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Original Research

Possible immune adverse events as predictors of durable response to BRAF inhibitors in patients with BRAF V600–mutant metastatic melanoma

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Abstract BRAF inhibitors (BRAFi) and MEK inhibitors (MEKi) are among the cornerstones of metastatic melanoma therapy demonstrating excellent response rates with duration of 7–12 m.

Long-term benefit from these agents was reported in patients with normal lactate dehydrogenase (LDH) and less than three disease sites. However, a treatment-dependent marker for long-term efficacy is lacking. Data suggest that immune-related adverse events (irAEs) are associated with clinical benefit in patients treated with immunotherapy and that response to BRAF/MEK therapy may have an underlying immune mechanism. We hypothesised that AEs with an underlying immune mechanism may be associated with a durable response to targeted therapy.

We retrospectively identified a cohort of 78 BRAF V600–mutant metastatic melanoma patients treated with BRAFi or BRAFi + MEKi between November 2010 and November 2013. Four treatment-related AEs including vitiligo, uveitis, erythema nodosum and keratitis sicca were defined as irAEs of interest. Retrospective analysis of AEs in relationship to progression-free survival (PFS), disease burden and LDH levels was performed.

Median PFS (mPFS) for all patients was 7.5 months with responses ongoing in eight patients as of April 2017. Ten patients were identified with the AEs defined previously. Cox

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regression analysis revealed a very strong association between those AEs and PFS; mPFS was 42.8 m in patients with at least one AE versus 6.1 m in those without an AE (hazard ratio [HR] 0.22, p = 0.002). This association was independent of LDH levels and disease burden (HR 0.24, p = 0.035).

This analysis demonstrates a strong association between immune AEs and durable response to targeted therapy and may provide a treatment-related biomarker to estimate the outcome of therapy.

1. Introduction

Melanoma is the most lethal form of skin cancer with incidence rates increasing over the last three decades [1]. Most patients with melanoma are diagnosed at an early stage and are treated surgically. While some patients are cured, others progress to metastatic disease. A minority of patients present with metastatic disease. Systemic treatment for metastatic melanoma has advanced dramatically in recent years with an impressive increase in overall survival (OS) rate, from a median of 6–8 months in 2009 to 2-year landmark OS of >60% years in 2017 [2,3].

There are currently two main treatment strategies for metastatic melanoma: (1) targeted therapy directed against the mitogen-activated protein kinase (MAPK) pathway, which is constitutively activated in about 50% of the patients due to an activating mutation in position V600 of the BRAF kinase [4]. This mutation is more common with younger age, and its incidence significantly decreases in older patients [5]. While selective BRAF V600 inhibitors and MEK inhibitors (MEKi) are indicated as monotherapy, current standard of care targeted therapy regimen is a combination of BRAF inhibitor (BRAFi) and MEKi [6,7]. (2) Immunotherapy with monoclonal antibodies against the immune checkpoint proteins programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4). Each of the immune checkpoint inhibitors (ICIs) is indicated as an independent line of therapy, but PD-1–blocking antibodies are superior to CTLA-4 blockade [8] and have a better toxicity profile [8]. Combination of CTLA-4 and PD-1 blockade has a higher response rate (RR) than anti–PD-1 monotherapy, but this benefit still has not translated into improved OS, while substantially worsening the toxicity profile [9].

Therapy with ICIs leads to a wide array of immune-related adverse events (irAEs) secondary to enhanced immune activation [10]. The common AEs range from skin toxicity (vitiligo and rash), gastrointestinal toxicity (autoimmune colitis and hepatitis), endocrinopathies (thyroid, adrenal and pituitary gland) and pneumonitis to less common and rare autoimmune neurotoxicity and bone marrow toxicity [11–13]. The relationship between occurrence of irAEs and oncologic benefit is mostly based on retrospective data from small cohorts, e.g. vitiligo [14] or arthritis [15]. Furthermore, two retrospective analyses show that treatment discontinuation due to severe irAEs is associated with durable response even without rechallenge in metastatic renal cell carcinoma (RCC) patients treated with anti–PD-1/anti–programmed cell death ligand-1 (PD-L1) agents [16] or metastatic melanoma treated with ipilimumab–nivolumab combination [17]. Clinical efficacy is also associated with PD-L1 expression levels [8], mutational burden [18] and T-cell inflammation [19]. However, to this end, there are no reliable clinical or molecular markers predictive of prolonged clinical benefit from immune checkpoint blockade.

