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ORIGINAL RESEARCH

Sphingosine-1 Phosphate Receptor Modulators Increase In Vitro Melanoma Cell Line Proliferation at Therapeutic Doses Used in Patients with Multiple Sclerosis

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ABSTRACT

Introduction: $S1P_1$ receptor modulators ($S1P_1$-RM) are oral disease-modifying therapies (DMTs) for multiple sclerosis (MS). Several authorities have raised doubts that $S1P_1$-RM are responsible for an increased risk of melanoma in patients with MS. We studied the in vitro effects of $S1P_1$-RM on different melanoma cell lines to compare the effect of available $S1P_1$-RM on the proliferation of human melanoma cells.

Methods: Four $S1P_1$-RM were studied which are currently approved for managing MS, namely fingolimod (Gilenya®), siponimod (Mayzent®), ozanimod (Zeposia®), and ponesimod (Ponvory®). We tested these four drugs at different concentrations, including therapeutic doses (0.5, 1.6, 5.5, 18, and 60 μM), on human melanoma cell lines (501Mel cells, 1205LU cells, and M249R cells) to analyze in vitro cell proliferation monitored with the IncuCyte ZOOM live cell microscope (Essen Bioscience).

Results: At therapeutic doses, median confluence increased overall for all lineages: + 122% for ozanimod ($p < 0.001$), + 71% for ponesimod ($p < 0.001$), + 67% for siponimod (NS), and + 41% for fingolimod ($p = 0.094$). Ozanimod- and ponesimod-treated cells increased confluency in 501Mel, 1205LU, and M249R cell lines ($p < 0.001$).

Conclusion: These data suggest an increased proliferation of various melanoma cell lines with $S1P_1$-RM treatments used at therapeutic concentrations for patients with MS and should raise the question of increased dermatologic surveillance.

Keywords: Sphingosine-1 phosphate; Multiple sclerosis; Inflammation; Siponimod; Ozanimod; Ponesimod; Fingolimod; Melanoma cell lines

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Key Summary Points

There is some (but not sufficiently supported) evidence that drugs for MS such as fingolimod, siponimod, ozanimod, and ponesimod can increase the risk of malignancies, including skin cancers.

This problem is not well defined by data from randomized controlled trials, but several case reports have confirmed the occurrence of melanoma in patients treated with these new medications called $S1P_1$ receptor modulators ($S1P_1$-RM).

This study assessed the in vitro effects of $S1P_1$-RMs on different melanoma cell lines to compare their effect on the proliferation of human melanoma cells.

An increased proliferation of various melanoma cell lines was observed suggesting (and confirming) the advisability of a careful dermatologic surveillance of patients treated with this new family of drugs.

INTRODUCTION

Multiple sclerosis (MS) is the most common inflammatory immune-mediated demyelinating disease of the central nervous system (CNS) and represents the most common cause of neurological disability in young adults [1]. An early diagnosis of MS is essential to receiving timely treatment aimed at reducing permanent neurological disability. Many disease-modifying therapies (DMTs) with different immunomodulatory mechanisms have become available with a marked effect on MS inflammatory activity [2].

Early immunosuppression and immunomodulation have been the mainstay of therapeutic strategies in relapsing–remitting forms of MS (RRMS). Sphingosine 1-phosphate receptor modulators ($S1P_1$-RM) are a class of treatment that enables the sequestration of lymphocytes within lymphatic tissue [3]. The primary mechanism of action of $S1P_1$-RM in MS is through receptor binding on lymphocytes, resulting in the internalization of the receptor–drug complex resulting in the loss of responsiveness to the S1P gradient that drives lymphocyte egress from lymph nodes [4]. The reduction in circulating lymphocytes presumably limits inflammatory cell migration into the CNS [4]. Even if clinical trials or follow-up studies involving fingolimod, such as TRANSFORMS [5], FREEDOMS [6], and INFORMS [7], have not revealed any significant differences in the incidence of cancers, many pharmacovigilance cases have been reported recently [8], including lymphoma, HPV-related cancers, and cutaneous malignancies (Table 1). Several serious adverse events have been described due to the non-selective interactions with other receptors, specifically $S1P_{3–5}$ [9]. The development of new-generation $S1P_1$-RM with more enhanced selectivity, such as siponimod, ozanimod, and recently marketed ponesimod (March 2021) [10, 11], may result in improved safety and efficacy. Nevertheless, skin cancers such as melanoma remain a safety concern. Indeed, some publications have described new cases of skin cancer in patients treated with fingolimod [8, 12, 13] and ozanimod [14]. However, until now, no study has been performed to evaluate the safety of different doses of fingolimod, siponimod, ozanimod, and ponesimod and their effect on human melanoma cell line proliferation. This study analyzed the in vitro effects of $S1P_1$-RM through established melanoma cell lines.

