Ref: Ro Med J. 2022;69(2)
DOI: 10.37897/RMJ.2022.2.4

Impact of immunotherapy with pseudomonas serotip xv etanolic extract (Cantastim) on local recurrence and survival at 3 and 5 years in operated rectal cancer

Daniela Aurora Pesu[1], Eugenia Claudia Zarnescu[1,2], Narcis Octavian Zarnescu[1,2], Nicoleta Aurelia Sanda[1,2], Stejara Nicoleta Mihai[1], Aurora Salageanu[5], Ştefan Ilie Neagu[3,4]

[1] Bucharest Emergency University Hospital, General Surgery Clinic II, Bucharest, Romania
[2] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[3] Academy of Medical Sciences, Bucharest, Romania
[4] Academy of Romanian Scientists, Bucharest, Romania
[5] Research and Development Center, I.N.C.D.M.M. "Cantacuzino", Bucharest, Romania

ABSTRACT

Although most recurrences (approximately 80%) occur in the first three years after a curative resection, a recurrence of CRC can occur even ten years after the initial curative resection (dormant spread of cancer cells). Immunotherapy is an emerging therapy with high potential. The immune system plays a major role in the development of CRC. This has led to innovative new therapies, such as cancer vaccines and T-cell stimulation therapies. Cantastim belongs to the class of non-specific immunostimulators or immunomodulators, most of which are of bacterial origin and are used as mono- or polymicrobial suspensions. Cantastim is an ethanolic extract obtained from a pathogenic strain of Pseudomonas aeruginosa serotype XV. The beneficial effect of immunotherapy with Cantastim was more pronounced for the local developmental stages (I and II) than for the later stages.

Keywords: rectal cancer, cantastim, local recurrence, immunotherapy

BACKGROUND AND OBJECTIVES

Colorectal cancer ranks 3rd as a prevalence in the list of cancers worldwide [1] being responsible for 8% of all cancer deaths. Early diagnosis of colorectal cancer is the most important factor today in reducing mortality, survival rate to 5 years being 96% for stage I and 5% for stage IV [2].

The great advances made in the field of tumor cytology and genetics have led to the identification of oncological markers and the administration of targeted therapies, individualized according to the identified genetic mutations.

Three molecular subtypes have been identified in sporadic rectal cancer: microsatellite instability (MSI), chromosomal instability (CIN), and island methylator phenotype (CIMP-CpG). Microsatellites are short, repetitive sequences of distributed nucleotides along the AND chain. Approximately 15% of rectal cancers sporadically have MSI and therefore genetic hypermutability. MSI is thus a negative prognostic factor for sensitivity to SFU-based chemotherapy. On the other hand, MSS (microsatellite stability) has a significant beneficial effect on SFU monotherapy compared to MSI [3] .

A high MSI would recommend immunotherapy with monoclonal needles Nivolumab and Ipilimumab, antibodies that stimulate antitumor T lymphocytes. A recent study (Check-Mate-142) reported a 60% response rate to monoclonal needle therapy with a 7% complete response in patients with rectal tumors with high microsatellite instability (MSI-H) [4].

Corresponding author:
Daniela Aurora Pesu
E-mail: drdanielaaurora@yahoo.com

Article History:
Received: 7 June 2022
Accepted: 27 June 2022

Another useful parameter for individualizing immunotherapy is Ras mutations (Kras and Nras) present in 30-40% of rectal cancers and which gives them resistance to anti-EGFR immunotherapy, which is only indicated in metastatic rectal cancer of patients with wild-type Ras gene [5].

Local recurrence after operated rectal cancer is a particularly important aspect requiring periodic postoperative loco-regional surveillance by clinical examination and complex imaging examinations, MRI and PET-CT, if MRI indicates suspicion of recurrence. Periodic checks at 3 months in the first 2 years postoperatively, 6 months from 2 to 5 years and annually after 5 years can detect the occurrence of local recurrence making it possible to detect it, sometimes with chances of cure[6].

The current treatment of rectal cancer remains strictly surgical, which for the local and loco-regional stage of evolution is the only one able to achieve the healing of the patient, along with other therapeutic methods: radiotherapy, chemotherapy and immunotherapy. age and biological status. Thus the sequence can be: *preoperative radiotherapy and chemotherapy (neoadjuvant) + surgery + postoperative chemotherapy + immunotherapy* or it can be *surgery + postoperative radiotherapy and chemotherapy + immunotherapy* or *surgery + postoperative chemotherapy (adjuvant) + immunotherapy or postoperative surgery + surgery* [7].

