Effectiveness of Breast Cancer Recurrences Prophylaxis by Means of Exogenous Peptides: Results of 24 Months Supervision

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ABSTRACT

Introduction: The article deals with the current state of the problem of occurrence of relapses and early metastasis of breast cancer (BC) and provides the main molecular and cellular mechanisms for avoiding systemic immune surveillance by tumor cells. The work focuses on circulating tumor cells as the main factor of the unfavorable prognosis of the disease, as well as the results of integrating the Camelyn Bio (exogenous peptides) into the BC management scheme.

Objective: To study the effects of exogenous peptides on the clinical progression of breast cancer, using as a criterion disease-free survival within 24 months.

Materials and methods: The study included 76 women who had undergone subcutaneous radical mastectomy with the preservation of the skin and the nasal-arreolar complex at the stage of IIA (T1N1M0, T2N0M0). According to the recommendations of the ethics committee, the patients self-agreed to conduct the study. The statistical analysis was based on the use of descriptive statistics and comparative analysis. To compare the qualitative parameters of the studied groups, the chi-square (Pearson) criterion was used. For quantitative parameters, a preliminary assessment of the normality of distribution, which was the basis for the choice of parametric (t-test) or non-parametric criteria (Wilconson rank-sum (Mann-Whitney test), was performed. For the analysis of non-recurrent survival, Kaplan-Meier method was used. The analysis was performed using the STATA 12 statistical analysis package.

Results: In the group of patients receiving exogenous peptides, there was a harmonization of immune homeostasis, manifested by increased immune surveillance over the tumor process involving activated T-lymphocytes, as well as increased expression of TNF-α and IL-12. The analysis of survival curves showed that the 24-month non-recurring survival rate of the patients in the main group was 0.918 (95% CI: 0.847-0.989), and in the comparison group -0.763 (0.628-0.898). That is, in the group of patients receiving Camelyn Bio, during the 24 months of observation there was a significant increase in non-recurring survival and a reduction in the risk of recurrence of tumors and metastases for the indicated period by 71.8%-Hazard ratio (95% CI) = 0.292 (0.094-0.907), p=0.046.

Conclusion: The results of this work allow us to assume that the use of Camelyn Bio in patients with breast cancer leads to a significant improvement in the rate of disease-free survival in the 24-month period after surgical intervention.

INTRODUCTION

Breast cancer is the most common female's malignant tumor, though its timely detection has increased due to the improvement of diagnostic technologies, the survival rates have not changed for the past four decades [1-5]. Schemes optimization of the complex treatment of patients with breast cancer has been a subject of great discussion up till now. The systemic adjuvant therapy, including hormonal therapy, is used to reduce the rate of recurrences and the remote tumor centers development [2,6,7]. However, in many countries, cyclophosphamide, methotrexate and 5 fluorouracil still remain the standard of chemotherapy treatment, despite the new generation of antitumor drugs in the oncology arsenal. Surgery, chemotherapy, radiotherapy and hormonal therapy are the main oncology tools in treating breast cancer. However, breast cancer still accounts 20 percent of all deaths of women having cancer, and the overall survival of patients has remained comparatively unchanged over the last forty years [8-10]. Relatively little is known about the beginning of human breast cancer, that is, what exactly becomes a trigger for cellular transformations [11-16]. Hundreds of genetic influences and biochemical interactions have been studied, new hypotheses for launching the pathological processes on the basis of nuclear, cytoplasmic, membrane and trans membrane mechanisms has been proposed every year [17-22]. For example, research flow indicates the significant role of Toll- like receptors (TLRs) in carcinogenesis regulation. Various activated TLRs may play opposite roles in different conditions: either stimulate tumor development or suppress it [23,24].

It has been proved not once those tumors are able to interfere different links of the immune cascade to violate the regulation of T-cells recruitment and macrophages, to reinforce or vice versa, to weaken the cytokines expressiveness, depending on the desired effects. Being the same in biochemical content, but the opposite in result, interaction between membrane microstructures of tumor and immunocompetent cells occur continuously in the presence of mediators or inflammation inhibitors and other bioregulators - this is a reflection of the of the immune homeostasis system functioning. As it is known, conducting neo-adjuvant polychemotherapy in local breast cancer leads to a tumor node reduction in the mammary gland and contributes to an increase in the number of organ-saving surgery. Surgical treatment becomes less traumatic and provides better social adaptation of patients. But it should be noted that along with the popularization of organ-preserving surgical interventions, the problem of circulating tumor cells (CTC) has been discussed in different clinics of the world for the last decade and most important is the discussion of their quality impact on the disease prognosis [25,26].