METHODS

$S1P_1$-RM were prepared at different concentrations ranging from subtherapeutic to supratherapeutic levels, specifically 0.5, 1.6, 5.5, 18, and 60 μM compared to the control group (0 μM). We have determined the therapeutic concentration from the therapeutic doses at 1.6–4.06 μM [19], 0.48–3.9 μM [20], 4.3–43 μM [21], and 0.57–2.27 μM [20] for fingolimod,

Table 1 Prevalence of malignancies under $S1P_1$-RM treatment

Drug	Trials/duration	Malignancies prevalence
Fingolimod	[3]	• 0.5 mg group (n = 429)
	FREEDOMS	o Basal cell carcinoma, n = 4 (0.9%)
	24 months	• 1.25 mg group (n = 425)
	[5]	o Basal cell carcinoma, n = 1 (0.2%)
	TRANSFORM	o Breast cancer, n = 1 (0.2%)
	12 months	o Malignant melanoma, n = 1 (0.2%)
		o Bowen's disease, n = 1 (0.2%)
		• Placebo group (n = 418)
		o Basal cell carcinoma, n = 3 (0.7%)
		o Breast cancer, n = 3 (0.7%)
		o Malignant melanoma, n = 1 (0.2%)
		o Cervical carcinoma stage 0, endometrial cancer, n = 1 (0.2%)
		o Prostate cancer, n = 1 (0.2%)
		• 0.5 mg group (n = 429)
		o Melanocytic nevus, n = 28 (6.5%)
		o Basal cell carcinoma, n = 3 (0.7%)
		o Melanoma (including in situ), n = 3 (0.7%)
		o Breast cancer, n = 2 (0.5%)
		• 1.25 mg group (n = 420)
		o Melanocytic nevus, n = 42 (10.0)
		o Basal cell carcinoma, n = 2 (0.5%)
		o Melanoma (including in situ), n = 0
		o Breast cancer, n = 2 (0.5%)
		• Interferon beta-1a n = 431
		o Basal cell carcinoma, n = 1 (0.2%)
		o Melanoma (including in situ), n = 0
		o Breast cancer, n = 0

Table 1 continued

Drug	Trials/duration	Malignancies prevalence
Fingolimod	[7]	• 0.5 mg group (n = 336)
	INFORMS	o Basal cell carcinoma, n = 14 (4%)
	36 months	o Squamous cell carcinoma of skin, n = 6 (2%)
		o Malignant melanoma (including in situ), n = 1 (< 1%)
		o Breast cancer, n = 1 (< 1%)
		o Invasive lobular breast carcinoma, 0
		o Non-Hodgkin lymphoma, 1 (< 1%)
		o Lung neoplasm, malignant, 1 (< 1%)
		o Ovarian cancer, 1 (< 1%)
		o Prostate cancer, 1 (< 1%)
		• Placebo group (n = 487)
		o Basal cell carcinoma, n = 9 (2%)
		o Squamous cell carcinoma of skin, n = 1 (< 1%)
		o Malignant melanoma (including in situ), n = 0
		o Breast cancer, n = 0
		o Invasive lobular breast carcinoma, n = 1 (< 1%)
		o Non-Hodgkin lymphoma, n = 0
		o Lung neoplasm, malignant, n = 0
		o Ovarian cancer, n = 0
		o Prostate cancer, n = 1 (< 1%)
Siponimod	[15]	• 0.25 mg group (n = 50)
	BOLD	o None
	24 months	• 0.5 mg group (n = 29)
	[16]	o Cervix neoplasm n = 1, (3.4%)
	EXPAND	• 1.25 mg (n = 43)
	36 months	o Basal cell carcinoma, n = 1 (2.3%)
		• 2 mg (n = 29)
		o None
		• 10 mg (n = 33)
		o None
		• 0.5 to 2 mg (n = 1099)
		o Skin neoplasms, malignant and unspecified, n = 14 (1%)
		o Basal cell carcinoma, n = 11 (1%)
		• Placebo (n = 546)
		o Skin neoplasms, malignant and unspecified, n = 8 (1%)
		o Basal cell carcinoma, n = 6 (1%)

