Upregulation of IL-12 Signaling Genes Mediated by STAT4 Predicts a Complete Clinical Response in Patients with Squamous Cell Carcinoma of the Esophagus who Undergo Concurrent Chemoradiotherapy

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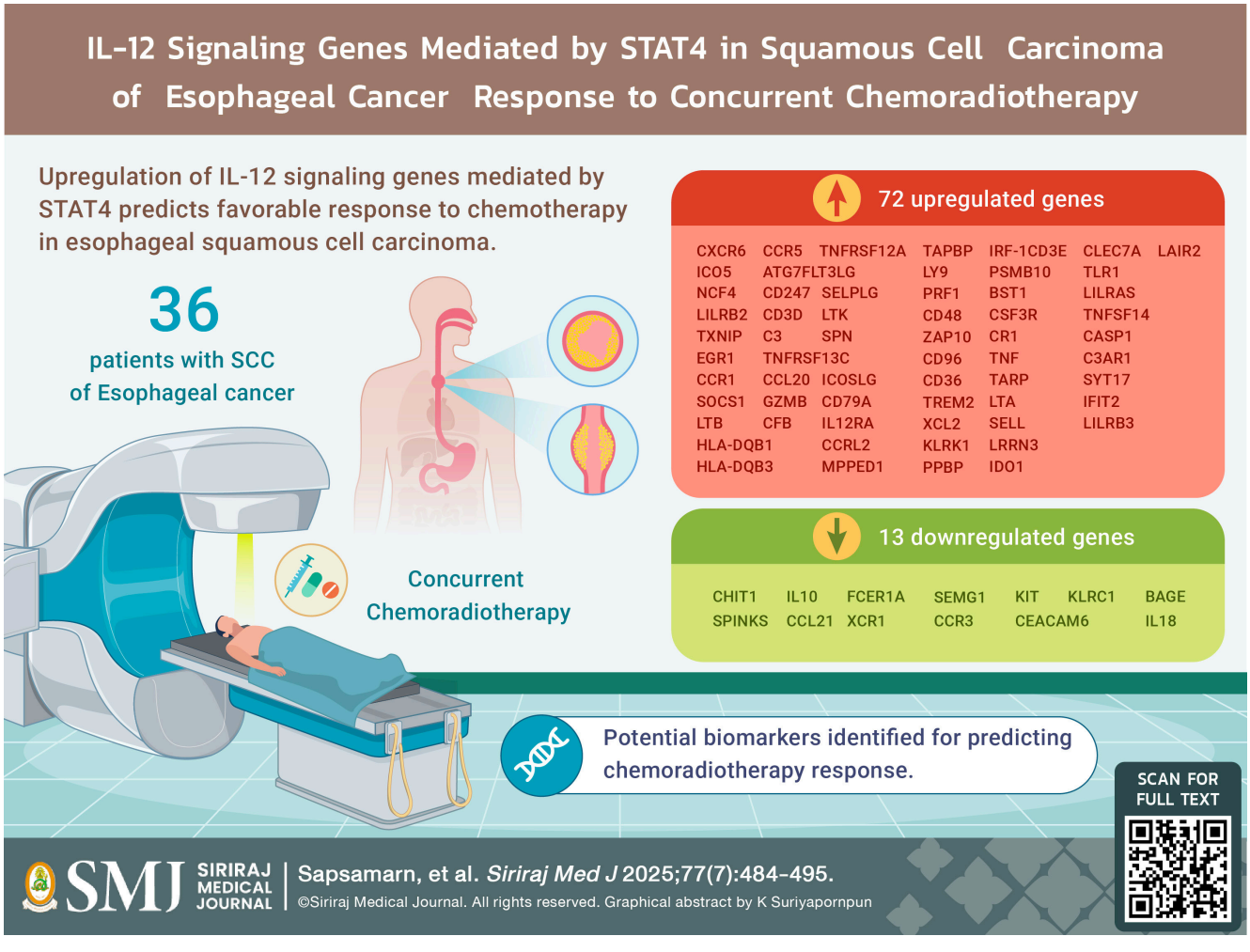

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ABSTRACT

Objective: This study compared immune transcriptome profiles between SCC patients achieving cCR and those with incomplete responses to CRT using the NanoString Ncounter® Pancancer Immune Profiling Panel.

Materials and Methods: A retrospective cohort of 36 SCC patients treated with CRT was analyzed. Clinical data and blood samples were collected, and immune transcriptome profiling was conducted. Differential gene expression analysis identified distinctions between cCR and non-clinical complete response (non-cCR) groups.

Results: Of the 36 patients, 20 achieved cCR, while 16 had non-cCR. Significant differences in immune transcriptomes were observed, particularly in IL-12 signaling mediated by STAT4. In the cCR group, 72 genes were upregulated, and 13 were downregulated, suggesting their role as predictive biomarkers for treatment response.

Conclusions: This study identified distinct immune transcriptome profiles in SCC patients with cCR versus non-cCR, highlighting genes related to IL-12 signaling as potential biomarkers. These findings emphasize the role of immune-related gene expression in determining patient outcomes and may support personalized therapeutic strategies to enhance CRT efficacy in SCC patients.

Keywords: Squamous cell carcinoma of the esophagus; Chemoradiotherapy; Interleukin 12 (Siriraj Med J 2025; 77: 484-495)

INTRODUCTION

Esophageal cancer is the seventh most common cancer worldwide. It ranks as the seventh most frequently occurring cancer in men and the thirteenth most common cancer in women.[1] In Thailand, esophageal cancer is the thirteenth leading cause of cancer-related death. There are two main pathological types of esophageal cancer: squamous cell carcinoma (SCC), which can occur in the cervical, thoracic, or abdominal regions of the esophagus, and adenocarcinoma, which can develop at the junction of the esophagus and stomach (esophagogastric junction). There are various multimodal treatment options available for esophageal SCC, including endoscopic resection, primary esophagectomy, neoadjuvant chemotherapy or chemoradiotherapy (CRT) followed by surgery, and definitive CRT. The treatment of choice depends on the location and stage of the tumor.

