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Mutation landscape in melanoma patients clinical implications of heterogeneity of BRAF mutations

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Background: The detection of V600E BRAF mutations has fundamental clinical consequences as the treatment option with BRAF inhibitors such as vemurafenib or dabrafenib yields response rates of ~48%. Heterogeneity with respect to BRAF mutation in different metastases has been described in single cases. As this has important implications for the determination of BRAF status and treatment of patients, it is essential to acquire more data.

Methods: A total of 300 tumour samples from 187 melanoma patients were analysed for BRAF mutations by pyrosequencing. Equivocal results were confirmed by capillary sequencing. Clinical data with respect to melanoma type, tumour site and survival were summarised for 53 patients with multiple analysed tumour samples (2–13 per patient).

Results: BRAF mutations were found in 84 patients (44.9%) and 144 tumour samples (48%) with BRAF mutations in 45.5% of primary tumours and 51.3% of metastases, respectively. In 10 out of 53 patients (18.9%) where multiple samples were analysed results were discordant with respect to mutation findings with wild-type and mutated tumours in the same patient. Mutations did not appear more frequently over the course of disease nor was its occurrence associated with a specific localisation of metastases.

Conclusion: As heterogeneity with respect to BRAF mutation status is detected in melanoma patients, subsequent testing of initially wild-type patients can yield different results and thus make BRAF inhibitor therapy accessible. The role of heterogeneity in testing and for clinical response to therapy with a BRAF inhibitor needs to be further investigated.

Discovery of the activating (oncogenic) V600E BRAF mutation that is present in ~41–50% of melanomas (Houben *et al*, 2004; Curtin *et al*, 2005) has paved the way to targeted therapy with BRAF inhibitors. The first BRAF inhibitor to gain approval, vemurafenib (Zelboraf), has demonstrated improvement of survival in patients with metastatic melanoma who present with the V600E mutation (Chapman *et al*, 2011) and another BRAF inhibitor, dabrafenib, has also shown to be effective (Hauschild *et al*, 2012). Other variant BRAF mutations (V600K) have been described and were shown to be associated with distinct clinicopathological features including differences in age distribution (higher rates in older patients), localisation (higher rates in tumours localised in the head and neck) and a worse distant metastasis-free survival (Menzies *et al*, 2012). However, this mutation type might be missed with some methods (Anderson *et al*, 2012; Heinzerling *et al*, 2013) and consequently these patients would be excluded from clinical trials with BRAF inhibitors or regular treatment with vemurafenib or dabrafenib (Flaherty *et al*, 2010). In previous studies ~6–30% of all BRAF mutations represented variant mutations distinct from the more common V600E genotype (Rubinstein *et al*, 2010; Beadling *et al*, 2011; Long *et al*, 2011; Lovly *et al*, 2012). In fact, among BRAF V600

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mutations, 79%, 12%, 5%, and 4% were V600E, V600K, V600R, and V600M, respectively (Lovly *et al*, 2012). Interestingly, *in vitro* and *in vivo* data indicate that BRAF inhibitors could be similarly effective in these patients (Rubinstein *et al*, 2010; Chapman *et al*, 2011).

As treatment of BRAF mutation-positive patients with BRAF inhibitors has a profound impact on disease and overall survival, with some evidence for tumour regression in up to 90% of patients (Long *et al*, 2011), the correct identification of the mutation status is crucial. Although there seems to exist a certain consistency in BRAF mutation status of multiple metastases within the same patient, variation of mutation findings between distant metastases, lymph node metastases or the primary tumours has been observed with, for example, higher mutation rates of 41–55% in metastases compared with 33–47% in primary tumours (Long *et al*, 2011). Interestingly, there is also variation dependent on the site of the tumour with mutations being detected more frequently in skin metastases compared with visceral lesions (Colombino *et al*, 2012). Branched evolution in metastatic disease has been shown to create a remarkable genetic heterogeneity among different metastases of one patient (Gerlinger *et al*, 2012; Yancovitz *et al*, 2012) and within single metastases (Lin *et al*, 2011).

