

## A medical nutriment supports dacarbazine treatment in stage III melanoma\*

Running title: Medical nutriment in melanoma

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\*Interim results of this study were presented at the 18th UICC International Cancer Congress. Oslo, Norway, 30 June – 5 July, 2002.

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### **ABSTRACT**

MSC (Avenar) is a medical nutriment, which has been shown to support anticancer treatments in colorectal cancer. This 1 year long open-label randomised study compared, in postsurgical adjuvant setting, dacarbazine plus 1 yr long continuous MSC administration (MSC group, 22 patients) vs dacarbazine alone (control group, 24 pts) in stage III malignant skin melanoma. At end-point, there were significantly more control patients with progressive disease (MSC: 36% vs control: 75%;  $P < 0.01$ ). Log-rank analysis (Kaplan-Meier estimate) showed significant difference in time-to-progression (median, days) in favour of the MSC group (MSC:  $366 \pm 53$  vs control:  $231 \pm 77$ ;  $\chi^2 = 8.21$ ;  $P = 0.0042$ ). Continuous supplementation of dacarbazine treatment with MSC is beneficial to stage III melanoma patients in terms of progression-free survival.

**Keywords:** fermented wheat germ extract; medical nutriment; stage III melanoma; dacarbazine; progression-free survival

MSC (Avemar) is a standardized fermented wheat germ extract which has been registered as a medical nutriment for cancer patients in Hungary (reg. no. 503), in the Czech Republic (reg. no. HEM-3512-13.103-1178) and in Bulgaria (reg. no. 05180/2003). Previously it had been reported that synchronous application of MSC (*per os*, *po*) and 5-fluorouracyl (5FU) injection (intraperitoneal, *ip*) significantly reduced metastatic spread of C38 colorectal carcinoma in mice (Hidvégi *et al*, 1999) and, later on, it was shown that MSC, applied as a supplementary nutriment, was beneficial regarding progression-free and overall survivals in colorectal cancer patients (Jakab *et al*, 2003). As it had also been reported that MSC (*po*), when used synchronously with dacarbazine (DTIC) injection (*ip*) in B16 melanoma bearing mice, blocked the dissemination process (Hidvégi *et al*, 1999) by completely inhibiting the development of lung metastases, it was aimed if this nutriment had any supportive value in skin melanoma patients receiving DTIC chemotherapy. Because the majority of the UICC (International Union Against Cancer) stage III malignant skin melanoma patients, treated by the standard anticancer therapies, will eventually develop progressive disease, a comparative, randomised clinical study was initiated to test the possible supportive value of MSC in this condition.

## **PATIENTS AND METHODS**

An open-label, randomised clinical trial was conducted to assess the supportive value of MSC in postsurgical adjuvant setting, given together with adjuvant DTIC chemotherapy, and continued alone for up to 12 months, in high risk stage III malignant skin melanoma patients.

Chemotherapy naïve, postoperative patients were randomised to either DTIC plus MSC (MSC) or to DTIC alone (control) groups. DTIC (400 mg per m<sup>2</sup> body surface) was given in short infusions. Each cycle lasted for five consecutive days, and was repeated monthly for up to 4 times or until disease progression. Beyond the adjuvant cytostatic monotherapy, patients of the MSC group took 9 grams of MSC, dissolved in 150 ml of water, orally once daily uninterruptedly and continuously from study entry for up to 12 months. To be eligible for this study, patients had to have histologically proved malignant skin melanoma with also histologically proved lymphatic but, lack of distant metastases (stage III disease); a World Health Organisation performance status of 0, 1, or 2; adequate organ functions; and life expectation of at least 12 months. All of the patients had to undergo radical surgery including complete removal of the primary tumour with further complete resection of the regional nodal disease (lymphatic metastases) resulting in macroscopically disease-free state. Accrual and randomisation were done within one month following the establishment of histological diagnosis. Exclusion criteria were: history of other type of cancer; pregnancy; lactation. The institutional review board approved the protocol, and all patients gave written informed consent before entering into the study. All patients were evaluated at baseline, at the end of each DTIC cycle, and 1, 5 and 9 months after completion of chemotherapy. Evaluation included physical examination, assessment of disease progression by imaging (radiographic, ultrasonic, or magnetic resonance) techniques, laboratory tests (haematology, chemistry and urinalysis), and toxicity monitoring according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Primary and/or nodal disease recurrence and new lymphatic and/or distant metastatic disease occurrence were reckoned as progression related events. The length of the study was planned to last for 12 months. Time-related events were measured from the date of entry into the trial. The end-point of this study was to compare progression-free survivals of the two groups. For this case, two-tailed, unstratified log-rank test (Kaplan-