The sign that circulating tumor cells do not enter the cell cycle (in the resting state - G0), do not proliferate and therefore do not undergo apoptosis and the action of cytotoxic agents, seems to be important [26-28]. They can circulate in the form of single cells or unite into circulating tumor microembolias in the bloodstream that have a high proliferative potential. Tumor microembolias are resistant to apoptosis and have the properties of aggressive metastasis. But what is more significant and may worsen the disease prognosis is that a lot of tumor cells retain the ability to circulate in the bloodstream for a very long time. Evading from immune observation, cancer cells are found in the breast cancer patients' blood even in 10 or more years after the completion of radical treatment [25,29,30]. One of the directions in modern antitumor immunotherapy is the use of regulatory exogenous proteins. Camelyn Bio preparation was created to solve the complex task of harmonizing the immune homeostasis, as well as to restore the complete antitumor immune observation. Camelyn Bio consists of biologically active substances, including exogenous peptides derived from mountain honey by controlled gel electrophoresis. Mountain honey peptides (MHP) represent one of the most evolutionarily ancient families of proteins. Their content in various types of honey is 0.08 - 1.9%. There is an assumption that MHP, entering the body, interact with the human regulatory proteins. It also has been proved that peptides of plants and animals are similar in their structure and functions to defenses and alarmins and are capable of activating body immune responses [31-36]. Particularly, some mountain honey peptides are similar to the antimicrobial peptide molecule element of the cathelicidin LL-37 and other regulatory peptides by their molecular structure and mass. The similarity in structure and molecular weight with human regulatory peptides opens the boundless potential for exogenous peptides to be used in medical practice [37-39]. Having the similar to cathelicidin LL-37 molecular weight, the MHP are able to act as its activator and, therefore, induces inactive T-lymphocytes to counteract pathogens effectively.

It is known that under conditions of the secondary local immunodeficiency, mutated cells are able to avoid proper immune surveillance and even with the onset of genotype radical changes, they maintain a structure identical to healthy cells [40-42]. Thus, it is very difficult, and sometimes even impossible for T-lymphocytes to carry out their main task of recognition danger. Being developed through surrounding tissues, malignancies make maximum to survive and keep their Toll-like receptors intact, that is, inactive. It is believed that by implementing the tissue accumulation effect, exogenous peptides can bind to cancerous cell intact receptors and disclose it. T-lymphocytes are able to identify a dangerous cell and begin work on its destruction, as well as licensing macrophages to capture and evacuate toxic residues [32,33,43-45]. It can be assumed that due to its evolutionarily programmed ability to react with other protein structures, the Camelyn Bio exogenous peptides join tumor cell membranes and thus provoke their more productive interactions with T-lymphocytes, NK-cells and macrophages. Macrophages synthesize TNF-α and license more macrophages and T cells to counteract the tumor, the intensity of antitumor interleukin-12 expression increases. This, in its turn, leads to increasing of the tumor immune surveillance **(Figure 1)**.

Objective

To study the effects of exogenous peptides on the clinical progression of breast cancer, using as a criterion disease-free

survival within 24 months.

