Variation of the T Lymphocytes According to Treatment in Breast Cancer

ANCA ZGURA[1], LAURENTIA GALES[1], ELVIRA BRATILA[1], CLAUDIA MEHEDINTU[1]*, BOGDAN HAINEALA[1], RAMONA ILEANA BARAC[1], AMORIN REMUS POPA[2], CAMELIA BUHAS[2], COSTIN BERCEANU[3],CRISTINA VERONICA ANDREESCU[1], RODICA ANGHEL[1]

[1] Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Str., 050474, Bucharest, Romania

[2] University of Oradea, Faculty of Medicine and Pharmacy, 1 Universitatii Str., 410087, Oradea, Romania

[3] University of Medicine and Pharmacy, 2 Petru Rares, 200349, Craiova, Romania

Breast cancer is a multifaceted disease whose varied phenotype recapitulates only partially the biological complexity. At present, there are new approaches to the diagnosis and treatment of this form of cancer, but research should also focus on identifying and implementing other individual prognostic factors, factors that may lead to improved clinical decision making with regard to the patient, in order to establish an individualized treatment.

Keywords: breast cancer, T-lymphocytes, chemotherapy, radiotherapy, hormonal therapy

Breast cancer is a multifaceted disease whose varied phenotype recapitulates only partially the biological complexity.

Due to very high absolute levels of incidence and, inevitably, mortality, breast cancer is one of the major forms of both prevention and treatment and, not for the sake of scientific research. Numerous efforts have been made over time to improve the survival rate through early diagnosis and multiple (combined) therapies. At present, there are new approaches to the diagnosis and treatment of this form of cancer, but research should also focus on identifying and implementing other individual prognostic factors, factors that may lead to improved clinical decision making with regard to the patient, in order to establish an individualized treatment.

Immunoediting is a dynamic process that consists of immunosuppression and tumor progression. Tumor progression has 3 phases: elimination, equilibrium and escape. In the elimination and balance phases, cancer cells are attacked by the CD8 + T lymphocytes, while the tumor escape phase inhibits the CD8 + T lymphocytes.

Experimental part

In order to better understand the effects of the treatment on the adaptive immune system, peripheral blood samples were collected from 50 patients diagnosed and treated at the Bucharest *Prof. Dr. Alexandru Trestioreanu* Oncological Institute, during 2012-2018, to determine the influence of T lymphocytes on tumor progression as possible prognostic factors in relation to the clinical and pathological parameters and their response to the adjuvant / neoadjuvant, hormonal or radiotherapy treatment.

Chemotherapy regimens were established according to the ESMO and NCCN guidelines.

The 50 patients included in the study underwent adjuvant cytostatic and neoadjuvant chemotherapy consisting of EC chemotherapy (Epirubicin 90 mg / m2 IV, Cyclophosphamide 600 mg / m2 IV) followed by Docetaxel 100 mg / m², CMF type (Cyclophosphamide 600 mg / Methotrexate 40 mg / m2 IV, 5-Fluorouracil 600 mg / m2 IV followed by Docetaxel 100 mg / m² IV administered every 21 days) or FEC chemotherapy (5-Fluorouracil 500 mg / m² IV, Epirubicin 100 mg / IV, Cyclophosphamide 600 mg / m² IV, administered every 21 days) followed by Docetaxel (100 mg / m2 IV, given every 21 days). Patients who had positive hormonal receptors followed hormone treatment (Tamoxifen or Anastrozole). For patients confirmed with Her2 in the IHC (7 patients), Trastuzumab (6 mg / kg IV every 21 days for 1 year) could be given. Of the total patients, 20 representing 35.71% performed radiotherapy. Table 1 presents the statistical correlation between age and lymphocite T values.

Age is negatively correlated with the total CD3 T lymphocytes (-0.012), but statistically significant.