This study investigates the frequency, type and intraindividual concordance of rare V600 BRAF mutations in primary tumours and different metastases of melanoma patients, compares different detection methods, and correlates the BRAF genotype with clinical characteristics and survival.

MATERIALS AND METHODS

Patients. A total of 300 tumour samples from 187 consecutive patients with metastatic stage IV melanoma consulting the University Hospital Erlangen were analysed within this study excluding patients with uvea melanoma. For patients with multiple tumour samples data on tumour type, treatment and course of disease were gathered from patient files. Primary tumour tissue was requested for all patients with multiple samples. However, for some of the patients tissue was no longer available. Survival data were obtained at the tumour registry Nürnberg–Erlangen if not accessible from the clinic. Patients had a median age of 60 years, 39% being female.

The investigations were approved by the local ethics committee of the University Erlangen.

DNA extraction and mutation analysis. Genomic DNA was extracted from 2–3.5 μm sections of formalin-fixed paraffin-embedded tissue blocks. The relevant tumour area was marked by a pathologist (AH, AA) and in some cases by the dermato-pathologist. After deparaffinisation, DNA was prepared as described recently (Heinzerling *et al*, 2013) using the NucleoSpin Tissue kit according to the manufacturer's instructions (Daniels *et al*, 2011).

DNA was amplified using the multiplex PCR-kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany) using the following primers: forward: 5′-TGA AGA CCT CAC AGT AAA AAT AGG-3′, and reverse: 5′-Biotin AAA ATG GAT CCA GAC AAC TGT TC-3′. The cycling was performed as follows: a single cycle of denaturation at 95 °C for 15 min, 42 cycles of 95 °C for 20 s, 61 °C for 30 s, and 72 °C for 5 min, and a final 5 min extension at 72 °C. For pyrosequencing (PyroMark Q24, Qiagen) single-stranded DNA was prepared from 40 μl biotinylated PCR product with streptavidin-coated sepharose and 0.5 μM of the sequencing primer: 5′-GGTGATTTTGGTCTAGC-3′ using the PSQ Vacuum Prep Tool (Qiagen). The set up for the pyrosequencing assay was selected with the following sequence in 'sequence to analyse': TACAGA/TGAAA. The underlined A/T describes the hot spot mutation site at codon 600 and primarily describes the V600E with a substitution of GTG (valine) by GAG (glutamic acid). The following dispensation order was used: GTACACGATG. The underlined 'C' was included as an internal control.

Statistical analyses. For analysing differences of survival times in the different groups the Mann–Whitney *U*-test was applied.

RESULTS

A total of 300 melanoma tissue samples from 187 patients were analysed. Tissue samples included primary tumours ($n = 44$), skin ($n = 137$), lymph node ($n = 20$), distant metastases (liver, lung, gastric, pancreas, brain, intestinal and soft tissue; $n = 37$) and tumour tissue of unknown origin ($n = 62$).

Variation in the mutation status detectable in a subset of patients. In 53 out of 187 patients multiple tumour samples (2–13 biopsies) were available for analysis. Tumour samples were obtained from primary tumours and skin, lymph node, soft tissue, lung, visceral organ and brain metastases. In a total of 10 out of these 53 patients (18.9%) both wild-type and mutation-positive metastases were found. There was one patient (patient #2; Table 1C), who showed different results in one tumour probe depending on the assay (wild type in pyrosequencing, V600E mutation-positive in capillary sequencing) and was regarded as wild-type for further analyses. Two patients showed both, tumours with V600E as well as rare BRAF mutations (patient #1 and #7; Table 1B). In 12 patients with multiple tumour probes only rare BRAF mutations were seen. From the remaining 41 patients 31 (75.6%) were concordant with respect to BRAF mutation status (Table 1A, B), whereas 10 patients showed differences in mutation status in different tumour samples, that is, with some tumours that were BRAF wild-type and some tumours that showed the BRAF mutation (Table 1C). In these discordant patients no clear association of mutation status with duration of disease was seen. Indeed when analysing the time of occurrence of mutation in metastases of patients with multiple metastases there was no accumulation of mutations over time (Figure 1B). For example, one patient (patient #1; Table 1C) showed a mutated primary tumour but multiple subsequent metastases revealed a wild-type genotype.