There are two main types of immune resistance mechanisms: innate immunity and adaptive immunity. The mechanism that primarily targets tumors is adaptive immunity. Tumors can trigger specific adaptive immune resistance, which may prevent host immune response to limit their growth and spread. This immune response largely involves T cells, particularly CD8+ cytotoxic T lymphocytes (CTLs). Many tumors are surrounded by infiltrates of mononuclear cells, including T lymphocytes and macrophages. Activated lymphocytes and macrophages can also be found in the lymph nodes that drain the areas where tumors are located. The key cells involved in important cancer prevention mechanisms are T cells, macrophages, and natural killer cells.[2]

T cells, specifically T helper cells, are categorized into two types: Th1 and Th2 cells. Th1 cells secrete several cytokines, including IFN-gamma, IL-2, IL-3, TNF-alpha, TNF-beta, and the adjustment factor IFN-gamma. In contrast, Th2 cells produce cytokines such as IL-4, IL-5, IL-9, IL-10, and IL-13, especially those that regulate IL-4, IL-5, and IL-13.[2]

Interleukin-12 (IL-12) is a heterodimeric cytokine composed of two subunits: p40 and p35.[3] It is considered a proinflammatory cytokine and is produced by antigen-presenting cells such as dendritic cells and macrophages. IL-12 plays a significant role in facilitating effective antitumor immune responses. IL-12 signals through its receptors, IL-12R1 and IL-12R2, which are expressed on target cells. This signaling pathway activates downstream pathways involving Jak2 and Tyk2, leading to the phosphorylation and homodimerization of STAT4.[3] These processes are essential for the recruitment and effector functions of CD8+ T cells and natural killer cells. Given its capabilities, IL-12 is a strong candidate for immunotherapy-based interventions, as it enhances the activity of tumor-specific cytotoxic natural killer and CD8+ T cells, both of which are critical for killing tumor cells. However, systemic administration of IL-12 can be quite toxic. Therefore, alternative administration methods are being explored.[3-9] IL-12 signaling, which is mediated by STAT4, is a crucial pathway in the immune system that regulates the response to infections. IL-12 is produced by dendritic cells and macrophages and then binds to its receptor on T cells and natural killer cells before activating JAK kinases. This activation leads to the

phosphorylation of STAT4, which then dimerizes and translocates into the nucleus to promote gene expression. This pathway enhances the differentiation of Th1 cells and stimulates the release of IFN-γ, which is crucial for eliminating pathogens. Dysregulation of this pathway is associated with autoimmune diseases but can also enhance anticancer responses, indicating potential implications for cancer therapy in the future.[10,11]

A clinical complete response (cCR) occurs when no tumor remnants are detectable through nonsurgical methods, as proven by imaging studies such as esophagography, computed tomography (CT) scans, endoscopy, or positron emission tomography-CT following CRT. Neoadjuvant chemoradiotherapy increase survival rates in patients with locally advanced esophageal carcinoma. According to Monjazeb et al., achieving a cCR after concurrent CRT (CCRT) can lead to a more favorable prognosis.[12] A pathological response study in another type of cancer, specifically CA rectum, reported that downstaging of the T-category was observed in 10 patients (55.6%), while downstaging of the N-category was noted in 14 patients (77.8%) following neoadjuvant chemoradiation.

MATERIALS AND METHODS

1. Study cohort

A total of 800 patients with SCC of the esophagus were enrolled in this study. All patients were over 18 years of age at the time of diagnosis. Among these patients, 36 met the study criteria. The remaining patients were excluded from our analyses. The datasets collected included various aspects of patient information and clinical data, which were part of the inclusion criteria. These datasets comprised demographic details such as age at disease onset, sex, and underlying conditions; treatments received following CCRT, categorized as surgical or nonsurgical; and laboratory results obtained prior to CCRT, including complete blood count, absolute neutrophil count, absolute lymphocyte count, neutrophil-lymphocyte ratio, absolute eosinophil count, platelet count, and platelet-lymphocyte ratio. Additional information included tumor location within the thoracic region (classified as upper, middle, or lower), tumor differentiation based on biopsy results (poorly differentiated, moderately differentiated, or well-differentiated), tumor staging, and lymph node status as per the National Comprehensive Cancer Network (NCCN) guidelines, details on preoperative radiation (dose and fractionation), preoperative chemoradiotherapy, and recurrence following CCRT. All patients included in the study were diagnosed with Stage 3 disease, meeting the inclusion criteria as defined by the NCCN guidelines.

Sample selection and specimen retrieval

The protocol used for the selection and retrieval of specimens was as follows:

- All samples must come from patients who meet the inclusion criteria.
- Clinical data for potential samples will be collected through reviews of outpatient department and inpatient department documents.
- Patient clinical information from operative records, as well as outpatient department and inpatient department documents, will be reviewed. Slides from formalin-fixed, paraffin-embedded (FFPE) blocks of tissue biopsies obtained from esophagogastroduodenoscopy will be obtained from the pathology department.

2. Treatment

All patients underwent upper gastrointestinal endoscopy along with a biopsy of the lesion to confirm a diagnosis of SCC. Patients received a combination of chemotherapy and radiation therapy. Imaging studies were subsequently conducted via posttreatment sequential CT scanning at approximately 4- to 6-week intervals. After being diagnosed with esophageal SCC, patients were treated with a combination of chemotherapy and radiation therapy. The chemotherapy regimens were divided into three different formulas: Formula 1: paclitaxel and carboplatin; Formula 2: cisplatin and 5-FU; and Formula 3: carboplatin and 5-FU. Radiation therapy was administered at doses ranging from 50–60 Gy. Following the chemotherapy and radiation treatments, patients who underwent CT scans were categorized into two groups on the basis of their response: (1) clinical complete response (cCR) and (2) non-clinical complete response (non-cCR). These categories were further classified according to specific criteria.

3. Laboratory methods for RNA extraction from FFPE tissue

Deparaffinization

FFPE slides were cut into 5-micron-thick sections, resulting in a total of 5 cuts. Deparaffinization was performed by adding 1000 µL of xylene, followed by vortexing the mixture. Next, 400 µL of absolute ethanol was added, and the mixture was vortexed again. The sample was then centrifuged in RCF mode at 16000 × g for 2 minutes. The supernatant was discarded. Subsequently, 1000 µL of absolute ethanol was added to the pellet, which was vortexed. The sample was centrifuged a second time in RCF mode at 16,000 × g for 2 minutes, and the

supernatant was discarded. Finally, the samples were dried in a heating box at 55 °C for 50 minutes.