Age is negatively correlated with CD4 + T (-0.102), statistically insignificant, i.e. younger patients have elevated CD3 + CD4 + T values. CD8 (-0.256) is correlated with age, has a poor but statistically significant correlation, suggesting that younger patients have higher CD8 + T values. The statistical correlation between age and ratio is only 10%, suggesting that older patients have a higher CD4 / CD8 ratio.

Table 2-4 shows the maximum and minimum values of analyzed T lymphocytes at the I, II, and III evaluation.

The first evaluation was performed on a total of 15 patients with the following lymphocyte counts:

-For CD3 + T, the minimum value was 24.18%, the maximum value was 75.13% and the average value was 52.77%.

-For CD4 + T the minimum value was 13.35%, the maximum value was 42.30% and the average value was 28.58%.

- For CD8 + T the minimum value was 8.08, the maximum value was 19.81 and the average value was 19.81.

-For the CD4 + / CD8 + ratio the average value was 1.61 (minimum 0.71 and maximum 4.65).

A second evaluation was performed on a number of 15 patients who had the following values:

-For CD3 + T, the minimum value was 22.99%, the maximum value was 68.44% and the average value was 50.28%.

- For CD4 + T the minimum value was 10.84%, the maximum value was 44.03% and the average value was 27.23%.

- For CD8 + T the minimum value was 8.85%, the maximum value was 32.05% and the average value was 19.56%.

- For the CD4 + / CD8 + ratio, the mean value was 1.61 (minimum value 0.76 and maximum value 4.25)

For the evaluation we had a total of 4 patients who had the following values:

* email: claudiamehedintu@yahoo.com, Phone: +40 722312976 *All the authors have equal contribution at this original article*

Table 1
STATISTICAL CORRELATION BETWEEN AGE AND LYMPHOCYTE T VALUES

LYMPHOCYTE T		CD3.1	CD4.1	CD8.1	DP.1	DN.1	CD4_CD8.1	Age
CD3.1	Correlation Coefficient	1.000	.510**	.512**	.180	.182	-.097	-.212*
	Sig. (2-tailed)		.000	.000	.066	.063	.323	.031
	N	50	50	50	50	50	50	50
CD4.1	Correlation Coefficient	.510**	1.000	.078	-.073	.042	.348**	-.102
	Sig. (2-tailed)	.000		.427	.456	.670	.000	.299
	N	50	50	50	50	50	50	50
CD8.1	Correlation Coefficient	.512**	.078	1.000	.119	.088	-.575**	-.256**
	Sig. (2-tailed)	.000	.427		.225	.371	.000	.009
	N	50	50	50	50	50	50	50
DP.1	Correlation Coefficient	.180	-.073	.119	1.000	.080	-.162	.003
	Sig. (2-tailed)	.066	.456	.225		.417	.099	.973
	N	50	50	50	50	50	50	50
DN.1	Correlation Coefficient	.182	.042	.088	.080	1.000	-.030	-.198*
	Sig. (2-tailed)	.063	.670	.371	.417		.757	.044
	N	50	50	50	50	50	50	50
CD4_CD8.1	Correlation Coefficient	-.097	.348**	-.575**	-.162	-.030	1.000	.170
	Sig. (2-tailed)	.323	.000	.000	.099	.757		.085
	N	50	50	50	50	50	50	50
Age	Correlation Coefficient	-.212*	-.102	-.256**	.003	-.198*	.170	1.000
	Sig. (2-tailed)	.031	.299	.009	.973	.044	.085	
	N	50	50	50	50	50	50	56

LYMPHOCYTE T	Minimum	Maximum	Mean	Std. Dev.
CD3.1	24.18	75.13	52.77	10.31
CD4.1	13.35	42.30	28.58	6.98
CD8.1	8.08	30.84	19.81	6.30
DP.1	.12	9.41	1.32	1.60
DN.1	.60	8.12	2.86	1.80
CD4_CD8.1	.71	4.65	1.61	0.79

Table 2
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (I EVALUATION) (N=50)

