swiss medical weekly

Original article | Published 15 December 2023 | doi:https://doi.org/10.57187/s.3504
Cite this as: Swiss Med Wkly. 2023;153:3504

Characteristics of long-survivor metastatic melanoma after polychemotherapy and interferon: a retrospective study

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Summary

BACKGROUND: The development of immunotherapy and tyrosine kinase inhibitors dramatically improved the prognosis of metastatic melanoma. Consequently, chemotherapy is now rarely used. Here, we describe the characteristics of long-surviving patients with metastatic melanoma treated with immunochemotherapy.

MATERIAL AND METHODS: We retrieved retrospective clinical and pathological data for patients diagnosed with metastatic melanoma between January 1993 and December 2015 who received the CVD-INF (cisplatin, vinblastine, dacarbazine, and interferon α-2b) regimen at the Hôpitaux Universitaires de Genève. We estimated their progression-free survival and overall survival. This ad hoc study's primary aim was to describe the clinical and biological characteristics of long-term survivors, defined as patients surviving more than two years after immunochemotherapy initiation. The spatial distribution pattern of $CD8^+$ T cells (inflamed, excluded, or desert) was immunohistochemically determined.

RESULTS: Ninety patients received CVD-INF. Their median age at metastatic melanoma diagnosis was 55 years (20–75). Their median progression-free survival was 2.8 months, and median overall survival was 7.2 months. Eleven (12%) patients were long-term survivors. In multivariate analysis, central nervous system metastases (hazard ratio [HR]: 2.66; 95% confidence interval [CI]: 1.43–4.95; p = 0.001), multiple metastases (HR: 1.82; 95% CI: 1.01–3.29; p = 0.047), and elevated lactate dehydrogenase (LDH) (HR: 1.92; 95% CI: 1.12–3.30; p = 0.016) were independently associated with shorter survival. Most long-survivors (6/8; 75%) had a tumour-inflamed pattern compared to 25% of non-long survivors (5/20; Fisher's test p = 0.030).

CONCLUSIONS: A subset of patients with metastatic melanoma and a tumour-inflamed phenotype treated with CVD-INF survived over two years. Factors associated with prolonged survival are consistent with those previously reported in metastatic melanoma.

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Introduction

Until the 2010s, the prognosis of patients with metastatic melanoma was dismal, with less than 10% survival at five years. The standard of care was dacarbazine, with an overall response rate of less than 20% and median survival not exceeding six months [1]. Several dacarbazine-based regimens were evaluated to prolong survival. The Dartmouth regimen (dacarbazine, carmustine, cisplatin, and tamoxifen) showed a higher response rate (40–50%) than dacarbazine alone but failed to demonstrate a survival benefit. Moreover, toxicity rates were higher in the combination arm [2]. Similarly, the triple CVD (cisplatin, vinblastine, and dacarbazine) regimen showed an increased response rate of up to 40% and a median survival of nine months [3]. The immunochemotherapy (biochemotherapy [BCT]) regimen combining CVD with double immunotherapy (interleukin [IL]-2 and interferon α-2b) increased the response rate and median progression-free survival at the cost of substantially increased toxicities and without survival benefit [4–6]. This regimen, excluding interleukin-2, which is unavailable in Switzerland, was introduced as CVD-INF in 1993 in our institution and used until 2015.

The management of metastatic melanoma has dramatically changed in the last decade with the development of B-Raf proto-oncogene serine/threonine kinase (*BRAF*) and mitogen-activated protein kinase (*MAP2K/MEK*) inhibitors and immune checkpoints inhibitors (i.e. anti-cytotoxic T-lymphocyte associated protein 4 [CTLA4] ipilimumab, followed by anti-programmed cell death 1 [PDCD1/PD1] nivolumab and pembrolizumab). Blocking signal transduction and/or enhancing the immune response leads to an unprecedented survival improvement. Nonetheless, most patients will ultimately relapse, and alternative and/or combined strategies are still needed [7, 8]. Here, we retrospectively analysed our historical cohort of patients with metastatic melanoma treated with CVD-INF. Our main aim was to describe the clinical and biological characteristics of long-term survivors, defined as patients surviving more than two years after immunochemotherapy initiation.

Methods

Patient population

We retrospectively retrieved the clinical data of patients with metastatic melanoma treated at Hôpitaux Universitaires de Genève over 22 years (from 1993 to 2015). We selected patients who received a 21-day immunochemotherapy regimen with CVD-INF (cisplatin, vinblastine, dacarbazine, and interferon α-2b). All consecutive patients were included (figure 1). The inclusion criteria were patients with metastatic melanoma who completed at least one CVD-INF cycle. The exclusion criterion was limited available clinical and pathology data. Dacarbazine was administered at 800 mg/m^2 on day 1, cisplatin at 20 mg/m^2 daily (days 2–5), vinblastine at 1.6 mg/m^2 daily (days 1–5), and interferon α-2b at 5 mio UI daily (days 1–5), adapted from [4, 5]. This study was conducted according to the declaration of Helsinki and the Swiss Law (HRA Art.34/HRO) that authorises the reuse of clinical and biological samples. The research protocol was approved by the Ethics Committee of Geneva (CCER 14-268) and amended in 2016 to allow genomic and immunohistochemistry (IHC) analyses. The Ethics Committee approved a waiver of informed consent due to the death of most patients.