Currently, there are two Food and Drug Administration-approved BRAFi and MEKi combinations—vemurafenib with cobimetinib and dabrafenib with trametinib. These agents have comparable efficacy with RRs nearing 70% and an even higher disease control rates [20,21]. A third combination—encorafenib and binimetinib—has recently shown similar results and awaits approval [22]. The median progression-free survival (mPFS) of BRAF + MEK inhibition therapy is around at 11–12 months, while only a minority of the patients develop a durable response that may last few years [23]. On the other hand, failure of targeted therapy may manifest as rapid progression that may prove to be insensitive to second-line immunotherapy. Attempts to identify the patient population who may develop a durable response to targeted therapy have so far been only partially successful. A comprehensive retrospective analysis published by Long *et al.* demonstrated that patients with low disease burden defined as a normal baseline lactate dehydrogenase (LDH) and less than three disease sites exhibit durable response (3yPFS = 33%, 3yOS = 70%) [23]. Nevertheless, there is still no reliable biochemical or clinical marker that predicts a prolonged response while on treatment. Here we hypothesise that development of possible irAEs to targeted therapy may predict a highly durable response.

2. Materials and methods

2.1. Study cohort

This is a retrospective cohort study of BRAF V600–mutant metastatic melanoma patients. Between the years 2010 and 2013, we identified metastatic melanoma patients bearing BRAF V600 mutation, who participated in clinical trials of targeted therapy for first-line metastatic melanoma or received targeted therapy outside of clinical trials at that time. Individual patient records were fully reviewed.

2.2. Covariates/primary exposure

For each patient, the following baseline characteristics were recorded: age, gender, Eastern Cooperative Oncology Group performance status (PS), disease stage (AJCC 7th edition), number of disease sites, LDH levels and the therapeutic regimen. AEs during therapy were defined and graded according to the Common Terminology Criteria for Adverse Events, version 4. Four rare AEs were retrospectively defined as having a probable underlying immune mechanism in this cohort of patients: vitiligo, uveitis, erythema nodosum and keratitis sicca.

2.3. Outcomes

Objective response was defined according to Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1. Efficacy measures included RR (defined as partial and complete RRs) and PFS.

2.4. Statistical analysis

Patients were retrospectively divided into two groups according to the development of possible irAEs. Analysis of correlation between occurrence of probable irAEs and PFS was performed. Single continuous variables and categorical variables were examined with *t*-test and chi square, respectively. Multivariate Cox regression was used to compare PFS between patients who developed irAEs and those who did not, and the analysis was adjusted for the number of disease sites and LDH level. Statistical analysis was performed using STATA, v. 13. All tests were two tailed. Statistical significance was determined by p value <0.05. This study was approved by Institutional Review Board of Sheba Medical Center (4387-17-SMC).

3. Results

3.1. Cohort characteristics

Between November 2010 and November 2013, 78 metastatic melanoma patients initiated therapy with either a BRAFi or a combination of BRAFi and a MEKi. Sixty-seven patients were treated with vemurafenib, and 11 patients were treated with dabrafenib. Sixteen patients received a combination therapy, seven patients, vemurafenib + cobimetinib and nine patients, dabrafenib + Trametinib. Forty-one percent were female (32/78), and the median age was 56 years (range 19–91). All patients harboured either a BRAF V600E or a BRAF V600K mutation. Most patients had stage IV M1c disease (86%). Median number of disease sites was three (range 1–8) with 42% of patients presenting with low disease burden of less than three disease sites. Twenty-one patients (28%) presented with higher than normal LDH, ten (13.8%) with >X2, the normal range. Most patients initiated treatment as first-line (70%) therapy (Table 1).