LYMPHOCYTE T	Minimum	Maximum	Mean	Std. Dev.
CD3.2	22.99	68.44	50.28	13.01
CD4.2	10.84	44.03	27.23	7.79
CD8.2	8.85	32.05	19.56	7.70
DP.2	.22	2.29	1.02	0.78
DN.2	.51	6.53	2.47	1.90
CD4_CD8.2	.76	4.25	1.61	0.91
Valid N (listwise)	-	-	-	-

Table 3
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (II EVALUATION) (N=15)

- For CD3 + T, the minimum value was 11.42%, the maximum value was 71.11% and the average value was 45.76%.
- For CD4 + T, the minimum value was 11.42%, the maximum value was 38.61% and the average value was 25.67%.
- For CD8 + T the minimum value was 9.21%, the maximum value was 29.55% and the average value was 17.15%.
- For the CD4 + / CD8 + ratio the average value was 1.75 (minimum value 0.84 and maximum 3.71).

Table 5 depicts the average values for patients with 2 evaluations.

For CD3 + T the mean value was 50.89%. For CD4 + T mean value was 28.04%.

For CD8 + T the mean value was 19.68%. For the CD4 + / CD8 + ratio, the mean value was 1.61.

Table 6 shows the statistical correlation between T lymphocyte evaluations.

The first and second evaluations are strongly correlated statistically positive ($p < 0.05$) (table 7).

LYMPHOCYTE T	N	Minimum	Maximum	Mean	Std. Deviation
CD3.3	4	25.86	71.11	45.76	18.85
CD4.3	4	11.42	38.61	25.67	12.85
CD8.3	4	9.21	29.55	17.15	8.76
DP.3	4	.78	3.77	1.68	1.41
DN.3	4	1.37	2.05	1.87	0.34
CD4_CD8.3	4	.84	3.71	1.75	1.32

Table 4
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (III EVALUATION)

Paired Samples Statistics		Mean	Std. Deviation	Std. Error Mean
Pair 1	CD3.1	50.8920	7.1348	1.8422
	CD3.2	50.2793	13.0109	3.3594
Pair 2	CD4.1	28.0480	6.4588	1.6676
	CD4.2	27.2293	7.7876	2.0107
Pair 3	CD8.1	19.6840	6.0553	1.5635
	CD8.2	19.5640	7.6994	1.9880
Pair 4	DP.1	.9853	0.9929	0.2564
	DP.2	1.0247	0.7801	0.2014
Pair 5	DN.1	2.1807	1.2990	0.3354
	DN.2	2.4733	1.8966	0.4897
Pair 6	CD4_CD8.1	1.6413	0.9646	0.2491
	CD4_CD8.2	1.6140	0.9110	0.2352

Table 5
AVERAGE VALUES FOR PATIENTS WITH 2 EVALUATIONS (N=15)

Paired Samples Correlations		N	Correlation	Sig.
Pair 1	CD3.1 & CD3.2	15	.436	.104
Pair 2	CD4.1 & CD4.2	15	.602	.018
Pair 3	CD8.1 & CD8.2	15	.700	.004
Pair 4	DP.1 & DP.2	15	.800	.000
Pair 5	DN.1 & DN.2	15	.944	.000
Pair 6	CD4_CD8.1 & CD4_CD8.2	15	.899	.000

Table 6
STATISTICAL CORRELATION BETWEEN T LYMPHOCYTE EVALUATIONS

Table 7
CORRELATION BETWEEN EVALUATIONS

Paired Samples Test		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	CD3.1-CD3.2	.61267	11.79969	3.04667	-5.92179	7.14712	.201	14	.844
Pair 2	CD4.1-CD4.2	.81867	6.46429	1.66907	-2.76114	4.39847	.490	14	.631
Pair 3	CD8.1-CD8.2	.12000	5.53504	1.42914	-2.94520	3.18520	.084	14	.934
Pair 4	DP.1-DP.2	-.03933	.59629	.15396	-.36955	.29088	-.255	14	.802
Pair 5	DN.1-DN.2	-.29267	.79686	.20575	-.73395	.14862	-1.422	14	.177
Pair 6	CD4_CD8.1-CD4_CD8.2	.02733	.42570	.10992	-.20841	.26308	.249	14	.807