Immunotherapy as a combination of other methods of treating rectal cancer may be specific and nonspecific. In non-specific immunotherapy, preparations are used that combine vitamins (C, B6, E, D3) with microelements (Zn, Se, Fe, Ca, Mg), herbal preparations (white mistletoe, aloe, marigold, ginseng, rosehips, echinacea, mate) and propolis[2].

More recently specific immunotherapy uses microbial preparations to stimulate / modulate the immune system (Polidin polymicrobial vaccine, Corynebacterium parvum for ovarian cancer, BCG for bladder cancer) and tumor autovaccine and the latest tumor messenger RNA vaccine for T lymphocyte transformation in NK (natural killer) with a role in the destruction of malignant cells. The latest method of immunotherapy of malignant tumors uses monoclonal antibodies, produced in the laboratory and specifically coupled with antigens from the surface of malignant cells, in various cancers (Transtuzumab for HER2-positive tumors of the breast, stomach, esophagus), Bevacizumab for cancer with receptors for vascular endothelial growth factor, VEGF, cervical, colorectal and ovarian cancer, Rituximab for non-Hodgkin's lymphoma , Cetuximab, which blocks the receptor for epidermal growth factor , EGFR on malignant cells and colorectal cancer and those in the head and neck region[7].

More recently, a number of studies have sought to determine the factors that influence the response to immunotherapy so that the agent or the maximum potential effect on the tumor process can be selected, with individualized oncoimmunological therapy[8].

Cantastim belongs to the class of non-specific immunostimulators or immunomodulators, most of which are of bacterial origin and are used in the form of mono- or polymicrobial suspensions. Cantastim is an ethanolic extract obtained from a pathogenic strain of Pseudomonas aeruginosa serotype XV. Experimental studies have found that it has a mitogenic effect in vitro for mouse lymphocytes, but not for guinea pig or human lymphocytes, being heat-resistant, non-immunogenic and non-allergenic and activating the cytostatic functions of macrophages. Biochemically, it is composed of over 80% phospholipids and a low percentage of protein (5-7%) and carbohydrates (3-4%). Synthesis of Ig M and Ig G antibodies has also been shown experimentally to delay the development of Ehrlich's ascitogenic tumor and protect animals from severe infections[9].

The biological activity of Cantastim is due to its phospholipid component, which is demonstrated by the loss of biological properties through the selective degradation of the lipid portion by phospholipase C[10]. It is also an activator of macrophage secretory and phagocytic functions and proliferative lymphocyte response.

MATERIALS AND METHODS

In the period 2003-2006, in 45 patients operated for rectal cancer, we administered postoperatively the treatment with the immunomodulator Cantastim, produced by the Cantacuzino Institute, in terms of local recurrence.

RESULTS

We reported the results obtained in a similar sample in terms of biological (sex, age) and pathological (grading, tumor stage) parameters from 2006-2008 to objectify the effect of immunotherapy on results in terms of local recurrence and survival at 3 and 5 years (Table I). Cantastim was administered starting with the 15th day postoperatively in subcutaneous injections 1 ampoule / week for 10 weeks. We did not noticed any major side effects, we rarely noticed a local inflammatory response, limited to the first administrations and remitted in a few days.

In the group of patients treated with Cantastim (A) there were 26 men and 19 women, the average age being 65.5 years, in the group without treatment (B) there were 25 men and 20 women, the average age being 67 years (Table I)

The staging of the cases corroborated with the presence of recurrence for group A showed for 8

Table 1. Results ,stage ,survival of recurrence

Cantastim treatment	n=45	
Women	n=19	
Men	n-26	
Average age	65,5 years old	
With recurrence	n=9	20%
Stage I	n=8	
Stage II	n=13	14%
Stage III	n=20	
Stage IV	n=4	25%
3 years survival	n=23	49%
5 years survival	n=12	26%

A

No Cantastim treatment	n=45	
Women	n=20	
Men	n=25	
Average age	67 years old	
Cu Recidiva	n=14	31%
Stage I	n=12	
Stage I	n=13	28%
Stage III	n=13	
Stage IV	n=19	35%
3 years survival	n=19	42%
5 years survival	n=9	20%

B

cases in stage I no recurrence, for 13 cases in stage II 3 recurrences, for 20 cases in stage III 5 recurrences and 4 cases in stage IV a single recurrence, in total 9 recurrences.