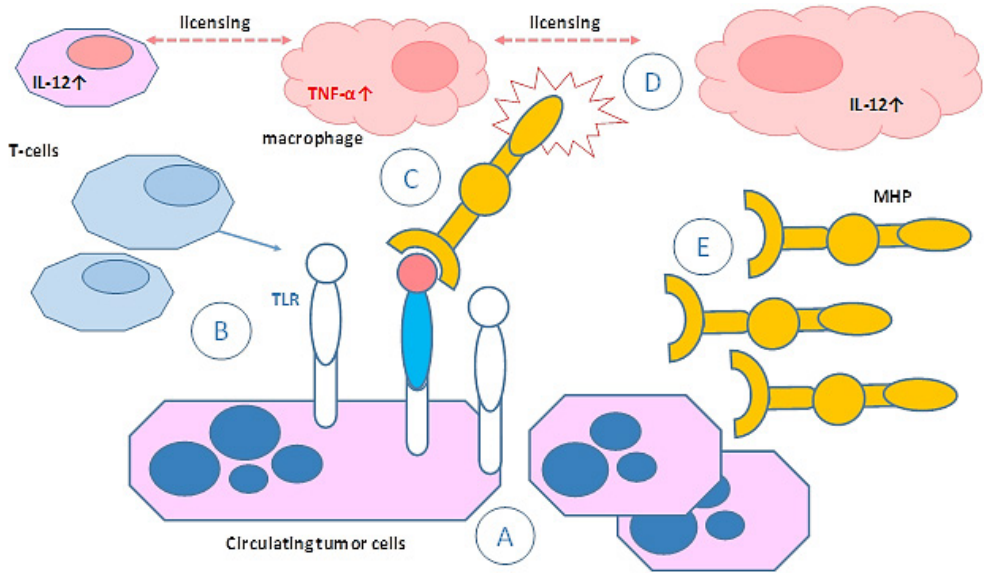

Figure 1. Tissue accumulation effect of exogenous peptides. A) Circulating tumor cells are trying to create a metastatic implant. **B)** Using inactive Toll-like receptors (TLRs), cancer cells avoid immune surveillance from T-lymphocytes. **C**) Exogenous peptides (MHP) attach to the tumor cell membrane structures and provoke its interaction with the macrophage; **D**) The macrophage synthesizes TNF-α and licenses other macrophages and T-lymphocytes to destroy the tumor cell, including the synthesis of interleukin-12 in them; **E)** Exogenous peptides interfere with the adhesion of circulating cells in a new location and prevent the formation of a metastatic lesion.

MATERIALS AND METHODS

During 2015- 2016 in the National Research Center for Radiation Medicine of NAMS of Ukraine at the clinic of radiation-induced oncological diseases 76 patients had subdermal radical mastectomy with preserving skin and nipple-areola complex on the stage II A (T1N1M0, T2N0M0). The criteria for patients selections were: node form of a tumor; mono- and multicentric growth; absence of tumor affection of the skin and nipple-areola complex, breast muscles; non-central tumor location; the distance between a tumor to the edge of the areola is not less than 4 cm; the size of the tumor is from 1,5 to 5 cm; slow and moderate rates of the tumor growth; absence of conglomerated metastases in regional lymph nodes; absence of distant metastases and serious related diseases.

After the surgical treatment the adjuvant chemotherapy was prescribed to the female patients and in accordance with the prescription, it was combined with hormonal therapy, following the FAC or CMF + anastrozole schemes. To estimate the role of exogenous peptides in strengthening the immune control over the tumor process, the patients were divided into 2 groups: in the Main group (n=38), besides prescribing the approved therapy protocol, the preparation Camelyn Bio was recommended for the role of the immune homeostasis harmonizer (1 capsule 3 times a day beginning from the 8th day after the surgery and for the next 20 days, after that 1 week of break, afterwards a refresher course); in the reference group (n=38) the female patients received only antitumoral polychemotherapy and anastrozole. The basic comparative characteristic of the groups is given in **Table 1**.

Table 1. Baseline characteristics of the study participants.

Characteristics	Main group (n=38)	Reference group (n=38)	p
Mean age (SD)	52.7 (10.9)	54.1 (9.9)	0.556[1]
BMI (SD)	27.7 (4.5)	26.2 (4.2)	0.141[1]
Parental status. Having children (yes) n (%)	32 (84.2)	31 (81.6)	0.761[2]
Hormone therapy (yes) n (%)	28 (73.7)	30 (79.0)	0.589[1]

Note: Abbreviations: SD - standard deviation; BMI - body mass index; Baseline characteristics did not differ significantly between the 2 groups (p>0.05), p1 – t-test; p2– Pearson's chi-squared test

The statistical analysis was based on the use of simple descriptive statistic and comparative analysis. The criterion chi-square (Pearson) was used to compare qualitative parameters of the groups under study. A preliminary assessment of distribution normality was made for quantitative parameter, that was the base for the choice of parametric (t-test) or non-parametric criteria (Wilcox on rank-sum (Mann-Whitney test). Kaplan-Meier method was used for analysis of recurrence-free survival. The analysis

was carried out by using statistical analysis STATA 12.