Table 8
MEAN T LYMPHOCYTE FOR PATIENTS UNDERGOING HORMONAL TREATMENT (N=34)

LYMPHOCYTE T	Range	Minimum	Maximum	Mean	Std. Deviation
CD3 (%)	50.95	24.18	75.13	53.5079	11.09944
CD4 (%)	28.95	13.35	42.30	28.1724	6.97379
CD8 (%)	23.97	8.08	32.05	20.6638	6.63040
CD4/CD8	2.236793	0.709654	2.946447	1.494621	0.562676

Of all the patients, 34 are hormone-treated and the mean values for CD4 + T were 28.17%, for CD8 + T 20.66% and a value of 1.49.

In the group of patients undergoing radiotherapy, the mean values for CD4 + T were 31.55%, for CD8 + T it was 17.06% and the CD4 / CD8 ratio was 2.5.

For the patients undergoing CHT + Transtuzumab treatment the mean value for CD4 + T was 23.78, for CD8 + T 16.73 and the ratio of 1.48 (table 10). Table 11 shows the results for the statistical correlation of T lymphocyte values in patients undergoing hormone and radiotherapy. Table 12 presents the mean values of T lymphocyte in the case of the patients undergoing treatment with Transtuzumab + HT and RT.

From a statistical point of view, there is no statistical difference between the CD4 + T and CD8 + T values in the group of patients undergoing hormone treatment and those undergoing radiotherapy.

LYMPHOCYTE T	Range	Minimum	Maximum	Mean	Std. Deviation
CD3 (%)	12.97	47.39	60.36	52.3383	4.35802
CD4 (%)	19.46	22.46	41.92	31.5567	8.26869
CD8 (%)	20.19	8.58	28.77	17.0617	8.58743
CD4/CD8	3.785372	0.909267	4.694639	2.515384	1.772185

Table 9
MEAN VALUES OF T LYMPHOCYTE FOR PATIENTS UNDERGOING RADIOTHERAPY (N=6)

LYMPHOCYTE T	Range	Minimum	Maximum	Mean	Std. Deviation
CD3 (%)	32.97	22.99	55.96	43.9856	11.24280
CD4 (%)	20.26	10.84	31.10	23.7844	7.46628
CD8 (%)	15.67	8.85	24.52	16.7333	5.19339
CD4/CD8	1.721059	0.950762	2.671821	1.480460	0.521595

Table 10
MEAN VALUES OF T LYMPHOCYTE FOR PATIENTS UNDERGOING TREATMENT WITH TRANSTUZUMAB

Group Statistics					
RT/HT		N	Mean	Std. Deviation	Std. Error Mean
CD4 (%)	3	6	31.5567	8.26869	3.37568
	9	34	28.1724	6.97379	1.19599
CD8 (%)	3	6	17.0617	8.58743	3.50580
	9	34	20.6638	6.63040	1.13710
CD4/CD8	3	6	2.5154	1.77218	0.72349
	9	34	1.4946	0.56268	0.09650

Table 11
STATISTICAL CORRELATION OF T LYMPHOCYTE VALUES IN PATIENTS UNDERGOING HORMONE TREATMENT AND RADIOTHERAPY

LYMPHOCYTE T		N	Mean	Std. Deviation	Std. Error Mean
CD4 (%)	2	9	23.7844	7.46628	2.48876
	3	6	31.5567	8.26869	3.37568
CD8 (%)	2	9	16.7333	5.19339	1.73113
	3	6	17.0617	8.58743	3.50580
CD4/CD8	2	9	1.48046	0.52160	0.17387
	3	6	2.51538	1.77218	0.72349