For group B, in stage I there were 12 cases with 2 relapses, in stage II there were 13 cases with 5 relapses, in stage III there were 13 cases with 5 relapses, and in stage IV there were 7 cases with 2 relapses, total 14 recurrences.

However, if we group stage I with stage II and stages III with IV and analyze the occurrence of local recurrence, we find a significant reduction in the use of Cantastim for the local stages of disease (I and II) in which the local recurrence rate was 14, 35%, compared to 28% as it was in stages I and II for the cases in which the immunomodulator was not administered. (Fig 2)

The local recurrence rate in the local evolutionary stages was 2 times higher for the group without immunotherapy. (P = 0.027)

For the stages of advanced local evolution, III and IV, the difference between the rates of local recurrence was maintained, being 25% for group A and 35% for group B, but not so important from a statistical point of view (p = 0.03) .(fig 3)

For all cumulative stages, the local recurrence rate was 20% (9 cases out of 45) for group A and 31.1% (14 cases out of 45) for group B, the decrease in the incidence of recurrence being 35.72% statistically insignificant (p = 1.000)

The analysis of the time interval until the recurrence shows that it was higher for the group treated with immunomodulator, compared to the group without treatment, the average being 32.2 months and 20.2 months respectively, all stages cumulated, the difference being statistically insignificant (p = 0.227).

In the first 2 years, recurrence occurred in 5 out of 9 cases (55.5%) in group A and in 11 out of 14 cases in group B (78.5%).

