ANTICANCER RESEARCH *43*: 1407-1413 (2023)
doi:10.21873/anticanres.16289

Preclinical Activity of 4-Demethyl-4-cholesteryloxycarbonylpenclomedine in Melanoma

PHILIP FRIEDLANDER[1], LEE ROY MORGAN[2], EDMUND N. BENES[2], ANDREW H. RODGERS[2] and BRANKO JURSIC[3]

[1]*Department of Hematology and Medical Oncology, Mount Sinai School of Medicine, New York, NY, U.S.A.;*
[2]*DEKK-TEC, Inc., New Orleans, LA, U.S.A.;*
[3]*Department of Chemistry, University of New Orleans, New Orleans, LA, U.S.A.*

Abstract. *Background/Aim: Temozolomide plays a role in treating melanoma refractory to immunomodulatory and mitogen-activated protein kinase-targeted approaches, but its efficacy is limited. 4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a polychlorinated pyridine cholesteryl carbonate. Its mechanism of action is considered to be via alkylation/adduct formation with N^7-guanine. It demonstrated activity in intracranial implanted human glioma and breast cancer xenograft mouse models. The activity of DM-CHOC-PEN in melanoma models was assessed. Material and Methods: B-16 melanoma cells were exposed to DM-CHOC-PEN at different concentrations to assess proliferation and survival. B-16 cells were implanted subcutaneously into the flank of adult female C57BL mice which were then were treated with 200 mg/kg DM-CHOC-PEN intraperitoneally daily for 5 days in the setting of palpable subcutaneous tumor. Survival was compared to mice treated with temozolomide or saline. Five mice were treated per group. Results: In vitro, the respective half-maximal inhibitory concentrations of DM-CHOC-PEN and temozolomide were 0.5 and ≥3.0 μg/ml. Floating, heavily melanotic cells formed and these cells were separated, analyzed, and contained 10-90 ng DM-CHOC-PEN per 10^5 cells. The improvement in survival of mice treated with DM-CHOC-PEN or temozolomide relative to saline controls was 142% and 78%, respectively. Conclusion: Longer survival was seen with DM-CHOC-PEN in a C57BL murine model relative to temozolomide and saline-treated controls, supporting the development of clinical trials assessing the efficacy of DM-CHOC-PEN as treatment for metastatic melanoma.*

Correspondence to: Philip Friedlander, Mount Sinai Hospital, 10 E. 102nd Street, Box 1128, New York, NY 10029, U.S.A. Tel: +1 2128248584, e-mail: philip.friedlander@mssm.edu

Key Words: DM-CHOC-PEN, melanoma, melanin, alkylation.

Melanoma is a neoplasm which develops through genetic alteration of melanocytes. Melanocytes are unique cells that synthesize the pigment melanin which protects the cells from reactive oxygen species generated following exposure to ultraviolet radiation. Melanin is a naturally occurring pigment synthesized in a regulated multistep fashion from L-2,3-dihydroxyphenyalanine (DOPA), which is a normal pathway in melanocytes, but can be increased in malignant melanomas (1, 2).

When stage IV melanoma develops, the prognosis is very poor, with long-term survival seen in fewer than 20% of patients (3). Until 2011, the only treatments approved by the Food and Drug Administration for the management of stage IV melanoma were the alkylating agent dacarbazine and the cytokine interleukin-2, neither of which confer any overall survival benefit (4).

Recently there have been significant advances in the treatment of stage IV melanoma through the use of immune checkpoint modulators which target cytotoxic T-lymphocyte-associated protein 4, lymphocyte activation gene 3, and programmed cell death protein 1 (5-7). Ipilimumab inhibits cytotoxic T-lymphocyte-associated protein 4 leading to durable benefit in 22% of patients (8). Programmed cell death protein 1 inhibitors pembrolizumab and nivolumab confer responses in approximately 40% of treated patients (9, 10). The response rate following treatment with the combination of nivolumab and ipilimumab is approximately 60% (11, 12). Approximately 50% of melanomas possess a mutation at position 600 of the serine/threonine-protein kinase B-Raf (*BRAF*) gene leading to activation of the mitogen-activated protein kinase pathway (13). Dual inhibition of the mitogen-activated protein kinase pathway confers high response rates and survival benefits in these patients (14-16).

While immunomodulatory and BRAF-targeted approaches confer survival benefit, there are patients for whom immune

checkpoint modulators do not provide sufficient efficacy or are not tolerated. BRAF-targeted therapies are only indicated as treatment in patients with melanoma expressing a V600 *BRAF* mutation and efficacy is limited by the development of resistance, with median progression-free survival of approximately 10-15 months (14, 15, 17). Despite important recent treatment advances, additional therapeutic options are desperately needed.