Data collection

Clinical and treatment characteristics were extracted from the medical records of eligible patients at the Hôpitaux Universitaires de Genève. Clinical characteristics included age, sex, site of the primary tumour, site and number of metastases, American Joint Committee on Cancer (AJCC) 8th edition stage, performance status estimated with the Eastern Cooperative Oncology Group (ECOG) scale, serum lactate dehydrogenase (LDH) levels, and toxicities.

Outcome measures

Tumour response was evaluated with contrast-enhanced computed tomography or magnetic resonance imaging. Clinical tumour response was assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1) criteria as follows: a complete response was defined as the disappearance of disease evidence, a partial response as a more than 30% decrease in tumour size without the appearance of new disease, progressive disease as a more than 20% increase in tumour size or the appearance of a new lesion, and stable disease as neither a partial response nor progressive disease [9]. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (CTCAE, version 5.0). The clinical characteristics of long-term survivors, defined as surviving more than two years after immunochemotherapy initiation, were analysed.

Immunohistochemistry

Tumour infiltration by CD8$^+$ T cells (DAKO; cloneC8/144B, 1:50 dilution) and tumour cell expression of PD-L1 Cell Signaling Technology; clone E1L3N, 1:200 dilution) were immunohistochemically determined in 28 patients with available biopsies: 8 long-survivors (≥2 years) and 20 non-long survivors (<2 years). The tumour immune phenotype was determined based on the spatial distribution of CD8$^+$ T cells in the tumour core and stroma (inflamed, excluded, or desert [10]). Inflamed tumours were defined by CD8$^+$ T cells in the tumour core. Excluded tumours were defined by CD8$^+$ T cells exclusively in the stroma adjacent to or within the tumour. Desert tumours were defined by a low prevalence of CD8$^+$ T cells. PD-L1 positivity was defined as at least 5% of tumour cells showing PD-L1 staining of any intensity on their surface [11]. *BRAF* V600E status was available for 47 patients (Ventana, clone VE1).

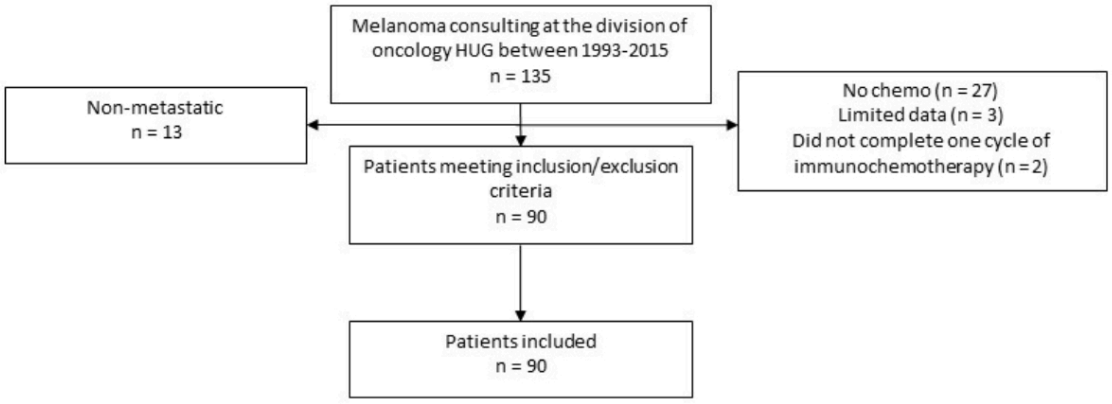
Genomic analyses

Next-generation sequencing of a custom panel of 443 genes was performed in three long-survivors.

Statistical analyses

This ad hoc study's primary aim was to describe the clinical and biological characteristics of long-term survivors, defined as patients surviving more than 2 years after immunochemotherapy initiation. Overall survival was measured from immunochemotherapy initiation to death or last follow-up. Progression-free survival was defined as the interval from immunochemotherapy initiation to clinical or radiological progression or death. Long-term survivors were defined as patients surviving more than 2 years

Figure 1: Flowchart of patient selection. HUG: Hôpitaux Universitaires de Genève.



after immunochemotherapy initiation. Survival was modelled with the Cox proportional hazards model, and the association of different variables with survival was tested with the log-rank and log-ratio tests. Univariate analysis of overall survival was conducted for the following variables: age, sex, serum LDH level, *BRAF* V600E status, primary tumour site, central nervous system metastases, and number of metastatic sites. Variables significantly associated with survival in the univariate analyses were included in a multivariate Cox regression. The Kaplan-Meier method was used to plot survival curves and calculate median survival and follow-up loss. The proportional hazard assumption was confirmed by testing the Schoenfeld residuals against transformed time with the cox.zph() function. P-values <0.05 at a two-tailed alpha were considered statistically significant. All analyses were conducted with the R statistical software (version 4.0.2).