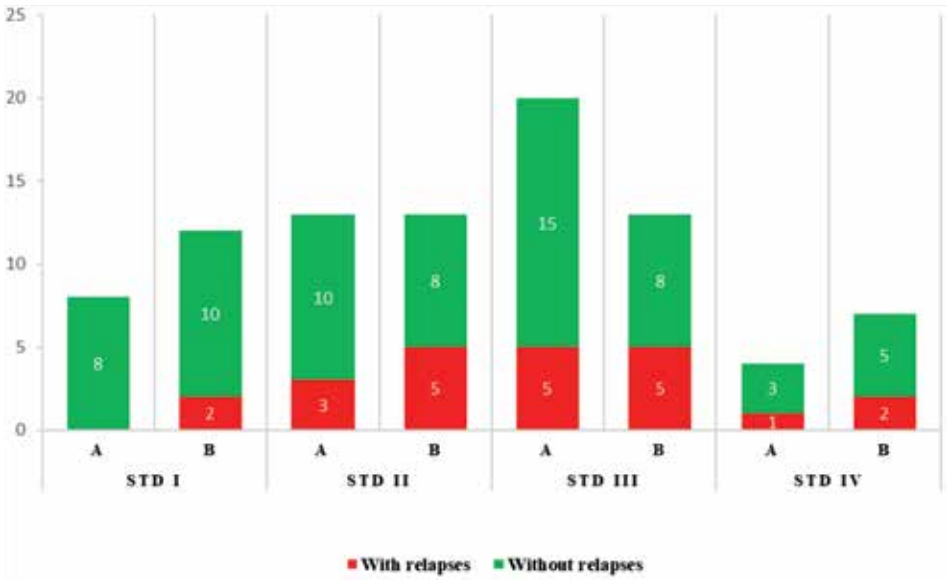

FIGURE 1. Distribution of cases according to stage

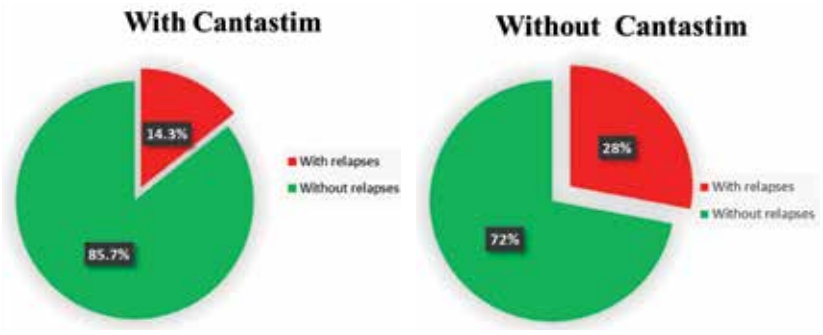

FIGURE 2. The rate of local recurrence in the incipient stages of the disease

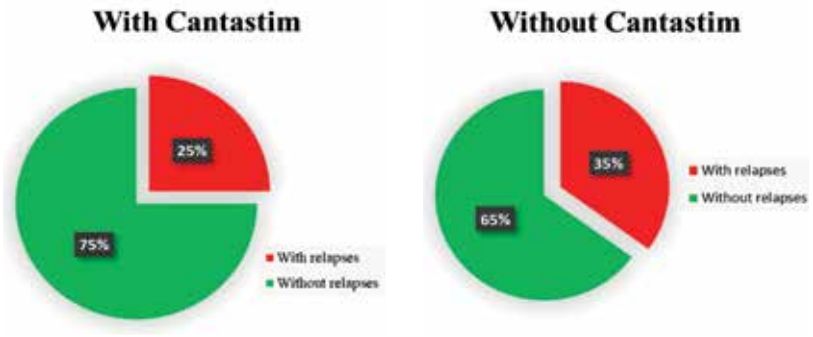

FIGURE 3. The rate of local recurrence in the advanced stages of the disease

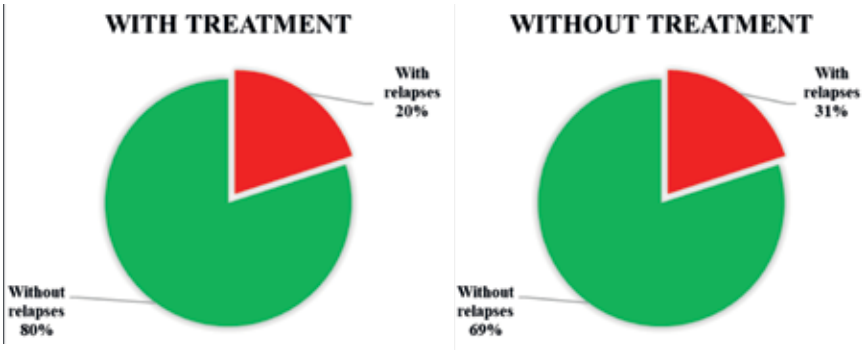

FIGURE 4. Incidence of local recurrence in the group treated with Cantastim and in the group without Cantastim