In accordance with known published data [6,7], mPFS for all patients was 7.5 months (range 3 m–78 m). Remarkably, however, responses were still ongoing in eight patients as of April 2017. Toxicity was common and on par with the known toxicity profile of single-agent BRAFi or of a combination of BRAFi and MEKi. Rash developed in 21/78 patients (27%), arthralgia in 31/78 (39%) with signs of arthritis in two patients. During the course of therapy, 7.5% (6/78) of patients developed fever and only a minority of the patients (3/78, 3.7%) developed diarrhoea (Table 2). Collectively, the

Table 1
Baseline patient and disease characteristics.

Characteristic	Percentage of patients(n = 78)
Performance status	
0	64% (50)
1	24% (19)
2	4% (3)
Unknown	8% (6)
LDH	
<ULN	64% (50)
>X1- < X2	14% (11)
>X2	14% (10)
Unknown	8%(7)
CNS involvement	
Yes	17% (13)
No	83% (65)
Disease stage(AJCC#7)	
M1a	8% (6)
M1b	6% (5)
M1c	86% (67)
BRAF inhibitor	
Vemurafenib	86% (67)
Dabrafenib	14% (11)
MEK inhibitor	
With	20% (16)
Without	80% (62)
Line of therapy	
1	70% (55)
2	22% (17)
3	8% (6)

LDH, lactate dehydrogenase; CNS, central nervous system; ULN, upper normal limit.

Table 2
Adverse events according to therapy.

Adverse event	BRAFi single agent		BRAFi + MEKi	
	Grade I–II	Grade III–IV	Grade I–II	Grade III–IV
Arthralgia/arthritis	34 (51%)	5 (7.5)	4 (30%)	0
Skin	26 (39%)	7 (12%)	2 (16%)	1 (8%)
CPK elevation	1 (1.5%)	1 (1.5%)	1 (8%)	1 (8%)
Hepatic toxicity	4 (6%)	3 (4.5%)	0	2 (16%)
Creatinine elevation	3 (4.5%)	1 (1.5%)	0	0
Fatigue	14 (21%)	1 (1.5%)	4 (30%)	0
CHF	1 (1.5%)	0	0	1 (8%)
Fever	6 (9%)	3 (4.5%)	3 (25%)	0
Diarrhoea	4 (6%)	0	0	0
Alopecia	8 (12%)	1 (1.5%)	0	0

BRAFi, BRAF inhibitor; MEKi, MEK inhibitor; CPK, Creatine phosphokinase; CHF, congestive heart failure.

combination therapy caused less skin toxicity but induced higher pyrexia rates (Table 2). The median time to occurrence of all AEs was 1 month.

3.2. Occurrence of suspected immune AEs and relation to efficacy

Ten of 78 patients (12.8%) developed a suspected irAE: four patients had developed significant vitiligo, four patients had developed uveitis, one patient developed erythema nodosum and one patient developed keratitis sicca. The characteristics of all ten patients are described individually in Table 3. The median time to occurrence of irAEs was 6.3 months compared with 1.7 months for non-irAEs. All the irAEs were of grade I–II and did not require or were resolved after a short course of low-dose corticosteroids, with the exception of one patient who developed grade III uveitis. In this patient, therapy was temporarily held but resumed after successful resolution of uveitis with topical and high-dose oral corticosteroids. In accordance with known published data [24], non-irAEs occurred in 8/10 (80%) of the patients in whom irAEs occurred, similar to the percentage of all non-immune AEs in the 68 patients who did not develop irAEs. Median age, LDH levels and M1 subgroup stage were similar among the two groups (Table 4). The number of disease sites was slightly lower in patients with irAEs (Table 4). Notably, 90% of patients with suspected irAEs received BRAFi therapy as first line in contrast to only 66% of patients without suspected irAEs.

Table 4
Patient and disease characteristics according to the occurrence of irAE.

irAE	Age (median)	LDH level (median)	Disease sites (median)	First line (%)	M1c stage (%)	Time to AE onset (median)
Yes (n = 10)	55 y	192	2	90	90	6.3 m
No (n = 68)	57.5 y	235	3	66	85	1.7 m
P value	0.78	0.15	0.235	0.72	0.23	<0.0001

irAE, immune-related adverse event; AE, adverse event; LDH, lactate dehydrogenase.
*One patient with irAE did not have recorded baseline LDH.