As the main criteria of adequate setting of antitumor immune response the following issues were taken into consideration: a level of expressiveness of tumor necrosis factor alpha (TNF-α), a level of expressiveness of interleukin-12 (IL-12), a number of activated T-lymphocytes (cells/μl) at the beginning of the late postsurgical period (the 10th day after a surgery) it is point 0, in 2 weeks (point 1), in 3 weeks (point 2), in 8 weeks (point 3), in 10 weeks (point 4) and in 12 weeks (point 5). The duration of relapse-free survival period (RFS) of the disease was also analyzed during the whole observation period (24 months).

RESULTS AND DISCUSSION

During the observation, the growth of average numbers of expressiveness level of TNF-α from monitoring point 1 to point 2 was noticed in both groups of female patients, which corresponds to the second and third weeks of the postsurgical period. In the Reference group this number was gradually decreasing later, while in the Main one it had decreased by the 8th week and it remained unchangeable till the 10th week and by the 12th week, it grew to 2.1 pg/ml **(Figure 2)**.

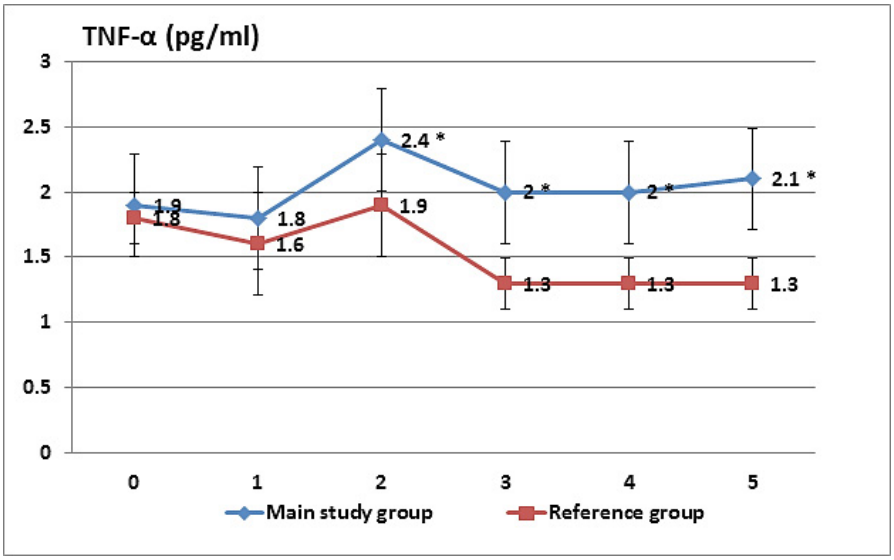

Figure 2. Dynamics of mean values of expression level TNF-α in patients of the Main study group compared to the reference group; mean (95% CI); (*- p<0.05; Mann-Whitney test).

Both in the Main and in the Reference group there was a gradual increase of an average number of interleukin expressiveness level -12 from point 0 to point 3 of the monitoring, which corresponds to the first and eighth observation weeks. Unlike the Reference group, in the Main group, the average number of expressiveness IL-12 remained almost on the same level (94.2 to 93.5 pg/ml) during 10th and 12th weeks, while in the Reference group it decreased from 72.7 to 54.8 pg/ml **(Figure 3)**.