RNA extraction and qualification

The process of extracting RNA from FFPE tissue via a Veracyte RNA Extraction Kit (Veracyte, San Francisco, CA, USA) began with the preparation of essential solutions. This involved adding ethanol to the wash solution and reconstituting DNase I with nuclease-free water, which was kept on ice. During the isolation phase, lysis buffer and proteinase K were added to the tissue sample. The mixture was then incubated at 56 °C and 80 °C to facilitate tissue breakdown and RNA release. Afterward, the sample was cooled on ice until it reached room temperature, followed by brief centrifugation to prepare for DNase treatment. Finally, DNase I was added to eliminate any DNA contaminants, ensuring high RNA purity for subsequent analyses. The RNA concentration and purity were measured via a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA purity was assessed via the A260/A280 ratio, which was required to be not less than 2.0.

Gene expression and experiments

This study utilized an nCounter Pan-Cancer Immune Profiling Panel (NanoString Technologies, Seattle, WA, USA). This panel includes 40 reference genes as well as 730 targets related to immuno-oncology that feature 109 cell surface markers and represent 14 immune cell types.

After RNA extraction, immune transcriptome profiling was performed via the nCounter PanCancer Immune Panel. NanoString assays were performed with 100 ng of RNA, 8 µL of reporter probe, and 2 µL of capture probe per sample. The hybridization reaction was performed for 24 h at 65 °C in a thermocycler.

NanoString data analysis

The nSolver Analysis Software (version 4, NanoString Technologies) was utilized to assess quality control parameters, including binding density, limit of detection, and positive controls. No quality control issues were identified, allowing all 36 samples to proceed for further analysis. The advanced analysis module of the nSolver software was employed for housekeeping gene selection, data normalization, differential expression analysis, and calculation of immune-oncology-related scores. Differential gene expression analysis was conducted to identify the transcriptomic differences between patients with cCR and those with incomplete clinical responses. Rosalind software (NanoString Technologies) was subsequently used to compare the gene expression in the two patient groups. Differentially expressed genes were identified via the software. The threshold was set at a fold change greater than 1.5 combined with a p-value of < 0.05 to determine significant differentially expressed genes. To account for multiple t tests, the classical Benjamini–Hochberg method was applied to adjust p-values, controlling the false discovery rate and producing adjusted p-values.

Gene set, pathway, and function analyses

Gene set, pathway, and functional analyses were conducted via gene set analysis with a directed global significance score based on NanoString annotations version 46. Gene set analysis was used to evaluate the overall significance of functionally related gene sets, with the global significance score reflecting cumulative evidence for differential gene expression in a pathway through the square root of the mean squared t statistic. The directed global significance score considered the direction of over- or underexpression on the basis of t statistics. Biological networks were examined via Rosalind and Qiagen Ingenuity Pathway Analysis (Qiagen, Hilden, Germany). Fisher's exact test was used to calculate enrichment p values, whereas Z scores were used to predict the activation or inhibition of molecular functions.

4. Statistical analysis

Non-normally distributed data were presented using the median and interquartile range (IQR) and analyzed with the Mann-Whitney test. Categorical data were analyzed using the Chi-square test and reported as proportions (percentages). All statistical analyses were performed using Stata version 19.1.

RESULTS

Patient characteristics

The characteristics of the eligible patients are summarized in Table 1. The median age of patients in the cCR group and the non-cCR group was 64 years. Many of these patients had underlying conditions, including hypertension and diabetes. In terms of treatment, the cCR group consisted of 10 patients, some of whom underwent surgery. Similarly, the non-cCR group consisted of 8 patients, with a comparable distribution of surgical and nonsurgical treatments.

Data collection from laboratory results revealed no statistically significant differences between the two groups. The laboratory findings included the complete blood count, absolute neutrophil count, absolute lymphocyte count, neutrophil-lymphocyte ratio, absolute eosinophil count, platelet count, and platelet-lymphocyte ratio.

TABLE 1. Characteristics of patients with a cCR and those with a non-cCR.

	Clinical complete response (n=20)	Non-clinical complete response (n=16)	*p* value
Sex			0.106
Male	17 (85%)	16 (100%)	
Female	3 (15%)	0 (0%)	
Age, mean ± SD.	63.9 ± 9.04	64.13 ± 8.69	0.94
U/D	13 (65%)	12 (75%)	0.517
HT	8 (40%)	8 (50%)	0.549
DM	3 (15%)	1 (6.3%)	0.406
Other U/D	9 (45%)	9 (56.3%)	0.502
Treatment			1.000
Surgical	10 (50%)	8 (50%)	
Nonsurgical	10 (50%)	8 (50%)	
CBC, median (IQR)	7900 (6430, 10280)	8040 (6380, 12100)	0.386
Absolute neutrophil (10*3/ul), median (IQR)	5.19 (3.82, 6.01)	4.7 (4.05, 7.71)	0.800
Absolute lymphocyte (10*3/ul), median (IQR)	1.71 (1.24, 2.28)	2.03 (1.64, 2.43)	0.278
NLR, median (IQR)	3 (2, 4)	3 (2, 4)	0.955
Absolute eosinophile, median (IQR)	0.17 (0.11, 0.28)	0.22 (0.12, 0.61)	0.262
Platelet (10*3/ul), median (IQR)	280 (230, 330)	317 (271, 408)	0.148
PLR, median (IQR)	195.87 (104.79, 229.6)	171.82 (120.35, 220.85)	0.942
Tumor location			0.782
Upper thoracic	5 (25%)	4 (25%)	
Middle thoracic	7 (35%)	4 (25%)	
Lower thoracic	8 (40%)	8 (50%)	
Tumor differentiation			0.351
Poorly differentiated	2 (10%)	0 (0%)	
Moderately differentiated	16 (80%)	13 (81.3%)	
Well differentiated	2 (10%)	3 (18.8%)	
Clinical stage			N/A
3	20 (100%)	16 (100%)	
Clinical tumor stage			0.657
2	1 (5%)	1 (6.3%)	
3	18 (90%)	15 (93.8%)	
4	1 (5%)	0 (0%)	

TABLE 1. Characteristics of patients with a cCR and those with a non-cCR. (Continue)

