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## A Rational for Novel Anti-NeuroOncology Drugs

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Additional information is available at the end of the chapter

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### 1. Introduction

Glioblastoma multiforme (GBM) is the most prevalent and lethal type of primary central nervous system tumors with a medium survival of 10-12 months, even with aggressive surgery, radiation and advanced chemotherapy [1]. Poor prognosis of patients with GBM has recently been connected with elevated expressions of a sphingosine kinase [2].

Although, GBM is an extremely lethal cancer, metastatic cancers to the brain from other organs are the most common intracranial tumors, out numbering GBM and other primary brain tumors by at least 10-fold [3]. Lung, breast and melanoma are the 1, 2, 3 most common cancers that metastasize to the brain [4]. The prognosis for these patients is worse than for primary CNS malignancies – 3.7 *vs.* 10-12 months for GBM [5].

Intracranial tumors, primary as well as metastatic, are increasing at an alarming rate since many patients with excellent quality of life who appear to have 'beaten the odds' develop metastatic disease to the brain after years of remission from breast and other cancers [3-6]. The development of CNS metastasis results in progressive physical and cognitive impairment and culminates in death within a few months of diagnosis.

In the US alone, the incidence of brain metastasis is expected to exceed 200,000 cases in 2012 – more than 20 times the incidence of primary high grade brain tumors. The median survival time for metastatic cancer to the brain is 2-3 months and with aggressive therapy – maybe 4-12 months [6]!

Over the past 25 years, approximately 4-25 MM cancer patients have died with brain metastasis! These staggering facts strengthen a plea for developing new treatments for both primary and secondary cancers of the brain.

There are many reasons so few drugs are available to treat CNS cancers. Classically patients with brain metastasis have been excluded from clinical trials because of their poor

prognosis; the FDA has always had concerns regarding patient safety during Phase I trials with unknown drugs that may cause CNS toxicity and finally once a new drug is identified to have activity in non-CNS cancer involvement, few sponsors want to risk the potential hazards of toxicity that could result from treating patients with CNS involvement – primary or metastatic. Thus, there have been limited attempts to develop drugs for patients with brain metastasis, as well as primary brain tumors. The problem has been compounded by the lack of attention to CNS drug distribution and penetration of brain tumors, with reduction in toxicities.

Thus, there is a major need for the development of unique anticancer molecules that are able to cross the blood brain barrier (BBB), initiate anticancer activity in the CNS tumors and are non-neurotoxicity.

## 2. Landscape analysis of drugs available for brain tumors

- **Temodar** (temozolamide, TMZ (Schering-Plough,)) is currently approved and marketed by Schering-Plough for the treatment of high-grade glioblastoma multiforme and refractory anaplastic astrocytoma in both in combination with radiation.
- **Gliadel** (BCNU wafer, Eisai) is an implantable wafer also used for the treatment of primary brain tumors.
- **Avastin** (bevacizumab, Genentech/Roche) is an anti-angiogenic inhibitor that is being combined with other agents that can penetrate the BBB. Combinations such as *Avastin* plus *Camptosar* (*irinotecan/CPT-11*) +/- *Temodar* are in trials.
- **Tarceva** (erlotinib, OSI Pharmaceuticals), was granted FDA orphan drug designation in August 2003 for patients with malignant gliomas, a recent study of Tarceva found 16% of patients with malignant gliomas who were given Tarceva alone or in combination with Temodar showed tumor shrinkage. The main side effect was an acne-like rash.
- **Iressa** (gefitinib, AstraZeneca) and has been approved for relapsing non-small cell lung cancer. A recent phase II trial of Iressa in patients with relapsed glioblastoma resulted in one of 52 patients achieving a partial response and 22 patients achieving stable disease. Side effects were limited to rash and diarrhea.
- **Zarnestra** (tipifarnib, Johnson & Johnson Pharmaceutical) is in early phase trials for brain tumors. The drug is also being studied in patients with leukemia and breast or lung cancers.
- **81C6** is a tenascin radioactive monoclonal antibody that is injected into a cavity created by the neurosurgeon after removal of the tumor. The antibodies deliver radiation to kill the tumor cells with less radiation to normal brain tissue than conventional radiotherapy. The product is under development at Duke Comprehensive Cancer Center.
- **BMS-247550** (Bristol Myers Squibb) is in Phase II/III @ Memorial Sloan-Kettering.

- **Ipilimumab** (yervoy, Bristol Myers Squibb) a monoclonal antibody to CTLA-4 has activity in melanoma with improved PFS.
- **Vemurafenib** (zelboraf, Genentech) is a BRAF inhibitor approved to treat melanoma, however, CNS mets were excluded in the trials, so the drug is being checked formally now.
- Cisplatin + etoposide, capecitabine (Xeloda), etc. – TTP/PFS - 3 mos max
- Dabrafenib and trametinib have delayed melanoma progression (GlaxoSmithKline)
- And others ..... [7-9]

### 3. A new approach to new novel agents for – 1<sup>o</sup> and 2<sup>o</sup> brain tumors

The current trend to develop ‘personalized therapy’ based on the presence of specific phenotypic or genotypic pathways is an improved approach over the structure activity relationships (SAR) of the 20<sup>th</sup> century medicinal chemists [10]. However, the management of unresectable brain tumors has not improved. There is still a deficit in drugs that cross the blood brain barrier (BBB), are not recycled out of the brain, are effective anticancer agents in the CNS and do not require hepatic activation. Most of the 21<sup>st</sup> century ‘*personalized medicine*’ drugs are large molecules that are given with other drugs and are ionizable chemicals that cannot penetrate the BBB and/or recycled out of the brain.

**A suggested platform for new novel agents** – that might be effective therapies for brain tumors comes from the earlier 20<sup>th</sup> century literature that focused on – ‘*the energy of cancer cells vs. normal cells*’ [11-13].

Brain tumor biochemistry began in 1930 with Warburg's publication - *The Metabolism of Tumors* [12]. The fundamental finding of this publication was that embryonic and tumor tissues have in common a high glycolytic metabolism even under aerobic conditions; in contrast to most normal tissue where oxidative metabolism predominates. The oxidative metabolism in cells is generally 16 x more economical in respect to energy than the glycolytic pathway. The Embden-Meyerhof glycolytic pathway is phylogenetically the more primitive form [12]. *In rapidly growing de-differentiated tissue, this pathway prevails over oxidative metabolism.*

These concepts are of interest because the energy for a healthy cell is generated from glucose where 36 ATP molecules are produced from 1 glucose molecule. Hydrolysis of ATP results in a free energy change of ~ -16,000 cal/ATP molecule, depending on the conditions.

Classically cancer cells generate 2 ATP/1 glucose molecule *vs.* 36 ATP/1 glucose molecule (for healthy normal cells) – with the end product of pyruvate, rather than CO<sub>2</sub> and water as occurs in healthy cells [12]. Otto Warburg explained the latter as a result of the cancer cell's energy-transfer conversion to aerobic glycolysis, a major shift in energy available for cellular work [12].

Albert Szent-Gyorgyi referred to the energy differences of cancer cells *vs.* normal cells as – the *alpha* ( $\alpha$ ) state with  $\uparrow\Delta S$  and  $\downarrow\Delta F$  *vs.* the *beta* state ( $\beta$ ), with  $\downarrow\Delta S$  and  $\uparrow\Delta F$ , resp. [13].

The  $\alpha$  state is the ground state of life! The most stable state of any system is with minimum  $\Delta F$  and maximum  $\Delta S$ . If the cellular organization becomes unstable, cells will return to an  $\alpha$  state and remain there. The more complete the return – the harder it will be to reverse.

The  $\beta$  state is where there is a high degree of differentiation and potential for release of F if the cells react and initiate differentiation. Here the energy present allow cellular regulation and multicellular organisms replication; not a mass of undifferentiated cells – cancer.

[Where, S = entropy or potential (stored capacity) for a reaction to occur and F (free energy), a state of function depending only on the initial and final states of a system [14].

The importance of  $\Delta F$  is that the value immediately provides quantitative information about the potential ability of a substance to undergo a chemical or physical transformation. A reaction may proceed spontaneously without external energy only if the  $\Delta F$  is negative (energy dissipated) [14]. Chemical thermodynamics tells us that “*F needs to be a released in order for the cell to be productive and do work, not just replicate with a low productive reactivity*” [13].

