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# Clinical and genetic analysis of melanomas arising in acral sites

Anne Zaremba [a,1], Rajmohan Murali [b,1], Philipp Jansen [a], Inga Möller [a], Antje Sucker [a], Annette Paschen [a], Lisa Zimmer [a], Elisabeth Livingstone [a], Titus J. Brinker [c,d], Eva Hadaschik [a], Cindy Franklin [e], Alexander Roesch [a], Selma Ugurel [a], Dirk Schadendorf [a], Klaus G. Griewank [a,f,1], Ioana Cosgarea [a,g,*,1]

[a] *Department of Dermatology, University Hospital Essen, West German Cancer Center, University Duisburg-Essen and the German Cancer Consortium (DKTK), Essen, Germany*
[b] *Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
[c] *Department of Dermatology, University Hospital Heidelberg, University of Heidelberg, Heidelberg, Germany*
[d] *National Center for Tumor Disease (NCT), German Cancer Research Center (DKFZ), Heidelberg, Germany*
[e] *Department of Dermatology and Venerology, University of Cologne, Cologne, Germany*
[f] *Dermatopathologie bei Mainz, Nieder-Olm, Germany*
[g] *Dermatological Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom*

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**Abstract** ***Study aim:*** Melanomas arising in acral sites are associated with a poorer prognosis than other melanoma subtypes. The aim of this study was to evaluate clinical-pathological and genetic characteristics as well as therapeutic responses of a larger cohort of patients with melanomas arising in acral sites.
***Methods:*** Clinical data of 134 patients with melanomas arising in acral sites from the Dept. of Dermatology Essen were collected and analysed with regard to clinicopathological characteristics and treatment responses. Genetic analysis with targeted next-generation sequencing was done on 50 samples.
***Results:*** In our cohort, *BRAF* (30%), *NRAS* (28%), *TERT* promoter (26%), *NF1* (14%) and *KIT* (6%) were frequently identified mutations. Comparing tumours situated on palms and soles with melanomas arising on dorsal acral sites, a higher frequency of *NRAS* (39.1% versus 25%) and *NF1* (17.3% versus 0%) and lower frequencies of *BRAF* (21.7% versus 75%) and *TERT* promoter (8.6% versus 50%) mutations were observed. MAPK activating

* *Corresponding author*: Department of Dermatology, University Hospital Essen, Hufelandstrasse 55, Essen, 45147, Germany.
*E-mail address:* Ioana.cosgarea@newcastle.ac.uk (I. Cosgarea).
[1] These authors contributed equally.

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mutations were identified in 64% of tumours. Overall survival was longer in patients treated with immune checkpoint inhibitors as first-line treatment than in patients receiving other systemic therapies (i.e. BRAF/MEK inhibitors and chemotherapy).
***Conclusion:*** Our data suggest that the genetics of melanomas arising in acral sites varies by tumour location and may influence biological behaviour.


## 1. Introduction

Melanomas arising in acral locations are generally identified at a later stage resulting in a poorer prognosis and different clinicopathological and genetic characteristics [1–3] compared with other cutaneous melanoma (CM) subtypes [2]. While some authors define acral melanomas (AMs) as tumours diagnosed histologically as acral lentiginous melanomas (ALMs) arising on the palms, soles and nail bed [4], other studies also include melanomas arising on dorsal acral sites [1,2,5].

Surgical excision with clear margins is of paramount importance in the treatment of early-stage melanoma. However, in melanomas arising in acral sites, the recommended surgical margins are not always achievable because of constraints imposed by anatomic location and a desire to preserve function [4]. Systemic therapy for advanced disease is also challenging in this melanoma subgroup. *BRAF* mutations are the most common activating mutation in CM and represent an important therapeutic option in advanced disease. In AM, *BRAF* mutations are less frequent [6,7], and patient responses to BRAF-inhibitors are modest, with reported progression-free survival (PFS) and overall survival (OS) of 3.6 and 6.2 months, respectively [8]. Activating mutations and copy number gains of *KIT* are present in AM [9], but targeted therapies with inhibitors such as imatinib generally demonstrate poor or non-durable responses [10]. Immune checkpoint blockade therapies, in particular antibodies targeting PD-1, have shown great potential in CM [11,12]. PD-L1 expression in the tumour microenvironment has been associated with improved objective response rates and PFS following anti-PD-1/PD-L1 monotherapy [13–15]. PD-L1 expression was observed in only 31% of AMs compared with 62% in non-acral chronic sun-damaged melanomas [16]. This may explain why although AM respond to anti-PD-1/PD-L1 monotherapy, the response rate is lower than in non-acral CM [17].

AM represents an aggressive melanoma subtype rarely harbouring currently known targetable mutations. A better understanding of this tumour subgroup will be critical to improve patient outcome. In this study, we evaluated the clinicopathological and genetic characteristics as well as therapeutic responses in a cohort of patients with melanomas arising in acral locations to better understand the pathogenesis and biological behaviour of these tumours.

## 2. Materials and methods

### 2.1. Data collection and sample selection

Patient medical history and data were retrieved from the medical databases/documentation system of the University Hospital Essen for a time period of 10 years (January 2009–February 2019). Included were all melanomas arising in acral sites (volar, dorsal, interdigital, subungual, periungual location). Fifty samples were retrieved from the biobank of the Department of Dermatology, Essen, Germany, for further genetic analysis. The study was performed after approval of the institutional ethical committee of the medical faculty of the University Duisburg-Essen (IRB protocol number 17-7904-BO).