Overall, wild-type BRAF was present in 60.4% of patients (113 patients) and 52.0% of samples (156 samples) and mutant BRAF in 44.9% of patients (84 patients) and 48.0% of samples (144 samples). In primary tumours, the BRAF mutation rate was 45.5%, whereas in metastases mutations were detected in 51.6%. Out of the BRAF-mutated patients 69 (82.1%) were V600E in a total of 94 tumour samples, whereas rare BRAF mutations were found in 17 patients (20%) in a total of 50 tumour samples.

There was no association of mutation status with localisation of primary tumour or metastases. In the patients with multiple analysed tumours, BRAF was detected as mutated in 100% of primary tumours on head/neck (3 out of 3), 83.3% on the trunk (5 out of 6) and 62.5% on the extremities (5 out of 8). For metastases these numbers were 42.5% for skin (34 out of 46), 0% for lymph node (0 out of 6) and 0% for visceral metastases (0 out of 6), respectively (Table 1A, B, C).

Patients with discordant mutation status showed a tendency towards better median survival when compared to concordant BRAF-mutated patients. Patients with discordant mutation status showed a median survival of 35.5 months (Figure 2) as compared with 14 months in patients with BRAF mutations in all tumour probes. Though this difference was not statistically significant ($P = 0.112$), it shows a tendency towards better median survival of

Table 1A. Patients with concordant BRAF wt results in analyses of multiple tumour samples and clinical characteristics

Patient-ID	Gender	Age	Primary melanoma (ALM, NM, SSM)	Localisation of primary melanoma	Region of primary melanoma	BRAF status	Metastases examined	Localisation of metastases	Time of occurrence	Survival in stage IV (months)	Number of probes analysed
1	M	55	Desmoplastic melanoma	Right cheek	Head and neck	No data			08/2007	30	2
						wt	No data	No data	No data		
						wt	Liver	Liver parts ii, iii, iv	08/2010		
2	M	81	SSM	Right heel	Extremities	wt			06/2004	20	3
						wt	Skin	Right lower leg, medial	05/2005		
						wt	Skin	Right heel	12/2008		
3	M	54	Unknown primary	—	—	—				87	2
						wt	Skin	Axilla	01/2007		
						wt	Lymph node	Cervical lymph node	06/2010		
4	F	>82	ALM	Plantar of the left foot	Extremities	no data			11/2007	>11	2
						wt	Lymph node	Inguinal, left	04/2009		
						wt	No data	No data	02/2013		
5	M	43	NM	Right thigh	Extremities	no data			12/2007	10	2
						wt	Skin	Right thigh	09/2008		
						wt	Skin	Right thigh	07/2009		
6	M	70	Ulcerated ALM	Right dorsum of the foot, interdigital	Extremities	wt			03/2007	5	4
						wt	Skin	Left thigh	01/2009		
						wt	Skin	Right lower leg, lateral	02/2009		
						wt	No data	No data	no data		
7	F	70	Nodular SSM	Left upper arm	Extremities	wt			07/2008	6	2
						wt	Skin	Abdomen	07/2009		
8	M	29	Unknown primary	—	—	—			—	51	2
						wt	No data	No data	05/2011		
						wt	Skin	Left upper arm, lateral	10/2011		
9	M	48	Unknown primary	—	—	—			—	59	5
						wt	Lymph node	Right axilla or cervical, right	11/2003		
						wt	No data	No data	11/2003		
						wt	Skin	Sternal	08/2005		
						wt	Skin and soft tissue	Right breast	12/2005		
						wt	Skin	Pectoral, right	05/2007		
10	F	>46	Ulcerated NM	Bottom lip	Head and neck	No data			05/2011	>12	2
						wt	No data	No data	no data		
						wt	Lung	Left inferior lobe	06/2012		
11	M	60	Mucosal melanoma	Penis	Mucosa	wt			07/2009	15	2
						wt	Skin	Right groin	04/2010		
12	M	53	NM	Back	Trunk	wt			02/2006	8	2
						wt	Skin	Left breast	02/2010		
13	F	69	Unknown primary	—	—	—			—	55	4
						wt	Skin	Back, left	10/2006		
						wt	Skin	Left upper arm	12/2006		
						wt	Skin	Right scapula	01/2007		
						wt	skin	Left upper arm, dorsal	01/2007		

