

***The commercial nutraceutical Metabolic Cell-Support (MC-S<sup>TM</sup>)  
inhibits cancer cell growth***

<sup>1</sup>D. A. Clark and <sup>2</sup>M. C. Adams, 2007

<sup>1</sup> TUNRA (The University of Newcastle Research Associates) Limited, Industry development Centre, University Drive, Callaghan NSW 2308 Australia

<sup>2</sup> School of Environmental and Life Sciences, Faculty of Science and IT, University of Newcastle, Callaghan 2308, NSW, AUSTRALIA

***Abstract:***

The therapeutic *in vitro* and *in vivo* use of active compounds derived from medicinal herbs and fungi in cancer research is well documented. The combining of nutraceuticals to synergistically enhance efficacy has not only an application in immune stimulation, but also in the inhibition of cancer cells. Metabolic Cell Support (MC-S) is an over the counter immune system supplement developed in Australia and formulated using a pharmacological approach to natural therapies. Rather than using individual bioactive chemicals, a combined highly standardised multi polysaccharide enriched blend of medicinal mushrooms, the herb *Astragalus membranaceus* and ascorbic acid was formulated so as not to exclude important components that may play a role in clinical outcomes. To examine if this pharmaceutical preparation inhibits cancer cell growth, an *in vitro* study of the proliferation of four cancer cell lines was undertaken at varying doses of MC-S. **Conclusion: These promising results demonstrate that within a broad range of doses, MC-S significantly inhibits the growth of breast, prostate, melanoma and colon cancer cells. Published data indicates MC-S is selective in its action by inhibiting cancer cell proliferation while enhancing immune cell production at comparable doses. This indicates MC-S could offer additional adjunctive benefits to standard oncology / cancer therapies.**

### ***Introduction:***

Cancer is one of the leading causes of human disease-related death in the world today [1]. The major methods for treating cancer include surgery, radiation, chemotherapy, and immunotherapy [1, 2]. Conventional cancer chemotherapy is used to kill or disable tumor cells while preserving the normal cells in the body by the application of synthetic compounds [1, 3]. These agents have a narrow safety margin, and the therapy can fail due to drug resistance and dose-limiting toxicities [1]. In Asia herbal preparations have been increasingly used for cancer therapy, in an attempt to assist in the killing of tumor cells and to reduce the toxicity of combined chemotherapeutic agents [1, 4-6]. Today many clinically effective anti-cancer drugs have been derived from natural sources, e.g. paclitaxel (Taxol) from *Taxus brevifolia* L. and vincristine (Oncovin) from *Cantharanthus roseus* G.Don. [7]. Plants and fungi extracts continue to offer a wide range of compounds with diverse structures and activity which will continue to occupy an important role in modern cancer treatments.

The medicinal properties of mushrooms and herbs, has long been utilized in traditional medicine for healing and health. In the past decades scientific validation of the clinical efficacy of medicinal therapies has encouraged modern medicine to investigate the active ingredients of medicinal mushrooms and herbs. Unfortunately this approach may in some circumstances resulted in products that have reduced functionality when compared to their natural sources [8]. It is known that a range of active compounds found in medical mushrooms and herbs can stimulate the human immune system and/or inhibit tumor growth. As these compounds can vary in bioactivity and biospecificity, it may be possible to utilize a range of the compounds to symbiotically stimulate the immune system leading to a more efficacious response and inhibit tumor growth [9]. The formulation of MC-S (Clinical Health Pty Ltd., Newcastle Australia) is based upon this principle, and is composed of three medicinal mushrooms, *Ganoderma lucidum*, *Lentinus edodes* (mycelia) and *Coriolus versicolor*; a herb, *Astragalus membranaceous* (from the root) and ascorbic acid. The individual components of this commercially available product have demonstrated immune stimulating potential and anti tumor properties [9,10].

Combined in its present formulation MC-S has demonstrated to have a proliferative effect on PBL's *in vitro* [11] and an inhibitory effect on colds, flu and secondary infections *in vivo* [12].

***Ganoderma Lucidum*** (*Lingzhi* or *Reishi*) is a highly regarded traditional Chinese medicine for enhancing the body's resistance to disease and consolidating the constitution of patients (13). Studies on the polysaccharide extracts of *Ganoderma lucidum*, mainly in the form of (1→3)-β-D-glucans, have demonstrated mitogenicity and activation of immune effector cells (9, 14) as well as a stimulating effect on the production of cytokines (9, 15). *In vitro* studies have also shown inhibitory effects on breast cancer cells (16-19), prostate cancer cells (16, 18, 19, 21, 22), leukemia, lymphoma, myeloma cells (16, 23) and colon cancer cell lines (16, 24).