### 2.2. DNA isolation and targeted sequencing

Genetic analysis was performed in the subgroup of patients, of whom tumour tissue was available, hereafter referred to as the 'mutational analysis subgroup'. DNA isolation, targeted sequencing, and data analysis was performed as previously described [18], applying a custom-designed amplicon-based sequencing panel covering the *TERT* promoter and the coding regions of 29 genes that are recurrently mutated in cutaneous and uveal melanoma (Supplemental Table 1).

### 2.3. Statistical analysis

Statistical analyses were conducted using SPSS 23.0 (IBM., Armonk NY). Associations between covariates were investigated using chi-squared tests and Fisher's exact tests as appropriate. Univariate survival data were generated using the Kaplan-Meier method with log-rank tests. OS was determined from time of initial diagnosis to last follow-up or death, whichever occurred first. The p values of $p \leq 0.05$ were interpreted as being statistically significant.

## 3. Results

### 3.1. Clinical characteristics and therapeutic treatment of melanomas arising in acral sites

We identified 134 patients with melanomas arising in acral sites, 74 (55.2%) female and 60 (44.8%) male (Table 1). Median age at diagnosis was 67 years (range 19–93 years). Location of the primary tumour was lower extremity/foot in 125 (93.3%) patients and upper extremity/hand in 9 (6.7%) patients. The histological subtypes and the tumour location (volar, dorsal and other) are shown in Table 1.

At the time of initial diagnosis, 20 patients (14.9%) were stage IA, six (4.5%) IB, 14 (10.4%) IIA and IIB, respectively, 18 (13.4%) IIC, 10 (7.5%) IIIA, 14 (10.4%) IIIB, 23 (17.2%) IIIC, two (1.5%) IIID and five (3.7%) stage IV. Stage at diagnosis was unknown for three patients (2.2%); five patients (3.7%) had a melanoma *in situ* (Table 1).

### 3.2. Systemic therapy

Adjuvant treatment was received by 41 (30.6%) patients, and 55 (41.0%) patients received systemic treatment for advanced metastatic disease (23 of which had previously received adjuvant treatment) (Table 2). Of these 55 patients, eight (14.6%) were stage IIIB or IIIC at start of first systemic treatment, 44 (80%) patients were in stage IV at start of therapy and in three patients, the stage was unknown. Immunotherapeutic agents included PD-1 inhibitors (n = 29), CTLA4-inhibitors (n = 19), a combination of PD-1 and CTLA-4 inhibitors (n = 3) or more than one line of immunotherapy (n = 9). A total of 17 patients received targeted therapy consisting of BRAF-inhibitor alone (n = 4), MEK-inhibitor alone (n = 7) and MEK-inhibitor in combination with a BRAF-inhibitor (n = 6). Single or multiagent cytotoxic chemotherapy was given to 25 patients (Table 1). A total of 34 (25.4%) patients received two or more treatment options. Therapy sequences included (1) chemotherapy followed by immunotherapy (n = 4), targeted therapy (n = 1) or other chemotherapies (n = 6), (2) immunotherapy followed by targeted therapy (n = 1), other immunotherapy (n = 7), chemotherapy (n = 1) or therapy within a clinical trial/others (n = 4), (3) targeted therapy followed by other targeted therapies (n = 3), immunotherapies (n = 1) or therapy within a clinical trial (n = 1). In five patients, the first-line treatment was within a clinical trial followed by targeted therapy (n = 1), immunotherapies (n = 2) or other therapies (n = 2). Patients in stage III received PD-1 (n = 1) or CTLA-4- inhibitors (n = 1), BRAF (n = 1) or MEK-inhibitors (n = 1), chemotherapy (n = 1) or treatment within a clinical trial (n = 3).

Table 1
Characteristics of the patients in the full cohort and in the mutational analysis subgroup.