Table 1A. (Continued)

Patient-ID	Gender	Age	Primary melanoma (ALM, NM, SSM)	Localisation of primary melanoma	Region of primary melanoma	BRAF status	Metastases examined	Localisation of metastases	Time of occurrence	Survival in stage IV (months)	Number of probes analysed
14	M	40	Nodular SSM	Left shoulder	Trunk	no data			07/2002	12	2
						wt	Skin	Left shoulder	08/2008		
						wt	Skin	Trunk, left, proximal	12/2008		
15	M	>85	Ulcerated NM	Left calf	Extremities	no data			05/2002	>72	2
						wt	Soft tissue	Abdomen	06/2007		
						wt	Soft tissue	Abdomen	06/2012		
16	M	68	Naevoid malignant melanoma	Back, left lumbal	Trunk	no data			06/2001	59	2
			SSM	Left elbow	Extremities	no data			04/2004		
						wt	Skin	Back	08/2005		
						wt	Lymph node	Right axilla	11/2007		
17	M	61	Mucosal melanoma	Oral cavity	Mucosa	No data			09/2006	3	3
						wt	Skin	Cervical, left	07/2007		
						wt	Skin	Cappilitum	07/2007		
						wt	Skin	Cervical, left	09/2009		
18	M	64	Unknown primary	—	—	—			—	52	4
						wt	Skin	Left axilla	05/2008		
						wt	Skin	Right thorax	04/2009		
						wt	Lymph node	Right axilla	02/2010		
						wt	Lymph node	No data	01/2011		
19	F	53	NM	Above right popliteal fossa	Extremities	No data			03/2006	5	2
						wt	Skin	Right thigh, medial	03/2007		
						wt	Skin	Right thigh, medial	03/2007		
20	M	43	Partly NM, partly SSM	Right heel	Extremities	No data				12	2
						wt	Skin	Right thigh	11/2010		
						wt	Skin	Thigh	05/2011		

Abbreviations: ALM = acrolentiginous melanoma; F = female; M = male; NM = nodular melanoma; SSM = superficial spreading melanoma; wt = wild-type.

patients with discordant mutation status. Interestingly, two patients showed a rare mutation (V600K) in their primary tumours and the more common V600E mutation in their metastases (patient #1 and #7; Table 1B). Patients with a BRAF V600E mutation (discordant and concordant mutated; $n = 21$) showed a median survival of 18 months (Table 1B, C) as compared with 13.5 months in patients with wild-type BRAF ($n = 20$; Table 1A). This difference was not statistically significant ($P = 0.695$). These comparable survival times result from the relatively long median survival of patients with discordant BRAF mutation status and the relatively short survival time of patients with a concordant mutation status.

There was no accumulation of mutations in the course of disease. Occurrence of mutations did not correlate with time from diagnosis. Also all patterns of occurrence of wild-type and mutated tumours were observed. See representatively Figure 1A (patient #10; Table 1C), whose metastases are distributed on head (V600E), upper extremity (wild-type) and trunk (V600E). Indeed, we found four patients with V600E-mutated primaries that did not show the mutation in any of the analyses of subsequently developed metastases (Table 1C; patients #1, #4, #5, #7 and #9) and simultaneously developed metastases with different mutation status (Figure 1A; patient #8).

Response to therapy with BRAF inhibitors. There was only one patient treated with a BRAF inhibitor since at the time of sample collection BRAF inhibitors were not yet available. This patient with discordant mutation status experienced a partial remission under treatment with vermurafenib (patient #3). Interestingly, in this patient tumour nodes that were evaluated as wild-type also regressed under treatment with vemurafenib.

