Article

The Influence of c-Kit and NRAS Mutation on Patients' Survival in Metastatic Melanoma Receiving Immune Checkpoint Inhibitors and Chemotherapy

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Abstract: The high metastasis and mortality rates of melanoma in the era of chemotherapy have decreased significantly over the last 10 years. The success is owed largely to the introduction of targeted therapy of oncogenes and immunotherapies, such as checkpoint inhibitors. The aim of the present retrospective, monocentric study is to investigate the impact of chemotherapy or immunotherapy in 550 patients with metastatic melanoma between the years of 2010 and 2019, looking at overall survival while considering BRAF/NRAS/c-KIT mutation status. A total of 17 patients were found to have a c-KIT mutation in exon 11, 13 or 17, including 58.3% with acral lentiginous melanoma, with 53% localized primarily in the lower limbs. In 13.3% of the 231 NRAS-mutated melanomas, primary tumor location was found to be in UV-exposed skin such as on the head and neck, thus about 50% lower than in the 302 patients with wild-type (BRAF-/NRAS-/cKIT-negative) melanoma. Patients with NRAS-mutated melanomas had a significantly lower probability of survival compared to patients with wild-type melanomas, irrespective of the recommendations of the clinical guideline on drug therapy for metastatic melanoma that have been in force since 2010. In contrast to patients with wild-type melanoma who showed a higher probability of survival receiving immune checkpoint inhibitors, the overall survival of patients with NRAS-mutated metastatic melanoma was not more favorable after therapy with immune checkpoint inhibitors compared to chemotherapy treatment.

Keywords: melanoma; NRAS; c-Kit; cKit; immunotherapy; anti PD-1; long-term survival data; immune checkpoint inhibitors

1. Introduction

Malignant melanoma has the highest mortality rate of all skin diseases [1]. Between 1970 and 2013, the incidence of malignant melanoma in Germany increased more than fivefold [2]. Due to the high risk and the increasing number of cases, effective therapy is becoming increasingly important. In the last ten years, the drug therapy spectrum, which was previously and predominantly characterized by chemotherapies, has expanded to include immune checkpoint inhibitors (ICIs), as well as targeted therapy of a number of detectable and characterized oncogenic mutations. Both forms of therapy have been repeatedly shown to significantly improve patient survival [3]. Common mutations that lead to the growth of malignant melanoma are activating mutations in the MAPK and PI3K/AKT pathways, such as mutations in the BRAF, NRAS and c-Kit genes, of which the NRAS and c-Kit mutations are investigated in this study. NRAS and c-Kit mutations lead to over-activation of the signaling pathways, causing malignant degeneration of the cell. Targeted therapy attempts to intervene in the signaling pathway and inhibit it. The natural immune response can also be disturbed in the presence of a tumor. Given that the T-cells are permanently exposed to antigens, the T-cell activity and especially the memory T-cell differentiation is disturbed (so-called T-cell exhaustion). This leads to dysfunction of

the T cells, expression of inhibitory receptors, altered expression of transcription factors, metabolic disorders and thus to a loss of effectiveness of the T cells. T-cell exhaustion contributes to the immune system's loss of control over the tumor environment [4,5]. Counteracting this mechanism and making the T-cells "ready for action" again is the basis of immune checkpoint inhibitors. Over-expressed inhibitory factors, such as CTLA-4 and PD-1, are blocked by monoclonal antibodies [4,6,7]. Chemotherapy is similarly an established method of cancer therapy. However, studies have only been able to confirm a modest benefit [8]. Nowadays, chemotherapies should only be offered to patients if superior therapy regimens such as BRAF/MEK inhibitors (targeted therapy) or PD-1 antibodies (immune checkpoint inhibitors) are no longer an option [9].