Results

Patients' demographic and clinical characteristics

We identified 90 patients with metastatic melanoma who received at least one cycle of CVD-INF between 1993 and 2015 (median = 3, range = 1–6). Fifty-five patients (61%) were male. The median age at metastatic melanoma diagnosis was 55 years (20–75). The primary tumour site was cutaneous in 63 patients (70%), mucosal in 6 patients (7%), and acral in 2 patients (2%). Nineteen patients (21%) had melanoma with unknown primary (MUP). The *BRAF* V600E mutational status was available for 47 patients, of whom 59.6% had the mutation. Most patients presented with a good performance status: 83 (92%) had an ECOG score of ≤1. Almost all patients (88/90) were in stage IV. The number of metastatic sites was 1 in 15 patients (17%), 2–3 in 46 patients (51%), and ≥4 in 29 patients (32%). Twenty-nine patients (32%) had central nervous system metastases at diagnosis. Almost two-thirds of the patients (59%) had elevated serum LDH. Patients' demographic and clinical characteristics are summarised in table 1.

Survival analyses

The median follow-up was 102.6 months, the median progression-free survival was 2.8 months, and the median overall survival was 7.2 months (figure 2A). The study period was defined as the interval (in years) between the start of the data collection period, corresponding to the first patient, and the inclusion of each successive patient. Since data collection spanned two decades, this variable was established to examine the evolution of the treatment effect

Table 1:
Patient characteristics in the entire cohort.

Variable		**n = 90**	**%**
Age at diagnosis, *years*, median (25^{th}–75^{th})		*52* (20–72)	
Age at metastatic relapse, *years*, median (25^{th}–75^{th})		*55* (20–75)	
Sex	Male	56	62
	Female	34	58
ECOG performance status	0	54	60
	1	29	32
	2	1	1
	3	2	3
	NA	4	4
Primary site	Cutaneous	63	70
	Acral lentiginous	2	2
	Mucosal	6	7
	Melanoma with unknown primary	19	21
Stage (AJCC 7^{th})	IIIC	2	2
	IV	88	98
LDH	≤ULN	20	22
	>ULN	53	59
	≤2 × ULN	49	54
	>2 × ULN	24	27
	NA	17	19
Central nervous system metastases	Yes	29	32
	No	61	68
Metastatic sites	≤2 metastatic sites	38	42
	≥3 metastastic sites	52	58
BRAF V600E status	Yes	28	31
	No	19	21
	NA	43	48
Best response	Complete response	13	14
	Partial response	16	18
	Stable disease	10	11
	Progressive disease	44	49
	NA	7	8

NA: unavailable; LDH: lactate dehydrogenase; AJCC: American Joint Committee on Cancer, 7^{th} edition stage; ULN: upper limit of the normal range; ECOG: Eastern Cooperative Oncology Group.