	Clinical complete response (n=20)	Non-clinical complete response (n=16)	*p* value
LN stage			0.439
Negative	4 (20%)	5 (31.3%)	
Positive	16 (80%)	11 (68.8%)	
cM stage			N/A
0	20 (100%)	16 (100%)	
Preop RT dose Gy, mean ± SD	52.3 ± 8.31	51.53 ± 3.5	0.73
Preop RT Fr, mean ± SD.	27.95 ± 1.88	26.81 ± 1.94	0.084
Preop CMT			0.271
Paclitaxel and carboplatin	9 (45%)	4 (25%)	
Cisplatin and 5FU	6 (30%)	9 (56.3%)	
Carboplatin and 5FU	5 (25%)	3 (18.8%)	
Recurrence			
Local recurrence	2 (10%)	6 (37.5%)	0.049*
Single metastasis	1 (5%)	6 (37.5%)	0.014*
Multiple metastases	2 (10%)	3 (18.8%)	0.451

Abbreviations: 5FU, 5-Fluorouracil; CBC, Comeplete Blood Count; cM, clinical Metastasis; CMT, Chemotherapy; DM, Diabetes Mellitus; Fr, Fraction; Gr, Gray; HT, Hypertension; IQR, Interquatile Rang; LN, Lymph node; NLR, NeutrophiL-Lymphocyte Ratio; PLR, Platele-Lymphocyte Ratio; RT, Radiotherpy; SD, Standard Deviation; U/D, Undelying Disease

In the cCR group, the majority of tumors were located in the upper and middle thoracic areas. In terms of tumor differentiation, moderate differentiation was most prevalent in the cCR group, with 16 patients, compared with 13 patients in the non-cCR group.

All patients in this study were at clinical stage 3. The most common tumor stage in both groups was T3. The lymph nodes were categorized into clinical lymph node–positive and lymph node–negative subgroups. There were 20 patients (55%) in the cCR group and 16 patients (44%) in the non-cCR group. None of the patients had metastasis before treatment.

The radiation dose for patients in the cCR group was 52 Gy. For those in the non-cCR group, the dose was 51 Gy. Three chemotherapy regimens were used: (1) paclitaxel and carboplatin were administered to 9 patients (25%) in the cCR group and 4 patients (11%) in the non cCR group; (2) cisplatin and 5-FU were given to 6 patients (16.6%) in the cCR group and 9 patients (25%) in the non cCR group; and (3) carboplatin and 5-FU were provided to 5 patients (13.89%) in the cCR group and 3 patients (8.33%) in the non-cCR group.

In terms of follow-up after treatment, there was a statistically significant difference in local recurrence ($p = 0.049$) and single recurrence ($p = 0.014$). The incidence of recurrence was greater in the non-cCR group than in the cCR group.

Table 2 presents a list of differentially expressed genes organized into upregulated and downregulated groups. On the left side, the "Up" section features 72 genes whose expression increased (highlighted by the red background). Some of these genes are Interleukin 12 Receptor Subunit Alpha *(IL12RA)*, C-X-C Motif Chemokine Receptor 6*(CXCR6)*, C-C Motif Chemokine Receptor 5*(CCR5)*, Tumor Necrosis Factor Receptor Superfamily Member 12A *(TNFRSF12A)*, and Inducible T Cell Costimulator*(ICOS)*. On the right side, the "Down" section highlights 13 genes with decreased expression (indicated by a green background). Examples of these genes are Chitinase 1*(CHIT1)*, Interleukin 10*(IL10)*, Fc Fragment of IgE

Receptor IA *(FCER1A)*, and Semenogelin1*(SEMG1)*. This table helps identify key genes that may contribute to different biological responses, highlighting them as potential targets for further research

The heatmap in Fig 1 displays the expression levels of various genes across patient groups. The horizontal axis represents the patients, with the blue axis indicating those who achieved a cCR and the orange axis denoting the non-cCR group. The vertical axis represents the individual genes analyzed in the study. The blue shading

UP=72							DOWN=13						
CXCR6	CCR5	TNFRSF12A	TAPBP	IRF-1CD3E	CLEC7A	LAIR2	CHIT1	IL10	FCER1A	SEMG1	KIT	KLRC1	BAGE
ICOS	ATG7FLT3LG		LY9	PSMB10	TLR1		SPINK5	CCL21	XCR1	CCR3	CEACAM6		IL18
NCF4	CD247	SELPLG	PRF1	BST1	LILRA5								
LILRB2	CD3D	LTK	CD48	CSF3R	TNFSF14								
TXNIP	C3	SPN	ZAP10	CR1	CASP1								
EGR1	TNFRSF13C		CD96	TNF	C3AR1								
CCR1	CCL20	ICOSLG	CD36	TARP	SYT17								
SOCS1	GZMB	CD79A	TREM2	LTA	IFIT2								
LTB	CFB	IL12RA	XCL2	SELL	LILRB3								
HLA-DQB1		CCRL2	KLRK1	LRRN3									
HLA-DRB3		MPPED1	PPBP	IDO1									

TABLE 2. In patients with a cCR, the differentially expressed genes were categorized into an upregulated group (72 genes, shown in red) and a downregulated group (13 genes, shown in green).

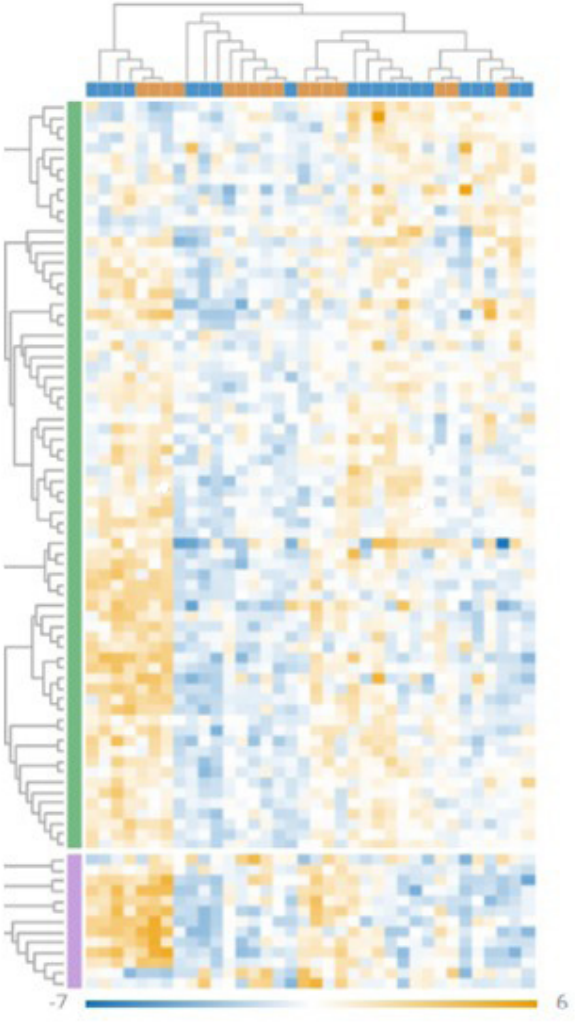

Fig 1. A heatmap displaying gene expression across patients, where blue represents clinical complete response (cCR) and orange indicates non-clinical complete response (non-cCR). The blue shading indicates downregulated genes, the orange shading represents upregulated genes, and the white shading signifies no change in expression.