Dacarbazine is an alkylating agent approved by the US Food and Drug Administration as therapy for the management of stage IV melanoma and is metabolized to the active metabolite 3-methyl-(triazen-1-yl)imidazole-4-carboximide (MTIC) (18, 19). Temozolamide is an orally active alkylating agent belonging to the imidazotetrazine class which is structurally similar to dacarbazine and is also metabolized to MTIC (20, 21). MTIC is a reactive methyl diazonium ion that is rapidly formed, resulting in the methylation of guanine present in DNA (22-25). The result is an interaction with DNA and the formation of O^6- and N^7-methylguanine, which facilitates the cytotoxic effects of temozolomide and dacarbazine (25).

4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a poly-chlorinated pyridine cholesteryl carbonate that was developed as part of a series of pyridine carbonates and carbamate analogs of 4-demethylpenclomedine that are lipophilic, electrically neutral, and able to cross the blood–brain barrier. The chemical structure of DM-CHOC-PEN is presented in Figure 1. DM-CHOC-PEN acts as a non-classical alkylating agent, binding with 4-benzylpyridine across the 6-trichloromethane group. DM-CHOC-PEN directly interacts with N^7-guanine DNA through a carbonium radical moiety derived from the trichloromethane group to form adducts with DNA (22, 25, 26). Tumor cell esterase can alter DNA adducts, leading to interstrand DNA cross-linking (22, 26). Given the activity of dacarbazine and temozolomide in the clinical management of stage IV melanoma and the activity of DM-CHOC-PEN by alkylating N^7-guanine, we assessed the anticancer activity of the latter *in vitro* and *in vivo* employing melanoma cell line and murine models. Preclinical studies with DM-CHOC-PEN in melanoma models are presented here.

Materials and Methods

Drugs/chemicals. DM-CHOC-PEN (DEKK-TEC, Inc., New Orleans, LA, USA) was dissolved in a soybean oil/egg yolk lecithin emulsion (2 mg/ml) and administered by intraperitoneal injection. Temozolomide (All Saints Pharmacy, Kenner, LA, USA) was administered orally as a 2 mg/ml saline suspension. For tissue culture studies, DM-CHOC-PEN and temozolomide were dissolved in dimethyl sulfoxide or tetrahydrofuran; cisplatin, doxorubicin (DOX), 4-hydroxyifosfamide (4-HOOI), and actinomycin D (All Saints Pharmacy) were dissolved in RPMI 1640 tissue culture medium.

In vitro studies. B-16 melanoma cells (obtained from the American Type Culture Collection, Manassas, VA, USA) were grown in wells at 1×10^4 cells/ml of complete RPMI media (RPMI with 10% fetal bovine serum) for 12 h and incubated at 36˚C with 5% CO_2. B-16 melanoma cells were incubated with DM-CHOC-PEN, cisplatin, doxorubicin, 4-hydroxyifosfamide, or actinomycin D at concentrations ranging from 0.5-2 μg/ml for 12 h. The cells were then washed, and fresh medium was added. Cytotoxicity was measured 24 h later employing a Beckman counting chamber. The results are reported as half-maximal inhibitory concentration (IC_{50}) for each agent. Three cultures from each group were monitored for 3-5 days post treatments for changes in the cytological appearance and morphology of treated cells relative to each other and to untreated cells were recorded.

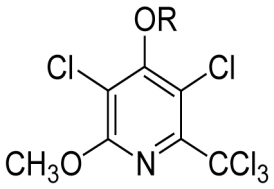

Figure 1. *Penclomedine analogs – PEN (R=CH_3); demethyl (DM)-PEN (R=H); 4-DM-4-cholesteryloxycarbonyl-PEN (R=-CO_2 -cholesteryl).*

Determination of intracellular DM-CHOC-PEN. After treatment of B-16 cells (1×10^4) in complete RPMI medium with DM-CHOC-PEN for 12 h, the cells were harvested, washed, and extracted with dichloromethane and a high-performance liquid chromatography analysis was performed to determine the intracellular concentration of DM-CHOC-PEN.

In vivo studies. B-16 murine melanoma cells (1×10^6) in suspension were implanted subcutaneously into the flank of adult C57BL male mice (age 7-9 weeks; Harlan Labs, Indianapolis, IN, USA). Groups of five mice per group were treated with DM-CHOC-PEN, temozolomide, or saline once the tumors were palpable 5-8 days post implant. DM-CHOC-PEN was administered intraperitoneally at dose of 200 mg/kg daily for 5 days. Temozolomide was administered orally per gavage once daily at a dose of 60 mg/kg/day for 7 days. Dosing was based on prior assessment of multiple doses in murine tumor models (22). Changes in the size of the subcutaneous tumors, survival of the mice and toxicity from treatment were measured initially 24 h later and monitored until all mice died. Histological changes of the subcutaneous tumors following treatment with saline *versus* DM-CHOC-PEN treatment were reviewed by a pathologist and compared.