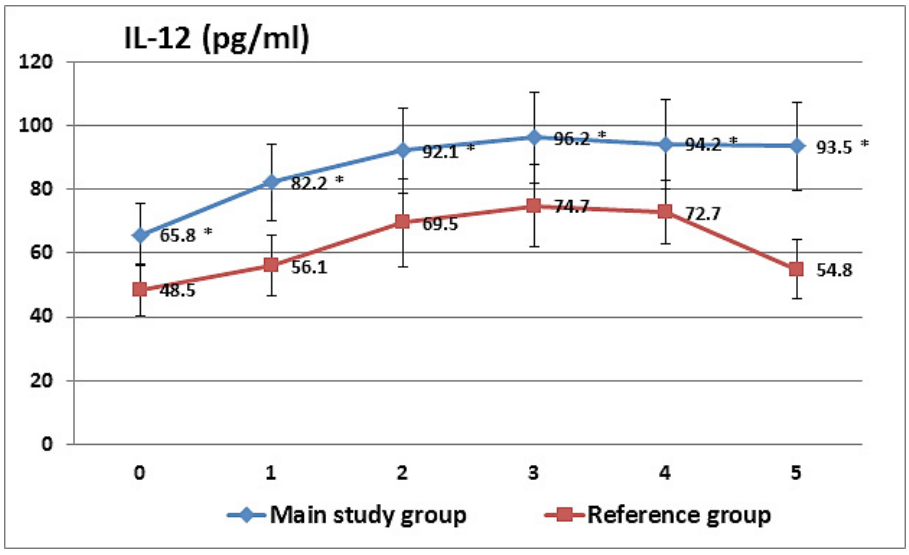

Figure 3. Dynamics of mean values of expression level IL-12 in patients of the Main study group compared to the reference group; mean (95% CI); (*- p<0.05; Mann-Whitney test).

This dynamics of TNF-α and IL-12 expressiveness in the group under study is understandable if to pay attention to the number of activated T- lymphocytes of $CD3^+/CD25^+$ subpopulations that female patients had. In contrast to the Reference group, the number of activated T- lymphocytes increased from the first to the eighth week of observation: on average from 98 to 203 cells/μl (Δ=105, $p<0.05$) among the patients who had the BC management scheme with included Camelyn Bio. At the same time, this rate in the Reference group increased to 171 cells/μl on average during the second week of monitoring and its gradual decrease from 167 to 122 cells/μl was recorded **(Figure 4)** at the subsequent monitoring points.

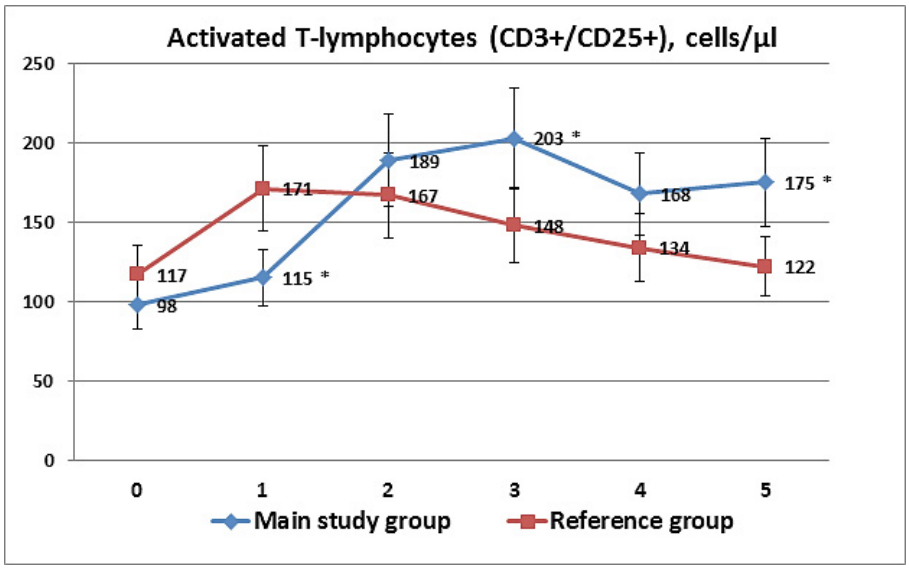

Figure 4. Dynamics of mean values of the number of activated T-lymphocytes in patients of the Main study group compared with the reference group; mean (95% CI); (*- p<0,05; Mann-Whitney test).

It is becoming clear that the harmonization of immune homeostasis with the help of exogenous peptides (Camelyn Bio) provides a more productive interaction of immunocompetent cells with circulating tumor cells. Obviously, this leads to the activation of interleukin-12 and TNF-α synthesis by means of macrophages and T-lymphocytes, and also contributes to additional recruitment of T cells. The experience of our observations shows that the immune homeostasis harmonization by means of exogenous peptides contributes to the improvement of patients' disease-free survival. During the 24 months of the observation period in the Main group (Camelyn Bio), no relapses or metastases were recorded among 35 (92.1%) female patients. In the Reference group, a relapse-free course of disease was noted among 29 (76.3%) of patients **(Figure 5)**. One patient in the Main group had a tumor relapse, and two patients had metastasis of the tumor in the contra-lateral mammary gland. In the Reference group, relapses were detected in 3 patients, 6 patients had metastases in different organs. The analysis of Kaplan-Meier relapses free survival lines is shown in **Figure 6**.

