Effects on Melanoma Cell Lines Suggest No Significant Risk of Melanoma Under Cladribine Treatment

Christine Lebrun-Frenay, Ilona Berestjuk, Mikael Cohen, Sophie Tartare-Deckert

Neurol Ther
https://doi.org/10.1007/s40120-020-00204-5

BRIEF REPORT

Effects on Melanoma Cell Lines Suggest No Significant Risk of Melanoma Under Cladribine Treatment

Christine Lebrun-Frenay · Ilona Berestjuk · Mikael Cohen · Sophie Tartare-Deckert

Received: May 31, 2020

ABSTRACT

Cladribine is an oral synthetic purine analog that depletes lymphocytes and induces a dose-dependent reduction of T and B cells. It was approved for the therapy of highly active relapsing-remitting multiple sclerosis. Given cladribine's mechanism of action, an increased risk of malignancies was suspected from the number of cancers that occurred in the 3.5 mg/kg-treated arm (CLARITY study). We showed that cladribine inhibits cell proliferation on three melanoma cell lines tested, irrespectively of their mutational oncogenic status and invasive/metastatic potential. Aggregated safety data demonstrated that the risk of melanoma is not confirmed.

Digital Features To view digital features for this article go to https://doi.org/10.6084/m9.figshare.12662237.

M. Cohen
Université Côte d'Azur, URRIS UR2CA, Nice, France

M. Cohen
Centre de Ressources et de Compétence Sclérose En Plaques, CHU de Nice, Hôpital Pasteur2, Nice, France

C. Lebrun-Frenay (✉) · I. Berestjuk · S. Tartare-Deckert
Université Côte d'Azur, INSERM, C3M, Nice, France
e-mail: lebrun-frenay.c@chu-nice.fr

Keywords: Cladribine; Immunosuppressors; Melanoma; Multiple sclerosis

Key Summary Points

There is literature relating immunosuppressive drugs used for autoimmune diseases to the risk of cancer

Clinical studies have raised some doubts about cladribine being responsible for an increased risk of melanoma in multiple sclerosis patients

Our study demonstrates that at either low or high cladribine concentrations, there is no proliferation or differentiation of melanocytes

These results could affect future clinical practice for neurologists using cladribine for their MS patients and could influence monitoring

Data from registries are mandatory to confirm our in vitro study

INTRODUCTION

Cladribine (2-chloro-2′-deoxyadenosine, 2-CDA) is a synthetic purine nucleoside analog

Published online: 25 July 2020

cytotoxic to lymphocytes and, to a lesser degree, monocytes and hematopoietic cells. As a result, cladribine induces a dose-dependent reduction of T and B cells lasting months to years. The 96-week, placebo-controlled CLARITY study showed that treatment with oral cladribine is clinically and radiologically effective in patients with relapsing-remitting multiple sclerosis (MS) [1]. However, US and European regulatory authorities expressed concerns about the risk-benefit profile of the drug, and the sponsor initially decided not to continue its further development in MS. Patients included in the phase 3 randomized ORACLE MS trial from October 2008 to 2010 receiving either cladribine 5.25 mg/kg cumulative dose, cladribine 3.5 mg/Kg cumulative dose or placebo were offered participation in an extension period [2]. Three cases of cancer occurred in patients treated with 3.5 mg/kg cladribine (one cutaneous melanoma, one pancreatic cancer and one ovarian cancer). A meta-analysis of 11 phase III trials demonstrated that there was no evidence of a higher risk of cancer in patients, but they sustained a suspected increase of cancer risk, especially skin cancers, with cladribine [3, 4]. As of 2017, cladribine (Mavenclad®) has been approved by the EMA and the FDA for the treatment of active MS at the cumulative dose of 3.5 mg/kg per year. Cladribine is given orally in 10 mg tablets, 0.875 mg/kg per course for two courses the first year and then two other courses of cladribine 0.875 mg/kg during the second year. The Summary of the Product Characteristics (SmPC) provides information regarding increased cases of malignancies in treated MS patients compared with placebo-exposed MS patients, as in parallel, it has been shown that untreated MS patients do not have an increased risk of cancer, including cutaneous melanoma [5, 6].

Our work aimed to explore whether cladribine could be linked to the growth of skin melanoma cells and normal melanocytes at doses used in MS through in vitro studies.