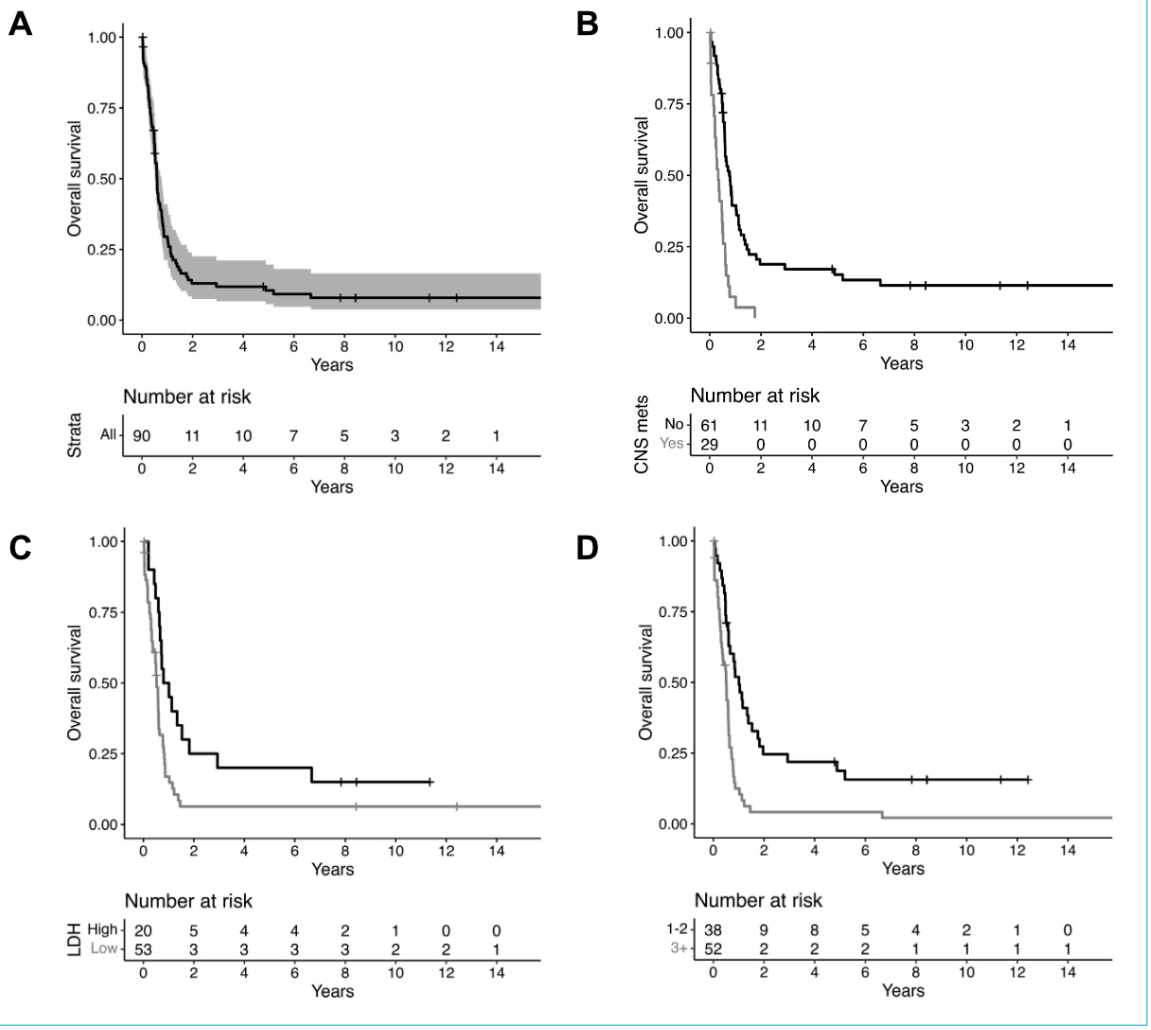
over the studied period. Overall survival did not vary as a function of the study period (hazard ratio [HR]: 1.00; 95% confidence interval [CI]: 0.96–1.04; p = 0.89). Univariate analysis of overall survival revealed that young age (<50 years), elevated LDH, central nervous system metastases, and multiple metastatic sites (≥3) were associated with worse prognosis (table 2). None of the variables associated with shorter overall survival changed over the study period (age, performance status, LDH, central nervous system metastases, and multiple metastases; all p >0.1). The paradoxical increase in hazard observed in younger patients was fully explained in the multivariate analysis by the presence of central nervous system metastases. Indeed, central nervous system metastases were more common in patients aged <50 years (45%) than those aged ≥50 years (25%), with a trend toward statistical significance (Fisher's test: odds ratio: 0.39; p = 0.06; see table S1 in the appendix). In the multivariate analysis, elevated LDH, multiple metastases, and central nervous system metastases were independently associated with shorter survival (figures 2B and 2C; table 2).

Long survivors

Eleven patients (~12%) survived more than 2 years. Their median survival was not reached at the end of the study period. Their clinical characteristics are summarised in table 3. Eight long-survivors achieved a complete response, one had stable disease, and two had progressive disease after CVD-INF. The 8 patients who achieved a complete response had cutaneous melanoma (n = 4) or MUP (n = 4). Only one patient among the 11 had previously received immune checkpoint inhibitors, and they progressed before the introduction of immunochemotherapy. One patient who achieved a complete response relapsed after 18 years and received a tyrosine kinase inhibitor (TKI). One of the two patients who had progressive disease after immunochemotherapy did not receive further standard therapy. They were lost to follow-up for 13 years and reappeared during the COVID-19 outbreak in complete response. They disclosed having been treated by a traditional healer.

None of the long-survivors had central nervous system metastases (p = 0.014, Fisher's test), and 75% had normal LDH levels (p = 0.052, Fisher's test). Eight of the nine long-survivors (89%) had the *BRAF* V600E mutation compared to 11 of the 26 non-long-survivors (42%) (table 4).

Figure 2: Overall survival of patients with metastatic melanoma treated with CVD-interferon α-2b. (**A**) Overall survival of the entire cohort. (**B**) Overall survival by the presence of central nervous system (CNS) metastases. (**C**) Overall survival by lactate dehydrogenase (LDH) level. (**D**) Overall survival by the number of metastatic sites. CVD: cisplatin, vinblastine, dacarbazine.



Of the patients without central nervous system metastases at baseline (n = 61), five (8%) survived at least 8 years, and six were censored. Three long-survivors developed vitiligo after immunochemotherapy.

