

## Changes in immunity characteristics in patients with small (T1) choroidal melanoma after a course of 810-nm diode laser TTT delivered using the developed methodology

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**Background:** Immune response is an important factor for efficacy of choroidal melanoma (CM) treatment. Adequate uveal melanoma (UM) organ-saving treatment and treatment efficacy monitoring requires determining the interaction pattern between immune and tumor cells.

**Purpose:** To investigate changes in immune characteristics in patients with small T1 CM (measuring  $\leq 3$  mm in thickness and  $\leq 12$  mm in basal dimension) after a course of 810-nm diode laser transpupillary thermotherapy (TTT) delivered using the developed methodology.

**Materials and Methods:** We determined immune system characteristics in 35 patients (9 men (25.7%) and 26 women (24.3%); mean age, 53.9 (12.1) years) with small (T1) CM before and after a course of 810-nm diode laser TTT delivered using the developed methodology.

**Results:** Some characteristics (absolute white blood cell count, absolute and relative CD8+ T cell counts, absolute and relative phagocytic neutrophil activity, absolute and relative CD19+ B cell counts, IgA, IgM, and IgG) decreased, whereas others (relative white blood cell count, absolute and relative CD3+ T cell counts, absolute and relative CD4+ T helper counts, CD4+/CD8+ T-cell ratio, and absolute and relative CD16+ NK cell counts) increased after treatment. However, these changes were not statistically significant, excepting that relating to CD4+/CD8+ T-cell ratio ( $F=7.9$ ;  $p = 0.05$ ).

**Conclusion:** 810-nm diode laser transpupillary thermotherapy (TTT) delivered using the developed methodology results in a shift in specific antitumor immune response patterns with a suppression of cytolytic CD8+ T cell response, which requires administering immunocorrective therapy as early as the initial stage of the disease.

## Introduction

In recent years, there have been reports of new ideas of pathogenetic tumor progression mechanisms mediated by the interaction between immune and tumor cells [1-9]. There is a notion that under certain conditions, the immune system does not reject the tumor, but is involved in its development and progression [10].

Uveal melanomas (UM) are highly malignant tumors, and up to 90% of them develop in the choroid. Small choroidal melanomas (CM) (those with a height of up to 3.0-4.0 mm [19-21]) account for 5-21% [11-18] of all CM. According to the 2009 American Joint Committee on Cancer (AJCC)/ Union for International Cancer Control (UICC) staging system, CM measuring  $\leq 3$  mm in thickness and  $\leq 12$  mm in basal dimension are classified as T1 (small), whereas those measuring 3.1 mm to 6 mm in thickness and  $> 12$  mm in basal dimension are classified as medium [22]. T1 CM is an initial state of the disease, but there is a substantial difference in treatment strategies between patients with small CM and those with medium CM. Adequate and prompt treatment for CM has been found efficient with regard to visual function saving and improved survival prognosis [11-13, 17, 19, 21, 23-29].

Immune response is an important factor for efficacy of CM treatment. Correlations have been reported between cellular and humoral immunity characteristics, on the one hand, and UM patient's resistance to cancer and disease prognosis, on the other hand [30-33]. Adequate UM organ-saving treatment and treatment efficacy monitoring requires determining the interaction pattern between immune and tumor cells.

The purpose of the study was to investigate changes in immune system characteristics in patients with small T1 CM (measuring  $\leq 3$  mm in thickness and  $\leq 12$  mm in basal dimension) after a course of 810-nm diode laser transpupillary thermotherapy (TTT) delivered using the developed methodology.

## Materials and Methods

We determined immune system characteristics in 35 patients (9 men (25.7%) and 26 women (24.3%); mean age, 53.9 (12.1) years) with small (T1) CM (measuring  $\leq 3$  mm in thickness and  $\leq 12$  mm in basal dimension) before and after a course of 810-nm diode laser TTT delivered using the developed methodology [34].

Immune system characteristics were investigated using conventional methodologies [35, 36].

Statistical analyses were conducted using Statistica 10 (StatSoft, Tulsa, OK, USA) software.