METHODS

Cells and Reagents

Melanoma cell lines and their maintenance were described previously [7]. 501mel cells were a gift from Dr. R. Halaban (Yale University School of Medicine, New Haven, CT, USA), M249R cells were from Dr. R. Lo (UCLA Dermatology, Los Angeles, CA, USA), and 1205Lu cells were from Rockland (USA). Cell lines were used within 6 months between resuscitation and experimentation and were authenticated via short tandem repeat (STR) profiling (Eurofins Genomics). Cells were routinely tested for the absence of mycoplasma by PCR. The experiments using melanoma cells derived from human tissue samples were conducted according to the Declaration of Helsinki Principles and 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 μg/ml). Culture reagents were purchased from Thermo Fisher Scientific. Cladribine (2-CDA) and DAPI were from Sigma. Vemurafenib (PLX4032) was from Selleckem. Cell growth assays using a live cell imager were used to determine melanoma cell viability in response to cladribine. Different nuclear-labeled fluorescent melanoma cells were treated with various concentrations of cladribine. We used a simple rationale for clinical dose to use 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 3 days. Confluency and number of nuclei (red cell objects) were quantified by IncuCyte software. Measuring normal melanocyte proliferation was performed by counting DAPI-stained nuclei.

RESULTS

We assessed the effect of cladribine on proliferation on two melanoma cell lines that harbor the oncogenic BRAF mutation, the most common somatic mutation found in cutaneous

melanoma. These cell lines display distinct gene expression patterns and phenotypic behavior; 501Mel cells show a predominantly proliferative and melanocytic differentiation phenotype and 1205Lu cells an invasive and de-differentiated state [7]. Cladribine markedly reduced the proliferation and survival of both melanoma lines in a time- and dose-dependent manner (Fig. 1). Treatment of cells with 2 μmol/l of the BRAF inhibitor vemurafenib, a dose that induces a cytostatic effect, was used as a control. Interestingly, cladribine exhibited no