***Lentinus edodes*** is a common eatable mushroom. The noted extracts; LEM extract (from *Lentinus edodes mycelium*) and Lentinan, are both proven immuno-modulators augmenting activation and proliferation of peripheral mononuclear cells (25, 26). Lentinan shows no direct inhibition of growth on tumor cell lines *in vitro* (27).

***Coriolus versicolor*** contains two bioactive polysaccharides that are chemically similar; polysaccharide-peptide (PSP) and polysaccharide-K or Krestin (PSK). These differ by the presence of fucose on PSK and rhamnose and arabinose in PSP (9, 28). Both are potent immunomodulators and induce INF gamma and IL2 production (28, 29). Extracts of *Coriolus versicolor* have been shown to have inhibitory effects *in vitro* on gastric cancer, lung cancer, leukemia, lymphoma cell lines (30, 31). Similar results were obtained by others using another leukemic cell line, liver cancer and stomach cancer cell lines (30, 32-36). Finally, extracted polysaccharides and saponins from ***Astragalus membranaceus*** have been shown both *in vitro* and *in vivo* to stimulate NK cell activity and PBL proliferation (10, 37). Significant proliferation suppression was noted with macrophage-like, myeloid and lymphoid tumor cell lines (38) colon cancer cells (39).

***Objective:***

This study examines the immune modulating product, MC-S to determine whether its herbal based formulation has potential effect on a diverse range of human cancer cells lines *in vitro*. Each of the cancer cell lines represents a cancer class common to man. The types of cancer covered in this study are breast, prostate, colon and melanoma all of which have a profound impact world wide.

***Materials and Methods:******Chemicals and reagents:***

Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, trypsin/EDTA, and L- Glutamine 2mM were all Gibco, Invitrogen (Carlsbad California USA). Heat-inactivated fetal bovine serum (FCS) was purchased from Sigma (St Louis USA).

***Metabolic Cell- Support (MC-S):***

MC-S (supplied by Clinical Health Pty Ltd, Newcastle, Australia) was used in powdered form. The ascorbic acid was excluded from the *in vitro* testing due to its acidity and potential inhibitory effect on the tissue culture. Prior to testing a stock solution of MC-S was prepared in sterile water ( $100000\mu\text{g mL}^{-1}$ ). The test material was then prepared by diluting this stock to a final concentration of  $781.25\mu\text{g mL}^{-1}$  in DMEM complete with 10% FCS and sterile filtering through a 0.22 micron filter (Sartorius , Hannover Germany). Inhibitory efficacy of this test material was studied using 1:4 dilutions to a minimum concentration of  $0.0015\mu\text{g mL}^{-1}$  in DMEM complete with 10% FCS.

***Cell Culture:***

Four cancer cell lines were acquired for this study. Human melanoma cell line MM200 was kindly supplied by Oncology Immunology Newcastle Australia. Human breast cancer cell MCF7, human prostate cancer cell line DU145 and human colonic carcinoma cell line HT29 were all kindly supplied by Inter-K Newcastle Australia. All cell lines were seeded on to  $85\text{ cm}^2$  culture flasks (Cellstar Greiner Bio-one) and raised in DMEM and 10% FCS (v/v) till 95% confluency then harvested with Trypsin/EDTA, washed 3 times with PBS before being used in the proliferation assay at a concentration of  $1 \times 10^5\text{ mL}^{-1}$ .

### ***Cell Proliferation Assay:***

50 $\mu$ L of each dilution of MC-S test solution were added to six wells of a 96 well flat bottom culture plate (Nunc, Roskilde Denmark). To three wells of each dilution set, 50 $\mu$ L of Cancer cell suspension was added, giving a final concentration of  $5 \times 10^3$  cells per well. In the remaining three wells for each dilution set were added 50 $\mu$ L of DMEM complete media and 10% FCS (v/v) as a background control. Controls for this assay were non-treated (positive control). Plates were incubated at 37<sup>0</sup>C in a CO<sub>2</sub> incubator for 48 or 72 hours. Proliferation was measured using CellTiter 96 AQueous Non-Radioactive Cell proliferation assay kit (Promega Corporation, Madison Wisconsin USA) as per manufacturers' instructions with absorbance measured at 490nm using a Bio-Rad microplate reader model 680 (Bio-Rad Laboratories Hercules California USA). Absorbance data was calculated as a percentage of inhibition in comparison to non-treated control. Results were graphed as mean  $\pm$  SE of 3-4 separate assays.