Table 12
MEAN VALUES OF T LYMPHOCYTE FOR PATIENTS UNDERGOING TREATMENT WITH TRANSTUZUMAB + HT AND RT

Table 13
STATISTICAL CORRELATION OF T LYMPHOCYTE VALUES IN PATIENTS RECEIVING HT AND RT TREATMENT

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
LYMPHOCYTE T		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CD4 (%)	Equal variances assumed	.023	.882	-1.894	13	.081	-7.77222	4.10290	-16.63599	1.09154
	Equal variances not assumed			-1.853	10.056	.093	-7.77222	4.19394	-17.10987	1.56542
CD8 (%)	Equal variances assumed	2.642	.128	-.093	13	.927	-.32833	3.53399	-7.96306	7.30639
	Equal variances not assumed			-.084	7.458	.935	-.32833	3.90992	-9.45986	8.80320
CD4/CD8	Equal variances assumed	13.730	.003	-1.674	13	.118	-1.03492	.61810	-2.37024	.30039
	Equal variances not assumed			-1.391	5.583	.217	-1.03492	.74409	-2.88916	.81931

There is a statistically significant difference in the CD4 / CD8 ratio. The CD4 / CD8 ratio is increased in the group of patients undergoing radiotherapy. An additional study is needed in a larger group of patients.

For patients undergoing treatment with Transtuzumab and hormone therapy, the mean value for CD4 + T-lymphocytes was 23.78, for CD8 + 16.73 and the ratio was 1.48. For patients undergoing radiotherapy, the mean value for CD4 + T lymphocytes was 31.55 for CD8 + 17.06 and the CD4 + / CD8 + ratio was 2.51.

Table 13 presents the statistical correlation of T lymphocyte values in patients receiving HT and RT treatment, and table 14 shows the mean T lymphocyte for patients undergoing treatment with Transtuzumab + HT and HT

The higher CD4 + T and CD4 + / CD8 + ratio are observed in the group of patients undergoing radiotherapy, but statistically there are differences, but they are not statistically significant ($p > 0.05$).

LYMPHOCYTE T		N	Mean	Std. Deviation	Std. Error Mean
CD4 (%)	2	9	23.7844	7.46628	2.48876
	9	34	28.1724	6.97379	1.19599
CD8 (%)	2	9	16.7333	5.19339	1.73113
	9	34	20.6638	6.63040	1.13710
CD4/CD8	2	9	1.4805	0.5216	0.1739
	9	34	1.4946	0.5627	0.0965

Table 14
MEAN T LYMPHOCYTE FOR PATIENTS UNDERGOING TREATMENT WITH TRANSTUZUMAB + HT AND HT

For patients undergoing Transtuzumab + hormone therapy, the mean value for CD4 + T lymphocytes was 23.78, for CD8 + 16.73 and the ratio CD4 + / CD8 + of 1.48.

For patients undergoing hormone therapy, the mean value for CD8 + T lymphocytes was 28.17, for CD8 + 20.06 and the CD4 + / CD8 + ratio of 1.49.

Statistical analysis for the group of patients undergoing hormone therapy and the group of patients undergoing hormone therapy concluded that there are differences in CD4 + and CD8 + T lymphocytes, but they are not statistically significant ($p>0.05$).

Results and discussions

In breast cancer, the extensive tumor infiltration by cytotoxic CD8 T cells was strongly associated with patient survival and response to treatment. The presence of CD4 + T cells was associated with both good response to treatment and mitigation of the antitumor response [1-8]. Statistically, we did not find a statistical correlation between TILs grade and CD4 + and CD8 + T lymphocytes analyzed from the peripheral blood.

We noticed that chemotherapy used in different types of cancer [9-13] caused a short-term decrease in the values of all major subtypes of circulating lymphocytes (3-6 months) and prolonged (> 9 months) prolongation of CD4 + T cells. This is consistent with a smaller previous study showing a sustained decrease in the CD4 + T cells, but not CD8 +, after FEC breast cancer chemotherapy [3]. CD4 + and CD8 + T cells have opposite roles in the progression of breast cancer and in its evolution. From the analysis of CD4 + T cells in relation to the presence of metastases, it was revealed that elevated CD4 + values are associated with fewer metastases.