Table 1 continued

Drug	Trials/duration	Malignancies prevalence
Ozanimod	[17]	• 0.5 mg (n = 439)
	RADIANCE	o Malignant melanoma in situ, n = 1 (0.2%)
	24 months	o Medulloblastoma, n = 1 (0.2%)
	[18]	o Basal cell carcinoma, n = 1 (0.2)
	SUNBEAM	• 1.0 mg (n = 434)
	12 months	o Invasive breast carcinoma, n = 1 (0.2%)
		o Keratoacanthoma, n = 1 (0.2%)
		o Basal cell carcinoma, n = 1 (0.2%)
		o Brest cancer, n = 1(0.2%)
		• Interferon beta-1a (n = 440)
		o Chronic lymphocytic leukemia, n = 1 (0.2%)
		o Basal cell carcinoma, n = 1 (0.2%)
		• 0.5 mg (n = 453)
		o Invasive breast carcinoma and basal cell carcinoma, n = 2 (0.4%)
		• 1.0 mg (n = 448)
		o Testicular seminar, n = 1 (0.2%)
		• Interferon beta-1a (n = 445)
		o None
Ponesimod	[11]	• Ponesimod 20 mg (n = 565)
	OPTIMUM	o Skin malignant condition, n = 5 (0.9%)
	48 months	o Non skin malignant condition, n = 1 (0.2%)
		• Teriflunomide 14 mg (n = 566)
		o Skin malignant condition, n = 1 (0.2%)
		o Non skin malignant condition, n = 1 (0.2%)

siponimod, ponesimod, and ozanimod, respectively (Table 2). Cells and reagents for melanoma cell lines along with their maintenance were previously described in the literature [22]. Three cell lines harboring the activating oncogenic BRAF, a serine/threonine protein kinase mutation found in 50% of melanomas [23], were used in the experiments: 501Mel cells (from Dr. R. Halaban, Yale University School of Medicine, New Haven, CT, USA), M249R cells (from Dr. R. Lo, UCLA Dermatology, Los Angeles, CA, USA), and 1205LU cells (from Rockland, USA). These cell lines display distinct gene expression patterns and phenotypic behavior (Table 3): 501Mel cells show a predominantly proliferative and melanocytic differentiation phenotype, 1205LU cells are an invasive and de-differentiated state [22], and M249R are NRAS-mutated resistant to vemurafenib. Cell lines were used within 6 months between the resuscitation and experimentation and were authenticated via short tandem repeat (STR) profiling (Eurofins Genomics). The cells were routinely tested for the absence of mycoplasma by PCR. The experiments using melanoma cells derived from human tissue samples were

Table 2 Characteristics of $S1P_1$-RM modulators

Drug	Name	MW (g/mol)	Oral use (mg)	$S1P_1R$	Study/Trial	FDA approval date	Indications	Reported skin cancer in clinical trial
Fingolimod	Gilenya® FTY20	307.5	0.5–1.25	$S1PR_1$ $S1PR_3$ $S1PR_4$ $S1PR_5$	FREEDOMS FREEDOMS II [3]	March 2019	RRMS SPMS	0.2–6.2%
Siponimod	Mayzent® BAF 312	516.5	0.25–2	$S1PR_1$ $S1PR_5$	BOLD [15] EXPAND [16]	March 2019	RRMS SPMS	0–2.6%
Ozanimod	Zeposia® RPC 1063	404.5	0.23–0.92	$S1PR_1$ $S1PR_5$	RADIANCE [17] SUNBEAM [18]	March 2020	RRMS SPMS	0–0.45%
Ponesimod	Ponvory® ACT128800	460.9	2–20	$S1PR_1$	OPTIMUM [11]	March 2021	RRMS	0–0.9%

MW molecular weight, *RRMS* relapsing–remitting multiple sclerosis, *SPMS* secondary progressive multiple sclerosis, *FDA* US Food and Drug Administration

conducted according to the principles of the Declaration of Helsinki. They had institutional approval (agreement no. 2137 from the French ministère de l'Enseignement supérieur et de la recherche). For live imaging, cells were transduced with NucLight Red lentivirus reagent (Essen Bioscience) and selected with puromycin (1 mg/ml). Culture reagent was purchased from Thermo Fisher Scientific [24]. Cell growth assays using a live cell imager were used to determine melanoma cell viability in response to ponesimod, ozanimod, siponimod, and fingolimod. Different nuclear-labeled fluorescent melanoma cells were treated with various concentrations of $S1P_1$-RM, using clinical doses in people with MS for in vitro cell cultures. Cell proliferation was monitored with the IncuCyte ZOOM live cell microscope (Essen Bioscience), and images were taken every 4 h over 6 days. Confluency and number of nuclei (red cell objects) were quantified by the IncuCyte software. Normal melanocyte proliferation was measured by counting DAPI-stained nuclei.