Meier method), where  $p < 0.05$  indicates statistical significance, was used. For other comparisons the Fisher's exact test was applied.

## RESULTS

Between March 2000 and May 2002, 56 intent-to-treat patients entered to this study at the Melanoma Unit of the N. N. Blokhin Cancer Research Center, Moscow, Russia. In the MSC group 2 patients refused treatment due to nausea, and 3 patients had to receive anticancer therapies other than DTIC monotherapy due to the presence of progressive disease at or close to study entry. In the control group 2 patients refused treatment (1 due to nausea, 1 due to haematological toxicity), 2 patients were lost (probably due to disease progression), and 1 patient just entered the study at the time of the present evaluation. These patients were not included into data analysis. Baseline characteristics of the treated patients are shown in Table 1. At study entry there were no statistical difference in parameters of the two groups.

The administration of the medical nutriment was safe. No adverse events were reported after completion of chemotherapy, i.e. during the MSC only treatment (MSC group) or follow up (control). In the course of DTIC plus MSC or DTIC only treatments, the following acute adverse events were registered. Adverse event: NCI-CTC grade (number of adverse episodes in MSC/control groups). Nausea/vomiting: 1 (29/29), 2 (6/9), 3 (0/2), 4 (0/1); diarrhoea: 1 (12/23), 2 (2/0); fatigue: 2 (0/7); fever/infection: 2 (2/8); leukopaenia: 1 (0/2), 2 (0/1); thrombocytopaenia: 1 (0/1), 2 (0/1), 3 (0/1). It is to note that there were generally less toxic side effects in patients receiving the combined treatment than in those of the DTIC only group.

At end-point, majority of progression-related events were significantly more frequent in the control group (Table 2). Log-rank analysis (Kaplan-Meier estimate) also showed

significant difference in favour of the MSC patients in the cumulative probability of progression-free survival (Fig. 1), as well as in time-to-progression values (Table 3).

## DISCUSSION

Metastatic melanoma remains incurable. Thus, delay of disease progression in this condition is of high clinical importance. Because the medical nutriment, MSC, had previously been shown synergism with DTIC in the prevention of metastatic spread of melanoma cells in mice, an open-label, randomised clinical study was carried out to test whether MSC could act similarly in high risk stage III skin melanoma patients receiving adjuvant DTIC chemotherapy. The study results became significant: DTIC treatment in adjuvant setting plus 1 year long continuous MSC administration was superior to DTIC treatment alone in stage III melanoma patients concerning progression-free survival.

The explanation behind the benefits of MSC administration in melanoma may be based on the poly(ADP-ribose) polymerase (PARP) mediated apoptosis inducing activity of this nutriment. MSC has been shown to induce apoptosis in cancer cells by activating the caspase-3 catalysed cleavage of the PARP enzyme (Comín-Anduix *et al*, 2002). It has been proposed that PARP inhibition may increase the cytotoxic potential of DTIC in melanoma cells (Lonn and Lonn, 1987). This might also be true for our study. As the activity of PARP is accelerated in cancer cells, these cells can be selectively sensitised by PARP inhibitors (like MSC) to agents (like 5-FU or DTIC) inducing base-excisions or lesions in DNA (Virág and Szabó, 2002). Recently, it has been demonstrated that PARP-mediated trans-activation is essential for transcription of the melanoma growth stimulatory activity *CXCL1* gene, which is constitutively expressed during inflammation and progression of melanocytes into malignant melanoma (Nirodi *et al*, 2001). MSC, through inducing PARP cleavage, may also inhibit the expression of gene *CXCL1* thus, delaying further progression of melanoma cells.