We should view entropy as an index of a condition or character. It is an index of the capacity for spontaneous changes. By historical accident, the index was actually defined so that it *increased* as the capacity of an isolated system for spontaneous change *decreased*.

*Hence, entropy is an index of exhaustion.* The more a system has lost its capacity for spontaneous change, the greater is the entropy [14].

Yes, cancer cells represent a primitive, high random energy, low functional state with unbridled proliferation [13]. Cancer cells are not sick or weak, their vitality, as a wild type, may be even higher than that of a normal cell.

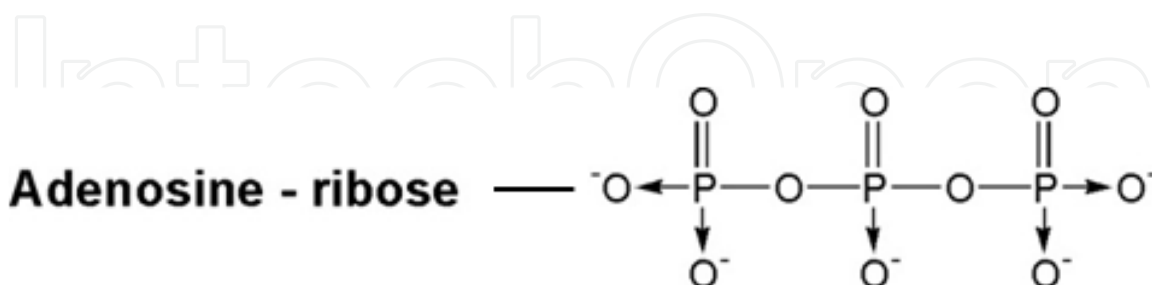
The cancer cell is unable to rebuild its beta state after it has completed its division and has to persist in the proliferative alpha state until death. In itself, the proliferative state would not be pathological – it is only its timing and irreversibility, which makes it so. *It is not pathological to be an infant, but it is pathological to be one and remain so x 50 years!*

Is cancer an outward manifestation resulting from depletion in cellular free energy within cells and increased entropy?

Although oxygen is considered essential for life, sulfur (S) and phosphorus (P) are the most common biological selections for energy transfer. The latter two elements are members of the Third Period of the Periodic Table and are capable of accepting an additional pair of electrons into unoccupied *3d* orbitals. This phenomenon is characteristic of the elements of the Third Period, which possess *d*, in addition to *s* and *p* orbitals, and thus have a place to hold electrons beyond the normal outer-shell octet. Molecules that possess P or S can store electrons (energy) and then release the energy during the transfer of shared electrons to other electron deficient molecules [15].

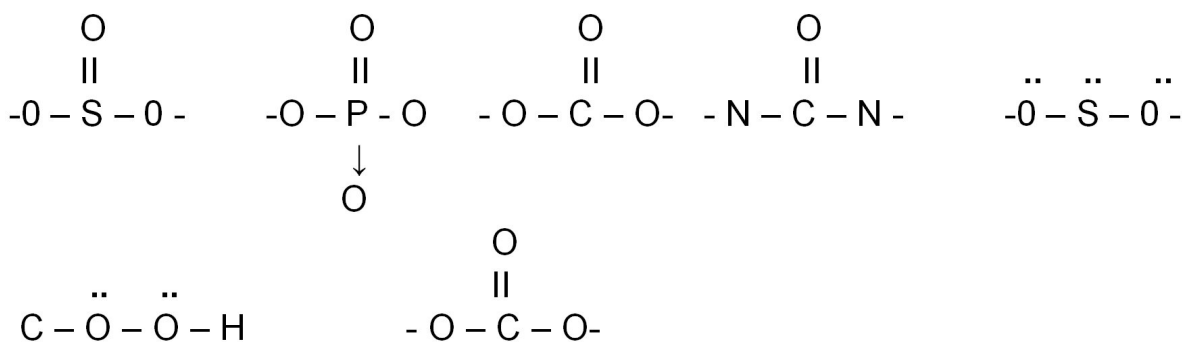
ATP (Fig. 1) is a unique example of how a 3<sup>rd</sup> Period element - ‘P’ participates in a key natural energy-rich compound, which upon hydrolysis generates ~ 8 kcal/mole upon hydrolysis

with a  $+\Delta F$ . When hydrolyzed in the presence of suitable transformers (transducing systems), the energy liberated may be used for cellular or tissue processes – cellular metabolism, nerve conduction, cell membrane transport, etc.



**Figure 1.** Adenosine triphosphate (ATP)

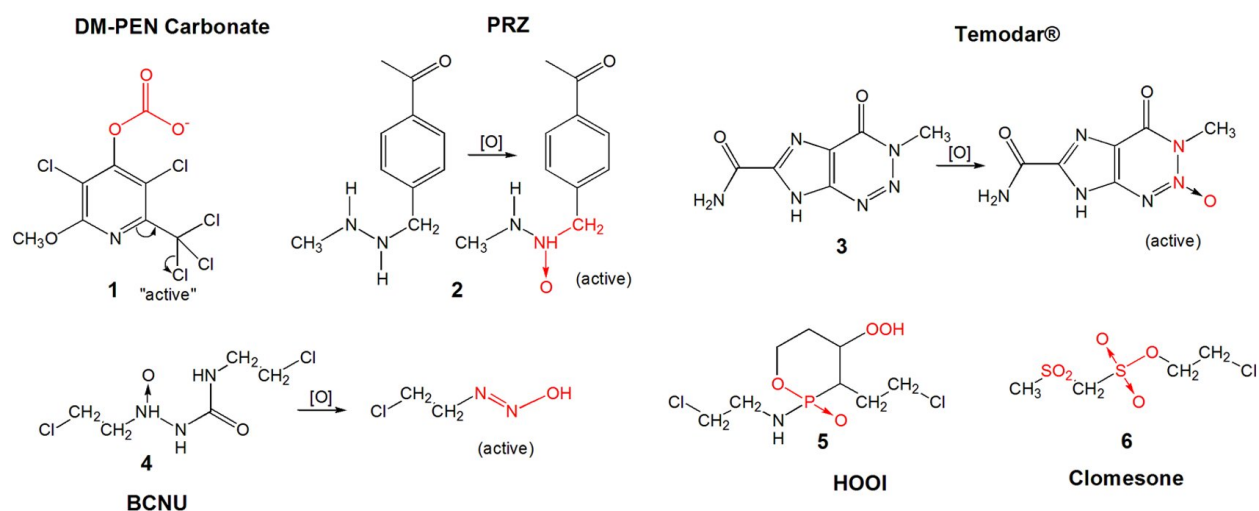
Energy rich phosphates usually possess either a second phosphoryl group or a resonating organic radical capable of entering into conjugation with the mobile electrons. This type of molecule usually contains a chain of at least three adjacent atoms, all bearing a net positive charge (deficient in  $\pi$  electrons), the chain sometimes extends over a five or even six atoms [Fig. 2], [16].



**Figure 2.** Examples of 3-atom high-energy moieties

Many of the small molecules used as anti-CNS cancer agents also possess 3-atom high energy moieties (in red) [3]





**Figure 3.** Small Molecules that are used to treat CNS cancer

Where: **DM-PEN carbonate**, 1 is a carbonate analog of 4-demethyl penclomedine; **PRZ**, 2 is dacarbazine; **Temodar®**, 3 is temozolamide (TMZ); **BCNU**, 4 is bis-(chloroethyl)nitrosourea; **HOOI**, 5 is 4-hydroperoxyifosfamide; **Clomesone**, 6 is a chloroethylating agent similar to BCNU – alkylates guanine-O<sup>6</sup> [17].

Compounds can be classified as “energy-rich” or “energy-poor” based on their  $\Delta F$ . Energy poor compounds do not possess more than two adjacent atoms bearing a positive charge and poorly absorbed by the CNS.

Thus, the design of “high-energy molecules” that are lipophilic, energetic and absorbed into a low free energy ( $\Delta F$ ) and high entropy ( $\Delta S$ ) cancer environment are timely. The latter is a unique therapeutic target – *the result of inefficient energy of cancer cells* [14].

Life is controlled by the transfer of energy. All living systems obey the 1<sup>st</sup> law of thermodynamics – which states that the sum of all energies in an isolated system remains constant. Energy may be converted into another form but the quantity can be neither created nor destroyed [18].