VigiBase® Query

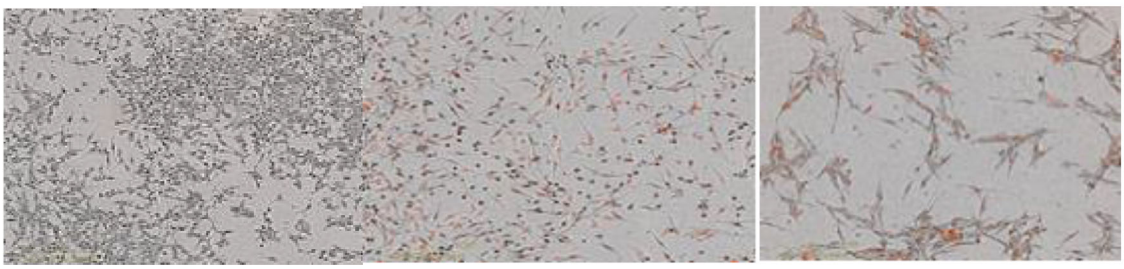
Since 1978, the World Health Organization (WHO) mandates the Uppsala Monitoring Centre (UMC) to monitor worldwide drug safety. Data issued by each of the 130 national pharmacovigilance networks feed into the UMC database. VigiBase® [25] collects spontaneous reports, ensuring the preservation of the anonymity of patients and notifiers.

Sociodemographic characteristics (age, sex, notifier's country) and details concerning the reported effect (suspected drugs, concomitant drugs, adverse drug reaction, and seriousness) are collected.

We queried VigiBase® for all notified cases of melanoma, and the following drugs: fingolimod, siponimod, ponesimod, and ozanimod, from November 14, 1967 (first cases reported) to November 20, 2022. In practice, the melanoma reports were defined by the Medical Dictionary for Regulatory Activities (MedDRA, version 25.1), with "melanoma of skin" as High

Table 3 Characteristics of melanoma cell lines

	501 Mel	1205lu	M249R
Phenotypic signature	Proliferative	Invasive	Proliferative
Mutation(s)	BRAFV600E	BRAFV600E/PTEN[a]	BRAFV600E/PTEN[a]/NRAS
Resistance	None	None	vemurafenib



Level Term (HLT) and ocular melanoma as Preferred Term (PT) [26].

Statistical Analysis

Numeric data were expressed as median (IQR) and discrete data as frequencies (percentages). The Shapiro–Wilk test and Levene's test assessed the normality and heteroskedasticity of data. Differences in the confluence between experiences were assessed with the Mann–Whitney's test for two groups and the Kruskal–Wallis's test for three or more groups. If the null hypothesis of the Kruskal–Wallis's test was rejected, post hoc pairwise analyses were performed using Dunn–Bonferroni's test considering p value adjustment for multiple comparisons. Alpha risk was set to 5% ($\alpha = 0.05$). Statistical analysis was performed using EasyMedStat (version 3.19; www.easymedstat.com) and GraphPad Prism software version 9.4.1 (GraphPad Software, San Diego, CA, USA).

RESULTS

Proliferation of All Melanoma Cell Lines Under $S1P_1$-RM at Therapeutic Doses

At therapeutic doses, median confluence increased overall for all lineages (Table 4): + 122% for ozanimod ($p < 0.001$), + 71% for ponesimod ($p < 0.001$), + 67% for siponimod (NS), and + 41% for fingolimod ($p = 0.094$).

At supratherapeutic concentrations, the drugs crystallized, resulting in a lack of adherence of cells. We could not detect if low confluency was due to cell death or high proliferation provoked by cell detachment (Figs. S1 and S2 in the supplementary material). A saturation point of confluency was reached at those supratherapeutic concentrations for all four drugs, resulting in a ceiling effect. Confluence decreased at 18 concentrations in all lineages but siponimod (Fig. 1 and Fig. S2).