Genomic alterations of tumours from long-survivors

We performed next-generation sequencing of a panel of 443 genes in biopsies from 3 long-survivors. All three patients had the *BRAF* V600E Glu mutation (confirmed by IHC), and two out of three had pathogenic *ARID1A* mutations (see table S2 in the appendix).

Table 2:
Univariate and multivariate analyses of overall survival.

			Univariate analysis		Multivariate analysis	
		n (%)	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Age	≥50 years	57 (63%)	1		1	
	<50 years	33 (37%)	1.70 (1.07–2.70)	0.023	1.48 (0.86–2.55)	0.153
Sex	Male	56 (61%)	1			
	Female	34 (39%)	1.31 (0.82–2.09)	0.247		
LDH	≤ULN	20 (27%)	1		1	
	>ULN	53 (73%)	2.22 (1.31–3.75)	0.002	1.92 (1.12–3.30)	0.016
Primary site	Cutaneous	63 (70%)	1			
	Acral lentiginous	2 (2%)	0.44 (0.11–1.84)	0.260		
	Mucosal	6 (7%)	0.78 (0.34–1.81)	0.560		
	Unknown primary	19 (21%)	0.77 (0.44–1.38)	0.390		
BRAF V600	No	19 (40%)	1			
	Yes	28 (60%)	0.59 (0.31–1.13)	0.110		
Central nervous system metastases	No	61 (68%)	1	$<10^{-3}$	1	0.001
	Yes	29 (32%)	3.23 (1.97–5.31)		2.66 (1.43–4.95)	
Number of metastastic sites	≤2 metastatic sites	38 (42%)	1		1	
	≥3 metastatic sites	52 (58%)	2.54 (1.58–4.07)	$<10^{-3}$	1.82 (1.01–3.29)	0.047

LDH: lactate dehydrogenase; ULN: upper limit of the normal range.

Table 3:
Clinical characteristics of long-survivors and non-long-survivors.

Clinical characteristic		**Long-survivor**	**Non-long-survivor**
		11 (%)	**79 (%)**
Age at diagnosis, *years*, median (25^{th}–75^{th})	*NA (n = 0)*	*54.4* (50.3–60.8)	*51.6* (40.6–58.4)
Age at relapse, *years*, median (25^{th}–75^{th})	*NA (n = 0)*	*55.0* (52.8–64.6)	*54.8* (43.2–62.3)
Sex	Female	5 (55%)	29 (37%)
	Male	6 (45%)	50 (63%)
Primary site	Cutaneous	6 (55%)	57 (72%)
	Acral lentiginous	1 (9%)	1 (1%)
	Mucosal	0 (0%)	6 (8%)
	Unknown primary (MUP)	4 (36%)	15 (19%)
ECOG performance status	0	6 (67%)	48 (61%)
	1	3 (33%)	26 (33%)
	2	0	1 (1%)
	3	0	2 (3%)
	NA (n = 4)		
TNM	N3	0	2 (3%)
	M1a	5 (45%)	5 (6%)
	M1b	2 (18%)	14 (18%)
	M1c	4 (36%)	58 (73%)
Stage	IIIC	0 (0%)	2 (3%)
	IV	11 (100%)	77 (97%)
Central nervous system metastases	No	11 (100%)	50 (63%)
	Yes	0 (0%)	29 (37%)
Best response	Complete response	8 (73%)	5 (6%)
	Partial response	0 (0%)	16 (20%)
	Stable disease	1 (9%)	9 (11%)
	Progressive disease	2 (18%)	42 (63%)
LDH	≤ULN	6 (75%)	23 (36%)
	>ULN	2 (25%)	42 (64%)
	NA (n = 3)		

NA: unavailable; MUP: melanoma with unknown primary; LDH: lactate dehydrogenase; ULN: upper limit of the normal range; ECOG: Eastern Cooperative Oncology Group.

Tumour immune phenotype in long-survivors

We compared the spatial distribution pattern of $CD8^+$ T cells in tumour compartments (core, stroma and margins) [10] in long-survivors (n = 8) and short-survivors (n = 20). We found that most long-survivors (75%; 6/8) had an inflamed pattern compared to 25% of short-survivors (5/20; p = 0.029, Fisher's test) (table 4 and figure 3). Two of the eight long-survivors expressed PD-L1 (≥5%) compared to none of the short-survivors (p = 0.074, Fisher's test) (table 4).

Subsequent therapies

Forty-four patients received at least one subsequent therapy, mainly for central nervous system metastases. Specifically, 30 received temozolomide, 19 received radiation therapy, 4 received TKIs, two received recombinant IL-2, two patients were included in a clinical trial of vaccine combined with recombinant IL-2, one received dacarbazine, and one received thalidomide.