Results

DM-CHOC-PEN inhibition of melanoma cell growth in vitro. *In vitro*, B-16 mouse melanoma cells grew well in culture (Figure 2). There were occasional cells that generated granules of melanin, a polymer of indole-5,6-quinone, in the growth phase but most replicating melanoma cells were amelanotic.

Incubating B-16 cells with DM-CHOC-PEN (0.25-1 μg/ml) in the growth phase resulted in cells accumulating with increased amounts of melanin. These cells separated the adhering colonies and died as floating clusters of heavily melaninated cells. Figure 3 demonstrates the morphology of B16 cells after 3 days of exposure to DM-CHOC-PEN. The

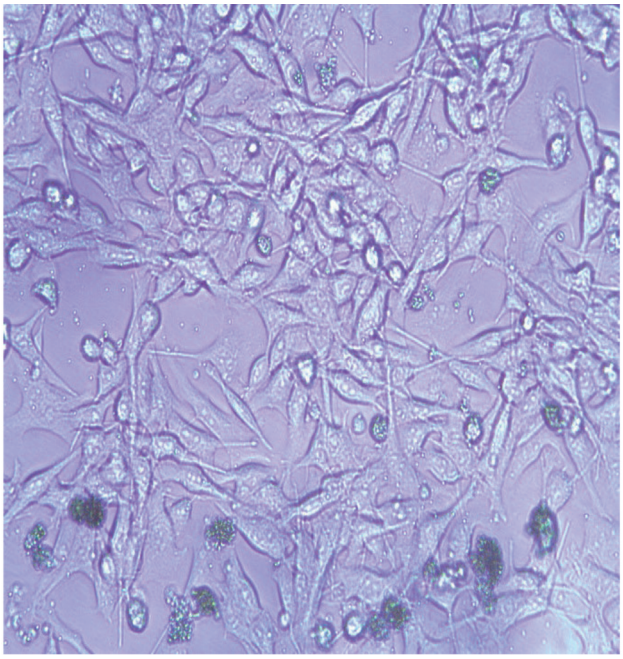

Figure 2. *Untreated B-16 mouse melanoma cells growing in a monolayer. Original magnification: 10×.*

heavily pigmented melanotic cells were collected, washed with saline, extracted with dichloromethane, and DM-CHOC-PEN was quantitated in concentrations of 0.01-0.06 μg/ml of packed B-16 cells. The IC_{50} for DM-CHOC-PEN *versus* B-16 melanoma was 0.5 μg/ml of culture fluid.

Comparative growth-inhibitory effect of DM-CHOC-PEN and other chemotherapeutic agents on B-16 melanoma in vitro. To appreciate the effects of other drugs, B-16 melanoma cells (10^4/ml) were incubated with temozolomide, 4-hydroperoxyifosfamide, *cis*-platinum, doxorubicin, or actinomycin D. The IC_{50} of these different chemotherapeutic agents are listed in Table I. In contrast to DM-CHOC-PEN, cells treated with the other agents in Table I died with only ghosts remaining, as can be seen in Figure 4. No melanin was generated, and the terminal pathway was quite different from that of the DM-CHOC-PEN-treated cells shown in Figure 3.

In vivo studies. Given the efficacy appreciated *in vitro*, the effect of DM-CHOC-PEN on the growth of melanoma was assessed *in vivo*. Murine B16 melanoma cells were implanted subcutaneously into the flank of C57BL mice, and the mice were treated daily with DM-CHOC-PEN (200 mg/kg) injected intraperitoneally for 5 days or temozolomide given orally at a concentration of 60 mg/kg/day for 7 days.

The survival profiles for mice treated with DM-CHOC-PEN, temozolomide or saline were compared as depicted in Figure 5. Median survivals were as follows: 12 days for the saline-treated mice, 15 days for temozolomide-treated mice and 19 days for DM-CHOC-PEN-treated mice (Figure 5). DM-CHOC-PEN was well tolerated up to doses of 200 mg/kg, with the mice continuing to gain weight.

Table I. *Half-maximal inhibitory concentration (IC_{50}) of different chemotherapeutic agents against B-16 mouse melanoma cells in vitro.*

Drug	IC_{50} (μg/ml)
DM-CHOC-PEN	0.5±0.01
Actinomycin D	0.5±0.02
cis-Platinum	1.5±0.1
4-HOOI	0.75±0.3
Doxorubicin	0.7±0.1
Temozolomide	>3.0

DM-CHOC-PEN: 4-Demethyl-4-cholesteryloxycarbonylpenclomedine; 4-HOOI: 4-hydroperoxyifosfamide.

Gross examination of the tumors treated with DM-CHOC-PEN demonstrated similar findings to those observed *in vitro* with DM-CHOC-PEN treatment – increased pigmentation of dead/residual cells. Increased pigmentation was not appreciated upon histological assessment of the melanoma tumor derived from mice treated with saline control (Figure 6).