RR for patients with irAEs was 90% (9/10 patients); 4/10(40%) had a partial response (PR) to therapy, 5/10 (50%) had a complete response (CR) to therapy and one patient had stable disease per RECIST but maintained that for a durable period of 40 months. Overall RR in patients who did not develop irAEs was 83% (57/68) with 21% (12/57) reaching a CR and the rest (81%) reaching a PR. There was a remarkable correlation between PFS and occurrence of suspected irAEs as the mPFS for patients with suspected irAEs was 42.8 months compared with 6.1 months for the rest of the patients (p = 0.002, hazard ratio [HR] = 0.22, Cox regression analysis; Fig. 1a). Expectedly, the irAE group was enriched with patients with known parameters for good prognosis such as PS 0, normal LDH and less than three disease sites (Table 4). Nevertheless, the association of irAEs with PFS remained strong after multivariate analysis for correlation with LDH level before therapy and the number of disease sites (p = 0.035 HR 0.24, Cox regression analysis; Fig. 1b). Notably, PFS was significantly longer for those patients with normal LDH and less than three disease sites compared with the total cohort population (mPFS 12 months versus 6

Table 3
Baseline, toxicity and response parameters in patients who developed irAEs.

No.	Gender	Age	Stage	Baseline LDH	Baseline ECOG PS	Baseline # of disease sites	irAE	Time from treatment	Treatment	Response	PFS	Progressed
1	Male	49	M1c	240	0	3	Uveitis	6	Vemurafenib	PR	6	Yes
2	Female	63	M1c	?	0	5	Vitiligo	4	Vemurafenib	CR	45	No
3	Male	57	M1c	213	?	2	Vitiligo	2	Vemurafenib	CR	40	Yes
4	Female	69	M1b	164	?	2	Erythema nodosum	2	Vemurafenib+ cobimetinib	PR	41.5	No
5	Female	48	M1c	286	0	3	Vitiligo	2	Vemurafenib	CR	62	No
6	Male	32	M1c	238	0	3	Keratitis sicca	12	Vemurafenib	PR	36	Yes
7	Male	49	M1c	173	0	2	Uveitis	13	Vemurafenib	CR	35	Yes
8	Female	54	M1a	201	0	2	Vitiligo	5	Vemurafenib	CR	61	No
9	Female	51	M1c	129	0	2	Vitiligo	8	Vemurafenib	SD	78	No
10	Male	65	M1b	212	0	2	Uveitis	12	Vemurafenib	PR	29	Yes

LDH, lactate dehydrogenase, irAE, immune-related adverse event, PR, partial response, CR, complete response, PFS- progression-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status.