Toxicity

Overall, 89% of patients had adverse events of any grade, and 50 (56%) had severe (grade 3–4) adverse events. The most frequent severe adverse events were haematological: neutropenia (40%), thrombopenia (18%), anaemia (9%), febrile neutropenia (20%), and fatigue (12%).

Discussion

This retrospective study described a cohort of patients with metastatic melanoma treated with the CVD-INF immunochemotherapy regimen between 1993 and 2015 at a single institution. Central nervous system metastases, multiple metastases, and elevated LDH were independently associated with shorter survival, consistent with the literature [12, 13]. These findings are consistent with those of a recent study describing a large cohort of patients with metastatic melanoma treated with chemotherapy [14]. We observed that central nervous system metastases were more common in young patients (aged <50 years), an observation consistent with a large AJCC study showing that decreasing age at diagnosis was an independent predictive factor for the occurrence of central nervous system metastases at relapse for patients with stage III melanoma [15]. In the context of our cohort, this increased frequency of central nervous system metastases may also reflect selection bias. Elderly patients with central nervous system metastases might have been considered unfit for chemotherapy and excluded from our cohort.

Interestingly, 12% of patients survived at least two years, which was achieved without exposure to TKIs or immunotherapy (except for two patients). The proportion of patients alive at two years was 18% (11 of 61) in those without baseline central nervous system metastases at diagnosis. This proportion is in the same range as the 20%-survival plateau observed with anti-CTLA4 ipililumab in large randomised trials that excluded patients with central nervous system metastases [11, 16]. In addition, retrospective studies have observed a survival plateau with ipililumab only in patients without elevated LDH [17]. Our cohort included many patients with central nervous system metastases (32%) and/or elevated LDH (73%), which are associated with particularly poor outcomes with any type of therapy [18, 19]. This finding indicates that the survival plateau we observed after polychemotherapy and interferon is not due to the selection of patients with melanoma with a good prognosis.

The long survival obtained with CVD-INF in a subset of patients and the appearance of vitiligo in three of them may suggest that this regimen has an immunomodulating effect [20]. Therefore, we explored the tumour immune phenotype of long-survivors. $CD8^+$ T cells are key effectors mediating tumour rejection [21, 22]. Three classes of tumour immune microenvironment were defined based on the spatial distribution of $CD8^+$ T cells within tumour compartments (core, stroma, and invasive margins) [10, 23–25]: (a) "*Immune-inflamed*" or "hot" tumours are characterised by high $CD8^+$ T cell infiltration, increased interferon-γ signalling, and PD-L1 expression; (b) "*Immune-excluded*" or "cold" tumours are characterised by T cells localised in the tumour margin along the border of the tumour which are prevented from infiltrating the tumour core by myeloid cells [23]; (c) "*Immune desert*" tumours are characterised by few or no $CD8^+$ T cells. Immune-excluded and desert tumours are associated with poor outcomes [24, 26–29] and reduced response to immune checkpoint blockade [21, 30–33]. We found that most long-survivors had an inflamed tumour immune phenotype. This pattern has been associated with a better response to immune checkpoint blockade [32, 33] and chemotherapy [34] in metastatic cancer. It would be interesting to explore the combination of immune checkpoint blockade and chemotherapy in patients with such a phenotype.

Table 4:
Pathological characteristics of long-survivors and non-long-survivors.