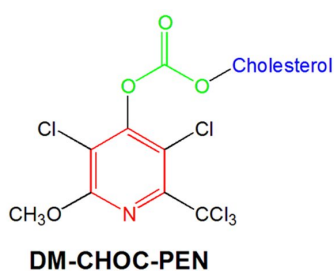
A question to be answered – are high energy compounds really attracted to low energy highly utilized regions in the body?

#### 4. Polyhalogenated pyridyl carbonates [16, 19-21]

The polyhalogenated pyridyl carbonates are neutral molecules that when coupled with lipids via high energy linkers – carbonates, phosphates, etc., fulfill the above requirements for novel high energy anticancer agents that could be attracted to and accumulate in CNS cancer tissue [16, 19-21]. The carbonate moiety like phosphate has the properties (resonance) of the high energy 3-atom moieties described in Fig. 3.

DEKK-TEC has designed a series of polychlorinated pyridyl carbonates (with 1 as a template, Fig. 3) that are linked with lipids and lipophilic moieties to promote CNS penetration [19]. DM-CHOC-PEN (4-demethyl-4-cholesteryloxycarbonylpenclomedine) is the most active and stable member of a large series of carbonates with improved activity vs. intracranially (IC) implanted human xenograft models – U251 and D54 glioblastoma and MX-1 breast cancer [Table 1] [19].

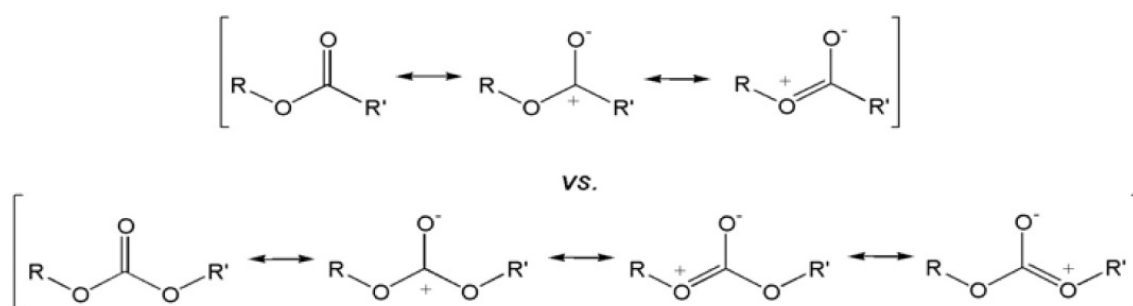
DM-CHOC-PEN is a unique molecule with several functional moieties that enable it to inhibit cancer replication. The structure contains the following moieties of interest:



- Lipophilic cholesterol link - blue.
- Carbonate linker that contains the properties seen in the elements of 2nd and 3rd period elements with higher energy storage potential - green.
- A pyridinium ring system that can transfer electrons via resonance into intermediary metabolism, ATP synthesis and substrate for the ABC cassette transport - red.

**Figure 4.** Lipophilic cholesterol link - blue. Carbonate linker that contains the properties seen in the elements of 2nd and 3rd period elements with higher energy storage potential - green. A pyridinium ring system that can transfer electrons via resonance into intermediary metabolism, ATP synthesis and substrate for the ABC cassette transport / red

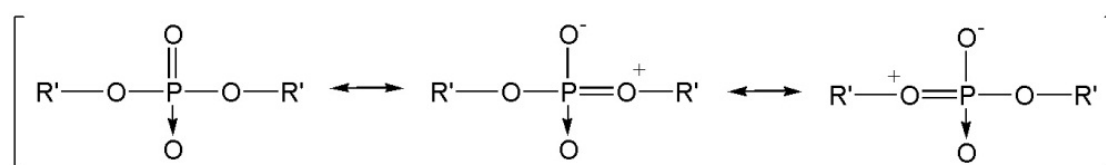
The importance of the carbonate moiety is its internal energy and ability to transfer electrons through multiple structures (resonance) (see Fig. 5):



**Figure 5.** Mesomeric forms of the carbonate; where R = pyridyl group & R'=cholesterol

The additional oxygen in the carbonates [Fig. 5, lower structures] stabilizes the moiety and allows for distribution of charge through resonance (Fig. 5). This chemical property exists in the phosphates and other oxides of the 3<sup>rd</sup> period elements [see below] [15].



**Scheme 1.**

In contrast, esters lack the degree of resonance/electron distribution [Fig. 5, upper structures].

As indicated in Fig. 5, a carbonate is not isosteric with an acyl or ester group nor is it a direct extension of an ester group. The carbonate group is unique and when combined with cholesterol its CNS penetration properties are enhanced [Table 1] [16].

Tumor	DM-CHOC-PEN			BCNU			TMZ		
	*Dose	%ILS	%LTS	*Dose	%ILS	%LTS	#Dos	%ILS	%LTS
U251 Glioblastoma	135	+54	+20(1/5 CR)	9	52	+20(1/5 CR)	120	+54	+20(1/5 CR)
D54 Glioblastoma	200	+3	+20(1/5 CR)	NA	NA	NA	NA	NA	NA
MX-1 Breast Cancer	50 100	+20 Toxic	17(1/6 CR)	60	12	0/5	NA	NA	NA

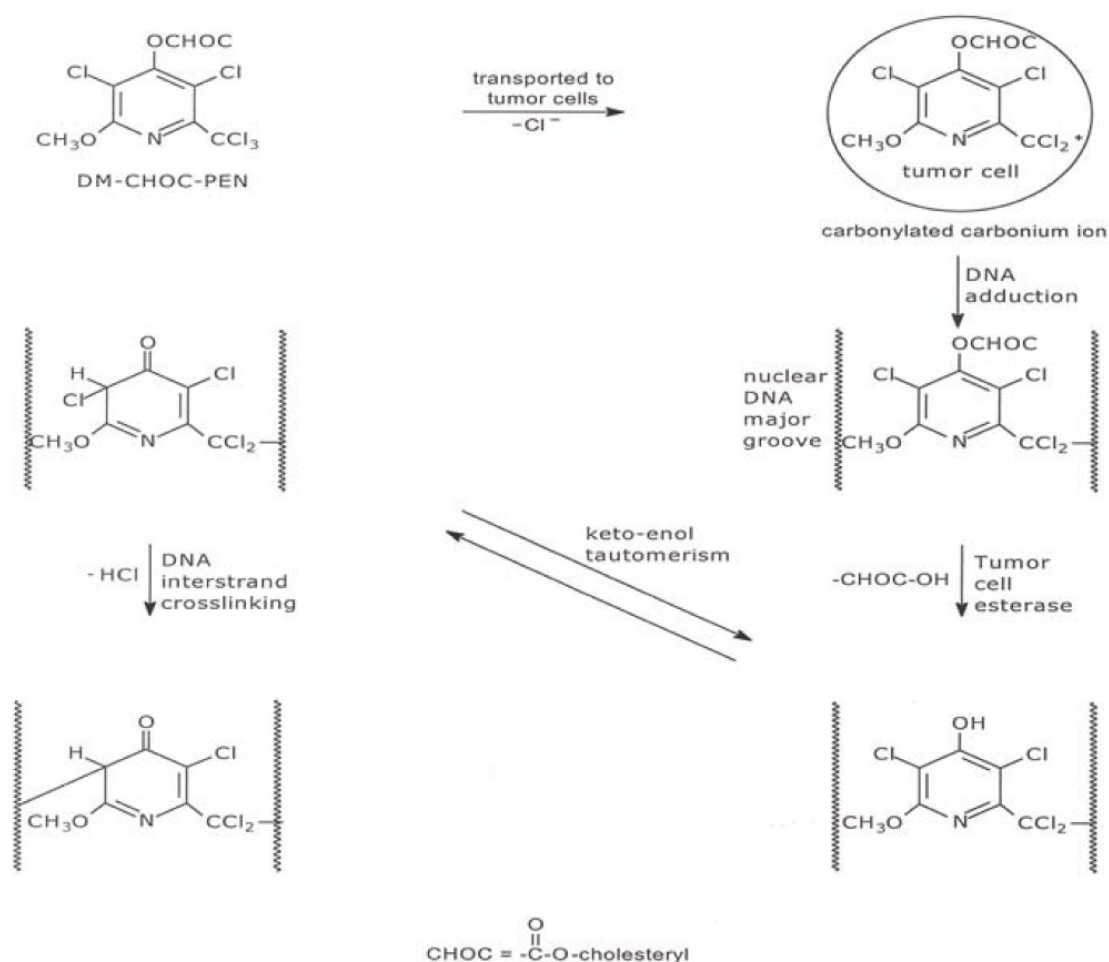
**Table 1.** Activity of DM-CHOC-PEN vs. Intracerebrally Implanted Xenograft Tumors in Mice