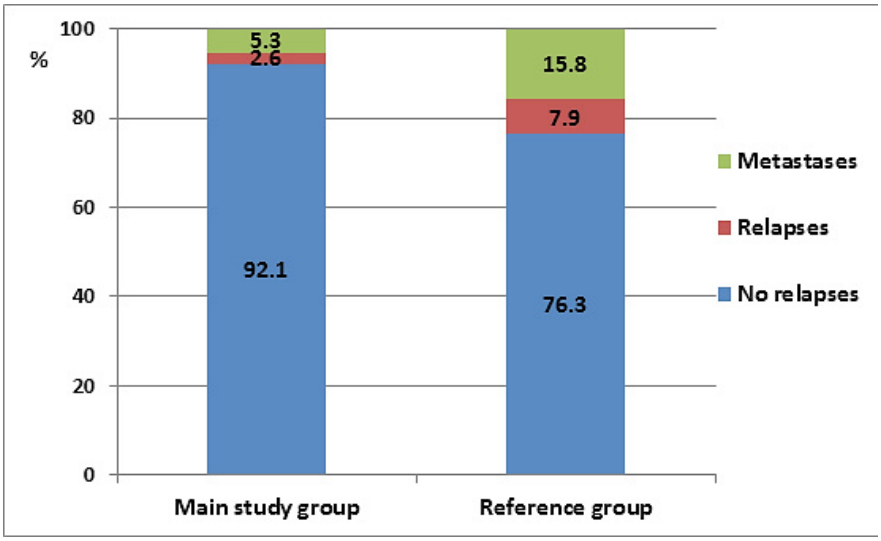

Figure 5. Distribution of patients according to the results of observation (24 months) in the study groups (%).

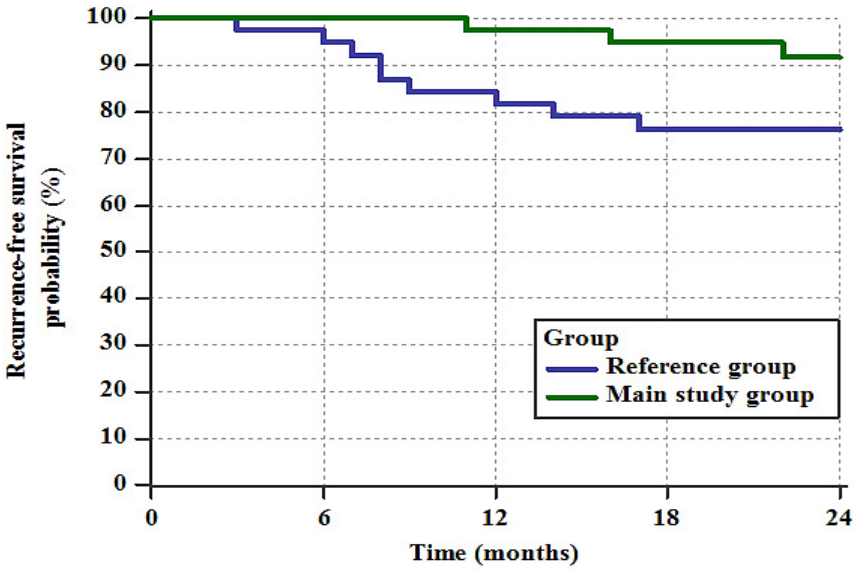

Figure 6. The curves of recurrence free survival of patients with breast cancer in the main study group (Camelyn Bio) and the reference group (Kaplan-Meier curves analysis).