Table 4 Median confluence increased overall for all lineages at therapeutic doses

Experience	Proliferation median (IQR) All lineages	*P* value compared to the control
Ozanimod control	28.6 (10.8)	–
Ozanimod 1.6 µM	63.5 (53.9)	< 0.001
Ponesimod control	38.7 (31.2)	–
Ponesimod 1.6 µM	85.7 (24.8)	< 0.001
Ponesimod 5.5 µM	81.9 (48.5)	< 0.001
Fingolimod control	45.6 (69.2)	–
Fingolimod 1.6 µM	67.1 (60.2)	0.094
Siponimod control	85.6 (67.6)	–
Siponimod 0.5 µM	81.5 (53.1)	0.71
Siponimod 1.6 µM	75.6 (43.3)	0.231

Proliferation of Melanoma Cell Lines Under $S1P_1$-RM at Therapeutic Doses

Median confluence values increased from 24.7 (IQR 3) to 34.6 (IQR 4) in M249R cell lines treated with fingolimod at therapeutic doses ($p < 0.001$) but did not increase in 501Mel ($p = 0.285$) and 1205LU ($p = 0.069$). Similar results were observed for siponimod-treated cells, with no increase in proliferation in 501Mel ($p = 0.553$) and 1205LU ($p = 0.553$) but increased confluency in M249R ($p < 0.001$). Conversely, second-generation $S1P_1$-RM ozanimod and ponesimod-treated cells increased confluency in 501Mel, 1205LU, and M249R cell lines ($p < 0.001$).

Dose–Response Relationship

We observed a dose–response effect for therapeutic doses of ponesimod on M249R lineages with increasing confluences between 0 and 0.5 µM [28.6 (0.5) vs 41.6 (3.6), $p = 0.005$] and between 0.5 and 1.6 µM [41.6 (3.6) vs 47.8 (3.8), $p = 0.028$].

Our experiments did not observe such a dose–response effect for other drugs on cell lineages (Fig. 2).

Characteristics of the Reports

As of November 20, 2022, 418 cases of melanomas were collected in VigiBase®, of which 412 were associated with fingolimod and 6 with siponimod. There is no case of melanoma reported with ponesimod or ozanimod to date in Vigibase®. Most cases concerned women: 286 (68.4%) among all reported melanomas. The most represented age range was the 45-to-64-year group, with 158 (37.8%) cases, with a median age of 47 years. More than a quarter of the notifications came from the USA (122 reports, 29.2%). Nearly all melanoma cases, 397 (95.0%) were deemed serious, with 346 reports (82.8%) medically serious and 4 fatal cases (1%). In 6 cases, melanoma was an ocular melanoma, the other preferred term reported was malignant melanoma. The most frequent co-reported MedDRA clinical terms were melanocytic nevus in 26 reports (6.2%), basocellular carcinoma in 21 reports (5.0%), and skin lesions in 17 reports (4.1%).

DISCUSSION

There are increasing reports in the literature about the development of cutaneous melanoma