Implant:  $10^6$  cells IC; Treatment Route: Intraperitoneal (- 5 days post IC implant); Schedule: \*IV q1d x 5d; +IV q1d x 3d; #po 1d x 5d; Species: Athymic NCr/nu mice – female, Charles River; Dose: mg/kg; %ILS = % increased life span; %LTS = long term survival; all studies were terminated at 54 days

Based on the preclinical antitumor activities in Table 1, DM-CHOC-PEN produced a moderate increase in life span (% ILS); however it was comparable to that seen with BCNU and TMZ. DM-CHOC-PEN did yield long-term survivors that were healthy with no weight loss. Even the highly resistant IC implanted D54 glioma was sensitive to DM-CHOC-PEN. The drug has minimal toxicity and has encouraging potential [16, 19, 21].

An *in vivo* activation mechanism scheme is shown in Fig. 6, where DM-CHOC-PEN is proposed to be acting as a non-classical alkylating agent. The scheme describes a dual carbonium ion - DNA cross-linking mechanism (in a G-X-C sequence) with tumor DNA in the major groove via N<sup>7</sup>- guanine [20]. A bi-molecular coupled product (*via* the trichloromethylene group) has been identified as the major microsomal metabolite of PEN

[20]. The trichloromethylene group is required, since the dichloromethylene analog of PEN is not active [does not react with 4-(*p*-nitrobenzyl) pyridine (NBP)] (Struck, personal communication).



**Figure 6.** Proposed mechanism of action of 4 - demethyl - 4 - cholesteroyl - penclomedine (DM - CHOC - PEN)

Mice with IC growing U251 gliomas were treated with DM-CHOC-PEN, 135 mg/kg daily x 5-days and after 3-days the animals were sacrificed and the brains with tumors removed. The tumors were easily separated from the normal brain tissue. Each was homogenized in phosphate buffer, extracted with dichloromethane, and evaporated to dryness. The residues were assayed by HPLC and DM-CHOC-PEN, a guanine-N<sup>7</sup>-CCl<sub>2</sub>-adduct with DM-CHOC-PEN involving the -CCl<sub>3</sub> moiety and the DM-CHCO-PEN dimer-Cl<sub>2</sub>C-CCl<sub>2</sub>- were identified in the glioma tissue [16]. Neither DM-CHOC-PEN nor metabolites could be identified in the normal brain tissue.

Thus, DM-CHOC-PEN alkylates DNA with adducts at guanine-N<sup>7</sup> that could be quantitated in tumor tissue. The above MOA is in contrast to TMZ, BCNU and others, which alkylate guanine-O<sup>6</sup> [22].

The polyhalogenated pyridyl carbonates are unique structures that cross the BBB, accumulate in CNS tumor tissue, not normal brain tissue and are not recycled out of the CNS are cytotoxic *vs.* intracranially (IC) implanted human tumor xenografts in mouse models; fulfilling all of the criteria of the preceding paragraphs [23].

The combinations of responses in mouse xenograft tumor models, and a lack of neurotoxicity in rat and dog biopsychology studies, have allowed a Phase I trial to be conducted with DM-CHOC-PEN in patients with advanced cancer, including CNS involvement - IND 68,876 [24].

DEKK-TEC and its clinical investigators have satisfied the FDA that they are capable of treating patients with Phase I drugs and diagnosing physical and mental/cognitive alterations that may be associated with CNS tumor growth *vs.* toxicity secondary to drugs.

## 5. Phase I clinical trial with a high energy drug – DM-CHOC-PEN

Weiner, Ware, Friedlander, et al. reported preliminary data from a Phase I trial involving 18-patients treated with DM-CHOC-PEN in single IV doses of 39-87.5 mg/m<sup>2</sup> once every 21-days [25]. Six [6] patients had either CNS or spinal nervous system (SNS) involvement. Most patients had multiple treatments. The longest survivor (a sarcoma w/ SNS involvement) is 19+ months with NED [who also received doxorubicin at a later time] [25].

Support for DM-CHOC-PEN crossing the blood brain barrier in humans is provided from the above trial with reported observations for – GBM, CNS melanoma and breast cancer (BC) having PFS 4-13 mos., objective reductions in tumor sizes and no CNS toxicity.

One patient with pelvic sarcoma spread to the spinal cord required debulking surgery 21-days post treatment with DM-CHOC-PEN (39 mg/m<sup>2</sup>). Tumor tissue was removed, assayed and DM-CHOC-PEN was quantitated in the spinal sarcoma tissue – 92 ng/g of tumor [25].

The only SLTs noted to date are hyperbilirubinemia in 2-patients with liver metastasis; patients with no liver pathology have not demonstrated SLTs. CNS toxicity has not been noted in any of the patients. Overall, the drug was reported as being tolerated very well [25].

## 6. CNS melanoma

Melanoma is the third most frequent cancer to metastasize to the brain. It is anticipated that there will be ~ 20,000 new cases of CNS stage melanoma diagnosed in 2012 [9]. Enrollment of these patients with CNS melanoma would be critical to appreciating the activity of the demethylpenclomedine carbonates in this stage of disease [9, 26]. Two of the patients enrolled and treated in the DM-CHOC-PEN Phase I trial had CNS melanoma; one patient had significant shrinkage of CNS lesions, the other stable (NC) disease [25].

To test the potential sensitivity of DM-CHOC-PEN as therapy for melanoma, the B-16 mouse melanoma was selected as a screening model and treated with DM-CHOC-PEN *in vitro* and *in vivo* [28].

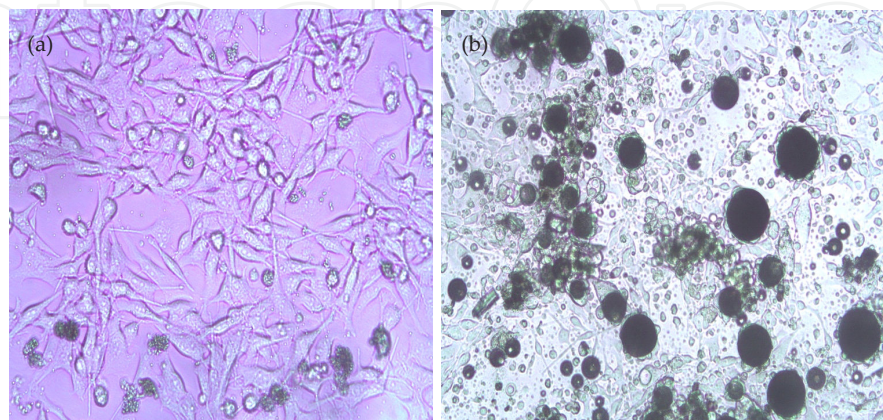
## 7. Experimental methods

**In Vitro** – B-16 mouse melanoma cells [obtained from ATCC, Menassas, VA] were assayed in culture wells ( $10^4$ /mL) using a complete RPMI media containing 10% FBS with pen/strep and maintaining cells @  $36^\circ$  C in a  $\text{CO}_2$  incubator. Drugs (in 1 mL volumes; in conc. - 0.1-5  $\mu\text{g}/\text{mL}$ ) were added to the cells in a growth phase and removed after 8-12 h; cultures were washed with fresh medium. For florescent studies - cells in growth phase were incubated with dichlorofluorescein diacetate (DCFDA, 3  $\mu\text{g}/\text{mL}$  RPMI) x 1-hr, washed and DM-CHOC-PEN added (1-5  $\mu\text{g}/\text{mL}$ ); incubation continued for an additional 5-hour. Cells were washed with fresh complete media and monitored with fluorescent microscopy. For the latter – RPMI without phenol red was used.