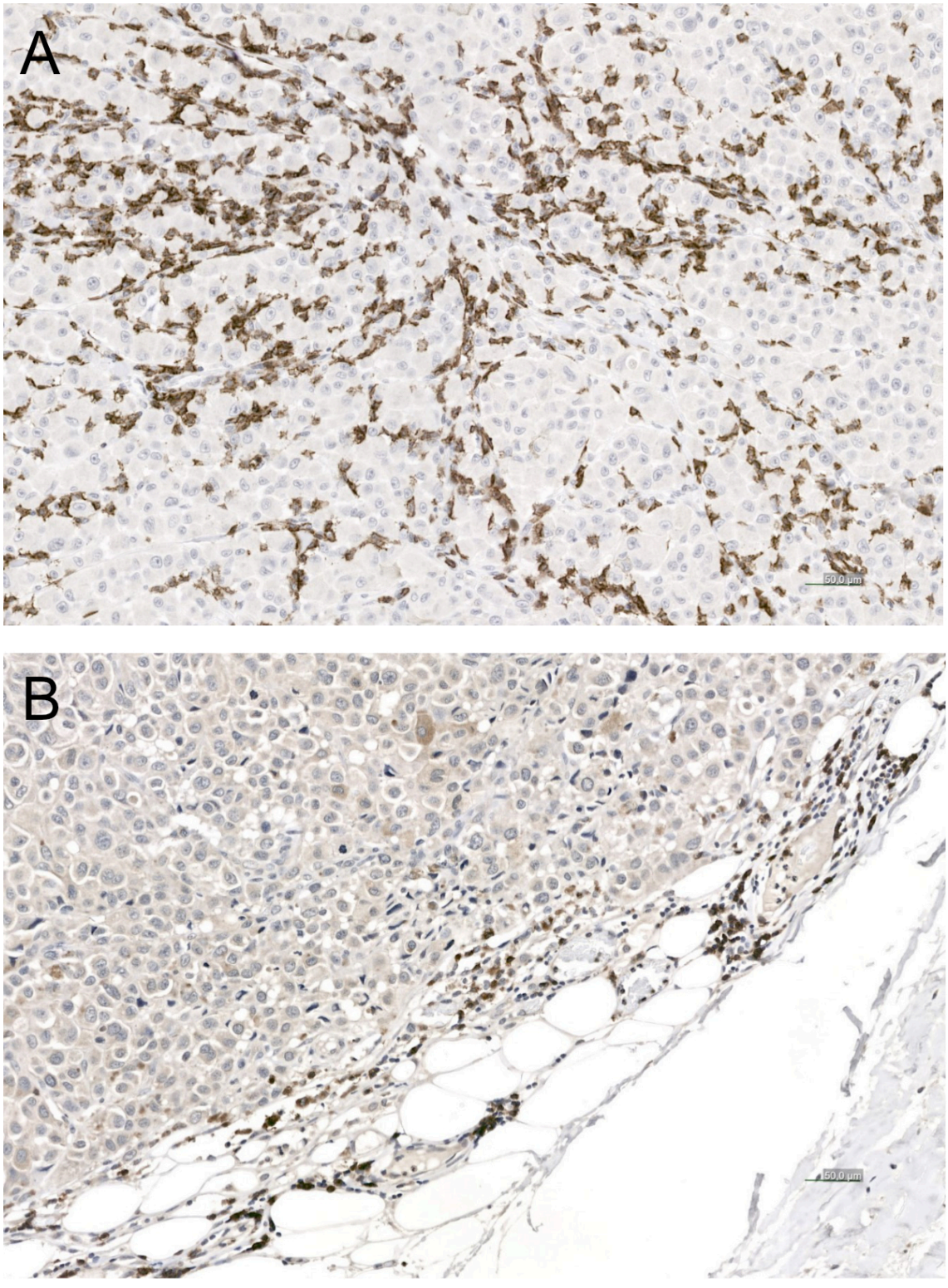
Pathological characteristic		Long-survivor	Non-long-survivor	p
		n = 9	n = 38	
		n (%)	n (%)	
BRAF V600	Yes	8 (89%)	20 (53%)*	0.064
	No	1 (11%)	18 (47%)	
PD-L1	>5%	2 (25%)	0 (0%)	0.074
	≤5%	6 (75%)	20 (100%)	
	NA	1	18	
Tumour immune phenotype	Inflamed	6 (75%)	5 (25%)	0.030
	Excluded/desert	2 (25%)	15 (75%)	
	NA	1	18	

PD-L1: programmed death ligand 1; NA: unavailable.

* One case had the *BRAF* V600K mutation.

An intriguing observation was the proportion of long-survivors with MUP (4 of 11), which represents 3% of newly diagnosed melanomas and 18% of stage IV melanomas [35]. The exact aetiology of MUP is unclear. It was suggested that MUPs are due to the regression of the primary lesion secondary to a robust immune response. They are more frequent in older patients, and males are disproportionally represented [36]. Stage IV MUPs have better outcomes than melanoma with known primary [35, 37] and may benefit more from immune checkpoint inhibitors [38]. Their mutational spectrum is similar to cutaneous melanoma with frequent mutations in *BRAF* and telomerase reverse transcriptase (*TERT*) [36, 39, 40]. Data on the

Figure 3: The spatial distribution pattern of CD8+ T cells. (**A**) A representative IHC CD8+ image of the fully-inflamed phenotype. (**B**) A representative IHC CD8+ image of the immune excluded phenotype. Magnification: 200×.



tumour microenvironment of MUPs are scarce, and they need further investigation.

The current frontline treatment for metastatic melanoma is immune checkpoint blockade or tyrosine kinase inhibitors, and these breakthrough therapies have led to unprecedented improvement in survival [41]. Nonetheless, half of patients with metastatic melanoma will progress and die from the disease within five years of diagnosis [41]. Adoptive T cell therapy with tumour-infiltrating lymphocytes is a new option for patients with metastatic melanoma resistant to PD1 blockade [42]. However, survival benefit is observed only in a subset of these patients. Alternative therapies are urgently needed. Combining chemotherapy with immunotherapy, such as immune checkpoint inhibitor blockade (reviewed in [43]) or intralesional injections of oncolytic virus, is currently being explored [44].

Our study suggests that a subset of patients with metastatic melanoma treated with immunochemotherapy who have a tumour-inflamed pattern show prolonged survival. These patients with a favourable immune phenotype are highly likely to respond well to any treatment. Indeed, the inflamed phenotype has been associated with better outcomes in other cancers treated with various therapeutic approaches [10, 45]. Whether chemotherapy may potentiate the benefit of immunotherapy should be explored in well-designed clinical studies, including in-depth translational analysis.