Immunohistochemistry showed intratumoural heterogeneity in a subset of patients. The V600E protein was stained by immunohistochemistry using the V600E mutation-specific antibody as previously reported (Heinzerling *et al*, 2013). Interestingly, some of the analysed tissue samples showed intratumoural heterogeneity (Figure 3).

DISCUSSION

This study characterises for the first time intraindividual heterogeneity of BRAF mutation findings in a larger melanoma patient

Table 1B. Patients with concordant V600-mutated results in analyses of multiple tumour samples and clinical characteristics

Pat-ID	Gender	Age	Primary melanoma (SSM, NM)	Localisation of primary melanoma	Region of primary melanoma	BRAF status	Metastases examined	Localisation of metastases	Time of occurrence	Survival in stage IV (months)	Number of probes analysed
1[a]	F	50	SSM	Left shoulder	Trunk	V600K			11/1992	101	4
			SSM	Retroauricular, right	Head and neck	V600K			04/1999		
						V600E	Skin	Supraumbilical	11/2009		
						V600E	Skin	No data	12/2009		
2	M	51	SSM	Thoracal, left	Trunk	No data			07/2002	22	5
						V600E	Skin	Left axilla	07/2005		
						V600E	Skin	Periumbilical, left	06/2006		
						V600E	Skin	Left lower abdomen	11/2006		
						V600E	Skin	Inguinal, right	11/2006		
						V600E	Skin	cervical	02/2007		
3	M	26	NM	Perineal	Trunk	V600E			08/2006	12	3
						V600E	Skin	Capillitum, centre	01/2008		
						V600E	Skin	Capillitum	02/2008		
4	F	70	Ulcerated NM	Right upper arm	Extremities	V600E			03/2007	11	3
						V600E	Skin	Axillary line	10/2007		
						V600E	Skin	back	02/2008		
5	F	86	MM	Right thigh	Extremities	No data			07/1965	12	2
						V600E	Skin	Right thigh	01/2006		
						V600E	Skin	Lumal	01/2007		
6	M	71	Polypoid melanoma	Paravertebral, right	Trunk	V600E			11/2004	10	3
						V600E	Skin	Right abdomen	04/2008		
						V600E	Skin	Right breast	04/2008		
7[a]	F	31	SSM	Capillitum, parietal, left	Head and neck	V600K			07/2006	14	4
						V600E	Skin	Left axilla	05/2010		
						V600E	No data	No data	06/2010		
						V600E	Skin	Collar, right	06/2010		
8	M	54	NM	Paravertebral, right	Trunk	—			07/2002	18	
						V600E	Skin	Right axilla, dorsal	09/2008		
						V600E	Skin	Back	02/2010		
9	M	62	Ulcerated NM	Right epigastrium	Trunk	V600E			04/2004	14	2
						V600E	Skin	Thorax, right	04/2007		
10	F	37	Unknown primary	—	—	—			—	27	5
						V600E	Skin	Pectoral, left	11/2005		
						V600E	Skin	Left axilla	12/2005		
						V600E	Skin and intramuscular	Left cheek	06/2007		
						V600E	Skin	Neck	06/2007		
						V600E	Skin	Frontal	06/2007		
11	F	44	Unknown primary	—	—	—			—	6	2
						V600E	Skin	Neck, left	06/2009		
						V600E	Skin	Mons pubis	07/2009		

Abbreviations: F = female; M = male; NM = nodular melanoma; SSM = superficial spreading melanoma; wt = wild-type.
[a]These patients showed different mutations (V600E and V600K).