Thus, the overall improvement in survival for the DM-CHOC-PEN-treated (200 mg/kg) group *versus* saline controls was 142%. Similarly, for the temozolomide-treated control *verses* saline-treated mice, survival improved by 59%. No unexpected or drug-associated deaths were noted in either of the treated groups. Mice tolerated both drugs, with weight gain until tumor size was ~2 cm in the greatest diameter. The maximum tolerated dose for DM-CHOC-PEN of 200 mg/kg was previously documented and reported (22).

Discussion

Preclinical *in vitro* and *in vivo* evidence is presented in support of the activity of DM-CHOC-PEN in a murine melanoma model using B-16 melanoma cells. DM-CHOC-PEN produced a significant improvement in long term survival which was superior to those for either saline or temozolomide controls. Of an associated significance is the degree of new melanin generated both *in vitro*, as well as *in vivo*.

Both malignant and benign melanocytes generate the pigment melanin. Melanin is a polymer of indole-5,6-quinone, an end-product of DOPA oxidation, which is highly electrophilic due to its conjugated structure and is capable of generating, transferring and storing electrons (27, 28).

The concept promoted here is that the melanoma–melanin system is a source of energy for the generation of electrons and electron-rich molecules. Due to the high redox potential between DOPA and DOPA quinone (+0.37 V), there is a

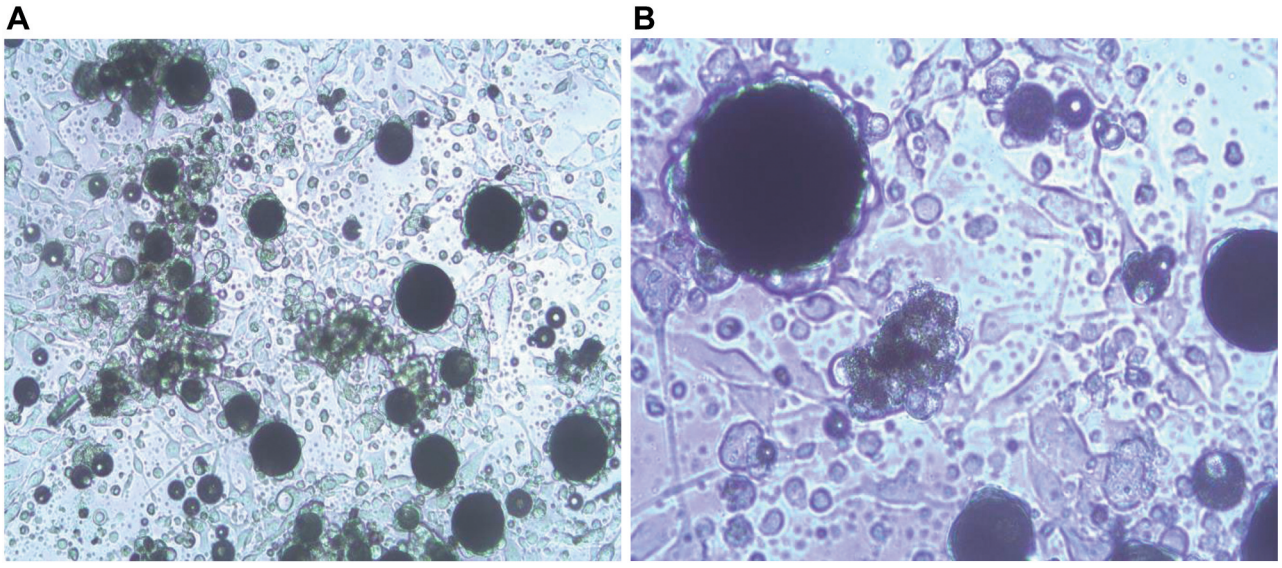

Figure 3. *4-Demethyl-4-cholesteryloxycarbonylpenclomedine-associated melanin production and cell death on day 3 of treatment. Original magnification: A: 10×; B: 20×.*

possible energy yield of –19.8 kcal/mole from the oxidation of one mole of DOPA; a significant amount of intracellular energy that could potentially be generated in melanoma cells.

The non-localized empty molecular orbitals associated with the copolymer chains of the indole quinoid units in the melanin polymer can also function as a two-dimensional semi-conductor, with bound protons producing electron traps (28). Further support for this notion is that poikilothermic vertebrates, *e.g.* turtles and alligators, actually store melanin in their livers and metabolize it as a source of energy during hibernation (29).

Theoretically, with the proper coupling factors and at least 49% efficiency, one ATP molecule (8.9 kcal) can be generated during the oxidation of one molecule of DOPA into a unit structure of melanin. Fourteen electrons would be generated per DOPA molecule, making it a 'mini-intracellular electron-beam generator'. The electrons and associated free-radical species generated during the oxidation of DOPA are potentially toxic to mitochondria and cell respiration, resulting in programmed cellular death (29). Thus, melanin is not inert, but is a highly conjugated polymer that generates an external highly charged 'aurora' capable of interaction with the intracellular microenvironment.