The analysis of survival lines showed that the 24-month recurrence-free survival of the patients in the Main group was 91,8% (95% CI: 84,7-98,9%), and in the Reference group– 76,3 (95% CI: 62,8-89,8%). Thus, during the 24 months of observation of the group of patients taking Camelyn Bio, the significant increase in non-recurrent survival and a reduction in the risk of tumor and metastases recurrence was noticed during the indicated period by71.8% - Hazard ratio (95% CI)=0,292 (0,094 – 0,907); p=0,046. All patients in the study groups have a 24-month observation period, but observations are ongoing.

CONCLUSION

It should be noted that primary reconstructive-plastic surgery is necessary for patients having breast cancer not only as a part of a therapeutic strategy but also as an opportunity to return to a fulfilling life. Complex treatment with the use of adjuvant chemotherapy allows achieving optimal results. Practice confirms that it is also very important to restore immune homeostasis of female patients in order to ensure the most effective immune control as a continual process of the immune-competent cells counteracting the spread of the disease. The obtained clinical results indicate that the harmonization of immune homeostasis by integrating Camelyn Bio into the breast tumor management scheme creates conditions for increasing the treatment efficiency of the disease. In the group of patients receiving Camelyn Bio, during the 24 months of observation a reduction in the risk of recurrence and metastases during the indicated period was noted at 71.8%. It should be emphasized that the study of immunological effects of Camelyn Bio should be continued in other studies, because the drug demonstrates the promising potential of application in clinical oncology.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

REFERENCES

1. Beije N, et al. Circulating tumor cell enumeration by the CellSearch system: the clinician's guide to breast cancer treatment? Cancer Treat Rev. 2015;41;144-150.
2. Keller PJ, et al. Defining the cellular precursors to human breast cancer. Proc Natl Acad Sci USA. 2012;109(8):2772-2777.
3. Wang D, et al. Molecular markers' progress of breast cancer treatment efficacy. J Cancer Res Ther. 2015;11:C11-15.
4. Yu F, et al. Let-7 regulates self-renewal and tumorigenicity of breast cancer cells. Cell. 2007;131:1109-1123.
5. Zhang M, et al. Selective targeting of radiation-resistant tumor-initiating cells. Proc Natl Acad Sci USA. 2010;107:3522-3527.
6. Baum M, et al. The ATAC Trialists Group: Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early stage breast cancer. Results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. Cancer. 2003;98:1802-1810.
7. Bian] L, et al. Prediction value for dynamic changes of circulating tumor cell in therapeutic response and prognosis of Chi-

nese metastatic breast cancer patients. Zhonghua Yi Xue Za Zhi. 2014;94:265-268.