(cool) indicates downregulated genes, whereas the orange shading (warm) indicates upregulated genes. The white cells presented no significant change in gene expression. The dendrograms on the top and left show clustering, with clustering for the top group based on the patient response profiles and clustering on the left based on the gene expression. The black markers highlight specific genes with notable expression changes and high statistical significance, suggesting potential key indicators for treatment response.

The volcano plot in Fig 2 compares gene expression between patients who achieved a cCR and those with a non-cCR. The x-axis represents the log2-fold change, indicating the ratio of gene expression between the cCR and non-cCR groups. Positive values on the right side of the x-axis represent genes with increased expression in the cCR group, whereas negative values on the left side denote genes with decreased expression. The y-axis displays the -log10 *p*-value, which represents the probability that the observed changes occurred by chance. Higher points on this axis signify changes that are statistically more significant.

The pathway interaction database in Fig 3 illustrates the signaling pathways in immune cells, with bars highlighting changes in pathway activity that are either upregulated (shown in red) or downregulated (shown in green) on the basis of adjusted p-values. The analyzed pathways included "IL12 signaling mediated by STAT4," "downstream signaling in naive CD8+ T cells," "TCR signaling in naive CD8+ T cells," "IL12-mediated signaling events," "caspase cascade in apoptosis," and "TCR signaling in naive CD4+ T cells." The length of each bar represents the adjusted *p* value, indicating the significance level of each pathway. This visualization emphasized the activity

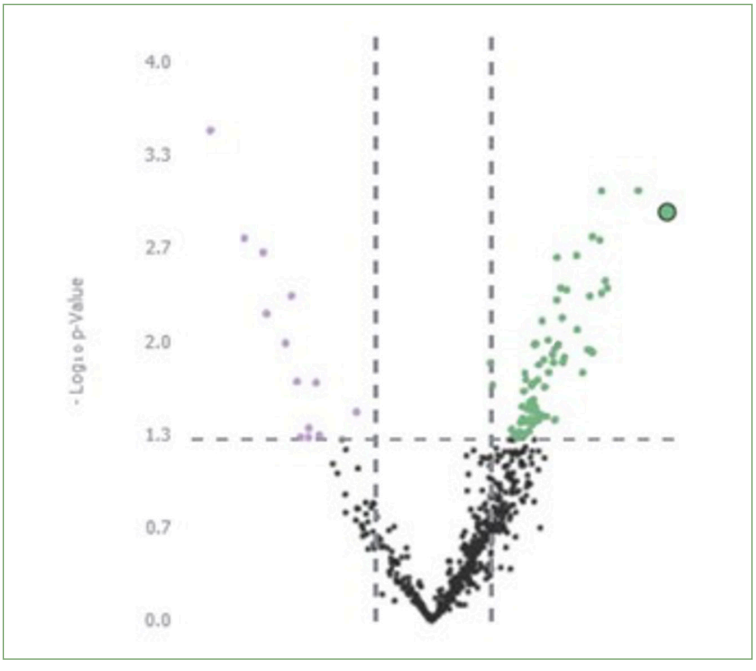

Fig 2. A volcano plot comparing gene expression between patients who achieved a clinical complete response (cCR) and those who did not achieve a complete response (non-cCR). Genes with increased expression in the cCR group are represented as green points on the right side of the plot, whereas genes with decreased expression are shown as purple points on the left side. The height of each point indicates the level of statistical significance, with taller points representing greater significance.

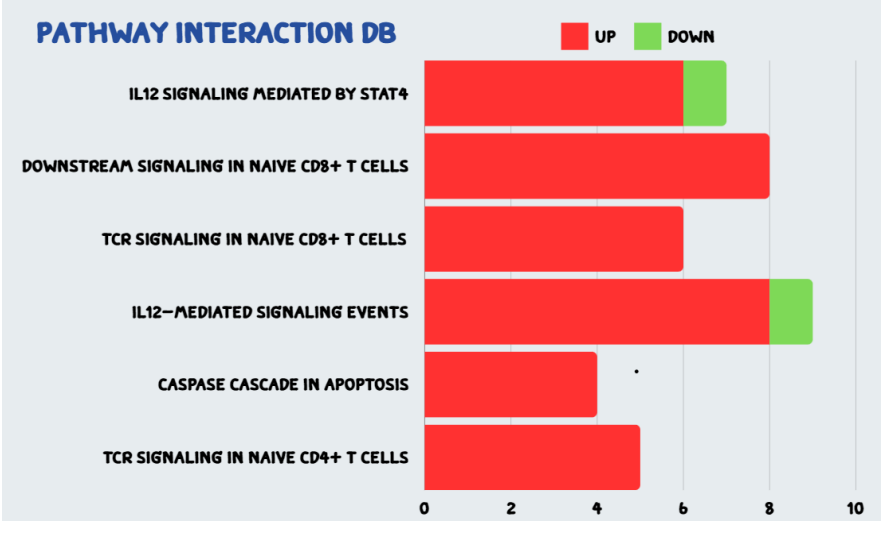

Fig 3. Pathway interaction database illustrating immune cell signaling pathways. The figure highlights upregulated activities in red and downregulated activities in green on the basis of adjusted *p*-values. The pathways that are represented include T-cell signaling and apoptosis, with the lengths of the bars indicating the significance levels of each pathway.

and suppression of specific pathways, particularly those involved in T-cell signaling and apoptosis.