| Variable | All patients (N = 134) | | Mutational analysis subgroup (N = 50) | |
|---|---|---|---|---|
| **Gender** | N | % | n | % |
| Female | 74 | 55.2 | 24 | 48 |
| Male | 60 | 44.8 | 26 | 52 |
| **Age, years** | | | | |
| ≤67 | 68 | 50.7 | 27 | 54 |
| >67 | 66 | 49.2 | 23 | 46 |
| **Histological subtype** | | | | |
| ALM | 65 | 48.5 | 23 | 46 |
| SSM | 9 | 6.7 | 4 | 8 |
| NM | 19 | 14.2 | 8 | 16 |
| U | 36 | 26.8 | 15 | 30 |
| Mis | 5 | 3.7 | 0 | 0 |
| **Stage at initial diagnosis** | | | | |
| IA | 20 | 14.9 | 3 | 6 |
| IB | 6 | 4.5 | 0 | 0 |
| IIA | 14 | 10.4 | 9 | 18 |
| IIB | 14 | 10.4 | 5 | 10 |
| IIC | 18 | 13.4 | 9 | 18 |
| IIIA | 10 | 7.5 | 5 | 5 |
| IIIB | 14 | 10.4 | 3 | 6 |
| IIIC | 23 | 17.2 | 13 | 26 |
| IIID | 2 | 1.5 | 2 | 4 |
| IV | 5 | 3.7 | 0 | 0 |
| U | 3 | 2.2 | 1 | 2 |
| Mis | 5 | 3.7 | 0 | 0 |
| **Tumour location** | | | | |
| Upper extremity | 9 | 6.7 | 4 | 8 |
| Lower extremity | 125 | 93.3 | 46 | 92 |
| **Anatomic location** | | | | |
| Dorsal | 12 | 9 | 4 | 8 |
| Volar | 54 | 40.3 | 23 | 46 |
| Other | 55 | 41 | 18 | 36 |
| U | 13 | 9.7 | 5 | 10 |
| **Adjuvant treatment** | | | | |
| Yes | 41 | 30.6 | 15 | 30 |
| No | 93 | 69.4 | 34 | 68 |
| **Systemic treatment** | | | | |
| Yes | 55 | 41.8 | 30 | 60 |
| No | 79 | 58.2 | 20 | 40 |
| **Treatment** | | | | |
| <2 treatment options | 98 | 73.1 | 39 | 78 |
| ≥2 treatment options | 34 | 25.4 | 11 | 22 |
| U | 2 | 1.5 | 0 | 0 |
| **Targeted therapy** | | | | |
| Yes | 17 | 12.7 | 7 | 14 |
| No | 113 | 84.3 | 43 | 86 |
| U | 4 | 3.0 | 0 | 0 |
| **Immune checkpoint inhibitors** | | | | |
| Yes | 37 | 27.6 | 25 | 50 |
| No | 97 | 72.4 | 25 | 50 |
| U | 0 | 0 | 0 | 0 |
| **Chemotherapy** | | | | |
| Yes | 25 | 18.7 | 9 | 18 |
| No | 109 | 81.3 | 41 | 82 |
| U | 0 | 0 | 0 | 0 |

ALM, acral lentiginous melanoma; Mis, melanoma *in situ*; NM, nodular melanoma; SSM, superficial spreading melanoma; U, unknown.

Table 2
Details of therapy.

| Adjuvant treatment | Systemic treatment | | | |
|---|---|---|---|---|
| | First-line therapy | Second-line therapy | Third-line therapy | Further therapies |
| Yes | PD-1 | CTLA-4 | PD-1 | |
| Total N = 41 | N = 6 | N = 2 | N = 1 | |
| - low-dose IFN N = 23 | | Others | PD-1 | |
| - high-dose IFN N = 10 | | N = 1 | N = 1 | |
| (1 switch to low-dose) | CTLA-4 | PD-1 | Chemo | Chemo |
| - others N = 6 | N = 3 | N = 2 | N = 1 | N = 1, then PD-1+CTLA-4 |
| - unknown N = 2 | | Others | | |
| | | N = 1 | | |
| | BRAF | MEK | | |
| | N = 3 | N = 1 (additional) | | |
| | MEK | CTLA-4 | Chemo | |
| | N = 2 | N = 1 | N = 1 | |
| | | Others | | |
| | | N = 1 | | |
| | Chemo | MEK | | |
| | N = 7 | N = 1 | | |
| | | CTLA-4 | | |
| | | N = 1 | | |
| | | Chemo | Chemo | Chemo |
| | | N = 2 | N = 2 | N = 1, then PD-1 |
| | Others | BRAF | CTLA-4 | |
| | N = 1 | N = 1 | N = 1 | |
| No | PD-1 | BRAF + MEK | BRAF + MEK | |
| Total N = 93 | N = 7 | N = 1 | N = 1 | |
| | | CTLA-4 | | |
| | | N = 1 | | |
| | | Chemo | Others | PD-1 + CTLA-4 |
| | | N = 1 | N = 1 | N = 1 |
| | | Others | Others | |
| | | N = 1 | N = 1 | |
| | CTLA-4 | PD-1 | | |
| | N = 2 | N = 2 | | |
| | PD-1+CTLA-4 N = 1 | PD-1+MEK | | |
| | | N = 1 | | |
| | BRAF | MEK | | |
| | N = 4 | N = 1 | | |
| | | BRAF + MEK | CTLA-4 | PD-1 |
| | | N = 1 | N = 1 | N = 1 |
| | BRAF + MEK | | | |
| | N = 1 | | | |
| | MEK | | | |
| | N = 2 | | | |
| | Chemo | CTLA-4 | PD-1 | |
| | N = 11 | N = 3 | N = 1 | |
| | | | Others | |
| | | | N = 1 | |
| | | Chemo | CTLA-4 | |
| | | N = 4 | N = 2 | |
| | Others | PD-1 | | |
| | N = 4 | N = 1 | | |
| | | PD-1 + CTLA-4 | Others | PD-1 |
| | | N = 1 | N = 1 | N = 1, then PD-1 + CTLA-4 |
| | | Others | PD-1 | |
| | | N = 2 | N = 1 | |
| | | | Others | |
| | | | N = 1 | |

### 3.3. Follow-up data of patients with melanomas arising in acral sites

At the time of analysis, 57/134 (42.5%) patients were alive, 53/134 (39.6%) had died and in 24/134 (17.9%) the status was unknown. Cause of death was documented as melanoma-related in 31/53 (58.5%), non-melanoma–related in 2/53 (3.8%) and unknown in the remaining cases. In total, 44/134 (32.8%) patients had reported locoregional or distant recurrent disease, 34/134 (25.4%) had no reported recurrence, leaving the remaining cases with unknown recurrence status.