Availability of materials and data

All data analysed in this study have been included in the manuscript (and its supplementary information files).

Acknowledgments

Author contributions: Conceptualisation: CP, SILG and PYD; methodology: PT and SILG; data collection: CP, GK and CDV; writing: CP, PT, SILG and PYD. All the authors reviewed and edited the manuscript. Author contributions: Conceptualisation: CP, SILG and PYD; methodology: PT and SILG; data collection: CP, GK and CDV; writing: CP, PT, SILG and PYD. All the authors reviewed and edited the manuscript.

Financial disclosure

The work of PT is funded by the “Ligue Genevoise contre le cancer”.

Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest related to the content of this manuscript was disclosed.

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Appendix: supplementary tables

Table S1:
Incidence of central nervous system metastases by age.

		Central nervous system metastases, n (%)	
		Yes	**No**
Age (years)	<50	15 (45)	18 (55)
	≥50	14 (25)	43 (75)
Total		29	61

Table S2:
List of mutations in the three long-survivors.

	Gene	Mutation	Allele frequency	Pathogenicity
Patient 1	*ABCB1*	leu784phe	40%	uncertain
	ARID1A	pro559ala	42%	uncertain
	BAI3	ala1292val	40%	uncertain
	BCL11B	pro66his	40%	uncertain
	BCORL1	pro968val	51%	uncertain
	BRAF	val600glu	32%	pathogenic
	CSMD3	gln771ter	45%	uncertain
	CYP2C9	c.820–1G>A	76%	uncertain
	DCC	gly490glu	87%	uncertain
	DCC	pro1168leu	30%	uncertain
	DNMT3A	glu37lys	39%	uncertain
	EPHA3	arg782lys	43%	uncertain
	ERBB4	asp813asn	42%	uncertain
	ETS1	arg287cys	45%	uncertain
	FBX011	his873tyr	34%	uncertain
	FGFR3	ser430phe	43%	uncertain
	GRIN2A	glu1301lys	44%	uncertain
	HSPH1	chr13:31736285:C>T	62%	uncertain
	IDH1	arg132his	5%	pathogenic
	KMT2D	c.14515+2T>A	48%	uncertain
	LRP1B	asp2961asn	46%	uncertain
	LRP1B	trp2657ter	40%	uncertain
	MTOR	ser2127phe	43%	uncertain
	NTRK3	ala469val	42%	uncertain
	NUP98	arg1127cys	44%	uncertain
	PARK2	glu321lys	43%	uncertain
	RSF1	arg1323gly	42%	uncertain
	RUNX1T1	arg160cys	40%	uncertain
	TET2	pro869leu	45%	uncertain
	TOP1	leu617phe	42%	uncertain
	TP53	delins	55%	pathogenic
	TYK2	ala813val	45%	uncertain
	UGT1A1	ala458val	45%	uncertain
	USP9X	pro2253	47%	uncertain
Patient 2	*ARID1A*	Arg1461Ter	13%	probably pathogenic
	ARID5B	Glu1048Val	17%	uncertain
	BRAF	Val600Glu	8%	pathogenic
	CDK12	Arg356Lys	17%	uncertain
	FLT3	leu520Pro	13%	uncertain
	GRM8	pro468his	24%	uncertain
	LRP1B	glu4333lys	13%	uncertain
	MED12	his182thy	13%	uncertain
	MTOR	ser2215phe	19%	probably pathogenic
	SETBP1	val1137ile	16%	uncertain
	sox11	pro132ser	16%	uncertain
	tbx3	ile241lys	22%	uncertain
	Col2a1	Pro28Ser	8%	uncertain
Patient 3	*ARID1a*	Gln1537Ter	39%	probably pathogenic
	ARID1B	pro1489Leu	74%	uncertain
	ARID2	Pro1497Leu	44%	uncertain
	ATM	Ser2859Phe	86%	uncertain
	BRAF	Val600Glu	57%	pathogenic
	CYP2B6	Asp469Asn	41%	uncertain
	DDR2	Gly235Ser	68%	uncertain
	EPHA3	Trp345Ter	37%	probably pathogenic
	EPHA3	Gln905Arg	40%	uncertain
	GREM1	gly63Arg	58%	uncertain
	IKZF1	pro172leu	56%	uncertain
	MTOR	thr1876ile	45%	uncertain
	PDGFRA	glu996lys	44%	probably benign
	POLE	His1810Tyr	39%	uncertain

	PTEN	p.His93Tyr	70%	pathogenic
	PTPRB	p.Ser862Phe	44%	uncertain
	SNCAIP	p.Met720Ile	48%	uncertain
	TERT	c.–146C>T	58%	pathogenic