### ***Statistics:***

Statistical analysis was performed using T-test analysis for two samples assuming equal variances, two tailed using means on percentage of decrease of each assay (Microsoft Excel). A p-value of less than 0.05 was considered to be significant.

### ***Results:***

The exposure of 4 cancer cell lines to varying doses of MC-S over a 48 hour and 72 hour period mediated an inhibitory response at most doses (Fig 1). The Melanoma cell line MM200 (Fig 1a) after 48 hours displayed significant inhibition of proliferation at all doses with peak inhibition at a dose of 390.625 $\mu$ g/ml with  $25.6 \pm 1.3$  %. After 72 hours incubation significant inhibition at all doses except 1.526 $\mu$ g/ml was observed peaking at 390.625 $\mu$ g/ml with  $37.7 \pm 2.7$  % inhibition. The Prostate cancer cell line DU145 (Fig 1b) after 48 hours exhibited significant inhibition at all doses above 0.006 $\mu$ g/ml in a dose dependant manner peaking at 390.625 $\mu$ g/ml with  $22.4 \pm 2.1$  % inhibition. At 72 hours showed significant inhibition at all doses in a dose dependant manner from 0.381 $\mu$ g/ml peaking at 390.625 $\mu$ g/ml with  $24.1 \pm 3.2$  % inhibition. The Breast cancer cell line MCF7

(Fig 1c) after 48 hours displayed significant inhibition at all doses with peak inhibition of  $19.7 \pm 2.7 \%$  at  $390.625\mu\text{g/ml}$ . After 72 hours significant inhibition at all doses except at  $1.526$  and  $6.104\mu\text{g/ml}$  peaking at a dose of  $24.414\mu\text{g/ml}$  with  $34 \pm 9.2 \%$  inhibition. The Colorectal adenocarcinoma cell line HT29 (Fig 1d) after 48 hours displayed significant inhibition at all doses except  $0.381$  and  $1.526\mu\text{g/ml}$  in a dose dependant manner peaking at a dose of  $390.625\mu\text{g/ml}$  with  $18.2 \pm 3.9 \%$  inhibition. After 72 hours data displayed significant inhibition at all doses in a dose dependant manner again peaking at a dose of  $390.625\mu\text{g/ml}$  with  $27.5 \pm 0.4 \%$  inhibition. Though there is an obvious increase in inhibition of proliferation from 48 hours to 72 hours incubation only a few doses showed statistically significant difference between the two time points. These are indicated on graphs (a) and (c) of figure 1.

### ***Discussion:***

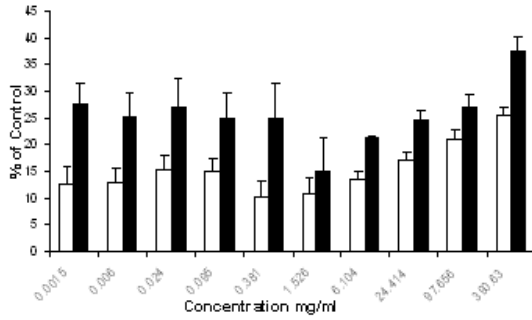
These experiments demonstrated that MC-S inhibits proliferation of all four cancer cell lines, exhibiting a statistically significant difference in most instances between non-treated control and MC-S treated cells. These findings compare favourably to the results achieved for the individual medicinal components of MC-S shown in other studies, however most research is usually directed specifically at concentrates of the isolates extracted from the whole fungi and plant making it difficult for direct comparisons. The highest dose of MC-S used in these experiments was  $390.63\mu\text{g/ml}$  in culture medium. The recommended human dose of MC-S ranges from two 900mg tablets per day for long term use to six tablets per day for short term use, resulting in a daily dose of  $1.8 - 5.4\text{g/day}$  of the MC-S concentrate bearing in mind that each tablet contains 250mg of Ascorbic acid. While it is infeasible to correlate human doses with cell culture concentration in the absence of data on serum concentrations of MC-S, we can hypothesize cell culture conditions ( $\mu\text{g/ml}$ ) are comparable to human therapeutic doses. [24]. In this study the concentration of the known active compounds of MC-S would be relatively low when compared to its purified counterparts, Lentinan, PSP and PSK. This makes the results of this study even more significant as it takes an extremely large quantity of mushroom or herb to gain a purified amount of an active compound [9].

