definiert, das etwa drei Monate nach Beginn der Therapie stattfand.

Weitere Ziele waren die Beurteilung der Patienteneigenschaften, das beste Ansprechen auf Immuntherapie, medianes Gesamtüberleben und Gesamtüberlebensraten sowie medianes progressionsfreies Überleben und progressionsfreie Überlebensraten.

Zusätzlich wurden prognostische und/oder prädiktive Faktoren der Primärresistenz gegen Immuntherapie untersucht.

Wir analysierten 530 Melanompatienten im Stadium IV, bei denen zwischen 2015 und 2018 ein metastasiertes Melanom diagnostiziert wurde. 347 Patienten wurden mit Immuntherapie behandelt, davon zeigten sich 144 als primär resistent, d.h. das beste Ansprechen war eine Progression, die zum Zeitpunkt des ersten Stagings beobachtet wurde.

Die für diese Analyse relevanten Daten wurden durch Recherchen in der Patientendatenbank des Universitätsklinikums Tübingen (SAP ISH GUI for Windows, Copyright 1993-2004) und der Datenbank des Zentralregisters Malignes Melanom (ZRMM) der DDG gewonnen. Die Daten wurden mit Epi Info™, einer Open-Source-Statistiksoftware, erhoben. Die Daten aus dem ZRMM und die Daten von Epi Info™ wurden in einer SPSS-Datei zusammengeführt.

Die gesamte Patientenpopulation wurde durch deskriptive statistische Analysen, Häufigkeitstabellen und Kreuztabellen charakterisiert. Kaplan-Meier-Kurven wurden verwendet, um die Überlebenswahrscheinlichkeiten und die mediane Überlebenszeit mittels Log-Rank-Tests auf statistische Signifikanz zu bestimmen. Unterschiede mit einem *p*-Wert von 0,05 wurden als signifikant erachtet. Darüber hinaus wurden die 1-, 2- und 3-Jahres-Überlebensraten mit einem Konfidenzintervall von 95% berechnet.

Die Follow-up-Zeit wurde definiert als die Zeit zwischen dem Datum der Stadium IV Diagnose des Melanoms und dem Datum des letzten Kontakts oder Todes. In den Gesamtüberlebens-Analysen wurden alle Todesursachen berücksichtigt.

Die signifikanten prognostischen Faktoren von Patienten mit Primärresistenz waren: Ausgangswert von S100, Ausgangswert von LDH, Anzahl von Organen mit Metastasen, sowie Vorhandensein von Lebermetastasen.

Patienten mit Primärresistenz hatten eine signifikant schlechtere Prognose als solche, die keine Primärresistenz gegen Immuntherapie zeigten (2-Jahres-Gesamtüberleben von 17% vs. 80,6%; 95% Konfidenzintervall: 9,9-24,1 und 73,4-87,6).

In Bezug auf die Art der erhaltenen Immuntherapie haben wir keinen Unterschied im Hinblick auf das Überleben zwischen kombinierter Immuntherapie und Monotherapie mit PD-1 gefunden (das mediane Gesamtüberleben lag bei 26 Monaten in beiden Gruppen). Dies ist wahrscheinlich darauf zurückzuführen, dass bei der Wahl der Art der erhaltenen Immuntherapie ein Selektionsbias vorhanden war. Bei der Analyse beider Gruppen zeigte sich, dass Patienten mit schlechteren prognostischen Faktoren häufiger mit kombinierter Immuntherapie behandelt wurden als mit Monotherapie mit PD-1 Antikörpern.

Unsere Analyse zeigt, dass sich das Überleben mit den neuen verfügbaren Therapien, der Immuntherapie, deutlich verbessert hat. Bei einem Vergleich mit anderen Publikationen, die Populationen mit einer kleinen Anzahl von Patienten analysierten, die mit PD-1-basierter Immuntherapie behandelt wurden, können wir deutlich sehen, dass unser Kollektiv besser abgeschnitten hat. (Forschner et al., 2017)

Zusammenfassend lässt sich feststellen, dass PD-1-basierte Immuntherapie bei Melanompatienten als Erstlinientherapie im Stadium IV in Betracht gezogen werden sollte. Das Vorhandensein einer BRAF-Mutation sollte berücksichtigt werden, obwohl es in unserer Analyse kein prognostischer Faktor war. Entscheidungen sollten immer in einem multidisziplinären Rahmen getroffen werden. Zukünftige Studien und präklinische Untersuchungen sollten sich auf die frühere Identifizierung von Patienten konzentrieren, die primär resistent gegen Immuntherapie sind. Dies sind diejenigen, die den geringsten Nutzen aus diesen Therapien ziehen und wahrscheinlich sollten andere Therapiemöglichkeiten als Erstlinientherapie in Betracht gezogen werden.

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8 Erklärung zum Eigenanteil

Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Die Planung und Konzeption dieser Arbeit erfolgte in Zusammenarbeit mit Herrn Prof. Dr. Claus Garbe, Leiter der Sektion Dermatologische Onkologie, Herrn PD Dr. Thomas Eigentler, Leiter des Studienzentrums der Dermatologischen Onkologie und Frau Dr. Teresa Amaral, Fachärztin für Onkologie und Mitarbeiterin in der Sektion Dermatologische Onkologie.

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Die retrospektive Datenerhebung wurde von mir eigenständig durchgeführt. Die statistische Auswertung erfolgte ebenfalls eigenständig von mir unter Anleitung von Dr. Amaral.

Tübingen, den 27.09.2019

Tonia Olivia Seeber

9 Veröffentlichungen

Teile der vorliegenden Dissertationsschrift wurden bereits in der folgenden Publikation veröffentlicht:

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