Table 1C. Patients with discordant results with respect to BRAF mutation status in analyses of multiple tumour samples and clinical characteristics

Patient-ID	Gender	Age	Primary melanoma (SSM, NM)	Localisation of primary melanoma	Region of primary melanoma	BRAF status	Metastases examined	Localisation of metastasis	Time of occurrence	Survival in stage IV (months)	Number of probes analysed
1	F	74	NM	Right upper arm, proximal	Extremities	V600E			07/2006	46	4
						wt	Skin	Right shoulder	07/2007		
						wt	Skin	Right shoulder	02/2008		
						wt	Mucosa	Cheek	04/2011		
2	M	58	Conjunctival melanoma	Right eye	Mucosa	No data			10/2005	6	2
						wt[a]	Skin	Back, right	05/2011		
						V600E	Skin	Right forearm	05/2011		
3	M	>31	Unknown primary	—	—	—				>73	3
						V600E	Skin	Cheek	02/2004		
						V600E	Skin	No data	03/2007		
						wt		Lung/pericard/ pleura	01 o. 02/ 2008		
4	F	71	SSM	Left vulva	Trunk	V600E			07/2004	31	2
						wt	Skin	Left, upper back	08/2010		
5	F	35	SSM	Right upper arm	Extremities	V600E			07/2002	12	6
						wt	Skin	No data	05/2005		
						wt	Skin	Occipital, left	06/2005		
						wt	Skin	Right clavicula	07/2005		
						wt	No data	No data	09/2005		
						wt	Skin	Interscapular	12/2005		
6	F	54	Mucosal melanoma	Nasal septum	Mucosa	No data			03/1999	40	3
						wt	No data	No data	12/2010		
						V600E	Relapse of primary tumour	Frontal sinus	07/2008		
						wt	Skin	Lumbal, right	05/2009		
7	M	74	ALM	Left middle finger, subungual	Extremities	V600E			08/2004	13	5
			NM	Tip of the nose	Head and neck	V600E			06/2006		
						wt	Skin	Right cheek	07/2008		
						wt	Skin	Retroauricular, right	07/2008		
						wt	Skin	Right cheek	07/2008		
8	F	76	Desmoplatisches MM	Right vulva	Trunk	No data			02/2004	44	2
						wt	Skin	Supraclavicular, left	03/2009		
						V600E	Skin	Right shoulder	04/2009		
9	M	75	Ulcerated ALM	Left foot, lateral	Extremitis	V600E			04/2006	42	5
						wt	Skin	Left calf	07/2008		
						wt	Skin	Left calf	12/2010		
						wt	No data	No data	04/2011		
						wt	No data	No data	No data		
10	M	50	NM	Left thigh	Extremities	No data			11/1996	20	4
						V600E	Skin	Abdomen	08/2007		
						wt	Skin	Left forearm	11/2007		
						V600E	Skin	Left jaw	02/2008		
						V600E	Skin	Right cheek	02/2008		

Abbreviations: F = female; M = male; NM = nodular melanoma; SSM = superficial spreading melanoma; wt = wild-type.
[a]Differential detection with different assays (wild type according to pyrosequencing; V600E according to capillary sequencing).