Analysis of the relationship between TIL density and patient age demonstrated that T lymphocytes and their CD4 + and CD8 + subgroups were directly associated with the age of the patient. Such a relationship has been investigated in several studies with discordant results performed by Marsigliante et al. [4] who found that only T cells were directly associated with the age of the patient, thus supporting our results in part. Instead, Menard et al. [5] did not report significant differences between the different age groups in terms of TIL frequency. More recently, Mahmoud et al. [6] showed that the CD8 + lymphocyte count was slightly inversely proportional to the age of breast cancer patients.

In the present study, the T lymphocyte analysis for patients undergoing radiotherapy indicated a mean value of 31% for CD4 + T and 17% for CD8 +, suggesting that the CD8 + T lymphocytes are more sensitive and more specific, also supported by the study of Mahmut Ozsahin et al., [7-8] which has prospectively confirmed that apoptosis of radiation-induced T lymphocytes has significantly predicted late effects [14].

Previous studies have shown that the CD4 / CD8 T cell response reflects the status of the immune system and can independently predict mortality from all causes. Shah et al. [15] in their study reported that the low CD4 / CD8 ratio was significantly associated with the worse prognosis of patients with cervical carcinoma, and in the study by Chang-Juan Tao et al. in 2016, it was shown that the higher CD4 / CD8 ratio (≥ 1.77) was associated with the free entry of the disease [16]. In the present study from the CD4 / CD8 ratio analysis for patients undergoing radiotherapy, an increase in values was observed, with an average of 2.5.

We analyzed the CD4 + and CD8 + T lymphocytes for patients undergoing hormone treatment (Tamoxifen or Anastrozole) resulting in an average of 28.17% for CD4 + and 20.67% for CD8 +, suggesting that hormone therapy helps recover populations of lymphocytes post-chemotherapy or radiotherapy, as confirmed by the study by Robinson et al. in 1999 [17].

Several studies have demonstrated the immuno-modulatory properties of radiotherapy (RT). RT induces the death of the immunogenic cells (ICD), increases the MHC-I expression in both normal and cancer cells, stimulates the chemotaxis and recruitment of T cells and T cells into the tumor by inducing intracellular adhesion molecules, cytokines and chemokines and inducing CTL primacy [18-22]. The higher CD4 + T and CD4 + / CD8 + ratio was observed in the group of patients undergoing radiotherapy, but statistically there are differences, but they are not statistically significant ($p> 0.05$).

Chemotherapy can enhance the immune response by improving the immune effector cells or by exhaustion of the immunosuppressive populations. In breast cancer, taxanes can enhance the function of NK and T cells according to Carson et al. 2004 [16], and the increase in the TIL percentage in the neoadjuvant context [23]. Docetaxel increases Th1-associated cytokine levels, while decreasing the inflammatory markers in metastatic disease, according to Tsavaris et al. 2002 [24]. Small doses of cyclophosphamide [25] and paclitaxel [26] can induce selective exhaustion of Tregs, while docetaxel [27] and gemcitabine [28] may reduce the number of myeloid-derived suppressor cells (MDSC). Paclitaxel, etoposide and 5-fluorouracil regulate the PDL-1 expression on cell lines in breast carp, thus promoting immune resistance [26]. Interference with the PD-1 / PD-L1 pathway with anti-PD-1 / PD-L1 immunotherapy could counteract this effect. Hormonal therapy can modulate and the immune system, e.g., letrozole in the neoadjuvant setting, reduces intratumoral FOX-P3 Tregs [29].

Conclusions

Although it has been considered that chemotherapy has immunosuppressive effects, contrary, it has also been shown to have immunomodulatory effects. The study demonstrated that the adaptive immune system is altered after chemotherapy for at least 9 months by assessing the CD4 + T lymphocytes, CD8 + T and the CD4 + / CD8 + ratio. Additional investigations will be needed to determine whether therapy should be modified to avoid the most serious effects on the immune system. Interestingly, for patients undergoing metastatic Capecitabine treatment, T cell antitumor reactivity was associated with lower

changes in the CD8 + and CD4 + ratios between the two evaluations.