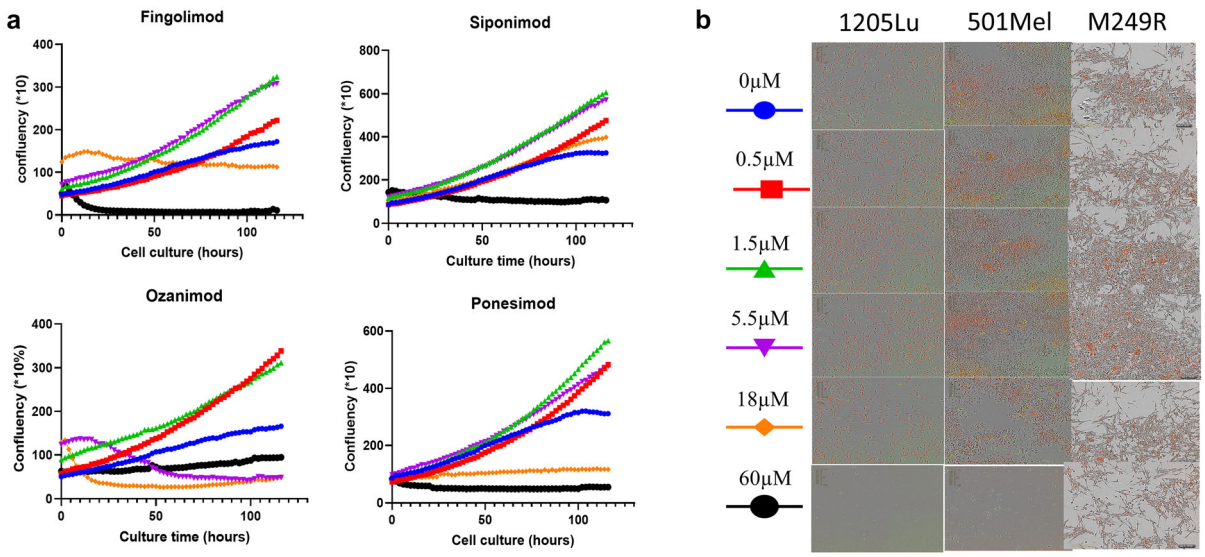


Fig. 1 Confluences of 501Mel, 1205LU, and M249R under treatment with $S1P_1$-RM over time. **a** Fingolimod, siponimod, ozanimod, and ponesimod induced growth of the non-metastatic 501Mel BRAF mutant melanoma cells, the metastatic 1205LU BRAF mutant, and M249R melanoma cells. Growth curves of 501Mel, 1205LU BRAF, and M249R melanoma cells labeled with the NucLight nuclear reagent were treated with vehicle (DMSO) or the indicated doses of $S1P_1$-RM. Data were acquired in triplicate for 6 days using the live-cell imager IncuCyte. **b** Microphotographs showing the survival of $S1P_1$-RM on the morphology of 501Mel cells (red nuclei, above) or the metastatic 1205LU BRAF mutant melanoma cells (below) at the end of the experimental course

following exposure to MS DMTs [3, 5, 12, 27–33] (Table 5) [33]. Indeed, previous studies [10] published real-world data that demonstrated an increased incidence of basal cell or squamous cell carcinoma or melanoma in those exposed to fingolimod and siponimod. However, the number of cases remains low compared to populational estimates, not reaching statistical significance. The occurrence of melanoma in these patients could be coincidental [28]. The relationship between cutaneous melanoma and exposure to MS DMTs is biologically plausible but approaches including the impact of these medications within in vitro studies of melanoma cell lines are lacking [34]. We tested the effects of incremental concentrations of $S1P_1$-RM (0, 0.5, 1.6, 5.5, 18, or 60 μM) on a panel of human melanoma cell lines (501Mel and 1205LU) sensitive to treatment and M249R resistant to treatment. Proliferative melanoma cells (501Mel, M249R) rapidly form tumors, whereas invasive melanoma cells (1205LU) take weeks longer to initiate tumor growth. Melanoma cells resistant to the BRAF inhibitor are characterized by a lower proliferation rate [35].

Our findings indicate that ponesimod and ozanimod increase melanoma cell proliferation irrespective of their oncogenic status, phenotypic behavior, and therapeutic susceptibility. Ozanimod and ponesimod, the new generation of $S1P_1$-RM modulators, have been marketed as having greater receptor selectivity, conveying better efficacy and safety, with no notable malignant events reported so far in the pilot studies [36]. Unexpectedly, these more recently approved $S1P_1$-RM showed higher proliferation and dose effects in melanoma cell lines than with fingolimod and siponimod. Our results also support a dose–concentration effect as melanoma cell proliferation was associated with increased concentrations of ponesimod. In the phase III OPTIMUM clinical trial, five people with MS receiving ponesimod developed malignant skin cancers (two were basal cell carcinomas, two with excision of preexisting