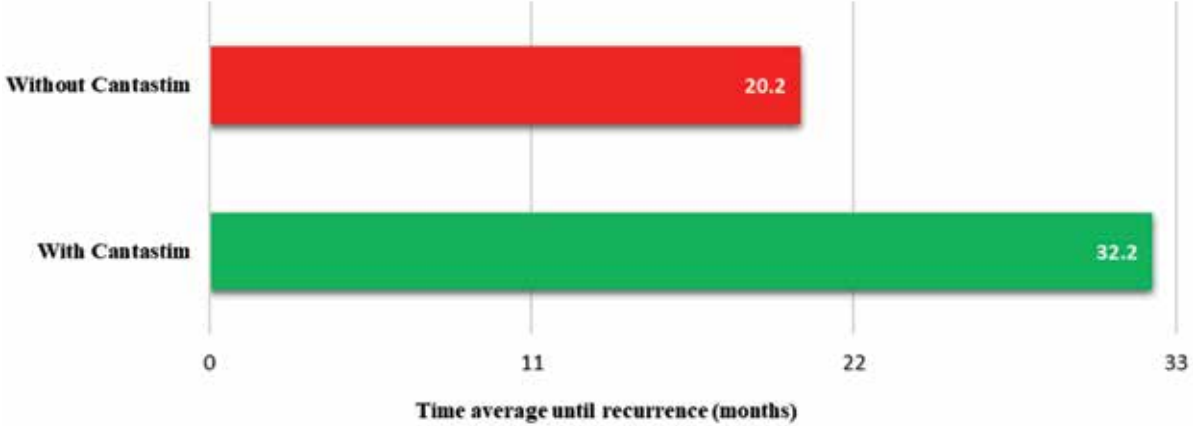

FIGURE 5. The free recurrence interval for the two studied groups

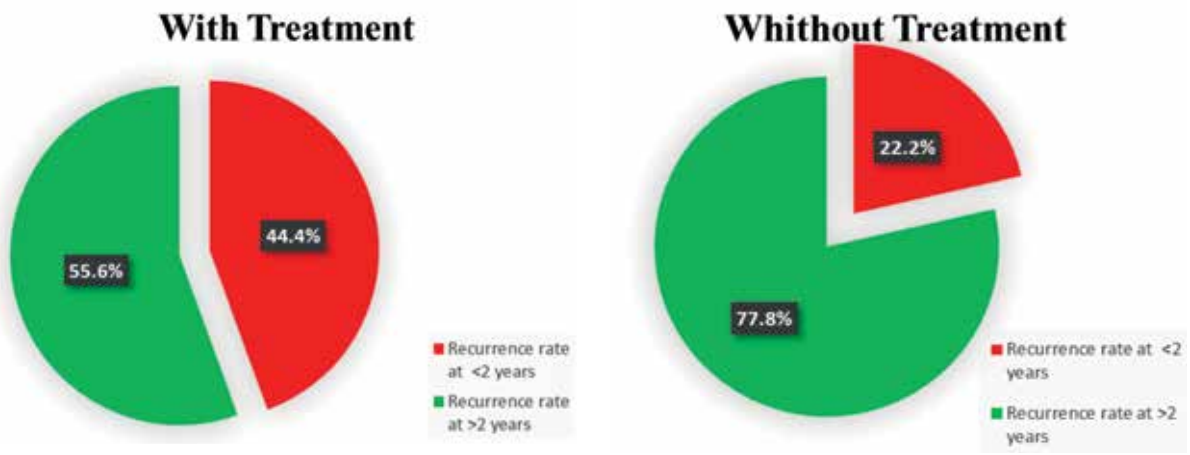

FIGURE 6. The rate of local recurrence occurred over 2 years

The local recurrence rate at more than 2 years was 44.4% for group A (4 out of 9 cases) and 22.2% in group B (3 out of 14 cases), the difference being important, the small number of cases not allowing but statistical assessments (fig. 6) (p = 0.227)

It can be seen the prolongation of the time when local recurrence occurs in the case of using the immunomodulator, knowing that 80% of recurrences occur in the first 2 years of postoperative evolution.

Analyzing the survival of patients at 3 and 5 years, it is found a correlation with the time at which the local recurrence occurred in the two groups. Thus, the 3-year survival was 49% (23 cases) for group A and 42% (19 cases) for group B. The 5-year survival was 26% (12 cases) for group A and 20% (9 cases) for group B.

Conclusion

Immunity is an important factor in the genesis and evolution of malignant processes. Immunotherapy in malignant tumors occupies an important place in complex oncological treatment, along with surgery, chemotherapy and radiation therapy.

If at first it was used in the form of poly or monomicrobial vaccines (Polidin, Cornybacterium parvum, BCG) lately, specific immunotherapy, which uses monoclonal antibodies, is gaining ground[11].

Most recently, LAG-3 antagonists (lymphocyte-activating gene 3) are being evaluated, and encouraging results are obtained by combining them with anti-PD-1[12].

PD-1 or CD 279 (programmed cell death protein 1 or cluster of differentiation 279) is a protein in the surface structure of activated T lymphocytes, which acts as a receptor for PD-L1 and PD-L2 ligands present on the surface of tumor cells, inhibiting this binding. immune process.

Lymphocyte activating gene 3 (LAG-3) is a cell surface inhibitory receptor and a regulator of immune homeostasis with multiple biological activities related to T cell functions[13].

LAG -3 is considered the new generation of immune control factors after PD-1 and CTLA -4, being the third inhibitory receptor used in anticancer immunotherapy. Several immunotherapies with LAG-3 antagonists are being evaluated at various preclinical and clinical stages. Encouraging results were obtained by combining LAG-3 blockade with PD-1[3,12].