**In Vivo** – B-16 mouse melanoma cells ( $10^6$ ) were implanted SC into the flank of adult female C57BL mice (age – 7-9 weeks, Harlan Labs.) and treatments with DM-CHOC-PEN, HOOI, and other drugs began were initiated when lesions were palpable - 3-5 days post implant.

**Drugs** – were dissolved in saline or in a soybean oil/egg yolk lecithin emulsion (2 mg/mL) and administer by IP injection. HOOI was administered as the L-lysine salt [27].

**Results** – B-16 cells were incubated with DM-CHOC-PEN and after ~ 6-8 hours became heavily melanotic, lost adherence and floated to the top of the wells. Isolation of the floating heavily pigmented melanotic cells that formed (Fig. 7b), followed by washing, extraction with dichloromethane, and HPLC assays revealed that the cells contained DM-CHOC-PEN in concentrations of 0.003-0.09  $\mu\text{g}/\text{mL}$  of packed B-16 cells. Attached amelanotic cells contained 0-20 ng/mL of DM-CHOC-PEN (significant) [28].

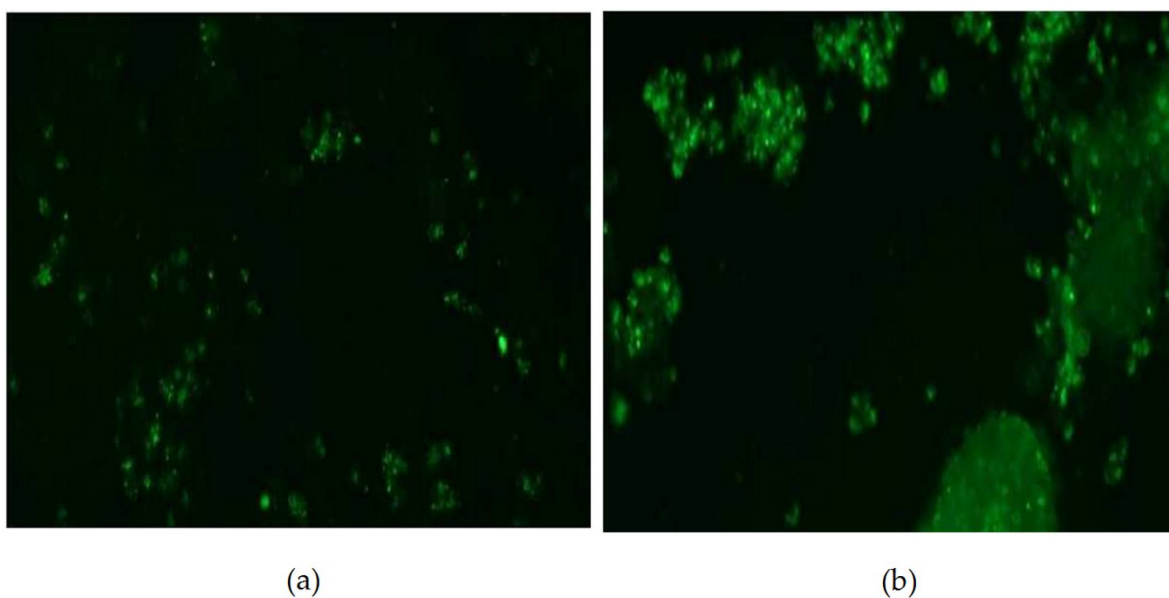


**Figure 7.** a) B-16 melanoma cells – untreated; b) After treatment with DM-CHOC-PEN

In comparison with other agents – DM-CHOC-PEN had an  $IC_{50}$  of 0.4  $\mu\text{g/mL}$  and TMZ, BCNU, DOX and actinomycin D (Act D), HOOI – 0.5-0.9  $\mu\text{g/mL}$ ; *cis*-platinum (*cis*-platin) – 1.5  $\mu\text{g/mL}$  resulted in  $IC_{50}$  cell death @ 36 hour post-intial exposure. With the exception of DM-CHOC-PEN, all treated cells died with ghosts remaining; excessive melanin formation only occurred with DM-CHOC-PEN; the terminal pathways were quite different for the other drugs.

Drug	$IC_{50}$ ( $\mu\text{g/mL}$ )
DM-CHOC-PEN	0.4 +/- 0.01
Actinomycin D	0.5 +/- 0.02
BCNU	0.9 +/- 0.1
<i>cis</i> -Platin	1.5 +/- 0.1
DOX	0.7 +/- 0.1
TMZ	"/>3.0
HOOI	0.8 +/- 0.2

**Table 2.** Drugs Responses vs. B-16 Melanoma ( $IC_{50}$ )



**Figure 8.** a)B-16 cells + DCF-DA; b)B-26 cells plus DM-CHOC-PEN + DCF-DA

Viewed with florescent microscopy – green florescent cells are generating ROS and the DCF is florescent.



Melanoma cell death due to DM-CHOC-PEN could be a result of increased intracellular melanin, as well as DNA alkylation. Addition of DCF-DA (dichlorofluorescein diacetate) to the culture medium (10 µg/mL) documented the formation of radical oxygen species (ROS) in the DM-CHOC-PEN treated cultures; green labeled cells (Fig. 8b). ROS release was documented with DCF, which is due to the hydrolysis and oxidized of DCF-DA by ROS with the release of fluorescent DCF. The generation of ROS was not observed with B-16 melanoma cells treated with *cis*-platin or actinomycin D and cultured under similar conditions. There is some background fluorescence and secondary to melanin-associated ROS formation [16].

**In Vivo Studies** – Adult female C57BL mice in groups of 5-6 mice with palpable SC nodules were dosed IP daily (175 or 200 mg/kg) for 5-days with DM-CHOC-PEN and monitored daily until death (Fig. 9a).

Mice treated with DM-CHOC-PEN (200 mg/kg/d x 5 days, IP) alone demonstrated an ILS of 142% (Fig. 9a). No drug related toxicity was noted – weight gain occurred until tumor growth was obvious.

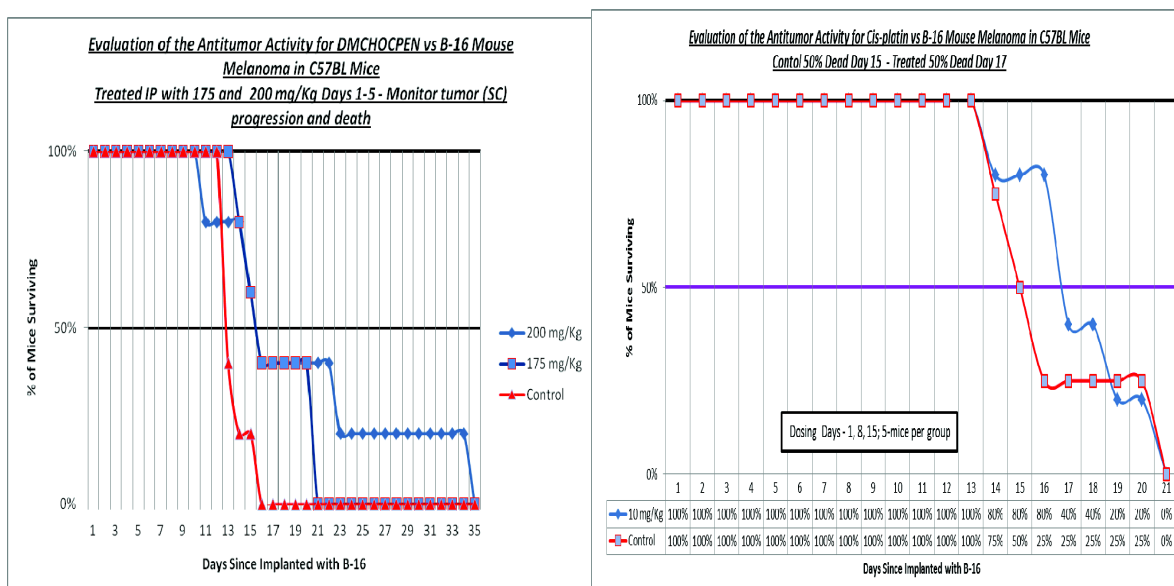
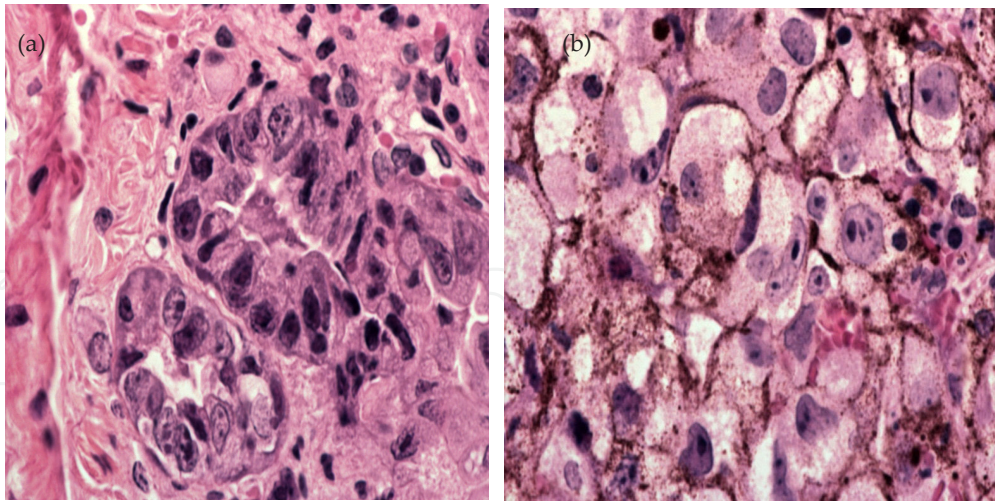