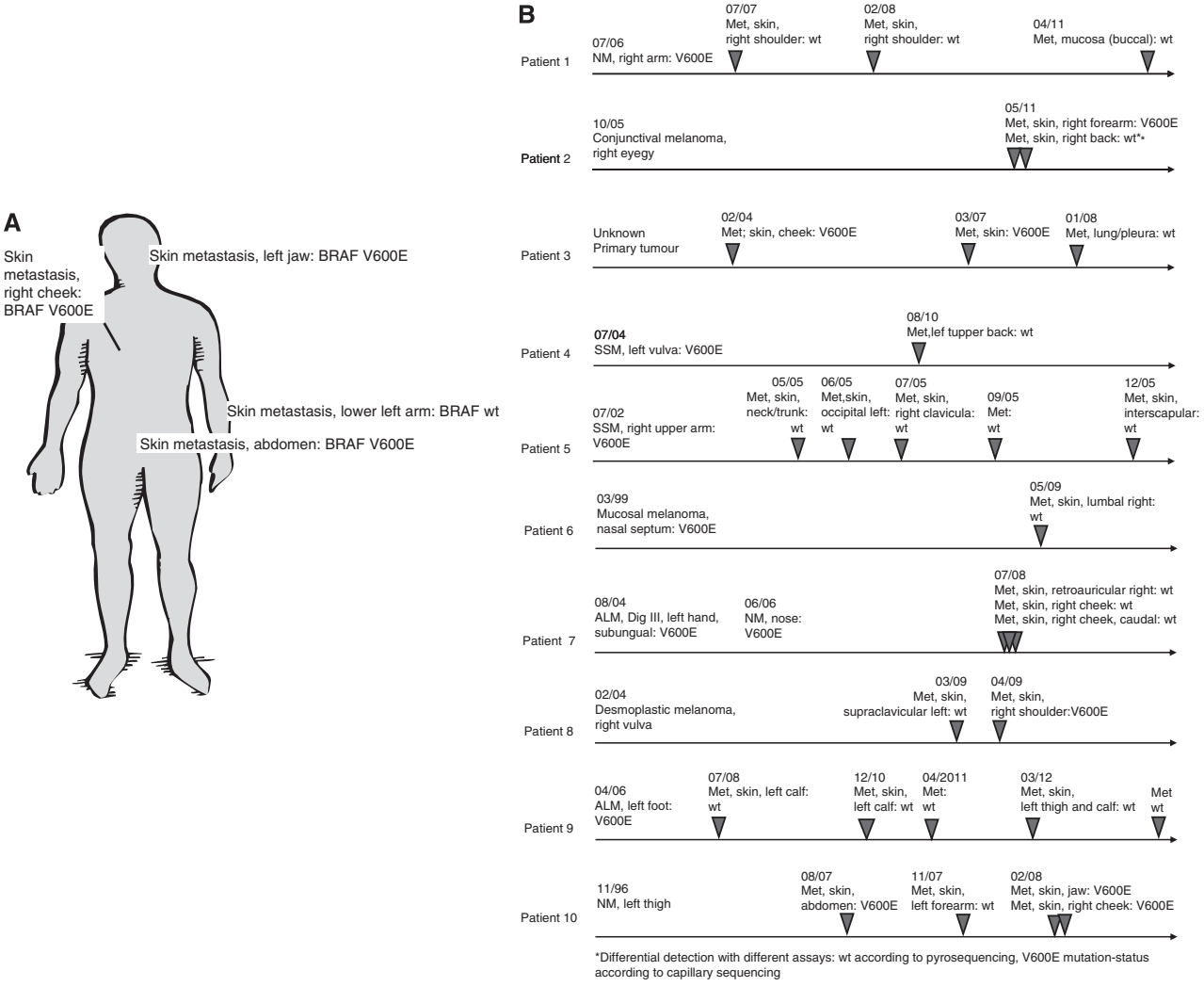

Figure 1. Distribution of tumours evaluated as BRAF-mutated and non-mutated in discordant patients. (**A**) Example of different localisations in patient #10 (**B**) Mutations appear over the course of disease (patients #1–10).

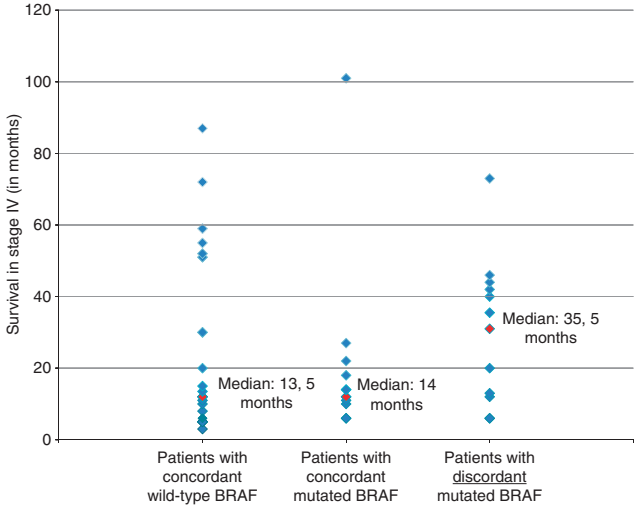

Figure 2. Survival of different patient groups. Each patient's survival is represented by a blue rhomb, median survival is marked with red rhomb.