Previously published data demonstrated MC-S at a comparable range of doses promoted PBL proliferation *in vitro* [11]. This would suggest that MC-S is selective in its action by inhibiting cancer cell proliferation while enhancing PBL (immune cell) proliferation. As mentioned in the introduction isolates of the individual components of MC-S have yielded similar results. Most of the major polysaccharides extracted from medicinal mushrooms have been subject to preclinical studies [9, 40]. The degree of safety testing on mushroom products is in general are more advanced than that for most herbal products [9,40,41]. Further to this, large-scale clinical trials utilizing Lentinan and other medicinal mushroom derivatives have not reported any serious adverse reactions or evidence of serious drug-drug interactions [9, 40].

In comparison to conventional chemotherapy and radiation-therapy, mushroom polysaccharides appear not to be harmful to health [40]. The clinical efficacy of the individual components of MC-S have been established and utilized clinically in Asian countries. The Western world has only in recent years realized the potential of complementary medicines. There is however the need for scientific validation of potential clinical applications. Therapeutic drug agencies such as the TGA in Australia and the FDA in the USA monitor claims made of complementary therapies, but still a gap exists between accepted applications of whole or parts of plants and fungi, isolated active agents and combinations of both. Efficacy re-trialing is again required when these products are turned into pharmaceutical preparations. Unfortunately world wide, there are many products on the market which could be considered inadequately scientifically reviewed.

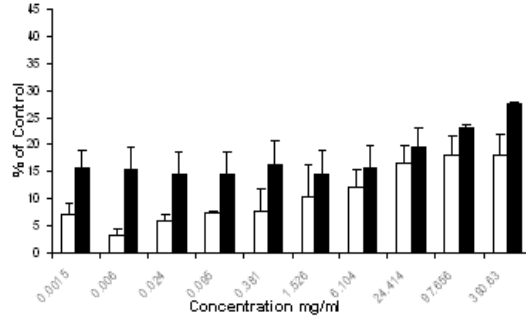
Breast, Prostate, Colon and Melanoma  
cancer cell growth were inhibited by an average of 34.25%

(A)



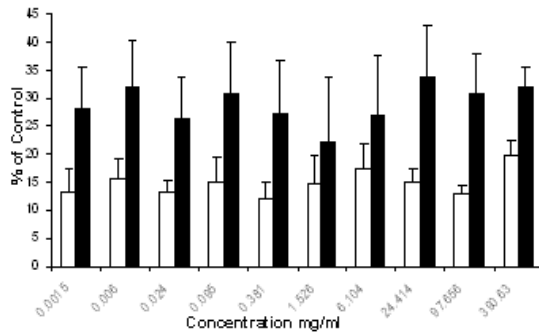
**Melanoma cancer was inhibited by 40%**

(B)



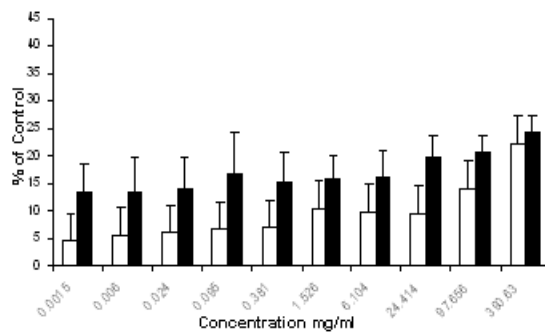
**Prostate cancer was inhibited by 28%**

(C)



**Breast cancer was inhibited by 43%**

(D)



**Colon cancer was inhibited by 26%**

% of cancer cell growth inhibited by MC-S at 48 hours   
 % of cancer cell growth inhibited by MC-S at 72 hours

Percentage inhibition in proliferation of Cancer cell lines when treated with MC-S™ at varying doses after 48 hours and 72 hours. Results represent the mean of 4 experiments performed in triplicate. (a) MM200 melanoma cell line (b) DU145 prostate cancer cell line (c) MCF7 breast cancer cell line (d) HT29 human colonic carcinoma cell line.

***Conclusion:***

**This study demonstrates that MC-S actively inhibits the growth of breast, prostate, melanoma and bowel cancer cells *in vitro*. In contrast historical data has shown MC-S to promote PBL (immune cell) proliferation *in vitro*, indicating that MC-S is selective in its action. This selectivity suggests that MC-S may offer additional benefits to standard cancer therapies.**

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