8. Kasimir BS, et al. Does primary neo adjuvant systemic therapy eradicate minimal residual disease? Analysis of disseminated and circulating tumor cells before and after therapy. Breast Cancer Res. 2016;18:20.
9. Sørlie T, et al. Gene expression patterns of breast carcinomas distinguishes tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869-10874.
10. Ursaru M, et al. Causes of death in patients with Stage 0-Ii breast cancer. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 2015;119:374-378.
11. Saloman DS, et al. The EGF-CFC family: novel epidermal growth factor-related proteins in development and cancer. Endocr Relat Cancer. 2000;7(4):199-226.
12. Shackleton M, et al. Generation of a functional mammary gland from a single stem cell. Nature. 2006;439:84–88.
13. Stingl J, et al. Deciphering the mammary epithelial cell hierarchy. Cell Cycle. 2006;5:1519-1522.
14. Stefani G and Slack FJ. Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol. 2008;9(3):219-230.
15. Strizzi L, et al. Epithelial mesenchymal transition is a characteristic of hyperplasias and tumors in mammary gland from MMTV-Cripto-1 transgenic mice. J Cell Physiol. 2004;201(2):266-276.
16. Sun Y, et al. Overexpression of human Cripto-1 in transgenic mice delays mammary gland development and differentiation and induces mammary tumorigenesis. Am J Pathol. 2005;167(2):585-597.
17. Ansieau S, et al. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. Cancer Cell. 2008;14:79-89.
18. Batlle E, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumor cells. Nat Cell Biol. 2000;2(2):84-89.
19. Satoh K, et al. Progesterone enhances branching morphogenesis in the mouse mammary gland by increased expression of Msx2. Oncogene. 2007;26(54):7526-7534.
20. Satoh K, et al. Up-regulation of MSX2 enhances the malignant phenotype and is associated with twist 1 expression in human pancreatic cancer cells. Am J Pathol. 2008;172(4):926-939.
21. Stingl J, et al. Phenotypic and functional characterization in vitro of a multipotent epithelial cell present in the normal adult human breast. Differentiation. 1998;63(4):201-213.
22. Storci G, et al. The basal-like breast carcinoma phenotype is regulated by SLUG gene expression. J Pathol. 2008;214:25-37.
23. Yang MH and Wu KJ. TWIST activation by hypoxia inducible factor-1 (HIF-1): implications in metastasis and development. Cell Cycle. 2008;7(14):2090-2096.
24. Yook JI, et al. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nat Cell Biol. 2006;8(12):1398-1406.
25. Magbanua MJ, et al. Circulating tumor cell analysis in metastatic triple-negative breast cancers. Clin Cancer Res. 2015;21:1098-1105.
26. Masuda T, et al. Clinical and biological significance of circulating tumor cells in cancer. Mol Oncol. 2016;10:408-417.
27. A Schramm, et al. Prevalence of circulating tumor cells after adjuvant chemotherapy with or without anthracyclines in patients with HER2-negative, hormone receptor-positive early breast cancer. Clin Breast Cancer. 2017.
28. Turker I, et al. Detection of circulating tumor cells in breast cancer patients: prognostic predictive role. Asian Pac J Cancer Prev. 2013;14(3):1601-1607.
29. Mocellin S, et al. Circulating tumor cells: the 'leukemic phase' of solid cancers. Trends Mol Med. 2006;12;130-139.
30. Ring IE and Smith M. Circulating tumour cells in breast cancer, Lancet Oncol. 2004;5:79-88.
31. Elssner A, et al. A Novel P2X7 Receptor Activator, the Human Cathelicidin-Derived Peptide LL37, Induces IL-1 Beta Processing and Release. J Immunol. 2004;172(8):4987-4994.
32. Kurchenko AI, et al. The role of exogenous peptides in the renewal of a full immune reaction in the conditions of the second immunodeficiency. Women's health. 2017;1(117):89-97.
33. Lytvynenko OO and Tatskyi OF. Effective prophylaxis of breast cancer recurrence: the role of exogenous peptides MHP in the restoration of the immune homeostasis. Allergol Infectol. 2017;3(100):51-57.

34. Rico MR, et al. Effect of antimicrobial peptides derived from human cathelicidin LL-37 on Entamoeba histolytica trophozoites. Exp Parasitol. 2013;133:300-306.

35. Wu WK, et al. Emerging roles of the host defense peptide LL-37 in human cancer and its potential therapeutic applications. Int J Cancer. 2010;127:1741-1747.

36. Zanetti M. The role of cathelicidins in the innate host defenses of mammals. Curr Issues Mol Biol. 2005;7:179-196.

37. Putsep K, et al. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. Lancet. 2002;360:1144-1149.

38. Ahmed S and Othman NH. Honey as a Potential Natural Anticancer Agent: A Review of Its Mechanisms. Evid Based Complement Alternat Med. 2013;2013:829070.

39. Hayashi T, et al. Immunological characterization of honey proteins and identification of MRJP 1 as an IgE-binding protein. Biosci Biotechnol Biochem. 2011;75(3):556-560.

40. De Marzo AM, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007;7(4):256–269.

41. Sutcliffe S, et al. Trichomonosis, a common curable STI, and prostate carcinogenesis--a proposed molecular mechanism. PLoS Pathogens. 2012;8(8):e1002801.

42. Sutcliffe S and Platz EA. Inflammation and prostate cancer: a focus on infections. Curr Urol Rep. 2008;9(3):243-249.

43. Melnikov SM and Tatskyi OF. A new look at endometriosis: the role of restoring of full immune surveillance over ectopic cells of the endometrium. Women's Health. 2017;7(123):99-107.

44. Ming S, et al. Application potential of toll-like receptors in cancer immunotherapy: Systematic review. Medicine (Baltimore). 2016;95:e3951.

45. Zaseda YI and Tatskyi OF. The effect of tissue accumulation of exogenous peptides is the key to understanding the mechanisms of harmonization of the immune homeostasis of patients with chronic bacterial prostatitis. Health of the Man. 2017;1(60):51-56.