Melanoma cells, as seen in Figure 2, represent classical cancer cells with a low resting free energy (ΔF) and high entropy (ΔS) – the *alpha state*. The interactions of DM-CHOC-PEN and DOPA induced the formation of melanin – a high-energy component/storage that results in an increase in ΔF and a decrease in ΔS, with the development of cells in a new, resting high-energy state – the *beta state* (Figure 3). The latter cells are well-differentiated and die (30).

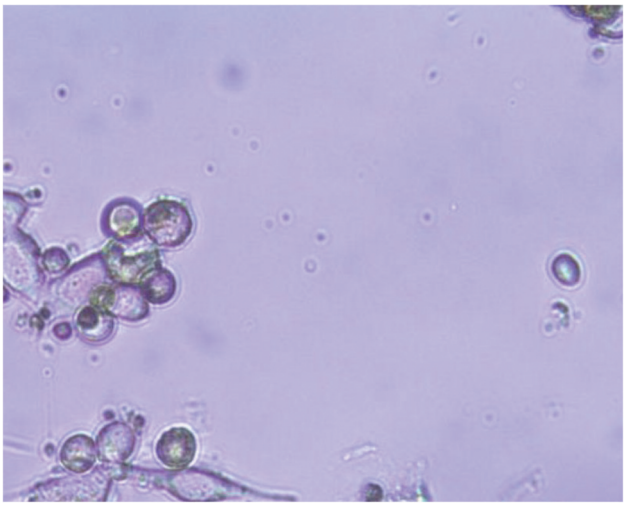

Figure 4. *A typical pattern of cell death as seen for B-16 cells treated with doxorubicin as compared with the cells shown in Figure 3. Similar findings were seen following treatment with 4-hydroperoxyifosfamide, actinomycin D, or cis-platinum.*

Although the generation of DM-CHOC-PEN-induced melanin-derived free radicals may be a mechanism of action for the drug's cytotoxicity, it is not considered to be the primary one.

DM-CHOC-PEN is an alkylating agent and its mechanism of action is currently considered to be *via* its trichloromethyl moiety producing a dichloromethylene carbonium ion; with

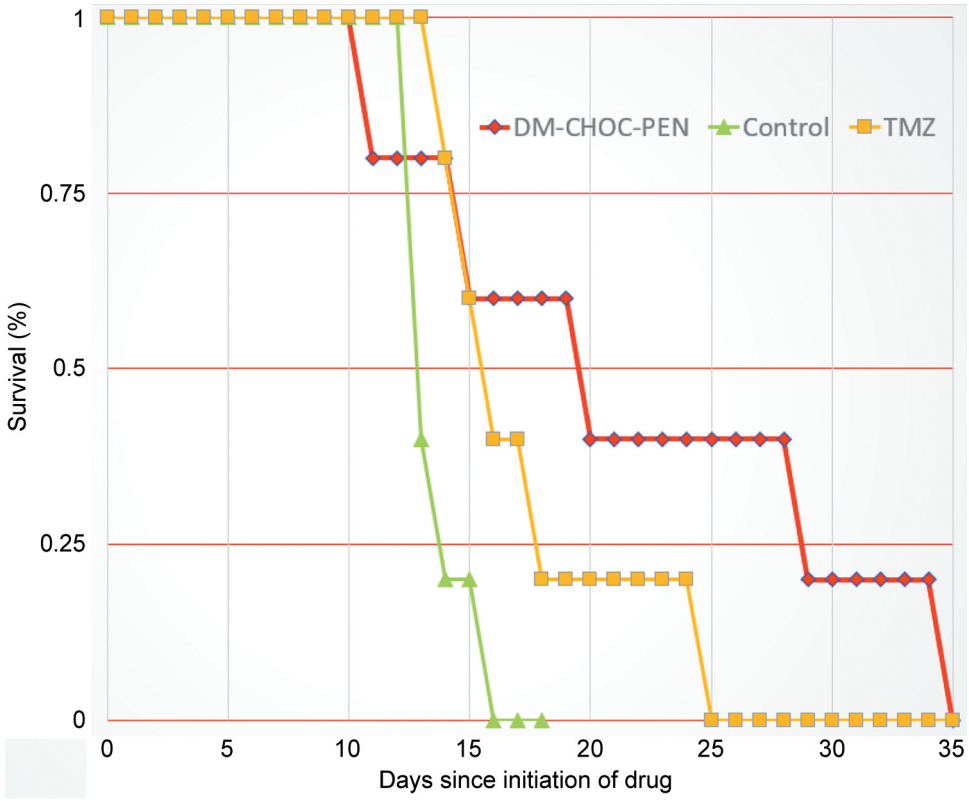

Figure 5. *B-16 mouse melanoma response in C57BL mice treated with 4-demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) at a dose of 200 mg/kg/day intraperitoneally for 5 days versus oral temozolomide at dose of 60 mg/kg/day for 7 days.*