In 1989, A. Olinescu et al demonstrated experimentally on mice the stimulating effect of the cellular and humoral mediated immune response of a microbial derivative derived from Pseudomonas aeruginosa, called Cantastim[9]. Regarding the clinical use of Cantastim, normalization of immune function was achieved in a case of chronic lymphocytic leukemia in 1988 [9], and subsequently encouraging results of T and NK (natural killer) cell stimulation were reported in 11 cases of SCLC (small cell long cancer) and breast cancer[10]. Increased TNF-alpha production was observed in all patients. Also, the lymphocyte expression detected by flow-cytometry was more pronounced in the case of patients compared to the control group.

Solid tumors are frequently infiltrated by T cells, B cells, natural killers, mast cells and macrophages. Although epidemiological studies have shown the active involvement of the inflammatory process in tumor progression, cases have been described in which the effect is inclined towards anti-tumor action. According to Klampfer L., stimulation of T cells with effect on IFNγ in patients with colon cancer is associated with the absence of metastases and better survival [11].

Malignant tumors are frequently infiltrated by T and B lymphocytes, NK, mast cells and macrophages, with an appreciation of the degree of infiltration that provides an important prognostic factor for tumor aggression. The more important the inflammatory infiltrate, the lower the aggressiveness of the tumor, the longer is the 5-year survival and the low-

er the potential for metastasis is [12,13] .By stimulating inflammatory infiltration, the immunomodulator Cantastim would reduce the decrease in tumor aggression with a favorable effect on survival and metastasis. peritumoral inflammation contributing in some situations to tumor progression. TAM is a key component of the tumor response stroma. In most (but not all) cases, their presence is associated with a very poor prognosis. Macrophages release cytokines and growth factors for tumor and endothelial cells and degrade the extracellular matrix[14]. This continuous remodeling of the stroma promotes the release of matrix-bound growth factors and triggers the processes of motility and invasion. Macrophages produce structural proteins such as osteopontin, osteoactinin, collagen and fibronetics, but also proteolytic enzymes: metalloproteinases, cathepsins, lysosomal proteases and plasminogenurokinase-type. These structures contribute to the formation and maintenance of an inflammatory peritumor atmosphere that could eventually promote tumor progression[9].

In our study, comparing the results obtained on the two groups of patients operated on for rectal cancer, one group being treated with Cantastim and one control group, without treatment we found a favorable effect in the case of immunomodulatory treatment on local recurrence after colorectal cancer. This occurred to a lesser extent, and when it did occur, it developed over a longer period of time. The beneficial action of Cantastim immunotherapy was more pronounced in the local developmental stages (I and II) than in the later stages.

The use of the Cantastim immunomodulator in the postoperative treatment of rectal cancer patients has resulted in a statistically significant decrease in the rate of local recurrence.

Regarding the survival at 3 and 5 years, we found an increase in it, but the low number of cases did not allow conclusions with statistical significance.