Statistically significant differences in OS were observed regarding stage at initial diagnosis and first line of treatment for stage IV melanoma (Table 3, Fig. 1). A median OS survival of 49 months was seen for stage I melanoma, 80 months for stage II, 67 months for stage III and 23 months for stage IV melanoma, $p = 0.009$. Patients receiving anti-PD-1/PDL1 ($n = 16$) or anti-CTLA-4 ($n = 5$) checkpoint inhibitors as first-line treatment had an OS of 98 months and 95 months, respectively, which was higher compared with other first-line therapies ($p = 0.02$) (Table 1). Associations of other clinical and pathological covariates (age, gender, tumour location, histologic subtype, tumour thickness and ulceration of the primary tumour) with survival were not statistically significant. The median follow-up was 41.5 (0.1–207.4) months.

Table 3
Associations of overall survival with clinico-pathological characteristics of the patients in the full cohort.

| Variable | | N | OS | p value |
|---|---|---|---|---|
| | | | Median (range) | |
| Age | ≤ 67 years | 61 | 84 (52–116) | 0.10 |
| | >67 years | 57 | 53 (41–66) | |
| Sex | Male | 56 | 80 (42–118) | 0.54 |
| | Female | 62 | 57 (32–82) | |
| Tumour location | Dorsal | 12 | 54 (17–91) | 0.85 |
| | Volar | 51 | 80 (50–110) | |
| | Other | 47 | 95 (39–150) | |
| Stage at diagnosis | Melanoma in situ | 1 | 73 (NA) | 0.009 |
| | Stage I | 20 | 49 (0–108) | |
| | Stage II | 42 | 80 (40–120) | |
| | Stage III | 47 | 67 (27–107) | |
| | Stage IV | 5 | 23 (0–52) | |
| Histologic subtype | ALM | 54 | 84 (50–118) | 0.26 |
| | SSM | 9 | 31 (0–73) | |
| | NM | 20 | 46 (18–73) | |
| First-systemic treatment | BRAF—inhibitor | 8 | 47 (13–81) | 0.02 |
| | MEK—inhibitor | 4 | 27 (12–41) | |
| | BRAF + MEK—inhibitor | 1 | 34 (NA) | |
| | PD1—inhibitor | 16 | 98 (41–155) | |
| | CTLA4—inhibitor | 5 | 95 (64–125) | |
| | PD1 + CTLA4—inhibitor | 1 | 10 (NA) | |
| | Chemotherapy | 18 | 31 (16–45) | |

ALM, acral lentiginous melanoma; NA, not applicable; NM, nodular melanoma; OS, overall survival; SSM, superficial spreading melanoma.

### 3.4. Mutational analysis

Tumour samples from 50 melanomas arising in acral sites patients were analysed with a targeted sequencing panel. Mutations in at least one of 29 tested genes were identified in 41 (82%) samples. In nine (18%) cases, no mutations were identified, and tumours were classified as *wild-type* (Table 4, Fig. 2).

Mutations were identified in *BRAF* in 15 (30%) samples, *NRAS* in 14 (28%) samples, *TERT* promoter mutations in 13 (26%) samples, *NF1* mutations in seven (14%) samples (three of which were clearly inactivating mutations) and *KIT* mutations in three (6%) samples (two being known activating mutations). One sample presented concurrent activating *BRAF* and *NRAS* mutations. Of 13 samples with *TERT* promoter mutations, seven (54%) also harboured *BRAF* mutations. Mutation distribution with regard to tumour location (dorsal, volar, other) is shown in Table 5a. Out of the four melanomas arising on dorsal acral sites, three exhibited a *BRAF* mutation, two TERT promotor mutations, one a *NRAS* mutation and one sample was wild-type. A significant difference was observed for *TERT* promotor mutations being more frequent in melanomas arising on dorsal acral sites and other acral locations compared with volar location, 2/4 and 7/18 versus 2/23 ($p = 0.0381$), respectively. No other statistically significant associations were observed between tumour site (dorsal, volar, other), gender and *BRAF, RAS* and *NF1* mutational status (Table 5b).

Other rare mutations were found in the following genes: *GNAQ, ARID1A, ARID2, IDH, PTEN, PIK3CA, SMARCA4, EZH2, BAP1, WT1, MAP2K2* and *TP53* (Table 4).

### 3.5. Clinical findings in the mutational analysis subgroup

In 46 (92%) cases, the primary tumour was localised on the foot and in four (8%) cases on the hand (Table 4). The different histological subtypes as well as the anatomic distribution volar, dorsal or other acral sites are demonstrated in Table 5a. Other acral sites comprise interdigital, digital, edge of the foot, periungual or subungual location. Histologically, melanomas arising on dorsal acral sites showed one SSM and two NM, and in one case, the histological subtype was unknown (Table 5a, b).

Adjuvant treatment was given to 15 (30%) patients, consisting of interferon (14/16 cases) and treatment within a clinical trial (1/16). Systemic treatment for advanced disease was given to 30 (60%) patients (Table 1).