The Pathways/BioPlanet chart in Fig 4 illustrates immune signaling pathways associated with the body's immune response, which are categorized into upregulated pathways (in red) and downregulated pathways (in green). All the signals presented a *p*-value of less than 0.05, indicating statistical significance. The analyzed pathways included "T-cell activation co-stimulatory signal," "leptin influence on the immune response," "interleukin-12/STAT4 pathway," "T-cell receptor and CD3 complex," "CTL-mediated immune response against target cells," and "PD-1 signaling". The length of each bar represents the significance level of either upregulation or downregulation in each pathway. The chart reveals that the "leptin influence on immune response" pathway is the most upregulated pathway, whereas the "interleukin-12/STAT4 pathway" is partially downregulated. This provides a comprehensive overview of variations in the immune response across different pathways, highlighting the importance of T-cell signaling and immune regulation.

Genes, biological processes, and signaling pathways

To further investigate the different molecular functions at each resection margin, we conducted a functional analysis of differentially expressed genes with *p*- values < 0.05 via IPA. Our analysis revealed a total of 85 significantly 72 upregulated genes (positive fold change values) and 13 downregulated genes (negative fold change values) compared with cCR and non-cCR.

The analysis highlights key genes and pathways involved in immune regulation and inflammation. Central genes such as *TNF, IL6, IL1A,* and *IL1B* play pivotal roles in cytokine-mediated signaling, particularly in pathways such as cytokine storm signaling and Th1/Th2 activation. These processes drive immune responses, including the activation of cytotoxic T cells, monocyte adhesion, and the release of reactive oxygen species, which are essential for inflammation and defense mechanisms. Additionally, genes such as *RELA* (part of the NF-kappa B pathway) and *IFNG/IL17A* underscore the importance of adaptive immunity. These are depicted in Fig 5.

DISCUSSION

This study demonstrated that patients who achieved a cCR had higher levels of IL-12 than those with a non-cCR. These findings suggest potential applications for these two groups of patients. The first group included patients who were not suitable for surgery due to their physical condition. For these patients, achieving a cCR could help reduce complications associated with the tumor mass, such as bleeding or difficulty swallowing.

The second group consisted of patients eligible for surgery, where IL-12 could be used as an adjuvant therapy. As a result, the present research could improve treatment options for both groups of patients. Further studies are necessary because this research was retrospective and limited by the availability of biopsy tissue, which must be collected before CCRT. This limitation may reduce the number of patients who meet the criteria. Future research could expand the patient sample size and use a prospective design, enabling the collection of both fresh and paraffin-embedded biopsy tissues. These samples could then be utilized for RNA extraction and further analysis via Geomax technology, allowing for more accurate calculations.

The current review highlights that IL-12 has been utilized in the treatment of several cancer types,[13] including melanoma,[4,14-22] based on certain studies, as well as in gynecologic cancer[10,12,22-29] and breast cancer,[8,14,25,30-40] as

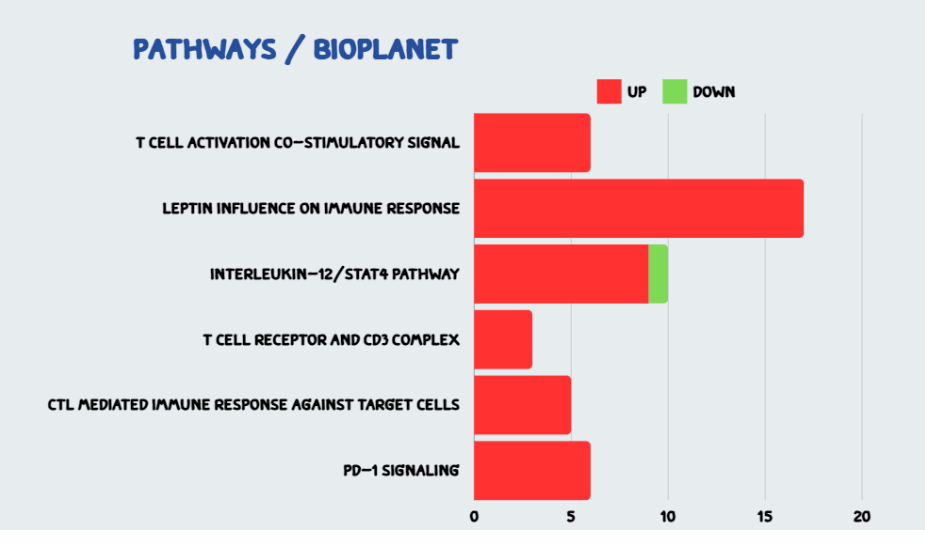

Fig 4. The Pathways/BioPlanet chart illustrates the immune signaling pathways involved in the body's immune response. Pathways that are upregulated are indicated in red, whereas those that are downregulated are shown in green. All signaling pathways had *p*-values less than 0.05. Notably, the "leptin influence on immune response" pathway was the most significantly upregulated pathway, whereas the "interleukin-12/STAT4 pathway" was partially downregulated.