It is necessary to continue the study in order to objectify the effect of immunotherapy with Cantastim, the postoperative treatment of rectal cancer, both in terms of local recurrence and survival at 3 and 5 years.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Prashanth Rawla ,Tagore Sunkara ,Adam Barsouk .Review paper,Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors *Gastroenterology Rev* 2019; 14 (2): 89–103 DOI: https://doi.org/10.5114/pg.2018.81072,Online publish date: 2019/01/06
2. Sanjoaquin MA, Choodari-Oskooei B, Dolbear C, Putcha V, Sehgal A, Key TJ, Møller H. Colorectal cancer incidence, mortality and survival in South-east England between 1972 and 2001. *Eur J Cancer Prev.* 2007 Feb;16(1):10-6. doi: 10.1097/01.cej.0000228398.30235.f5. PMID: 17220699.
3. Sanda, N., Ristea, R., & Neagu, Ștefan. (2019). SYNCHRONOUS AND METACHRONOUS TUMORS – A LITERATURE REVIEW. *Romanian Journal of Clinical Research*, *2*(1), 49-54. https://doi.org/10.33695/rjcr.v2i1.16
4. Lenz HJ, Van Cutsem E, Luisa Limon M, Wong KYM, Hendlisz A, Aglietta M, García-Alfonso P, Neyns B, Luppi G, Cardin DB, Dragovich T, Shah U, Abdullaev S, Gricar J, Ledeine JM, Overman MJ, Lonardi S. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. *J Clin Oncol.* 2022 Jan 10;40(2):161-170. doi: 10.1200/JCO.21.01015. Epub 2021 Oct 12. PMID: 34637336.
5. Mustachio LM, Chelariu-Raicu A, Szekvolgyi L, Roszik J. Targeting *KRAS* in Cancer: Promising Therapeutic Strategies. *Cancers* (Basel). 2021 Mar 10;13(6):1204. doi: 10.3390/cancers13061204. PMID: 33801965; PMCID: PMC7999304.
6. Purandare NC, Dua SG, Arora A, Shah S, Rangarajan V. Colorectal cancer - patterns of locoregional recurrence and distant metastases as demonstrated by FDG PET / CT. *Indian J Radiol Imaging*. 2010 Nov;20(4):284-8. doi: 10.4103/0971-3026.73545. PMID: 21423904; PMCID: PMC3056626.
7. Noguera-Ortega E, Guallar-Garrido S, Julián E. Mycobacteria-Based Vaccines as Immunotherapy for Non-urological Cancers. *Cancers* (Basel). 2020 Jul 5;12(7):1802. doi: 10.3390/cancers12071802. PMID: 32635668; PMCID: PMC7408281.
8. Olinescu A, Hristescu S, Sălăgeanu A, Manda G, Neagu M. Efectul imunostimulării nespecifice cu "Cantastim" asupra răspunsului imun mediat celular şi umoral la şoarece, apreciat prin teste in vivo şi in vitro [A nonspecific immunostimulant effect with Cantastim on the cellular and humoral immune responses in mice evaluated by in vivo and in vitro tests]. *Rev Ig Bacteriol Virusol Parazitol Epidemiol Pneumoftiziol Bacteriol Virusol Parazitol Epidemiol.* 1989 Oct-Dec;34(4):325-36. Romanian. PMID: 2641194.
9. Olinescu A, Hristescu S, Sălăgeanu A, Petrovici A, Grigoriu G. Normalizarea unor funcţii imune consecutiv terapiei cu Cantastim, într-un caz de leucemie limfatică cronică T (LLCT) [Normalization of immune function following therapy with Cantastim in a case of chronic T-cell lymphatic leukemia (CTLL)]. *Rev Ig Bacteriol Virusol Parazitol Epidemiol Pneumoftiziol Bacteriol Virusol Parazitol Epidemiol.* 1988 Jul-Sep;33(3):281-8. Romanian. PMID: 3249900.
10. Olinescu A, Hristescu S, Sălăgeanu A, Petrovici A, Grigoriu G. Normalizarea unor funcţii imune consecutiv terapiei cu Cantastim, într-un caz de leucemie limfatică cronică T (LLCT) [Normalization of immune function following therapy with Cantastim in a case of chronic T-cell lymphatic leukemia (CTLL)]. *Rev Ig Bacteriol Virusol Parazitol Epidemiol Pneumoftiziol Bacteriol Virusol Parazitol Epidemiol.* 1988 Jul-Sep;33(3):281-8. Romanian. PMID: 3249900.
11. Klampfer L. Cytokines, inflammation and colon cancer. *Curr Cancer Drug Targets.* 2011 May;11(4):451-64. doi: 10.2174/156800911795538066. PMID: 21247378; PMCID: PMC3540985.
12. Chocarro L, Blanco E, Zuazo M, Arasanz H, Bocanegra A, Fernández-Rubio L, Morente P, Fernández-Hinojal G, Echaide M, Garnica M, Ramos P, Vera R, Kochan G, Escors D. Understanding LAG-3 Signaling. *Int J Mol Sci.* 2021 May 17;22(10):5282. doi: 10.3390/ijms22105282. PMID: 34067904; PMCID: PMC8156499.
13. L.M. Roelofsen, P. Kaptein, D.S. Thommen, Multimodal predictors for precision immunotherapy, Immuno-Oncology and Technology, Volume 14, 2022, 100071, ISSN 2590-0188, https://doi.org/10.1016/j.iotech.2022.100071. (https://www.sciencedirect.com/science/article/pii/S2590018822000028)
14. Liguori M, Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages as incessant builders and destroyers of the cancer stroma. *Cancers* (Basel). 2011 Sep 28;3(4):3740-61. doi: 10.3390/cancers3043740. PMID: 24213109; PMCID: PMC3763394.