Determinations during hormonal treatment revealed that values increased after cytostatic treatment or radiotherapy. This observation suggests that hormone therapy helps in recovering lymphocyte populations after chemotherapy or radiotherapy. Hormone therapy also seems to help restore the T cell lymphocytes, thus the cellular immune response capacity, following the immune-induced immune suppression and chemotherapy. From analysis of the T lymphocyte percentages for radiotherapy patients, the mean CD4 + T was 31.55 for CD8 + 17.06 and the CD4 + / CD8 + ratio was 2.51.

References

1.ALIZADEH, D., LARMONIER, N., Cancer. Res., **74**, nr. 10, 2014, p. 2663.

2.SALGADO R, DENKERT C, DEMARIA S, SIRTAINE N, KLAUSCHEN F, PRUNERI G, WIENERT S, VAN DEN EYNDEN G, BAEHNER FL, PENAULT-LLORCA F, PEREZ EA, THOMPSON EA, SYMMANS WF, RICHARDSON AL, BROCK J, CRISCITIELLO C, BAILEY H, IGNATIADIS M, FLORIS G, SPARANO J, KOS Z, NIELSEN T, RIMM DL, ALLISON KH, REIS-FILHO JS, LOIBL S, SOTIRIOU C, VIALE G, BADVE S, ADAMS S, WILLARD-GALLO K, LOI S, *Annals Oncol.*, **26**, nr. 2, 2015, p. 259-271.

3.MOZAFFARI, F., LINDEMALM, C., CHOUDHURY, A., GRANSTAM-BJORNEKLETT, H., LEKANDER, M., NILSSON, B., et al., Cancer Immunol. Immunother., **58**, nr. 1, 2009, p. 111.

4.MARSIGLIANTE, S., BISCOZZO L., MARRA, A., NICOLARDI, G., LEO, G., LOBREGLIO, G.B., et al., Cancer Lett., **139**, 1999, p. 33.

5.MENARD, S., TOMASIC, G., CASALINI, P., BALSARI, A., PILOTTI, S., CASCINELLI, N., et al., Clin. Cancer Res., **3**, 1997, p. 817.

6.VATNER, R., COOPER, B., VANPOUILLE-BOX, C., DEMARIA, S., FORMENTI, S. Frontiers Oncol., **4**, 2014, 325.

7.GAMEIRO, S., ARDIANI, A., KWILAS, A., HODGE, J. Oncoimmunol., 2015, **3**, e28643.

8.KROEMER, G., GALLUZZI, L., KEPP, O., ZITVOGEL, L., Annu. Rev. Immunol., **31**, 2013, p. 51.

9.ABDEL-DAIM, M.M., ZAKHARY, N.I., ALEYA, L., BUNGAU, S.G., BOHARA, R.A., SIDDIQI, N.J., Oxid. Med. Cell. Longev., **2018**, 2018, ID 2098123, 2 pages.

10.ENDRES, L., TIT, D.M., BUNGAU, S., CIOCA, G., ABDEL-DAIM, M., BUHAS, C., POP, O., SAVA, C., Rev. Chim.(Bucharest), **69**, no. 12, 2018, p. 3675

11.ABDEL-DAIM, M.M., SHAHEEN, H.M., ABUSHOUK, A.I., TORAIH, E.A., FAWZY, M.S., ALANSARI, W.S., ALEYA, L., BUNGAU S., Environ. Sci. Pollut. Res. Int., **25**, nr. 24, 2018, p. 23909

12.MOGOANTA, S.S., COSTACHE, A., MUTIU, G., BUNGAU, S.G., GHILUSI, M., GROSU, F., VASILE, M., VILCEA, I.D., GHERGHINESCU, M.C., MOGOANTA, L., ION, D.A., Rom. J. Morphol. Embriol., **56**, nr. 2 Suppl., 2015, p. 511.