The focus of this study are the newly developed drugs and therapy approaches for the treatment of malignant melanomas in the last 10 years, such as targeted therapy or immune checkpoint inhibitors, which were also used in the Hornheide Specialist Clinic. In our study, the effects of the mutation on the disease course of melanoma are considered, as well as the clinical-histological characteristics with which they are associated. In addition, long-term results under the respective therapies will be investigated, in particular the overall survival (OS) of the patients from the Hornheide Specialist Clinic. Here, mainly statistical methods were used to determine whether the mutation of the tumor, and the drug therapies had a significant influence on the survival of the patients.

2. Materials and Methods

The study is a retrospective, monocentric, non-randomized clinical examination. Data from the years 2010 to July 2019 were used. Only data from patients who underwent a mutational analysis for histologically confirmed malignant melanoma at the Hornheide Specialist Clinic in the aforementioned period were used. Further selection was based on the mutation. Only patients with an NRAS or c-kit mutation and (NRAS/BRAF/c-kit) wild-type patients were compared. Patients with BRAF mutation were considered in the analysis of mutational frequency but were not included for further examinations. Of the 550, mainly Caucasian, patients studied, 315 (57.3%) were men and 235 (42.7%) were women. The median age was 76 years with an interquartile range of 64–82 years. There were no underage patients. There were also no occurrences of different mutations in the same patient. To assess the relationship between mutation and melanoma type, only patients in which the mutation and the melanoma type could be clearly determined were considered. Patients were further categorized by therapy, namely, "chemotherapy" and "immune checkpoint inhibitors". Patients who received other treatments, such as surgery only, radiotherapy, or another form of drug therapy, were not included.

Results were considered statistically significant if $p \leq 0.05$. A spreadsheet was created in Microsoft Excel® v16 to document the data collected, and the corresponding values were obtained from the patient records. Subsequently, the data were exported to IBM SPSS® v26 and subjected to statistical analyses. Both absolute and relative frequencies were reported for the parameters' frequency of mutations, localization, and melanoma type. The Chi-Square Test was used to infer the independence of two test variables. The main test was for independence or dependence of the test size on the different mutations. Overall survival was defined as the period between the initial diagnosis and death of any cause, or the last contact with the patient. In order to check the influence of the tumors mutation and type of therapy on overall survival, the Kaplan–Meier estimator was applied and the result validated by means of a log-rank test.

3. Results

Of 936 patients, 302 (32.3%) patients did not have a mutation in the BRAF, c-Kit and NRAS genes and were therefore categorized as wild-type (WT) patients. A mutation in the NRAS gene was detected in 231 (24.7%) patients, almost exclusively located at codon Q61 (95.2%). A mutation in the c-Kit gene occurred in only 17 (1.8%) patients and was localized mainly on exon 11 in 13 cases (72.2%), followed by 4 mutations (22.2%) on exon 13.

Furthermore, a significant correlation between localization and mutation was found ($p = 0.006$; $n = 541$). Notably, tumors in the head/neck region were observed more than twice as often in WT patients compared to patients with an NRAS mutation (WT: 27.2%; NRAS: 13.3%). The group of c-Kit patients had with 9 (53%) cases the highest proportion of tumors on the lower limb. In addition, melanomas on the vulva were affected by a c-kit mutation more often than average (2/5 or 40%).

A significant correlation between the mutation and melanoma type was found ($p = 0.011$; $n = 328$). In NRAS patients, nodular melanoma (NM) was the most common type with 48 (35%) cases. In patients with c-Kit mutation, acral lentiginous melanoma (ALM) had by far the highest proportion of melanoma types in this mutation group, with 7 (58.3%) cases. In terms of percentage, ALM was more than three times more common in c-Kit patients compared to NRAS or WT patients (NRAS: 13.9%; WT: 16.8%).