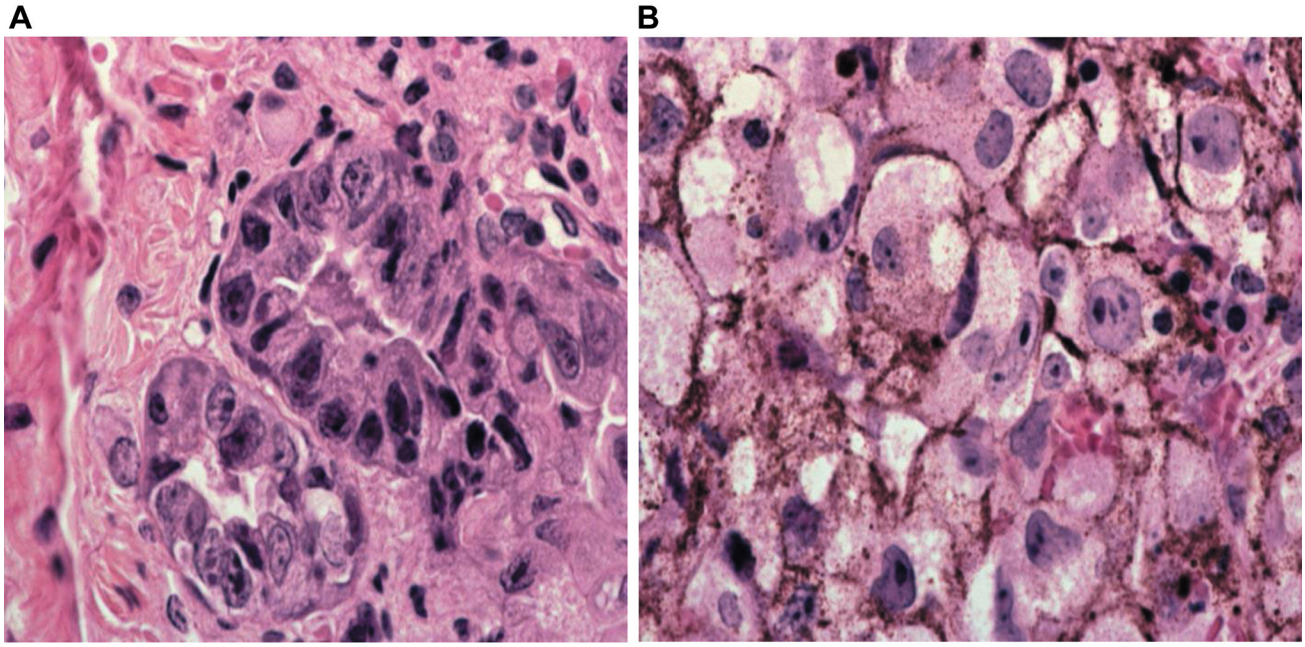

Figure 6. *Histology of tumors derived from B-16 cells in mice treated with saline as control (A) and in mice treated with 4-demethyl-4-cholesteryloxycarbonylpenclomedine (B). Note the pigment-laden cells in B are similar to those seen in in vitro culture.*

the latter binding to N^7-guanine DNA to form adducts and cause cell death (22). In addition to this mechanism, the current observations reported in this study support the ability of DM-CHOC-PEN to disrupt cellular metabolism *via* auto-oxidation of DOPA and the generation of free radical species, resulting in death – a second mechanism of action.

In conclusion, the *in vivo* studies plus *in vitro* observations indicate that DM-CHOC-PEN has anticancer activity in a melanoma murine model, supporting the development of clinical trials assessing its efficacy in patients with metastatic melanoma.

Conflicts of Interest

PF: Advisory board: Castle Biosciences. Consultant: DBV Technologies. Equity: Gilead Sciences, and Iovance Biotherapeutics. LRM, ENB, AR and BJ have no conflicts to declare.

Authors' Contributions

PF: Experimental design, data review, data interpretation, article preparation. LRM: experimental design, experimental work, data review, data interpretation, and article preparation. ENB: Experimental design, experimental work, data review, and data interpretation. AR: Experimental design, experimental work, data review, data interpretation and article preparation. BJ: experimental design, experimental work, data review, data interpretation and article preparation.

Acknowledgements

This study is supported by NCI/SBIR grant 2R44 CA132257-03.