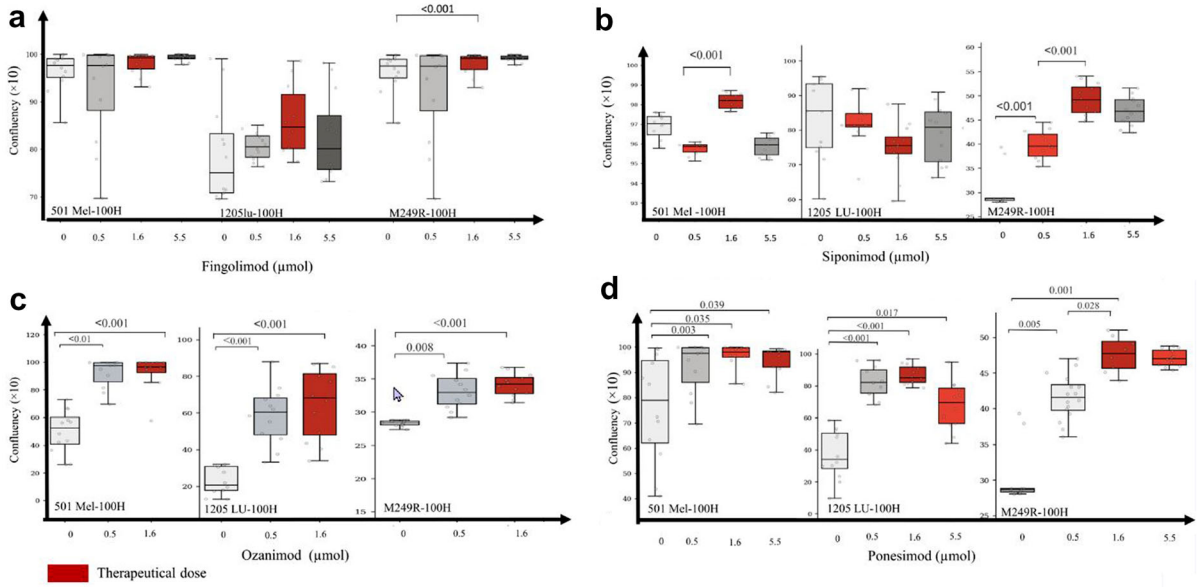


Fig. 2 Dose effect of $S1P_1$-RM on different melanoma cells lines. **a** Confluences of 501Mel, 1205LU, and M249R cell lines after 100 h of treatment with fingolimod at 0 μM, 0.5 μM, 1.6 μM, and 5.5 μM. Proliferation was stable from 0 to 5.5 μM in 501Mel cells line ($p = 0.17$). Median values of 1205LU cell line confluences steadily increased 45.62 (IQR 27.57), 57.84 (IQR 10.04), 67.11 (IQR 25.43), and 56.95 (IQR 25.32) ($p = 0.13$). Differences of proliferation were found at 0.5 μM vs 0 μM ($p = 0.006$), 1.6 μM vs 0 μM ($p < 0.001$), 5.5 μM vs 0 μM ($p < 0.001$), and 5.5 μM vs 0.5 μM ($p = 0.014$) in M249R. **b** Proliferation of 501Mel under siponimod was not linear from 0.5 to 5.5 μM but confluences increased significantly at 1.6 μM vs 0.5 μM ($p < 0.001$) whereas proliferation was stable from 0 to 5.5 μM, and no significant differences were found in 1205 cell lines. Proliferation of M249R cell lines increased for 1.6 μM vs 0 μM ($p < 0.001$), 1.6 μM vs 0.5 ($p < 0.001$), and 0.5 μM vs 0 μM ($p < 0.001$) under siponimod treatment. **c** Proliferation of 501Mel was not linear under ozanimod from 0.5 to 5.5 μM but confluences increased significantly at 1.6 μM vs 0.5 μM ($p < 0.001$). Proliferation was stable from 0 to 5.5 μM in 1205LU whereas proliferation of M249R cell lines increased for 1.6 μM vs 0 μM ($p < 0.001$), 1.6 μM vs 0.5 ($p < 0.001$), and 0.5 μM vs 0 μM ($p < 0.001$). **d** Under ponesimod treatment, proliferation of 501Mel increased at 0.5 μM vs 0 μM ($p = 0.035$), 1.6 μM vs 0 μM ($p = 0.003$), and 5.5 vs 0 μM ($p = 0.039$). Proliferation of 1205LU increased at 0.5 μM vs 0 μM ($p < 0.001$), 1.6 μM vs 0 μM ($p < 0.001$), 5.5 μM vs 0 μM ($p = 0.017$), and 5.5 μM vs 0.5 μM ($p = 0.046$). Proliferation of M249R increased at 0.5 μM vs 0 μM ($p = 0.005$), 1.6 μM vs 0 μM ($p < 0.001$), 1.6 μM vs 0.5 μM ($p = 0.028$)

benign lesions (nevus), and one with malignant melanoma) compared with one patient with basal cell carcinoma in the teriflunomide group [11, 32].

Fingolimod and siponimod increase the proliferation of M249R cell lines, which are resistant to treatment with BRAF and NRAS co-mutation and known to rapidly form tumors. A previous study found that fingolimod treatment, starting from a concentration of 0.5 μM, significantly impaired LCP-Mel proliferation whereas it did not affect the proliferation of both GR-Mel and WM115 cells (human melanoma cell lines) until a concentration of 5 μM fingolimod was reached [34]. In this study, treatment was performed only for 48 h whereas we observed differences in 100 h and impairing was identified at high doses of fingolimod.