In terms of overall survival, patients with an NRAS mutation were significantly less likely to survive than WT patients ($p = 0.009$), although the type of treatment did not differ significantly between the groups. First-line therapy was predominantly immune checkpoint inhibitors, mainly utilizing PD-1 antibodies such as nivolumab. In second-line therapy, chemotherapy was commonly used. Figure 1 shows the overall survival (OS) of the 525 patients examined (NRAS: 229; WT: 296) as a function of the mutation as a Kaplan–Meier plot. Furthermore, wild-type patients showed a significantly higher probability of survival receiving immune checkpoint inhibitors compared to WT patients receiving chemotherapy (69 (chemo) vs. 54 (ICIs); $p = 0.002$), whereas NRAS patients showed no difference in OS with either therapy (59 (chemo) vs. 49 (ICIs); $p = 0.19$). Figures A1 and A2 show the OS of NRAS and WT patients receiving chemotherapy and immune checkpoint inhibitors as a Kaplan–Meier plot.
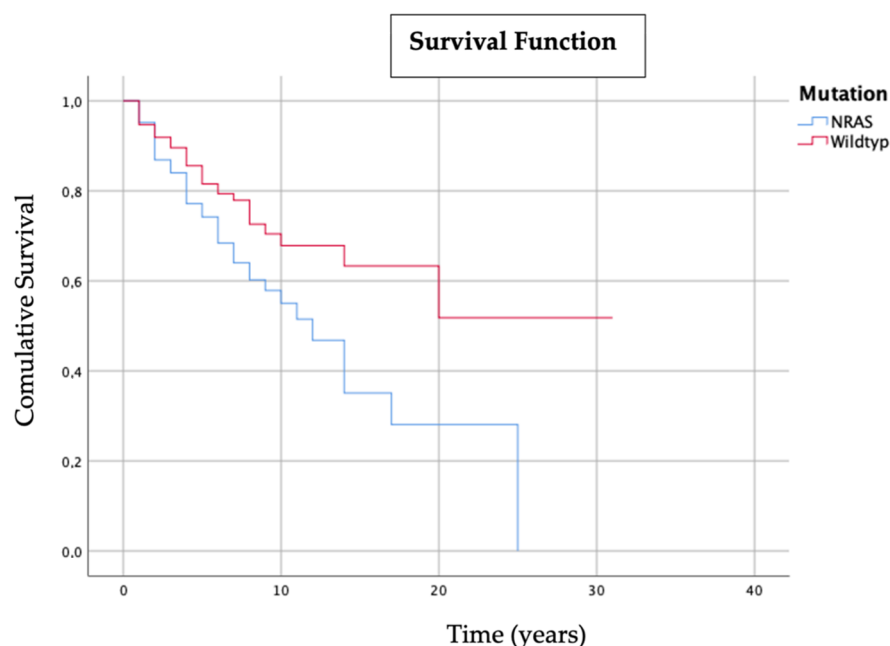

Figure 1. Survival function of patients with NRAS mutation and wild type.

4. Discussion

We show that particular mutations in melanomas are associated with distinct tumor characteristics. The majority of NRAS mutations (95.2%) were located in codon Q61, which confirms the results of other authors [10,11]. C-Kit mutations were predominantly located in exon 11 (72.2%). Here, too, there was agreement with the literature [12]. The NRAS mutation, c-Kit mutation and wild type were present with a frequency of 231 (24.7%), 17 (1.8%) and 302 (32.3%) cases, respectively. With a worldwide prevalence of 16.4% for the NRAS mutation and 10% for the c-Kit mutation, the incidence of the NRAS mutation was