References

1 Slominski RM, Zmijewski MA and Slominski AT: The role of melanin pigment in melanoma. Exp Dermatol *24(4)*: 258-259, 2015. PMID: 25496715. DOI: 10.1111/exd.12618

2 Sarna M, Zadlo A, Hermanowicz P, Madeja Z, Burda K and Sarna T: Cell elasticity is an important indicator of the metastatic phenotype of melanoma cells. Exp Dermatol *23(11)*: 813-818, 2014. PMID: 25180917. DOI: 10.1111/exd.12535

3 Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, Lazar AJ, Faries MB, Kirkwood JM, McArthur GA, Haydu LE, Eggermont AMM, Flaherty KT, Balch CM, Thompson JF and for members of the American Joint Committee on Cancer Melanoma Expert Panel and the International Melanoma Database and Discovery Platform: Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin *67(6)*: 472-492, 2017. PMID: 29028110. DOI: 10.3322/caac.21409

4 Wang H, Tran TT, Duong KT, Nguyen T and Le UM: Options of therapeutics and novel delivery systems of drugs for the treatment of melanoma. Mol Pharm *19(12)*: 4487-4505, 2022. PMID: 36305753. DOI: 10.1021/acs.molpharmaceut.2c00775

5 Au L, Larkin J and Turajlic S: Relatlimab and nivolumab in the treatment of melanoma. Cell *185(26)*: 4866-4869, 2022. PMID: 36563660. DOI: 10.1016/j.cell.2022.12.003

6 Aroldi F and Middleton MR: Long-term outcomes of immune checkpoint inhibition in metastatic melanoma. Am J Clin Dermatol *23(3)*: 331-338, 2022. PMID: 35359259. DOI: 10.1007/s40257-022-00681-4

7 Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen TT, Berman DM and Wolchok JD: Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol *33(17)*: 1889-1894, 2015. PMID: 25667295. DOI: 10.1200/JCO.2014.56.2736

8 Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A and Urba WJ: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med *363(8)*: 711-723, 2010. PMID: 20525992. DOI: 10.1056/NEJMoa1003466

9 Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V and Ascierto PA: Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med *372(4)*: 320-330, 2015. PMID: 25399552. DOI: 10.1056/NEJMoa1412082

10 Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A and KEYNOTE-006 investigators: Pembrolizumab *versus* ipilimumab in advanced melanoma. N Engl J Med *372(26)*: 2521-2532, 2015. PMID: 25891173. DOI: 10.1056/NEJMoa1503093

11 Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD and Hodi FS: Nivolumab and ipilimumab *versus* ipilimumab in untreated melanoma. N Engl J Med *372(21)*: 2006-2017, 2015. PMID: 25891304. DOI: 10.1056/NEJMoa1414428

12 Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, Lao CD, Schadendorf D, Wagstaff J, Dummer R, Ferrucci PF, Smylie M, Hill A, Hogg D, Marquez-Rodas I, Jiang J, Rizzo J, Larkin J and Wolchok JD: Nivolumab plus ipilimumab or nivolumab alone *versus* ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol *19(11)*: 1480-1492, 2018. PMID: 30361170. DOI: 10.1016/S1470-2045(18)30700-9

13 Garutti M, Bergnach M, Polesel J, Palmero L, Pizzichetta MA and Puglisi F: BRAF and MEK inhibitors and their toxicities: a meta-analysis. Cancers (Basel) *15(1)*: 141, 2022. PMID: 36612138. DOI: 10.3390/cancers15010141

14 Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, de la Cruz-Merino L, Dutriaux C, Garbe C, Sovak MA, Chang I, Choong N, Hack SP, McArthur GA and Ribas A: Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med *371(20)*: 1867-1876, 2014. PMID: 25265494. DOI: 10.1056/NEJMoa1408868

15 Dummer R, Flaherty KT, Robert C, Arance A, de Groot JWB, Garbe C, Gogas HJ, Gutzmer R, Krajsová I, Liszkay G, Loquai C, Mandalà M, Schadendorf D, Yamazaki N, di Pietro A, Cantey-Kiser J, Edwards M and Ascierto PA: COLUMBUS 5-year update: a randomized, open-label, phase III trial of encorafenib plus binimetinib *versus* vemurafenib or encorafenib in patients with BRAF V600-mutant melanoma. J Clin Oncol *40(36)*: 4178-4188, 2022. PMID: 35862871. DOI: 10.1200/JCO.21.02659

16 Atkins MB, Lee SJ, Chmielowski B, Tarhini AA, Cohen GI, Truong TG, Moon HH, Davar D, O'Rourke M, Stephenson JJ, Curti BD, Urba WJ, Brell JM, Funchain P, Kendra KL, Ikeguchi AP, Jaslowski A, Bane CL, Taylor MA, Bajaj M, Conry RM, Ellis RJ, Logan TF, Laudi N, Sosman JA, Crockett DG, Pecora AL, Okazaki IJ, Reganti S, Chandra S, Guild S, Chen HX, Streicher HZ, Wolchok JD, Ribas A and Kirkwood JM: Combination dabrafenib and trametinib *versus* combination nivolumab and ipilimumab for patients with advanced BRAF-mutant melanoma: The DREAMseq trial-ECOG-ACRIN EA6134. J Clin Oncol *41(2)*: 186-197, 2023. PMID: 36166727. DOI: 10.1200/JCO.22.01763