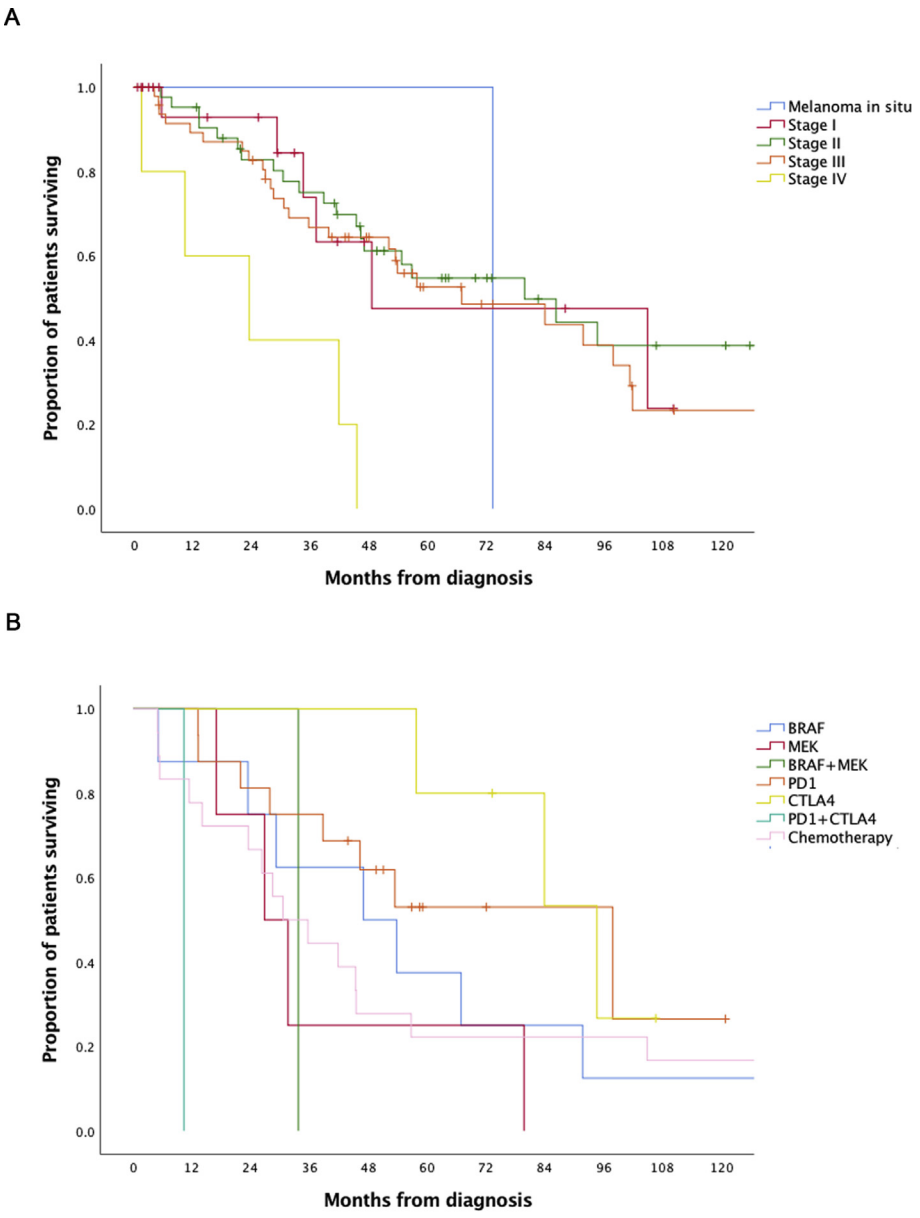


Fig. 1. **Survival analysis of the full cohort**. **(A)** Kaplan-Meier (KM) plot of overall survival (OS) by stage: melanoma *in situ*—73 months (n = 1), stage I melanoma—49 months (n = 20), stage II melanoma—80 months (n = 42), stage III melanoma—67 months (n = 47), stage IV melanoma—23 months (n = 5). **(B)** Kaplan-Meier (KM) plot of OS by first-line treatment: BRAF inhibitors—47 months (n = 1), MEK inhibitors—27 months (n = 4), BRAF and MEK inhibitors—34 months (n = 1), PD-1/PD-L1 inhibitor—98 months (n = 16), CTLA-4 inhibitor—95 months (n = 5), chemotherapy—31 months (n = 18). The median follow-up was 41.5 (0.1–207.4) months. The p values in stage at diagnosis and first systemic treatment might be substantially influenced by the groups Melanoma *in situ*, first-line combination treatment of BRAF and MEK inhibitors and first-line combination treatment of PD-1 and CTLA-4 inhibitors with n = 1.

### *3.6. Statistical analysis between patients without mutational analysis and the mutational analysis subgroup*

The two patient groups were statistically analysed regarding age, gender, primary tumour location, stage at initial diagnosis, histological subtype (ALM, SSM and NM), adjuvant treatment, systemic treatment for advanced metastatic disease and different treatment regimens for advanced metastatic disease. The only statistically significant difference identified regarded treatment; more patients in the mutational analysis subgroup received systemic treatment for stage IV melanoma (30/50, 60%) compared with the non-mutational analysis group (27/84, 32.1%; p = 0.002). More patients from the mutational analysis subgroup received treatment with anti-CTLA4 and anti-PD-1/PD-L1 inhibitors than in the non-mutational analysis group, 11/50 versus 8/76 (p = 0.045) and 22/50 versus 9/95 (p < 0.001), respectively (Supplementary Table 2).