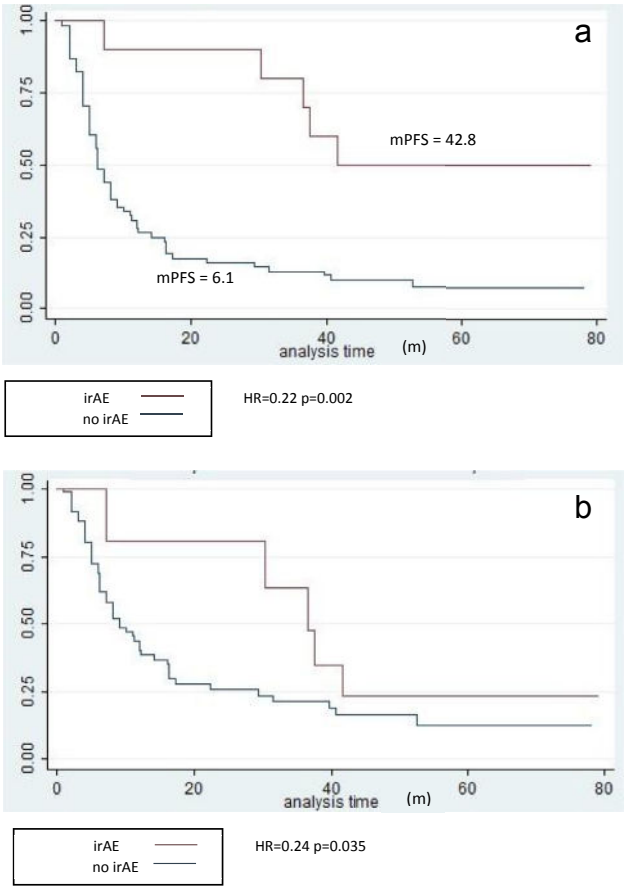

Fig. 1. a. Kaplan–Meier survival estimates in relation to occurrence of irAE. b. Kaplan–Meier survival estimates in relation to occurrence of irAE, corrected for LDH and the number of disease sites. irAE, immune-related adverse event; mPFS, median progression-free survival; HR, hazard ratio; LDH, lactate dehydrogenase.

months HR 0.52 p = 0.011). In addition, for 50% of the patients with a suspected irAE, the response is still ongoing at 6.5 years of follow-up. This is in contrast to 4% (3/68 patients) in the rest of the patients.

Four of the five patients who developed irAEs and had progressed received next-line therapy with different immunological agents. Two of those patients (50%) responded to subsequent therapy and two did not. One patient received ipilimumab and achieved progressive disease as best response; one patient received tumour-infiltrating lymphocytes (TILs) therapy and had rapidly progressed and died; another patient received multiple lines of immunotherapy (TILs, ipilimumab, pembrolizumab) and is currently in CR and another patient received nivolumab and reached CR as well. Interestingly, one of the five patients continued therapy with vemurafenib, despite late disease progression and is still deriving clinical benefit from the treatment. This is in contrast to 15% response in those patients who did not develop irAEs.

4. Discussion

Targeted therapy with MAPK inhibition regimens has dramatically changed the landscape of treatment of metastatic melanoma. However, despite accumulated experience of almost a decade, there is still no known on-treatment parameter, clinical or biochemical, to stratify for long- and short-term benefit. With the growing effectiveness of new immunotherapeutic agents and combinations, clinicians face a clinical dilemma regarding the sequence of therapy in metastatic patients bearing BRAF V600 mutation and how to provide data-driven patient reassurance. The latter point is expected to be even more important in the adjuvant setting, in which targeted therapy has recently proven effective [25], where treatment is actually given with no disease markers for monitoring. In this retrospective analysis, we provide evidence for a potential correlation between treatment-related possible irAEs occurring while on BRAF inhibitors or combined BRAF + MEK inhibition and durability of response to therapy.

An immunological basis seems to partly explain the efficacy of MAPK inhibition through various mechanisms, including enhanced CD8 cell recruitment [26], reduction in T-regulatory cell activity, increased expression of major histocompatibility complex (MHC) class I and melanoma antigens [27] and decrease in suppressive molecules such as PD-L1 [28] and carcinoembryonic antigen-related cell adhesion molecule (CEACAM1) [29]. This could also be supported by the reported benefit of targeted therapy in melanomas with high mutational burden [24]. However, these mechanisms do not deal with the potential effect of these inhibitors on elicitation of immune response beyond the tumour vicinity.

In immunotherapy, the likely association between irAEs and oncologic benefit is explained by treatment-induced overall enhanced immune activation, which could, therefore, be regarded as a potential surrogate. We, therefore, evaluated retrospectively the occurrence of possible irAEs in a cohort of 78 targeted therapy–-treated patients and its correlation with oncologic benefit. Importantly, our results suggest a strong association between possible irAEs and durability of response to targeted therapy, which is independent of disease burden and LDH (Fig. 1). Multivariate analysis confirmed irAEs as an independent predictor. Furthermore, we confirm the prognostic significance of these parameters with significantly longer PFS for patients with normal LDH and less than three disease sites compared with the entire cohort.