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**Table 1. Patient characteristic at baseline.**

		MSC (n = 22)		Control (n = 24)	
		#	%	#	%
sex <sup>1</sup>	male	13	59.1	15	62.5
	female	9	40.9	9	37.5
age <sup>2</sup> (years)	mean	49.9		49.4	
	range	25-73		17-72	
	< 40	5	22.7	4	16.7
	40-59	11	50.0	15	62.5
	> 60	6	27.3	5	20.8
WHO performance score <sup>3</sup>	0	1	4.5	5	20.8
	1	21	95.5	18	75.0
	2	0	0	1	4.2
primary site	head/neck	2	9.1	0	0
	arm/shoulder/chest	6	27.3	5	20.8
	leg/hip	8	36.4	7	29.2
	abdomen/back/waist	5	22.7	12	50.0
	unknown	1	4.5	0	0
Clark level <sup>4</sup>	III	1	4.5	1	4.2
	IV	6	27.3	8	33.3
	V	10	45.4	12	50.0
	unknown	5	22.7	3	12.5
positive nodes site	cervical	2	9.1	0	0
	axillary	11	50.0	15	62.5
	inguinal	9	40.9	9	37.5
time from histological diagnosis to study entry <sup>5</sup> (days)	mean	21.1		27.6	
	s.d. <sup>6</sup>	12.7		16.5	
	range	0-49		0-57	

<sup>1</sup>Not significant difference (ND). <sup>2</sup>ND. <sup>3</sup>ND (P=0.148). <sup>4</sup>ND. <sup>5</sup>ND (t[44]=1.475; P=0.147).

<sup>6</sup>Standard deviation.

**Table 2.** Progression-related events (end-point analysis<sup>1</sup>).

	MSC (n = 22)		Control (n = 24)	
	#	%	#	%
primary disease recurrence <sup>2</sup>	1	4.5	1	4.2
nodal disease recurrence <sup>3</sup>	1	4.5	9	37.5
new nodal disease occurrence <sup>4</sup>	3	13.6	9	37.5
first distant metastasis occurrence <sup>5</sup>	5	22.7	14	58.3
further distant metastasis occurrence <sup>6</sup>	0	0	5	20.8
overall events	10	-	38	-
patients with progressive disease <sup>7</sup>	8	36.3	18	75.0

<sup>1</sup>Fisher's exact test. <sup>2</sup>Not significant difference (ND) (P=0.733). <sup>3</sup>P<0.01. <sup>4</sup>ND (P=0.065).

<sup>5</sup>P<0.05. <sup>6</sup>P<0.05. <sup>7</sup>P<0.01.

**Table 3.** Time-to-progression [days] (Kaplan-Meier analysis).

	MSC		Control	
	mean (SE <sup>1</sup> )	median (SE)	mean (SE)	median (SE)
progression-free survival <sup>2</sup>	306 (21)	366 (53)	213 (21)	231 (77)
distant metastasis-free survival <sup>3</sup>	340 (12)	392 (28)	255 (23)	289 (40)

<sup>1</sup>Standard error. <sup>2</sup>Log-rank test:  $\chi^2 = 8.21$ ; P=0.0042. <sup>3</sup>Log-rank test:  $\chi^2 = 7.23$ ; P=0.0072.

**Figure 1.** Kaplan-Meier estimate of the cumulative probability of remaining free from disease progression in stage III malignant skin melanoma patients. Log-rank test:  $\chi^2 = 8.21$ ;  $P=0.0042$ .