Table 4
Details of mutations in the mutational analysis subgroup.

| Nr. | Age | Sex | Histological subtype | Location | Sample type | *BRAF* | *NRAS* | *NF1* | *KIT* | *TERT* promoter | Other mutations |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 45 | F | LE—ALM | Volar | U | V600E | | | | | |
| 2 | 56 | F | LE—U | Other | U | V600E | | | | | |
| 3 | 21 | M | LE—SSM | Dorsal | M | V600E | | | | | |
| 4 | 46 | M | LE—NM | Unknown | M | V600E | | | | | |
| 5 | 35 | M | LE—NM | Dorsal | U | V600E | Q61K | | | C250T | ARID1A G1375S |
| 6 | 58 | M | LE—ALM | Unknown | P | V600E | A155V | | | C250T | EZH2 D185H |
| 7 | 61 | F | LE—U | Unknown | P | V600E | | | | CC242-3TT | |
| 8 | 54 | F | LE—U | Other | U | V600E | | | | C250T | |
| 9 | 66 | M | LE—ALM | Other | U | V600E | | | | CC228-9TT C250T | SMARCA4 F1142S |
| 10 | 90 | F | UE—NM | Dorsal | U | V600E | | | | C225T | |
| 11 | 72 | F | LE—ALM | Volar | U | V600E | | | | CC228-9TT CC242-3TT | |
| 12 | 66 | M | LE—ALM | Volar | U | V600E | | | | | TERT R1086H, ARID1A N906K |
| 13 | 39 | F | LE—U | Volar | M | V600E | | | | | PIK3CA I391M |
| 14 | 71 | M | LE—ALM | Volar | U | V600E | | | | | SMARCA4 G101E, SMARCA4 R1640W |
| 15 | 69 | M | LE—SSM | Other | U | V600E | | | | | ARID1A P1710S, ARID2 Q397L, CDKN2A R10G CDKN2A I11fs IDH1 A141fs IDH1 Y183C |
| 16 | 80 | F | LE—NM | Volar | U | | Q61R | | | | |
| 17 | 79 | F | LE—U | Other | M | | Q61H | | | | |
| 18 | 76 | F | LE—U | Volar | M | | Q61K | | | | |
| 19 | 76 | F | LE—SSM | Volar | U | | Q61R | | | | |
| 20 | 67 | F | LE—ALM | Volar | U | | G12S | | | | |
| 21 | 60 | F | LE—ALM | Volar | P | | Q61R | M1461E | | | |
| 22 | 75 | M | LE—NM | Volar | P | | Q61R | V1308L | | | |
| 23 | 75 | F | LE—ALM | Other | P | | G12D | N2128K | | | ARID1A R1202Q ARID2 M428I PTEN Y318D |
| 24[a] | 69 | M | LE—ALM | Volar | U | | Q61R | | | | BAP1 S473M ARID1A R1551C ARID1A P1568S TERT P313L |
| 25 | 75 | F | LE—ALM | Other | P | | G12D | | | CC228-9TT | |
| 26 | 64 | M | LE—U | Volar | M | | Q61K | | | C250T | |
| 27 | 84 | M | LE—NM | volar | P | | Q61R | | | | GNAQ R183G |
| 28 | 72 | F | LE—ALM | volar | U | | | L1109[a] | | | |
| 29 | 74 | M | LE—ALM | unknown | U | | | T1565fs | | | |
| 30 | 68 | M | LE—U | other | R | | | C152fs | | | ARID1A R1202Q |
| 31 | 62 | M | LE—ALM | volar | P | | | R1337W | | | |
| 32 | 71 | M | LE—U | other | P | | | | L576P | | |
| 33 | 82 | F | LE—SSM | volar | P | | | | K642E | | |
| 34 | 57 | M | UE—U | other | U | | | | A829P | C250T | |
| 35 | 48 | F | LE—NM | other | U | | | | | CC242-3TT | |
| 36 | 64 | M | LE—ALM | other | P | | | | | C250T | |
| 37 | 63 | M | LE—ALM | other | P | | | | | CC242-3TT | |
| 38 | 74 | M | LE—U | Volar | P | | | | | | WT1 G317E |
| 39 | 28 | F | LE—ALM | Other | U | | | | | | IDH1 R222S |
| 40 | 72 | M | LE—NM | Volar | P | | | | | | TERT S803R |
| 41 | 57 | M | LE—U | Other | M | | | | | | MAP2K2 G286R WT1T221I SMARCA4 R842Q TP53 K120R |
| 42 | 55 | F | UE—ALM | Other | U | | | | | | |
| 43 | 34 | M | LE—ALM | Volar | P | | | | | | |
| 44 | 75 | M | LE—ALM | Unknown | U | | | | | | |
| 45 | 79 | F | LE—U | Volar | P | | | | | | |
| 46 | 61 | F | LE—ALM | Volar | U | | | | | | |
| 47 | 57 | M | LE—U | Dorsal | M | | | | | | |
| 48 | 69 | M | LE—ALM | Volar | M | | | | | | |
| 49 | 55 | F | UE—ALM | Other | U | | | | | | |
| 50 | 61 | F | LE—U | Other | P | | | | | | |

ALM, acral lentiginous melanoma; F, female; LE, lower extremity; M, male; Met, metastasis; NM, nodular melanoma, P, primary tumour; R, recurrence; SSM; superficial spreading melanoma; U, unknown; UE, upper extremity.

[a] Only mutations with a frequency >20% are displayed due to the high number of identified mutations.