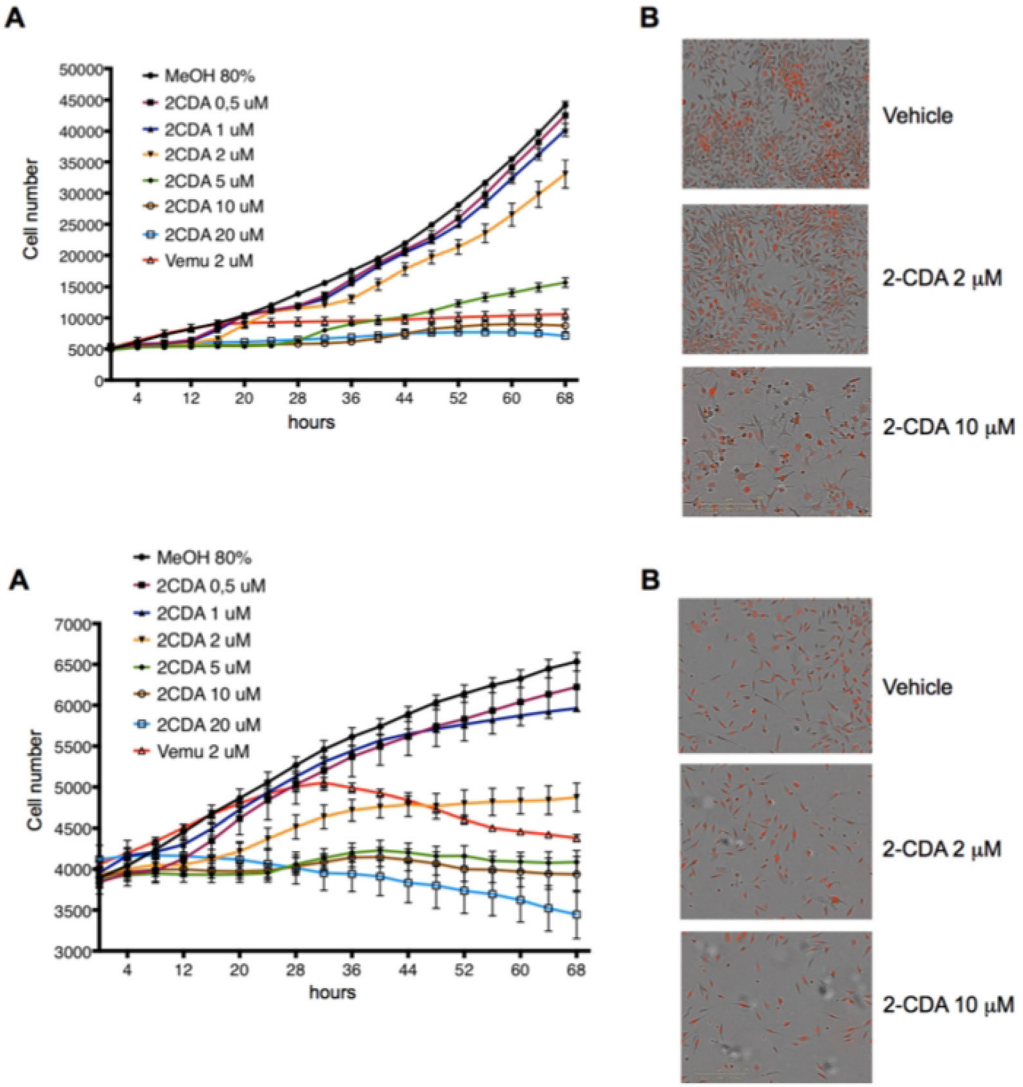

Fig. 1 Cladribine (2-CDA) induces growth inhibition and cytotoxicity of the non-metastatic 501Mel BRAF mutant melanoma cells (above) and of the metastatic 1205Lu BRAF mutant (below) melanoma cells. Growth curves of 501Mel and 1205Lu BRAF melanoma cells labeled with the NucLight nuclear reagent were treated with vehicle (MeOH 80%) or the indicated doses of 2-CDA in μM. Treatment with the BRAF inhibitor (vemurafenib, 2 μM) was used as a positive control of tumor cell proliferation inhibition. Data were acquired in triplicate during 3 days using the live-cell imager IncuCyte. Microphotographs showing the cytotoxic effet of 2-CDA 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

significant effects on normal human melanocyte proliferation (Fig. 2). We also demonstrated that cladribine (2-CDA) has a potent anti-melanoma effect in NRAS-mutated M249R cells that are resistant to vemurafenib (Table 1). The IC50 for 501Mel, 1205Lu or M249R cells was approximately 2.9, 2 or 6.3 μmol/l, respectively. Although the three cell lines exhibited a slight difference in drug sensitivity, these findings indicate that cladribine can inhibit melanoma cell proliferation irrespective of their oncogenic status, phenotypic behavior and therapeutic susceptibility.

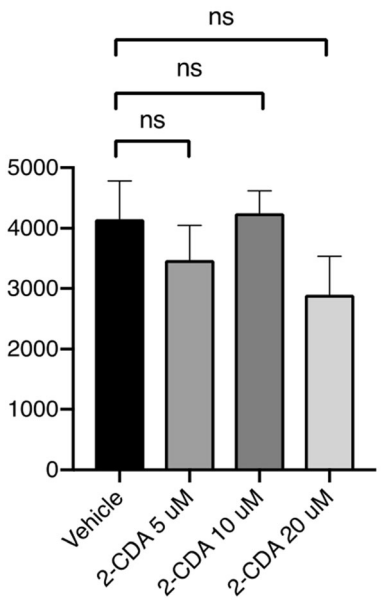

Fig. 2 (Supplementary data) No effect of cladribine (2-CDA) treatment on normal melanocyte proliferation. Human primary skin melanocytes were treated with vehicle or the indicated doses of 2-CDA. After 3 days, nuclei were stained with DAPI blue fluorescent dye. The number of cells was quantified by counting DAPI-stained nuclei using ImageJ software

Our study supports that in vitro cladribine displays anti-melanoma activities by itself, suggesting its therapeutic use in MS does not promote the progression of normal melanocytic cells or pre-existing melanoma lesions. Collectively, these results further support that cladribine does not increase the risk for nevus transformation and that it exerts anti-invasive and anti-migratory activities in vitro. Both interferences with DNA repair and reduced tumor surveillance could, in theory, lead to increased risk of malignancies during the treatment with cladribine but aggregated safety data and long-term extension follow-up (the PREMIERE registry: Long-term CLARITY cohort) demonstrate that the increased risk of malignancies reported in the pivotal trial seems to be caused by unexpectedly low numbers of malignancies in the placebo group [8, 9].