Figure 9. a)DM-CHOC-PEN treated; b)Cis-platin treated

Cis-platin was used as a drug control and administered (IP) once weekly x 3 (Fig. 9b); animals did not tolerate 3- or 5-day dosing schedules [28, 29]. Although active *in vitro*, *cis*-platin does not improve *in vivo* survival – as seen in Fig. 9b, although it is used in many melanoma clinical protocols [3].

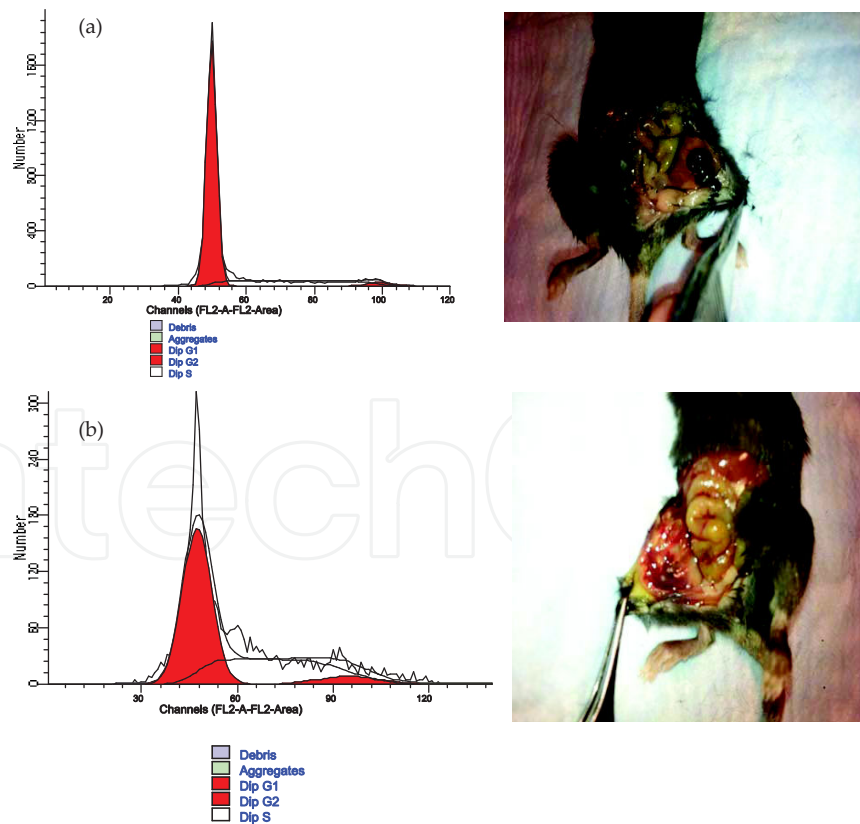
Tumor histology of the DM-CHOC-PEN treated B-16 melanomas are reviewed in Fig. 10. On the left (a) is the saline control and right (b) the DM-CHOC-PEN treated – 200 mg/kg IP daily x 5 days. *Note* - the melanin deposits in the cells with vacuoles, similar to what was seen in culture [29].





**Figure 11.** a)Histology of saline controls; b)Histology of DM-CHOC-PEN treated

Tumor tissue from the above DM-CHOC-PEN treated mice were assayed per cytofluorimetry using a BD FAC Scanner.



**Figure 12.** a) Mice bearing SC nodules – control animals. b) Mice bearing SC nodules dosed - 200 mg/kg; days 1-5 and sacrificed 3-days after the last treatment.

There were less viable cells available in the treated animals. DM-CHOC-PEN reduced the cellular concentration of the tumors and the cells accumulated in G<sub>1</sub> phase, with an inhibition in S-phase concentration of cells (Fig. 11b). Thus, more support for DM-CHOC-PEN's potential use in melanoma. Unfortunately, there was a significant amount of debris present.

**Discussion** – Mice bearing B-16 melanoma, tolerated DM-CHOC-PEN well when administered IP daily x 5 days or days 1 & 5 with improved overall survival (OAS) for both schedules; the former was the superior.

Histological examinations of the melanoma tissue from animals that had been treated with DM-CHOC-PEN revealed tumor cells encompassed in extracellular melanin, very similar to what was seen in tissue culture (Fig. 7b). DM-CHOC-PEN was extracted in up to 92 ng/g tissue quantities from the melanoma tissue removed in Fig. 11b [16].

DM-CHOC-PEN is a pseudo-alkylator that binds to DNA's guanine-N<sup>7</sup> via the trichloromethyl moiety → dichloromethylene carbonium ion and can form DNA-guanine adducts and cell death [Fig. 6] [9]. However, the current observations in a melanoma model support DM-CHOC-PEN's additional ability to disrupt cellular metabolism with death via autooxidation of DOPA to melanin and superoxide formation – an additional MOA for consideration – Fig. 12 [28, 29].

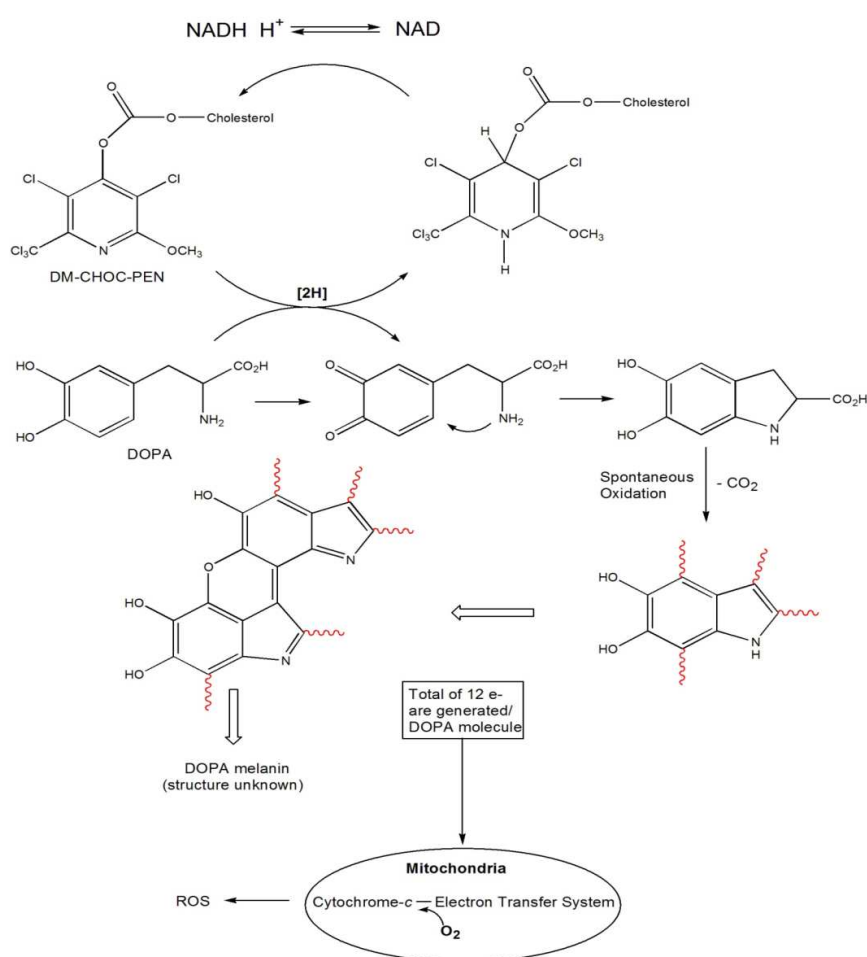
Electronic modeling studies support DM-CHOC-PEN's ability to act as a pyridinium co-factor oxidized by the pyridine nucleotides (NAD, etc.) with red-ox transfer of electrons from 3,4-dihydroxyphenylalanine (DOPA) to the mitochondria and cytochrome C/cytochrome oxidase transport system (Fig. 13). This result is the formation of 5, 6-indolequinone and melanin with a trail of electrons that can enter the mitochondrion intermediary pool resulting in all the same type of lethal changes seen with electron beam therapy for cancer [30, 31].