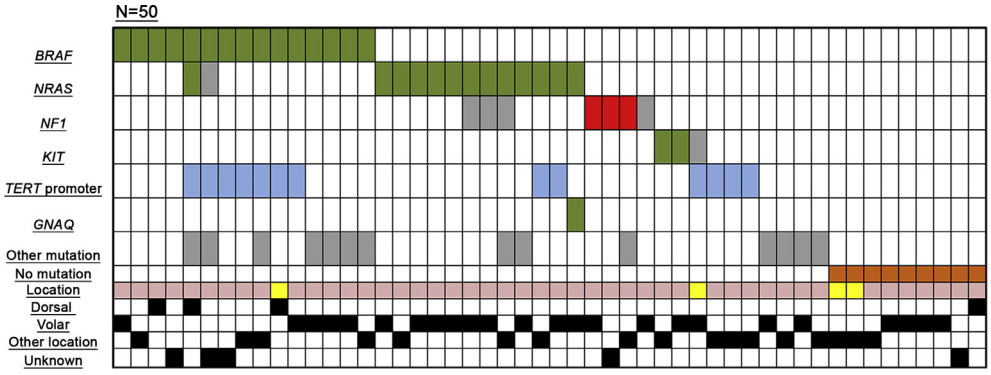


Fig. 2. **Oncoprint—Mutations identified by targeted next-generation sequencing in the mutational analysis subgroup**. Green: mutations known or assumed to be activating; red: loss of function mutations; blue: mutations in the *TERT* promoter region; grey: missense mutation (frequently with unknown functional consequences); orange: *wild-type* samples (showing no mutation in the analysed gene panel). Tumour location: yellow—upper extremities, light pink—lower extremities.

## 4. Discussion

ALM presents the most frequent, yet not the only histopathological type of melanoma in acral sites [5]. Interestingly, Carrera *et al.* could show that SSM subtypes located in acral sites were associated with red hair colour variants, thus indicating that pigmentation might have an impact even in melanomas arising on non–sun-unexposed body sites, although 50% of SSM were located on the dorsum/ankle/wrist [5]. Additionally, Nagore *et al.* showed that patients with ALM were older, did not remember severe sunburn at a higher frequency, had a lower number of common melanocytic nevi and had family histories of other non-cutaneous cancers compared with patients with SSM, NM or LMM [19]. Furthermore, it was shown that melanoma from dorsal acral sites more frequently were SSM (16/21 cases) compared with subungual and interdigital sites (0/13) or volar acral sites (3/9) [1]. Again, patients with melanomas arising on dorsal distal extremities were younger, more often Caucasian and more frequently harboured *BRAF*-mutations compared with those from other acral sites (volar, subungual/interdigital), supporting that melanomas arising on dorsal acral sites are distinct from AM from other sites [1]. This goes along with our findings and suggests that melanomas arising on dorsal acral sites and especially SSM should be considered differently in the evaluation of AM.

Comparing our single centre study of a large cohort of 134 melanomas arising in acral sites with existing data sets, the male to female relation of 55.2%–44.8% is comparable to the 59.6%–40.4% distribution in the data set of 2050 ALM patients from the Central Malignant Melanoma Registry (CMMR) of the German Dermatologic Society [2]. Our study found primary tumour location on the lower extremity/foot in 93.3% and in 6.7% of cases on the upper extremity/hand, comparable to 82% and 18%, respectively in the CMMR data set [2]. These data, with other studies [20], confirm that the

Table 5a
Clinical, pathological and genetic characteristics according to tumour location in the mutational analysis subgroup.

| Variable | Dorsal (N = 4) | Volar (N = 23) | Other (N = 18) | Unknown (N = 5) | Total (N = 50) |
|---|---|---|---|---|---|
| **Gender, no (%)** | | | | | |
| Female | 1 (25) | 12 (52.1) | 10 (55.5) | 1 (20) | 24 (48) |
| Male | 3 (75) | 11 (47.8) | 8 (44.4) | 4 (80) | 26 (52) |
| **Mutational status, no (%)** | | | | | |
| *BRAF* | 3 (75) | 5 (21.7) | 4 (22.2) | 3 (60) | 15 (30) |
| *NRAS* | 1 (25) | 9 (39.1) | 3 (16.6) | 1 (20) | 14 (28) |
| *NF1* | 0 | 4 (17.3) | 2 (11.1) | 1 (20) | 7 (14) |
| *KIT* | 0 | 1 (4.3) | 2 (11.1) | 0 | 3 (6) |
| TERT Promotor | 2 (50) | 2 (8.6) | 7 (38.8) | 2 (40) | 13 (26) |
| No mutation | 1 (25) | 4 (17.3) | 3 (16.6) | 1 (20) | 9 (18) |
| **Histological subtype, no (%)** | | | | | |
| ALM | 0 | 12 (52.1) | 8 (44.4) | 3 (60) | 23 (46) |
| SSM | 1 (25) | 2 (8.6) | 1 (5.5) | 0 | 4 (8) |
| NM | 2 (25) | 4 (17.3) | 1 (5.5) | 1 (20) | 8 (16) |
| U | 1 (25) | 5 (21.7) | 8 (44.4) | 1 (20) | 15 (30) |

ALM, acral lentiginous melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma; U, unknown.