significantly higher in the patients of the Hornheide Specialist Clinic, whereas the incidence of the c-Kit mutation was significantly lower at 1.8% [13]. The strong differences in the incidence rates of the c-Kit mutation could be related to the distribution of the subtype of melanoma in the patient population studied. Thus, we were able to show that the c-Kit mutation was disproportionately often detectable in ALM. At the same time, ALM had a low incidence of 56 out of 328 cases (17%) in our study population. According to studies, the incidence of ALM is rare in Caucasian populations, which most closely resemble ours, while it is the most common subtype in Asian or African populations [14]. An overall lower proportion of ALM would thus also imply a lower incidence of the c-Kit mutations. Similar mechanisms, such as a higher proportion of NM, could accordingly lead to an increased incidence of the NRAS mutation in our patient population. The location of the primary tumors showed a significant association with the mutation. As compared to the NRAS mutation, WT was found significantly more frequently in the head/neck area (WT: 27.2% in the head area; NRAS: 13.3% in the head area) and thus especially in UV-exposed skin areas. Tumors with c-Kit mutation were localized in the lower limbs in more than half of the cases (53%). A conclusion also supported by various authors [10,15,16]. The higher percentage of WT melanoma in the head/neck region (UV-exposed skin area) compared to NRAS tumors could be related to the non-specific cell damage caused by UV radiation, which tends to not induce specific point mutation in the NRAS gene but cause other non-specific malignant cell degeneration by direct or indirect exposure. When examining the histological subtype, significant differences were found in the mutation groups. For example, the distribution was very similar for the NRAS mutation and the WT, the NM was most common in these groups, while the ALM was represented by only 13.9% and 16.8%, respectively. Compared to this, the ALM was strongly overrepresented in the group of c-Kit patients with 7/12 cases (58.3%). Again, our results confirmed those of other authors, who similarly found a significant association between mutation and melanoma type [10,13,15].

Patients with NRAS-mutated melanomas had a significantly lower probability of survival compared to patients with wild-type melanomas, irrespective of the recommendations of the clinical guideline on drug therapy of metastatic melanoma that have been valid since 2010. In contrast to patients with wild-type melanoma who showed a higher probability of survival receiving immune checkpoint inhibitors, the overall survival of patients with NRAS-mutated metastatic melanoma was not more favorable after either therapy. The lower probability of survival of NRAS patients receiving immune checkpoint inhibitors could be explained by the fact that the NRAS mutation negatively influences the activity of the immune system and thus contributes to a lower probability of survival in this group. A review by Mandalà et al., which examined the effects of NRAS in melanomas, shows that, on the one hand, fewer antigen-presenting MHC complexes are expressed on the tumor surface due to the mutation and, on the other hand, that immunosuppressive regulatory T cells and myeloid suppressor cells are recruited. This results in an altered metabolism of the cancer cell, an altered tumor environment and thus a circumvention of the immune response, which contributes to the rapid metastasis process [17]. Thomas et al. were also able to determine a reduced rate of tumor-infiltrating lymphocytes in NRAS-mutated tumors [16]. Thus, NRAS-mutated melanomas present themselves as prognostically unfavorable, with a considerable lack of effective treatment options. Overall, it can be said that in our study, patients with NRAS mutated melanoma had a lower probability of survival than patients with WT melanomas. Potentially, this represents the result of minimal response to therapy, although much research remains to be done. Due to the limitations of the retrospective study design and the associated lack of evidence for causal links, this observation serves as an incentive to investigate the relationship between NRAS mutated melanomas and treatment response in further clinical studies.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. A positive ethical vote of the Medical Association Westphalen-Lippe and the University of Muenster dated 06.03.2020 is available (File No.: 2020-093-f-S).