Several theories have described how $S1P_1$-RM modulators may be related to skin cancer [10]: for example, fewer circulating lymphocytes possibly needed to identify and eventually eliminate malignant cells, activation of the IL-6/JAK/STAT3 identified to have a

Table 5 Reported cases of melanoma after fingolimod treatment in patients with multiple sclerosis

Cases sex (M/F)/age (years)	Melanoma type	Time of treatment (months)	Fingolimod (mg/day)	Skin phototype	Evolution	Familial history	Source
1 case	NA	24	1.25	NA	Treatment stopped	NA	[3]
1 case	NA	36	1.25	NA	NA	NA	[29]
3 cases	NA	NA	0.5	NA	NA	NA	[5]
F/41	Melanoma Ex-nevo	57	1.25	II	Treatment stopped	NA	[27]
M/51	SSM	48	0.5	NA	Treatment stopped	NA	[30]
1F/41	SSM	NA	0.5	II	Treatment stopped	NA	[12]
1 F/52	SSM	61	0.5	> 50 nevi	Treatment stopped	No history of skin cancer	[28]
1 case	NA	NA	0.5	NA	NA	NA	[7]
1 F/44	SSM	32	NA	NA	NA	NA	[31]
1 F/38	SSM	15	NA	NA	NA	NA	[31]
1 F/44	SSM	12	NA	NA	NA	NA	[31]
1 M/32	SSM	31	NA	NA	NA	NA	[31]
1 F/45	SSM	20	NA	NA	NA	NA	[31]
1 F/51	Nodular melanoma	48	0.5	I	Treatment stopped	Melanoma of her mother	[13]
1 F/52	Unclassifiable malignant melanoma	36	NA	II	Treatment stopped	No personal or family history of melanoma	[8]
1 F/48	Thin cutaneous melanoma	84	NA	NA	NA	Numerous nevi and reported of a familiarity with epithelial tumors	[34]

F female, *M* male, *MS* multiple sclerosis, *SSM* superficial spreading malignant melanoma, *NA* not applicable

protumorigenic effect, and the relationship with tumor microenvironment influencing the secretion of VEGF-A [31].

Our study is the first to test $S1P_1$-RM, including ponesimod, on various human melanoma cell lines for 6 days at different concentrations ranging from 0 to 5.5 µM. We could not analyze confluences for the supratherapeutic doses (18 and 60 µM).

This study has limitations. In vitro phenomena are often difficult to replicate in vivo as we do not have the dynamic interplay of

immunosurveillance, loss of in vivo microenvironment, stromal, vascular, and immune cellular populations [37]. Therefore, we have added clinical reports in our review of the literature. We could have tested $S1P_1$-RM on primary human melanocyte cells, but those cells do not proliferate as melanoma cell lines.

The results of this study, if correct, should alert patients to a potential increased risk of skin cancers, and the use of sunscreen or protective coverings to limit the impact of ultraviolet light exposure should be considered. The restricted use of $S1P_1$-RM in people with MS who have a prior history of skin malignancies may also be considered. Monitoring measures such as skin examination at baseline to screen for precancerous skin lesions or additional risk factors, regular dermatologic monitoring, and patient education for regular self-skin controls are indicated for patients with MS under treatment with $S1P_1$-RM.

CONCLUSION

Complementary studies are needed to evaluate whether the class of $S1P_1$-RM drugs results in an increased propensity for risk of melanoma or other skin cancers given the presence of atypical skin lesions. Pending further evidence, we asked healthcare professionals to report any suspected adverse reactions involving skin malignancies and to consult the risk management plan submitted to European medical agencies before $S1P_1$-RM administration. We suggest that skin examinations be regularly performed with close dermatological surveillance before and during therapy, and upon the identification of any suspicious lesions. People with MS treated with $S1P_1$-RM should be advised to follow the usual guidelines for people at high risk of skin cancer until further in vitro clinical research clarifies the link between these drugs and the potential risk of melanoma development. These findings need to be further evaluated, including evidence from people within real-world prospective cohorts from other regions.

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Disclosures. Caroline Ruetsch-Chelli, Darin Okuda, Fanny Rocher, Christine Lebrun-Frenay, Sophie Tartare-Deckert and Marcel Deckert have nothing to declare.

Compliance with Ethics Guidelines. The experiments using melanoma cells derived from human tissue samples were conducted according to the principles of the Declaration of Helsinki. They had institutional approval (agreement no. 2137 from the French ministère de l'Enseignement supérieur et de la recherche).

Data Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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