Table 5b
Associations of tumour site with sex and mutational status in the mutational analysis subgroup.

| Tumour site | Sex | | | Mutation status | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | p value | *BRAF* | WT | p value | *RAS* | WT | P value | *NF1* | WT | p value | *TERT* promoter | WT | p value |
| Dorsal | 1 | 3 | 0.53 | 3 | 1 | 0.072 | 1 | 3 | 0.547 | 0 | 4 | 0.600 | 2 | 2 | 0.038 |
| Volar | 12 | 11 | | 5 | 18 | | 9 | 14 | | 4 | 19 | | 2 | 21 | |
| Other | 10 | 8 | | 4 | 14 | | 3 | 2 | | 2 | 16 | | 7 | 11 | |

majority of melanomas arising in acral sites occur on the lower extremity.

One factor responsible for poor prognosis of melanomas arising in acral sites may be high recurrence rate. We documented locoregional and distant disease recurrence in 32.8% (44/134) of patients. Similar frequencies (35% [5] and 29% [2]) for relapse and local recurrence, respectively, were reported in other studies, consistent with melanomas arising in acral sites having a recurrence rate nearly twice as high as locoregional relapses of other melanoma subgroups, reported with 14.3% for non-ALM [2].

Owing to the relative rarity of AM, data are scarce concerning response to therapy. In our cohort, OS was significantly lower for patients receiving targeted therapy as either monotherapy or combination therapy and chemotherapy as first-line treatment compared with patients who received immunotherapies ($p = 0.02$).

In our analysis, OS for patients with *in situ* and stage I melanoma was lower than for patients with stage II and III melanoma (73 and 49 months versus 80 and 67 months, respectively). This finding is a result of sampling bias, as patients with melanoma *in situ*, stage I and some stage II melanomas are not followed-up in our skin cancer units but by primary care dermatologists; therefore, follow-up data from most of these patients are not available for inclusion in our analysis. Only if progression occurs are patients re-referred to our skin cancer unit, allowing documentation of follow-up data. Thus, sampling bias represents a limitation of our study. In our cohort, 91/134 patients (67.9%) either underwent sentinel lymph node (SLN) biopsy or had evidence of lymph node or distant metastases at initial diagnosis. In 48 (52.7%) patients, SLN biopsy was performed. The SLN was reported negative in 37/48 (77%) cases.

Some stage III patients enrolled in our study received systemic treatment with either a PD-1-inhibitor ($n = 1$), a CTLA-4-inhibitor ($n = 1$), a BRAF- or MEK-inhibitor (each $n = 1$) or chemotherapy ($n = 1$). Owing to small sample size, a possible influence on therapy sequence could not be further investigated.

The gene mutation profile of melanomas arising in acral sites differs from non-acral CM [9,21]. Melanomas situated on dorsal acral sites ($n = 4$) had a mutation profile reminiscent of CM with intermittent sun exposure, with 75% *BRAF*, 25% *NRAS* and 50% *TERT* promotor mutations. A similar distribution of 67% *BRAF* and 19% *NRAS* mutations in melanomas arising in acral sites was recently reported by Haugh *et al.* [1]. The number of *NRAS* mutations identified in volar AM (39.1%) is slightly higher than previously in literature, 33% (3/9) [1] and 27.9% [7]. The frequency of *BRAF* (21.7%) and *NF1* (17.3%) mutations was similar to the 21.3% and 14.8%, respectively, published by Yeh *et al.* [7]. Melanomas located on other acral sites harboured 22.2% *BRAF,* 16.6% *NRAS* and 38.8% *TERT* promotor mutations. The 6% *KIT* mutations ($n = 3$, 2 of which being known activating mutations) is lower than previously reported [7,9,22]. The 18% (9/50) of tumours where no mutation was identified is slightly lower than reported in the Korean cohort, 25% (16/64) [22].

In nine samples *TERT* promotor mutation coexisted with *BRAF* (7) or *NRAS* (2) mutations. *TERT* promotor mutations were significantly less frequent on volar sites compared with melanomas arising on dorsal and other acral sites. Half of melanomas arising on dorsal acral sites (2/4, 50%) had a *TERT* promotor mutation, a pattern reminiscent of non-acral CM [23,24]. An interesting finding was the high *TERT* promotor mutation frequency (38.8%) of melanomas arising in other locations such as periungual, interdigital, digital or subungual.

One sample harboured an activating *BRAF* as well as a *NRAS* mutation. The latter is a well-documented resistance mechanism and may have emerged as a result of the patient having received BRAF-inhibitor treatment [25].

Melanomas arising in acral sites are not only distinct from non-acral CM in terms of clinical and pathological characteristics but also genetically. The frequency of mutations identified in *BRAF, NRAS* and *KIT* is less than that described in some previous studies [1,9,21]. However, the overall presence of MAPK-activating mutations (i.e. mutations in *NF1, RAS, KIT, BRAF*) in 64% of tumours in our cohort highlights the central role of the MAPK pathway in the pathogenesis of melanomas arising in acral sites.

Our findings suggest that melanomas arising on dorsal acral sites genetically resemble non-acral CM and should be distinguished from AM arising on volar sites and other acral localisations such as subungual or interdigital sites. The varying genetic pathogenesis based on tumour location likely influences biological behaviour and therapeutic response.

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## Conflict of interest statement

E.L. has had intermittent advisory board relationships with Roche, BMS, Novartis and Actelion and has received travel grants and honoraria from Roche, BMS, MSD, Amgen, Novartis, Boehringer-Ingelheim and medac. E.H. received travel support and honoraria from Essex, Abbott, MSD, Janssen-Cilag, Novartis, Roche, BMS and La Roche Posay. A.R. received travel grants

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### Appendix A. Supplementary data

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