The 'melanin balls' that were seen *in vitro* are spheres of extracellular melanin polymer encapsulating colonies of melanoma cells that could induce a hostile microenvironment through ROS formation with death via oxidative stress and apoptosis (Figs. 10) [30]. Clusters of melanin laden cells contained significantly elevated concentrations of DM-CHOC-PEN, as compared to the amelanotic variant; a possible storage site for the drug [31]. The initial DOPA → DOPA quinone transformation is catalyzed by tyrosinase (DOPA oxidase) intracellular [32-35]. However, the red-ox potential for DM-CHOC-PEN is sufficient to catalyze the conversion with excess melanin formation per the Rapier Scheme below [32].

Both malignant and benign melanocytes generate melanin pigment via the sequence of chemical reactions as depicted in the classical Rapier Scheme in Fig.12 [32].

Melanin is far from being an end-product of oxidation [34-37]. At a glance the existence of energy bands are obvious from the highly conjugated heterocyclic indole quinone structure for melanin (Fig. 12). The hypothesis has been proposed that non-localized empty molecular orbitals are associated with the copolymer chain of the indole quinoid units and that the melanin polymer acts as a one-dimensional semi-conductor/trap with bound protons producing electron traps in the system [36].

Thus, melanin is a polymer of indole-5, 6-quinone which is highly electrophilic due to its conjugated structure and capable of attracting, storing and/or transferring electrons as electrical energy [30, 31, 34]. DM-CHOC-PEN treatment induced ROS formation *via* the melanin system resulting in cancer cell death – an end point.



**Figure 13.** Interaction of DM-CHOC-PEN with Rapier melanin cell cycle and mitochondrion [32]

Melanin is potentially a storage deposit of electrons and/or for electron-rich molecules (reported by our group years ago) [31]. Due to the high redox potential between DOPA and DOPA quinone, +0.37v, a possible yield of -19.8 kcal/mole occurs upon the oxidation of 1 mole DOPA. A total of 12 e<sup>-</sup> are generated per mole of DOPA oxidized to indole quinone which polymerize to melanin [Fig. 13 [31]]. A field of melanin encompassing a colony of cancer cells could generate a hostile electrical microenvironment and inhibit cellular metabolism and replication [33].

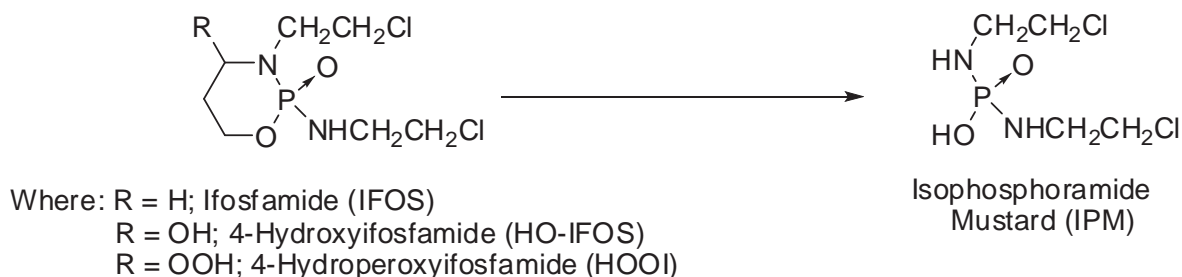
Melanoma cells are a classical representation of cancer cells with a resting low free energy ( $\Delta F$ ) and high entropy ( $\Delta S$ ) – the *alpha state* [14]. The interactions of DM-CHOC-PEN and DOPA induced the formation of melanin and ROS which resulted in an increased envi-

ronmental  $\Delta F$  and a decrease in  $\Delta S$ ; a new resting high energy state – the *beta state*. The cells that are contained in the melanin balls are in a *beta state* - undergo apoptosis and die [28, 30, 31].

Thus, DM-CHOC-PEN is a new drug entity that crosses the BBB and couples two new targets – the melanin cycle and superoxide formation with apoptosis plus DNA guanine –  $N^7$  adducts. DM-CHOC-PEN is a potential triple treat for CNS melanoma, the 3<sup>rd</sup> most common cancer that spreads to the CNS.

## 8. A 2<sup>nd</sup> High energy drug

Another high energy drug, 4-hydroperoxyifosfamide (HOOI) [Fig. 3, 14] and is a pre-activated form of ifosfamide (IFOS) which possesses the criteria for discussion [Fig. 14]. HOOI also contains a 3<sup>rd</sup> Period atom – 'P'; thus it has two centers of high energy – Fig 3, 14.



**Figure 14.** Ifosfamide and analogs

IFOS is a well-known anticancer agent that requires hepatic activation to 4-HO-IFOS which spontaneously undergoes hydrolysis with ring opening resulting in isophosphoramidate mustard (IPM) [Fig. 14], the latter is the active cytotoxic form of IFOS [37-39]. As part of the IFOS activation process, hepatic metabolic dechloroethylation releases chloroacetaldehyde, which has been proposed to be the major cause of IFOS associated neurotoxicity [40]. Acrolein is also released and has been implicated in dose limiting toxicities - hemorrhagic cystitis and 2<sup>o</sup> tumor promotion [41-45].

In contrast, HOOI does not require hepatic microsomal activation, crosses the BBB and is readily absorbed by cancer cells where it releases IPM *in situ* [46, 47]. The former does not generate chloroacetaldehyde (neurotoxic) during its conversion to IPM, as does IFOS. Neither hemorrhagic cystitis nor renal toxicity has been observed with HOOI in animal toxicology models. Pulmonary damage that could occur with peroxides – pulmonary air emboli was not observed in the dog HOOI study [46]. Hematological toxicity (bone marrow depletion) was the dose limiting toxicity in the dog study [47].

The potential therapeutic usefulness of HOOI is supported by *in vivo* activity in human tumor xenografts and cyclophosphamide (CPA)-resistant murine tumor models plus reduced toxicities.



*In vivo* in comparison to CPA and IFOS in rodent and dog species [Table 3] [47], a potentially improved safety profile is anticipated – with reduced systemic formation of acrolein and the absence of chloroacetaldehyde during the conversion of HOOI to IPM – *in situ* [47].

Drug	Dose (mg/kg/day)	No of Mice	MX-1* T- C (Days)	ZR-75-1* T- C (Days)	U251 (%ILS)**	P388/CPA (% ILS)**
HOOI	90 (<LD <sub>10</sub> )	10	>28.8 9 (33% LTS)	>46.1 (83% CR)	+54 (1/5 CR)	+209 <sup>+</sup>
DM-CHOC-PEN	135	10	>54 days (20% LTS) [Dose - 50]	NA	+54 (1/5 CR)	NA
IFOS	40 (MTD) <sup>++</sup>	10	8.6 (7% LTS)	>43.8 (17% CR)	50 (0/5 CR)	42
IPM	40 (MTD) <sup>++</sup>	10	2.1	NA	NA	+85
BCNU	9-15 (MTD)	5	12% LTS; 0 CR	NA	+52 (1/5 CR)	NA
TMZ	120 mg (MTD)	5	NA	NA	+54 (1/5 CR)	NA

Dose – qd x 5d (IP) – except TMZ – PO Q4D x 3; \*human breast cancer – SC implanted, \*\*human glioblastoma – IC implanted; \*\*\*CPA (cyclophosphamide) – resistant murine leukemia. For MX-1 – T-C (days) = difference in median times post implant for tumors of treated groups to attain an evaluation size compared to median of control group; +6-log cell kill; ++>40 mg/kg was too toxic; \*\*\*% ILS (increased length of survival – study terminated @ 54 days).