Indeed, the mPFS for the patients who did not develop a possible irAE was 6.1 months as compared with a remarkable PFS of 42.8 months for the patients who did develop a possible irAE. Moreover, 50% of patients still experience an ongoing response to therapy

at 6.5 years. This durable response could be due to an enhanced immune activation, reflected by the development of irAEs. It is interesting to note that the irAE involves the skin or the eye, areas that are rich in melanocyte antigens such as MART-1, gp100 and tyrosinase. Therefore, it could be speculated from a mechanistic point of view that successful immune induction by tumour cell death due to targeted therapy is at the basis of these irAEs. In a similar observation, to a certain extent, vitiligo was previously associated with good outcomes with dacarbazine/temozolomide [30]. Most of the patients who developed possible irAEs (90%) received the targeted therapy as a first line; therefore, it is not confounded by late manifestations of prior lines of immunotherapy. As the primary analysis end-point is PFS and not OS, the prolonged response is not confounded by subsequent lines.

The time to development of irAEs was longer than that of other AEs (6.3 months compared with 1.7 month, Table 4). The median onset of irAEs is earlier than the mPFS of the group that did not develop irAEs. This points out that irAEs cannot be attributed to prolonged drug exposure among those patients who experience durable response due to other reasons.

Clinical identification of irAEs could have a role in clinical decision-making, regarding whether to continue targeted therapy or switch to immunotherapy. Owing to resistance mechanisms that develop between 6 and 12 months into therapy with MAPK inhibitors, there are few clinical trials that test switching from targeted therapy to immunotherapy before progression (e.g. NCT03235245). A clinical marker such as appearance of irAEs, which may indicate on a long-term durable effect of MAPK inhibitors, could guide patient populations that may require this switch. Finally, as BRAF + MEK inhibition therapy was recently shown to be beneficial as an adjuvant therapy in stage III/IV (NED) melanoma [26], a clinical biomarker for potential efficacy is even further needed.

Interestingly two of the five patients who developed irAEs and had progressed eventually responded well to immunotherapy as next line of therapy. Both patients received anti–PD-1 agents and have reached a maintained CR as of the time of writing of this article. One patient received anti–CTLA-4 therapy and did not derive any benefit. These data may signal a different pathway of resistance and hence a better response to next-line immunotherapy with anti–PD-1 agents in patients who have an initial durable response to BRAF targeted therapy. This warrants further trials and to be validated in larger cohorts.

There are several limitations to this study: (a) retrospective design; (b) possible irAEs (vitiligo, uveitis, erythema nodosum and keratitis sicca) were defined based on clinical grounds from patient records with no pathological or laboratory confirmation. On the other hand, these are all well-established immunological conditions, and all side-effects were deemed as treatment related while documented; (c) selection bias may confound the interpretation. However, the patient population here is that of large clinical trials and is similar to that used in previous larger studies of durability of response to BRAF + MEK inhibition; (d) most of the patients included in this study received single-agent BRAFis, which is not the standard of care anymore. However, this actually provides a window of opportunities to study this phenomenon in a more defined manner and attribute the effect to the BRAFis.

In conclusion, despite its limitations, this study demonstrates the toxicity aspect of immune activation during targeted therapy with MAPK inhibition and its link to a durable response to therapy. It may provide a predictive clinical tool in aiding decision-making in the metastatic and adjuvant settings. This is a small retrospective study with remarkable results in our view. Larger datasets and prospective trials are warranted to validate our results, possibly using data from previous large phase III trials of BRAFis in melanoma.

Conflict of interest statement

Guy Ben-Betzalel received honoraria and travel support from Novartis, Roche, BMS, MSD and Medison; Yael Steinberg-Silman received honoraria and travel support from Novartis, BMS and MSD; Nethanel Asher received honoraria and travel support from BMS and MSD; Ronnie Shapira-Frommer received honoraria from Novartis, Roche, BMS, MSD and AstraZeneca; Jacob Schachter received honoraria from Novartis, Roche, BMS and MSD, serves on advisory boards of BMS, MSD and holds partial employment and shares at 4C Biomed; Gal Markel received honoraria from Novartis, Roche, BMS, MSD and Medison, research grant from Novartis, serves on advisory boards for MSD, BMS and Biond Biologics, holds IP and shares at FameWave, holds stock options at Biond Biologics and partial employment and shares at 4C BioMed.

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