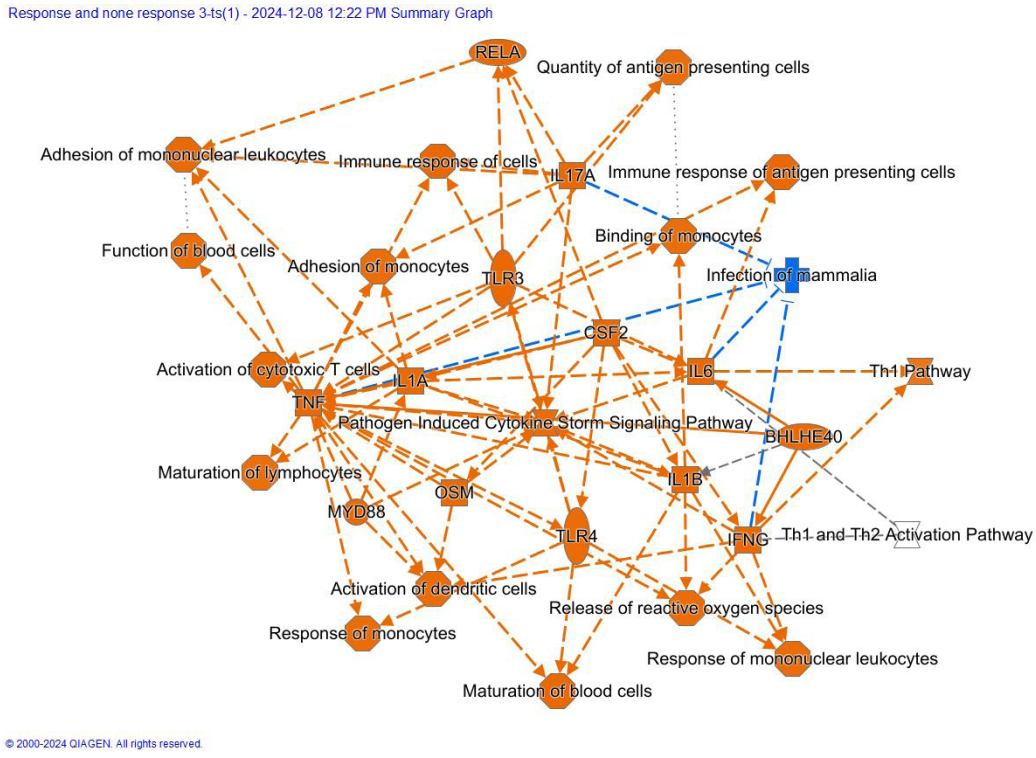

Fig 5. The diagram highlights critical immune pathways and key genes, such as *TNF, IL6, IL1A,* and *IL1B*, which regulate cytokine storms, Th1/Th2 pathways, and inflammation. These processes activate T cells, monocyte adhesion, and reactive oxygen species essential for immune responses.

indicated by other research. However, the use of IL-12 should be approached with caution due to potential side effects, such as flu-like symptoms, which some patients may find intolerable.[14] Various methods of administering IL-12 have been explored to mitigate these side effects.[36] Due to the severe side effects associated with systemic treatment via intravenous injections, alternative methods for administering IL-12, including intravenous injection, subcutaneous injection, intramuscular injection, and intratumoral injection, have been developed.[36] Future studies may focus on the intratumoral injection of IL-12, an adjuvant for cancer vaccines,[37] which could increase the effectiveness of treatment and potentially improve outcomes in patients with SCC of the esophagus. This recent discovery could prove beneficial in the future by improving treatment effectiveness for patients, particularly by increasing the rate of cCR.

CONCLUSION

This study highlights significant differences in immune transcriptome profiles between patients with a cCR and those with a non-cCR to CRT in patients with SCC of the esophagus. The findings indicate that the upregulation of genes involved in IL-12 signaling, particularly those mediated by STAT4, may serve as potential biomarkers for predicting favorable treatment responses. Patients who achieved cCR presented a lower incidence of distant recurrence than non- cCR patients did, indicating that immune transcriptomic characteristics may influence patient prognosis. The results provide a strong foundation for future research that leverages transcriptomic data to develop personalized treatment strategies, potentially improving patient outcomes in patients with esophageal cancer.

Data Availability Statement

The authors affirm that the data supporting the findings of this study are included within the article and its supplementary materials.

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DECLARATION

Grants and Funding Information

No grants or funding applied.

Conflict of Interest

All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

Registration Number of Clinical Trial

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Author Contributions

Conceptualization and methodology, N.S, V.T., A.M., P.T., T.T.; Specimen collection, A.M., J.S. and T.P.; Investigation, N.S., P.T., T.S., O.A., K.T.; Formal analysis, N.S. ,P.T. and T.S. ; Visualization and writing – original draft, N.S.; Writing – review and editing, N.S.,V.T. ; Funding acquisition, none ; Supervision, V.T. All authors have read and agreed to the final version of the manuscript.

Use of Artificial Intelligence

No artificial intelligence tools or technologies were utilized in the writing, analysis, or development of this research.

REFERENCES

1. The Globocan Cancer Observatory - Globocan 2020. The Globocan Cancer Observatory - Globocan 2020.
2. Abul K. Abbas M, Andrew H. Lichtman, MD, PhD, Shiv Pillai, MBBS, PhD. Cellular and molecular immunology. Edition, editor, 2015.
3. Stamm LM, Satoskar AA, Ghosh SK, David JR, Satoskar AR. STAT-4 mediated IL-12 signaling pathway is critical for the development of protective immunity in cutaneous leishmaniasis. Eur J Immunol. 1999;29(8):2524-9.
4. Byrne-Hoffman CN, Deng W, McGrath O, Wang P, Rojanasakul Y, Klinke DJ. Interleukin-12 elicits a non-canonical response in B16 melanoma cells to enhance survival. Cell Commun Signal. 2020;18(1):78.
5. Jia Z, Ragoonanan D, Mahadeo KM, Gill J, Gorlick R, Shpal E, et al. IL12 immune therapy clinical trial review: Novel strategies for avoiding CRS-associated cytokines. Front Immunol. 2022; 13:952231.
6. Lasek W, Zagożdżon R, Jakobisiak M. Interleukin 12: still a promising candidate for tumor immunotherapy? Cancer Immunol Immunother. 2014;63(5):419-35.
7. Liu J, Cao S, Kim S, Chung EY, Homma Y, Guan X, et al. Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression. Curr Immunol Rev. 2005;1(2):119-37.
8. Mirlekar B, Pylayeva-Gupta Y. IL-12 family cytokines in cancer and immunotherapy. Cancers. 2021;13(2):167.
9. Tugues S, Burkhard S, Ohs Ia, Vrohlings M, Nussbaum K, Vom Berg J, et al. New insights into IL-12-mediated tumor suppression. Cell Death Differ. 2015;22(2):237-46.
10. Su W, Ito T, Oyama T, Kitagawa T, Yamori T, Fujiwara H, et al. The direct effect of IL-12 on tumor cells: IL-12 acts directly on tumor cells to activate NF-κB and enhance IFN-γ-mediated STAT1 phosphorylation. Biochem Biophys Res Commun. 2001; 280(2):503-12.
11. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev. 2004;202:139-56.
12. Wang J, Qin J, Jing S, Liu Q, Cheng Y, Wang Y, et al. Clinical complete response after chemoradiotherapy for carcinoma of thoracic esophagus: Is esophagectomy always necessary? A systematic review and meta-analysis. Thorac Cancer. 2018;9(12): 1638-47.
13. Lu X. Impact of IL-12 in Cancer. Curr Cancer Drug Targets. 2017;17(8):682-97.
14. Agliardi G, Liuzzi AR, Hotblack A, De Feo D, Núñez N, Stowe CL, et al. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. Nat Commun. 2021;12(1):444.
15. Algazi AP, Twitty CG, Tsai KK, Le M, Pierce R, Browning E, et al. Phase II trial of IL-12 plasmid transfection and PD-1 blockade in immunologically quiescent melanoma. Clin Cancer Res. 2020; 26(12):2827-37.
16. Gao W, Pan J, Pan J. Antitumor activities of interleukin-12 in melanoma. Cancers. 2022;14(22):5592.
17. Gollob JA, Mier JW, Veenstra K, McDermott DF, Clancy D, Clancy M, et al. Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-γ induction is associated with clinical response. Clin Cancer Res. 2000;6(5): 1678-92.
18. Greaney SK, Algazi AP, Tsai KK, Takamura KT, Chen L, Twitty CG, et al. Intratumoral plasmid IL12 electroporation therapy in patients with advanced melanoma induces systemic and intratumoral T-cell responses. Cancer Immunol Res. 2020;8(2): 246-54.
19. Komel T, Bosnjak M, Brezar SK, De Robertis M, Mastrodonato M, Scillitani G, et al. Gene electrotransfer of IL-2 and IL-12 plasmids effectively eradicated murine B16. F10 melanoma. Bioelectrochemistry. 2021;141:107843.
20. Moretti S, Chiarugi A, Semplici F, Salvi A, De Giorgi V, Fabbri P, et al. Serum imbalance of cytokines in melanoma patients. Melanoma Res. 2001;11(4):395-9.
21. Sun Y, Jurgovsky K, Möller P, Alijagic S, Dorbic T, Georgieva J, et al. Vaccination with IL-12 gene-modified autologous melanoma cells: preclinical results and a first clinical phase I study. Gene Ther. 1998;5(4):481-90.
22. Chen X, Han S, Wang S, Zhou X, Zhang M, Dong J, et al. Interactions of IL-12A and IL-12B polymorphisms on the risk of cervical cancer in Chinese women. Clin Cancer Res. 2009;15(1): 400-5.
23. Kumar N, Vyas A, Agnihotri SK, Chattopadhyay N, Sachdev M, editors. Small secretory proteins of immune cells can modulate gynecological cancers. Seminars in Cancer Biology; 2022: Elsevier.
24. Marana HRC, da Silva JS, de Andrade JM. NK cell activity in the presence of IL-12 is a prognostic assay to neoadjuvant chemotherapy in cervical cancer. Gynecol Oncol. 2000;78(3):318-23.
25. Ruffell B, Chang-Strachan D, Chan V, Rosenbusch A, Ho CM, Pryer N, et al. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. Cancer Cell. 2014;26(5):623-37.
26. Whitworth JM, Alvarez RD. Evaluating the role of IL-12 based therapies in ovarian cancer: a review of the literature. Expert