Informed Consent Statement: Due to the study design, separate patient consent is not required.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.
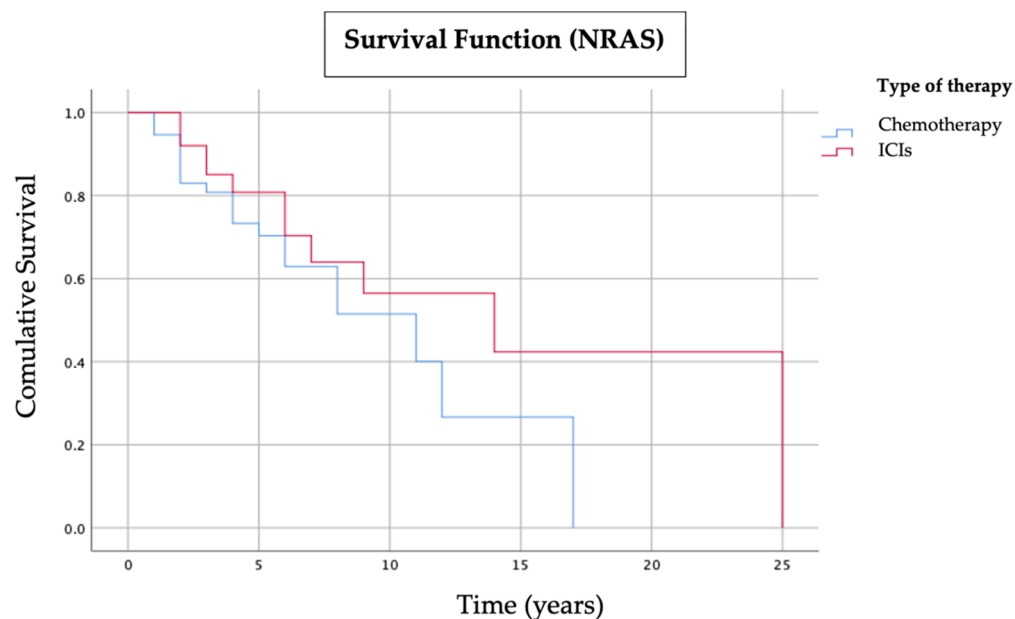
Appendix A

Figure A1. OS of NRAS patients receiving chemotherapy or immune checkpoint inhibitors (ICIs).
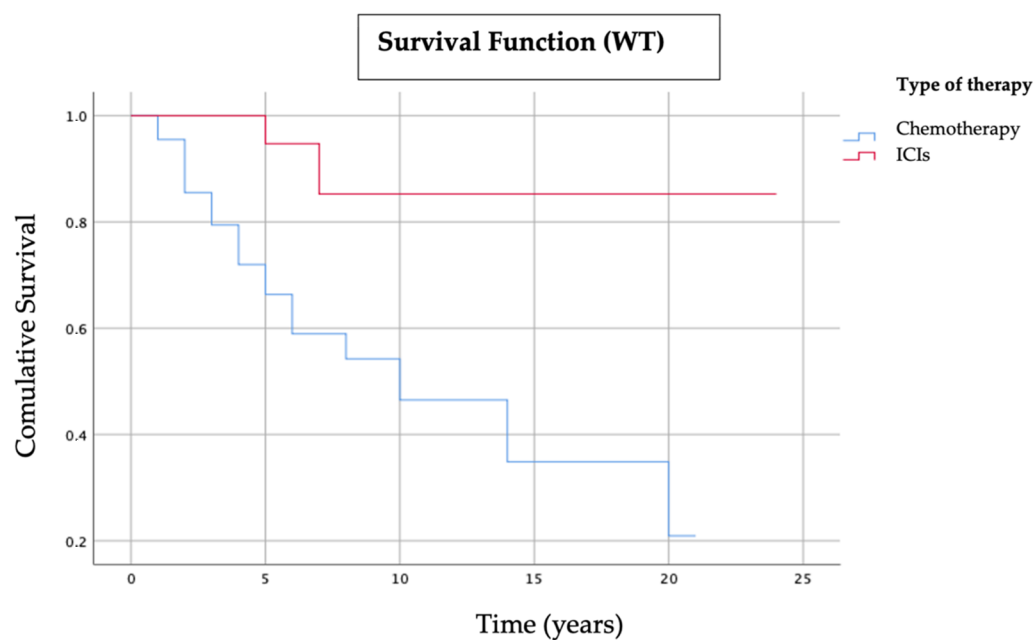

Figure A2. OS of WT patients receiving chemotherapy or immune checkpoint inhibitors (ICIs).

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