## Results and Discussion

Table 1 presents the results of the comparison of cellular and humoral immunity characteristics in patients with small (T1) CM before and after a course of TTT delivered using the developed methodology. When TTT exerted its effects on the tumor, CM patient's body responded by shifts in immunity. Some characteristics (absolute white blood cell count, absolute and relative CD8+ T cell counts, absolute and relative phagocytic neutrophil activity, absolute and relative CD19+ B cell counts, IgA, IgM, and IgG) decreased numerically, whereas others (relative white blood cell count, absolute and relative CD3+ T cell counts, absolute and relative CD4+ T helper counts, CD4+/CD8+ T-cell ratio, and absolute and relative CD16+ NK cell counts) increased. However, these changes were not statistically significant (excepting that relating to CD4+/CD8+ T-cell ratio;  $F=7.9$ ;  $p = 0.05$ ). This is possibly due to the short period between "before" and "after" studies, and actual changes have not yet been reflected completely by the immune system. In addition, we have found that, compared to healthy individuals, patients with small (T1) CM had high total numbers of T cells, T helpers and T suppressors [37, 38], which is in disagreement with those of Likhvantseva [9]. At the initial disease stage (when the tumor is small), the immune system is still active, despite the development of tumor process. The curative effect of TTT on small (T1) CM results in increased total numbers of T cells and T helpers and decreased cytotoxic CD8+ T cell counts. A general lymphocytopenia has been reported in patients with UM. In addition, it has been reported that lymphocytopenia became more severe as the tumor and metastases progressed, and was accompanied by variability with regard to T helpers and T suppressors [9]. Given these facts, in patients with UM, immunocorrective therapy should be administered as early as the initial stage of the disease, and the response to this therapy should be monitored over the course of organ-saving treatment.

## Conclusion

810-nm diode laser transpupillary thermotherapy (TTT) delivered using the developed methodology results in a shift in specific antitumor immune response patterns with a suppression of cytolytic CD8+ T cell response, which requires administering immunocorrective therapy as early as the initial stage of the disease.

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**Table 1.** Cell-mediated and humoral immunity characteristics (mean and standard deviations) in 35 patients with small (T1) CM before and after a course of 810-nm diode laser transpupillary thermotherapy (TTT) delivered using the developed methodology

Characteristic	Changes in characteristic		F	p	Δ%
	Before treatment, M (SD)	After treatment, M (SD)			
Absolute white blood cell count (thousands per $\mu\text{L}$ )	6.46 (1.76)	5.99(1.67)↓	1.10	0.25	7.28
Absolute CD3+ T cell count (thousands per $\mu\text{L}$ )	1.85 (0.59)	1.88(0.58)↑	1.03	0.84	1.62
Relative CD3+ T cell count (%)	30.06 (7.32)	31.71 ( 9.45)↑	1.67	0.41	5.49
Absolute lymphocyte count (thousands per $\mu\text{L}$ )	1248.68 (467.63)	1282.09 (557.14)↑	1.45	0.74	2.68
Relative lymphocyte count (%)	64.63 (10.74)	66.34 (13.56)↑	1.59	0.56	2.65
Absolute CD4+ T helper count (thousands per $\mu\text{L}$ )	887.57 (405.40)	987.69 (491.88)↑	1.47	0.36	11.28
Relative CD4+ T helper count (%)	46.03(12.27)	51.51 (16.57)↑	1.82	0.12	11.91
Absolute cytotoxic CD8+ T cell count (thousands per $\mu\text{L}$ )	306.63 (120.70)	273.46 (133.96)↓	1.23	0.28	10.82
Relative cytotoxic CD8+ T cell count (%)	16.43 (5.33)	14.77 (4.85)↓	1.21	0.18	10.1
CD4+/CD8+ ratio	3.11 (1.76)	4.45(3.85)↑	7.90	0.05	43.09
Absolute phagocytic neutrophil activity (thousands per $\mu\text{L}$ )	3015.11 (1178.34)	2687.11 (1144.11)↓	1.06	0.24	10.88
Relative phagocytic neutrophil activity (%)	66.29 (14.64)	64.00 (13.50)↓	1.18	0.50	3.45
Absolute CD19+ cell count (thousands per $\mu\text{L}$ )	283.14 (134.93)	255.06(156.97)↓	1.35	0.43	9.92
Relative CD19+ cell count (%)	15.23 (6.25)	13.46 (6.33)↓	1.02	0.24	11.62
Absolute CD16+ NK cell count (thousands per $\mu\text{L}$ )	187.83(87.04)	206.09 (95.83)↑	1.21	0.41	9.72
Relative CD16+ NK cell count (%)	9.97 (2.54)	11.06 (3.98)↑	2.46	0.18	10.93
IgA(0)	2.67 (1.16)	2.64 (0.10)↓	1.35	0.90	1.12
IgM(0)	1.02 (0.26)	0.92 (0.25)↓	0.12	0.12	9.8
IgG(0)	13.42 (2.87)	12.82 (3.11)↓	1.17	0.4	4.47

Notes: M, mean value; SD, standard deviation; p, significance of difference by Newman-Keuls test; F, Fisher's coefficient; Δ, percentage difference; ↑, the value increased after treatment; ↓, the value decreased after treatment