**Table 3.** Human Xenografts and Murine Tumors Growing in Mouse Models

HOOI possesses two [2] 3-high energy atom chains, thus the drug more than fits the criteria proposed earlier in this chapter (3). In addition, HOOI does not require activation by the liver and spontaneously undergoes conversion to IPM and cross-links with nuclear DNA via major groove alkylation – N<sup>7</sup>-guanine, forming G-X-C adducts [39].

Of significance is that HOOI was curative (54% LTS, with 20% CR) *vs.* the human U251 glioblastoma implanted IC as well as the MX-1 breast cancer IC model (Table 3) [47]. It should also be noted that BCNU – the gold standard for years in the treatment of gliomas produced no CRs and TMZ the current standard produced identical responses to HOOI.

Like DM-CHOC-PEN, HOOI accumulates in U-251 glioma tissue in 50 ng/g tissue content when the drug is administered IP to animals bearing IC implanted tumors [47]. No CNS/behavioral alterations or toxicity have been noted for HOOI [48].

Unlike ifosfamide (IFOS), HOOI is more lipophilic, activated *in situ*, with less extracellular acrolein, no chloroacetaldehyde released and no IFOS associated CNS or GU toxicity [48]. In dogs the principal toxicity with HOOI was bone marrow suppression that reversed with time [47, 48].

An early study reported that SK-MEL-31 human melanoma cells were sensitive to HOOI, which prompted the comparison of the drug with DM-CHOC-PEN in the B-16 melanoma model [47].

In 2012, the incidence of new cases of melanoma in the US alone is estimated at 76,250, an increase of ~ 20% during the past 10 years [9]. Of these cases, ~ 25% will metastasize to the CNS; thus our interest in the drug [26].

Fig. 15 describes the impact on survival of B-16 mouse melanoma when HOOI is administered alone and with DM-CHOC-PEN [49].

DM-CHOC-PEN (200 mg/kg/d) was injected IP daily x 5-days followed by HOOI (90 mg/m<sup>2</sup>) IP daily x 3 days (Day 6-8). The latter is a more classical mustard type agent with activity in the G<sub>1</sub> phase, as compared to DM-CHOC-PEN, which inhibited cell replication at the S phase – Fig. 12b [49].

The %LTS for the 2-drug combination was 173% vs. HOOI alone - 78%; for DM-CHOC-PEN alone - 142% (see Fig. 14).

No drug related toxicity was noted – weight gain continued until tumor growth was a burden and animals sacrificed. The use of 5-day dosing for HOOI was toxic and not considered [48].

Combination of the two -‘high energy’ agents selected for discussion as binary therapy has potential. It will be a while before these 2-drugs are administered together; however, other binary selections that incorporate DM-CHOC-PEN are in the planning stage for Phase II trials.

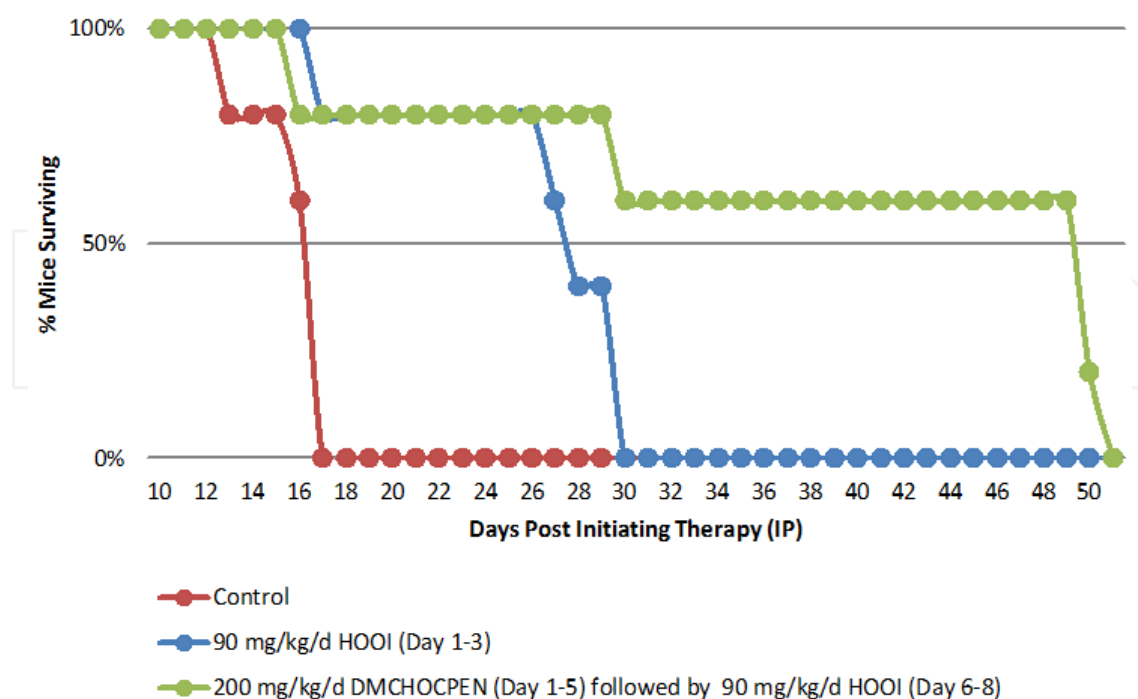


Figure 15. DM-CHOC-PEN plus HOOI in the Treatment of B-16 Mouse Melanoma



## 9. Conclusion

Melanoma cells, as seen in Fig. 7a represent classical cancer cells with a resting low free energy ( $\Delta F$ ) and high entropy ( $\Delta S$ ) – the *alpha state* [14]. The interaction of DM-CHOC-PEN and DOPA induced the formation of melanin – a high energy component/storage that resulted in an increase in environmental  $\Delta F$  and a decrease in  $\Delta S$ ; a new resting high energy state – the *beta state* [14]. Cells that are encompassed in the ‘melanin balls’ (Fig. 7b) were converted to a well differentiated state and die. The addition of HOOI to DM-CHOC-PEN inhibited those cells that reversed to the *alpha state* – survive and continue to replicate. Thus, the binary combination improved %LTS vs. either drug alone; supporting the *in vitro* observations.

Although, only two patients with CNS melanoma have been treated with DM-CHOC-PEN in the Phase I trial, the report that one patient did demonstrate an interval response and one patient had a no change response, both with no neurotoxicity is encouragement for the potential use of the drug in the treatment of CNS melanoma [25]. HOOI has not been used in clinical trials as yet.

There are many novel drugs that fall in to the ‘high-energy’ category that should be evaluated or re-visited. In particular – clomesone [Fig. 3], a drug developed at Southern Research Institute, US had potential in animal studies, but produced hematological toxicity in Phase I trials and discontinued from studies [17, 50]. The drug has novel chloroethylating properties and like BCNU forms adducts with DNA guanine-O<sup>6</sup> [50]. In addition, the drug contains two - S - atoms which are a 3<sup>rd</sup> Period element and able to expand its 3d orbital – a real ‘high-energy’ molecule. However, only one of the latter groups has the ability to resonant with energy storage.

With the two sets of 3-atom chain as a point of interest (Fig. 3, 6), clomesone should be revisited with dose modifications; it has potential [52].

In summary, three ‘high energy’ drugs have been reviewed as support that a novel approach to the treatment of CNS malignancies could be through the inefficient energy system of brain tumors [11, 12]. Anticancer agents that have the appropriate structure, can penetrate the CNS, not be recycled out and inhibit cancer growth.

Over all, DM-CHOC-PEN has been well tolerated in Phase I studies with advanced –breast cancer, sarcoma, glioblastoma multiforme, melanoma, pancreas, esophageal, non-small cell lung cancer colorectal cancer and cervical cancer and will obviously enter Phase II trials alone or as binary therapy [25].

Furthermore, HOOI is being readied for Phase I clinical trials and the results should be equally interesting.

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