13.PALLAG, A., ROSCA, E., TIt, D.M., MUTIU, G., BUNGAU, S.G., POP, O.L., Rom. J. Morphol. Embriol., **56**, nr. 3, 2015, p. 1103.

14.OZSAHIN M. CROMPTON NE, GOURGOU S, KRAMAR A, LI L, SHI Y, SOZZI WJ, ZOUHAIR A, MIRIMANOFF RO, AZRIA D., Clin. Cancer Res., **11**, nr. 20, 2005, p. 7426-33

15.SHAH, W, YAN, X., JING, L., ZHOU, Y., CHEN, H., WANG, Y., Cell. Mol. Immunol., **8**, 2011, p. 59.

16.CARSON, W., SHAPIRO, C., CRESPIN, T., THORNTON, L., ANDERSEN, B., Clin. Cancer. Res. 10, 2004, p. 3401.

17.ROBINSON, E., SEGAL, R., STRUMINGER, L. , FARAGGI, D. , EL'AD YARUM, R., MEKORI, T. Cancer, **85**, 1999, p. 2073.

18.MEHEDINTU, C., BRATILA, E., BERCEANU, C., CIRSTOIU, M.M., BARAC, R. I., ANDREESCU, C.V., BADIU, D.C., GALES, L., ZGURA, A., BUMBU, A.G., Rev. Chim.(Bucharest), **69**, no. 11, 2018, p.3133-3137

19.DIACONU, C.C., STANESCU, A.M.A., PANTEA STOIAN, A., TINCU, R.C., COBILINSCHI, C., DRAGOMIRESCU, R.I.F., SOCEA, B., SPINU, D.A., MARCU, D., SOCEA, L.I., BRATU, O.G., Rev. Chim.(Bucharest), **69**, no. 6, 2018, p. 1367.

20.DIACONU, C.C., DRAGOI, C.M., BRATU, O.G., NEAGU, T.P., PANTEA STOIAN, A., COBELSCHI, P.C., NICOLAE, A.C., IANCU, M.A., HAINÃRO'IE, R., STANESCU, A.M.A., SOCEA, B., Farmacia, 2018, **66**, nr. 3, 2018, p. 408.

21.DIACONU, C.C., MANEA, M., IANCU, M.A., STANESCU, A.M.A., SOCEA, B., SPINU, D.A., MARCU, D., BRATU, O.G., Rev. Chim.(Bucharest), **69**, no. 5, 2018, p. 1071.

22.RADULESCU, D., BALCANGIU STROESCU, A., PRICOP, C., GEAVLETE, B., NEGREI, C., BRATU, O., GINGHINA, O., VACAROIU, I., Rev. Chim.(Bucharest), **68**, no.1, 2017, p. 52.

23.DEMARIA, S., VOLM, M., SHAPIRO, R., YEE, H., ORATZ, R., FORMENTI, S. ET AL. Clin. Cancer Res., **7**, 2001, p. 3025.

24.TSAVARIS, N., KOSMAS, C., VADIAKA, M., KANELOPOULOS, P. AND BOULAMATSIS, D., Br. J. Cancer, **87**, 2002, p. 21.

25.GHIRINGHELLI, F., MENARD, C., PUIG, P., LADOIRE, S., ROUX, S., MARTIN, F., et al., Cancer Immunol. Immunother., **56**, 2007, p. 641.

26.ZHANG, L., DERMAWAN, K., JIN, M., LIU, R., ZHENG, H., XU, L. et al., Clin. Immunol., **129**, 2008, p. 219.

27.KODUMUDI, K., WOAN, K., GILVARY, D., SAHAKIAN, E., WEI, S., DJEU, J., Clin. Cancer. Res., **16**, 2010, p. 4583.

28.NOWAK, A., ROBINSON, B., LAKE, R., Cancer Res., **62**, 2002, p. 2353.

29.GENERALI, D., BATES, G., BERRUTI, A., BRIZZI, M., CAMPO, L., BONARDI, S. et al., Clin. Cancer Res., **15**, 2009, p. 1046

Manuscript received: 5.12.2018