Opin Biol Ther. 2011;11(6):751-62.
27. Xiong H-Y, Ma T-T, Wu B-T, Lin Y, Tu Z-G. IL-12 regulates B7-H1 expression in ovarian cancer-associated macrophages by effects on NF-κB signalling. Asian Pac J Cancer Prev. 2014; 15(14):5767-72.
28. Zeimet AG, Widschwendter M, Knabbe C, Fuchs D, Herold M, Müller-Holzner E, et al. Ascitic interleukin-12 is an independent prognostic factor in ovarian cancer. J Clin Oncol. 1998;16(5): 1861-8.
29. Zijlmans HJ, Fleuren GJ, Baelde HJ, Eilers PH, Kenter GG, Gorter A. Role of tumor-derived proinflammatory cytokines GM-CSF, TNF-α, and IL-12 in the migration and differentiation of antigen-presenting cells in cervical carcinoma. Cancer. 2007; 109(3):556-65.
30. Bekaii-Saab TS, Roda JM, Guenterberg KD, Ramaswamy B, Young DC, Ferketich AK, et al. A phase I trial of paclitaxel and trastuzumab in combination with interleukin-12 in patients with HER2/neu-expressing malignancies. Mol Cancer Ther. 2009; 8(11):2983-91.
31. Eliopoulos N, Francois M, Boivin M-N, Martineau D, Galipeau J. Neo-organoid of marrow mesenchymal stromal cells secreting interleukin-12 for breast cancer therapy. Cancer Res. 2008; 68(12):4810-8.
32. Gyorffy S, Palmer K, Podor TJ, Hitt M, Gauldie J. Combined treatment of a murine breast cancer model with type 5 adenovirus vectors expressing murine angiostatin and IL-12: a role for combined anti-angiogenesis and immunotherapy. J Immunol. 2001;166(10):6212-7.
33. Kaarvatn MH, Vrbanec J, Kulic A, Knezevic J, Petricevic B, Balen S, et al. Single nucleotide polymorphism in the interleukin 12B gene is associated with risk for breast cancer development. Scand J Immunol. 2012;76(3):329-35.
34. Kovacs E. The serum levels of IL-12 and IL-16 in cancer patients. Relation to the tumour stage and previous therapy. Biomed Pharmacother. 2001;55(2):111-6.
35. Mohamed Amin Z, Che Ani MA, Tan SW, Yeap SK, Alitheen NB, Syed Najmuddin SUF, et al. Evaluation of a recombinant Newcastle disease virus expressing human IL12 against human breast cancer. Sci Rep. 2019;9(1):13999.
36. Nguyen KG, Vrabel MR, Mantooth SM, Hopkins JJ, Wagner ES, Gabaldon TA, et al. Localized interleukin-12 for cancer immunotherapy. Front Immunol. 2020;11:575597.
37. Portielje JE, Gratama J, van Ojik HH, Stoter G, Kruit WH. IL-12: a promising adjuvant for cancer vaccination. Cancer Immunol Immunother. 2003;52(3):133-44.
38. Roszak A, Mostowska A, Sowińska A, Lianeri M, Jagodziński PP. Contribution of IL12A and IL12B polymorphisms to the risk of cervical cancer. Pathol Oncol Res. 2012;18(4):997-1002.
39. Sabel MS, Skitzki J, Stoolman L, Egilmez NK, Mathiowitz E, Bailey N, et al. Intratumoral IL-12 and TNF-α–loaded microspheres lead to regression of breast cancer and systemic antitumor immunity. Ann Surg Oncol. 2004;11(2):147-56.
40. Telli ML, Nagata H, Wapnir I, Acharya CR, Zablotsky K, Fox BA, et al. Intratumoral plasmid IL12 expands CD8+ T cells and induces a CXCR3 gene signature in triple-negative breast tumors that sensitizes patients to anti–PD-1 therapy. Clin Cancer Res. 2021;27(9):2481-93.