Because these capabilities of malignant cells correlate with their aggressive de-differentiated phenotype and metastatic potency, our findings provide evidence that cladribine might have a protective effect on melanoma development and give reassuring data for its use in treatment of MS. The suspicion of increased skin cancer risk from cladribine in MS needs to be followed up in prospective worldwide cohorts.

ACKNOWLEDGEMENTS

Funding. This work was supported by institutional funds from Institut National de la Santé et de la Recherche Médicale (Inserm), Université

Table 1 Cladribine (2-CDA) has a potent anti-melanoma effect irrespective of mutational status, gene expression signature and drug resistance

Cell Line	Phenotypic signature	Mutation(s)	Resistance	2-CDA I IC_{50} (μM)
501 Mel	Proliferative	BRAFV600E	None	2.9
1205lu	Invasive	BRAFV600E/PTEN[a]	None	2
M249R	Proliferative	BRAFV600E/PTEN[a]/NRAS	Vemurafenib	6.3

IC_{50} (μM) of 2-CDA treatment on melanoma cell proliferation was determined after 72 h as in Fig. 1

[a] Gene mutation or alteration

Côte d'Azur and the Ligue Contre le Cancer (Equipe labellisée 2016 to S. Tartare-Deckert). We also appreciate the financial support from ITMO Cancer Aviesan within the framework of the Cancer Plan, Conseil Départemental 06 and Canceropôle PACA. I. Berestjuk is a recipient of a doctoral fellowship from La Ligue Contre le Cancer. No funding or sponsorship was received for this specific study. No funding or sponsorship was received for this study or publication of this article.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

Disclosures:. Christine Lebrun Frenay has received personal compensation for consulting, serving on a scientific advisory board, speaking or other activities with Sanofi Genzyme, Novartis Pharmaceuticals and Roche. Ilona Berestjuk has nothing to disclose. Mikael Cohen has received consulting fees for advisory boards from Ad Scientiam, Biogen, Merck and Roche. Sophie Tartare-Deckert has received personal compensation for consulting activities with Bristol-Myers Squibb.

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

Data Availability. The dataset analyzed during the current study is available from the corresponding author on reasonable request.

REFERENCES

1. Giovannoni G, Comi G, Cook S, Rammohan K, Rieckmann P, Soelberg Sørensen P, Vermersch P, et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. N Engl J Med. 2010;362:416–26.

2. Leist TP, Comi G, Cree BAC, Coyle PK, Freedman MS, Hartung HP, Vermersch P, et al. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. Lancet Neurol. 2014;13(3):257–67.

3. Pakpoor J, Disanto G, Altmann DR, Pavitt S, Turner BP, Marta M, Juliusson G, et al. No evidence for higher risk of cancer in patients with multiple sclerosis taking cladribine. Neurol Neuroimmunol Neuroinflamm. 2015;2(6):e158.

4. Lebrun C, Rocher F. Cancer risk in patients with multiple sclerosis: potential impact of disease-modifying drugs. CNS Drugs. 2018;378(2):169-13.

5. Moisset X, Perié M, Pereira B, Dumont E, Lebrun-Frenay C, Lesage FX, et al. Decreased prevalence of cancer in patients with multiple sclerosis: a case-control study. PLoS One. 2017;12(11):e0188120-11.

6. Lebrun C, Vermersch P, Brassat D, Defer G, Rumbach L, Clavelou P, et al. Cancer and multiple sclerosis in the era of disease-modifying treatments. J Neurol. 2011;258(7):1304–11.

7. Girard CA, Lecacheur M, Ben Jouira R, Berestjuk I, Diazzi S, Prod'homme V, Mallavialle A, et al. A feed-forward mechanosignaling loop confers resistance to therapies targeting the MAPK pathway in BRAF-mutant melanoma. Cancer Res. 2020;80(10):1927–41.

8. Giovannoni G, Soelberg Sorensen P, Cook S, Rammohan K, Rieckmann P, Comi G, et al. Safety and efficacy of cladribine tablets in patients with relapsing–remitting multiple sclerosis: results from the randomized extension trial of the CLARITY study. Mult Scler. 2018;24(12):1594–604.

9. Cook S, Leist T, Comi G, Montalbán X, Giovannoni G, Nolting A, Hicking C, et al. Safety of cladribine tablets in the treatment of patients with multiple sclerosis. An integrated analysis. Mult Scler Relat Dis. 2019;29:157–67.