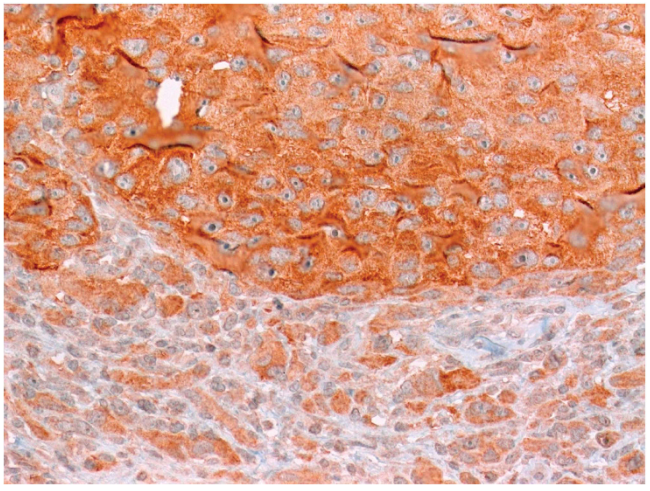

Figure 3. Intratumoural heterogeneity of BRAF V600E expression within melanoma metastasis. Example of a mutation-positive lymph node metastasis showed strong staining of a tumour clone (upper field) juxtaposed to nonstaining tumour tissue (lower field).

population with up to 13 analysed tumours per patient. It analyses clinical data and survival of melanoma patients with respect to BRAF V600 mutation status.

In clinical practice, mutation analyses are performed from a tissue sample available, preferably from a recently detected biopsied or resected metastasis. However, heterogeneity in BRAF

mutation findings has been documented between primary tumour and metastases (Houben *et al*, 2004) and between different metastases (Lin *et al*, 2011; Yancovitz *et al*, 2012). For example, discordant BRAF status among metastases was detected in 26% of patients (with 2 metastases each) and between primary tumour and one metastases in 33% (6/18 patients, Yancovitz *et al*, 2012). Though analysing a higher number of samples per patient (up to 13 tumour samples) our discordance rate was similar with 18.9% of patients showing discordant results (10 out of 53 patients). Discordance rate between primary tumours and metastases on the other hand was higher in our series with 44%. Analysis of the intratumoural mutation spectrum shows discordance in some instances (Yancovitz *et al*, 2012), so this observed heterogeneity could be a result of sampling or of the detection method used. However, it could also be associated with biological differences. The trend towards longer survival in the discordant group as well as the absence of these high discordance rate in patients with rare mutations of V600 (Richtig *et al*, 2012) would hint to the latter explanation.

In contrast to previous reports on three patients (Lin *et al*, 2011), we showed no correlation of mutation with progression of disease. This has profound implications for testing as the determination of the BRAF mutation is a prerequisite for treatment with the BRAF inhibitor. Selective BRAF inhibitors consistently produce an objective response rate of $\sim$50%. Furthermore, around 90% of treated patients show some evidence of tumour regression. Thus, it is especially alarming that according to our data potentially 18.9% of patients could be excluded from BRAF inhibitor therapy despite the presence of mutated metastases. Furthermore, a small subset of patients experience disease progression early during BRAF-targeted therapy. Although secondary resistance can be due to alternative mechanisms (pathways) and secondary mutations including activating *NRAS* mutations, *BRAF* gene amplification, overexpression of MAP3K8/COT, a kinase that directly activates MEK and ERK, and alternative splicing of BRAF mRNA (Romano *et al*, 2013; Sullivan and Flaherty, 2013) molecular heterogeneity with some wild-type metastases could be responsible for this early tumour progression under BRAF inhibition. However, data on this aspect are currently lacking as mutation testing in clinical practice usually is limited to one sample and sampling all metastatic lesions in one patient is not feasible. Thus, sampling a unifocal progressive disease would be of clinical relevance for patient treatment if new alternative drugs targeting resistant (BRAF wild-type) tumour clones are becoming available, particularly within clinical trials.

Our results demonstrate that single testing is not sufficient to identify all carriers of BRAF mutations, which could potentially benefit from the therapy.

Whether BRAF inhibitors have the same effectiveness in patients with concordant and discordant BRAF mutation findings has to be evaluated.

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