17 Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, Chiarion-Sileni V, Drucis K, Krajsova I, Hauschild A, Lorigan P, Wolter P, Long GV, Flaherty K, Nathan P, Ribas A, Martin AM, Sun P, Crist W, Legos J, Rubin SD, Little SM and Schadendorf D: Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med *372(1)*: 30-39, 2015. PMID: 25399551. DOI: 10.1056/NEJMoa1412690

18 Johnson RO, Metter G, Wilson W, Hill G and Krementz E: Phase I evaluation of DTIC (NSC-45388) and other studies in malignant melanoma in the Central Oncology Group. Cancer Treat Rep *60(2)*: 183-187, 1976. PMID: 769971.

19 Safgren SL, Reid JM, Rios R and Ames MM: Validated high-performance liquid chromatographic assay for simultaneous determination of dacarbazine and the plasma metabolites 5-(3-hydroxymethyl-3-methyl-1-triazeno)imidazole-4-carboxamide and 5-(3-methyl-1-triazeno)imidazole-4-carboxamide. J Chromatogr B Biomed Sci Appl *754(1)*: 91-96, 2001. PMID: 11318431. DOI: 10.1016/s0378-4347(00)00586-7

20 Kirstein MN, Panetta JC, Gajjar A, Nair G, Iacono LC, Freeman BB 3rd and Stewart CF: Development of a pharmacokinetic limited sampling model for temozolomide and its active metabolite MTIC. Cancer Chemother Pharmacol *55(5)*: 433-438, 2005. PMID: 15818507. DOI: 10.1007/s00280-004-0896-9

21 Middleton MR, Grob JJ, Aaronson N, Fierlbeck G, Tilgen W, Seiter S, Gore M, Aamdal S, Cebon J, Coates A, Dreno B, Henz M, Schadendorf D, Kapp A, Weiss J, Fraass U, Statkevich P, Muller M and Thatcher N: Randomized phase III study of temozolomide *versus* dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol *18(1)*: 158-166, 2000. PMID: 10623706. DOI: 10.1200/JCO.2000.18.1.158

22 Morgan LR, Struck RF, Waud WR, LeBlanc B, Rodgers AH and Jursic BS: Carbonate and carbamate derivatives of 4-demethylpenclomedine as novel anticancer agents. Cancer Chemother Pharmacol *64(4)*: 829-835, 2009. PMID: 19255760. DOI: 10.1007/s00280-009-0933-9

23 Denny BJ, Wheelhouse RT, Stevens MF, Tsang LL and Slack JA: NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. Biochemistry *33(31)*: 9045-9051, 1994. PMID: 8049205. DOI: 10.1021/bi00197a003

24 Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB and Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med *343(19)*: 1350-1354, 2000. PMID: 11070098. DOI: 10.1056/NEJM200011093431901

25 Hegi ME, Diserens AC, Godard S, Dietrich PY, Regli L, Ostermann S, Otten P, Van Melle G, de Tribolet N and Stupp R: Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clin Cancer Res *10(6)*: 1871-1874, 2004. PMID: 15041700. DOI: 10.1158/1078-0432.ccr-03-0384

26 O'Reilly S, O'Hearn E, Struck RF, Rowinsky EK and Molliver ME: The alkylating agent penclomedine induces degeneration of purkinje cells in the rat cerebellum. Invest New Drugs *21(3)*: 269-279, 2003. PMID: 14578677. DOI: 10.1023/a:1025456224751

27 Morgan LR and Singh R: Cytochrome oxidase-succinic dehydrogenase activities and the melanin pigment cycle in poikilothermic vertebrates. Comp Biochem Physiol *28(1)*: 83-94, 1969. PMID: 4304993. DOI: 10.1016/0010-406x(69)91323-1

28 Van Woert MH, Nicholson AR and Cotzias GC: Functional similarities between the cytoplasmic organelles of melanocytes and the mitochondria of hepatocytes. Nature *208(5012)*: 810-811, 1965. PMID: 5895704. DOI: 10.1038/208810a0

29 Pullman A and Pullman B: The band structure of melanins. Biochim Biophys Acta *54*: 384-385, 1961. PMID: 14489199. DOI: 10.1016/0006-3002(61)90389-4

30 Traub EF and Spoor HJ: Melanin and tyrosinase in skin pigmentation. *In*: Pigment Cell Growth, 3rd Conference on Biology of Normal and Atypical Pigment Cell Growth. Gordon M (ed.). New York, NY, USA, Academic Press, pp. 211-219, 1953.

Received January 3, 2023
Revised January 24, 2023
Accepted February 14, 2023