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Understanding the Anti-Tumor Properties Mediated by the Synthetic Peptide GK-1

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Abstract

Cancer exhibits adaptive features typical of complex systems, like resilience and robustness to environmental challenges through the emergent co-evolution of its components. These events promote carcinogenesis through dynamic interactions among numerous components and subsystems, including the immune system. During the past decade, our research group has provided substantial evidence that the peptide GK-1 has important immunomodulatory properties. In elderly mice, GK-1 acts as a potent adjuvant of the influenza vaccine through a mechanism that involves the activation of antigen-presenting cells (APCs) and an increased production of pro-inflammatory cytokines and chemokines (IFN- γ , TNF α , CCL2). To date, there is solid evidence supporting the antitumoral properties of GK-1 in murine cancer models. First, a lower occurrence and smaller size of spontaneous bronchiolar adenomas were found in elderly GK-1-treated mice compared to paired untreated mice. In two independent studies, GK-1 treatment reduced tumor growth and increased mouse survival in a murine model of melanoma and breast tumor. In the former model, a synergy between GK-1 and anti-PD-L1 treatment was observed, while in the latter, GK-1 alone controlled the metastatic burden. The effective activation of APCs induced by GK-1, restoring the antitumor-specific immunity, may underlie some of its antineoplastic effects.

Keywords: GK-1 peptide, melanoma, breast cancer, immunomodulator, antitumor

1. Introduction

1.1. Immunomodulation

Immunosurveillance comprises interactions between the immune system and cancer cells that take place even before the tumor formation [1, 2]. This process includes the recognition and control of transformed cells through antitumor immune responses, with three related outcomes: elimination, equilibrium, and escape [1–5]. In this regard, stimulating the innate immune system by immunogenic cells plays a role in the removal of incipient tumors, activating cells from the adaptive response like T and B cells, as well as promoting acute inflammation due to the concomitant production of immunostimulatory cytokines. Nevertheless, some transformed cells may not be eliminated. This escape phase of immunosurveillance is characterized by tumor growth promotion through a phenomenon called tumor-induced tolerance, which involves an increased expression of immunosuppressive components such as myeloid-derived suppressor cells (MDSC), regulatory T cells (Tregs), as well as T cell exhaustion and the production of immunosuppressive soluble factors [6, 7]. Indeed, some of these cells could be used as prognosis factors, since increased numbers of Treg and MDSC cells are related to a poorer outcome in cancer patients [8–13]; by contrast, a Th1 response is associated with a good prognosis in melanoma, breast, head, neck, colorectal, prostatic, and renal cancer [14–16].

The immune response can be modulated by compounds capable of enhancing (immunopotential) or diminishing (immunosuppression) the immune response, either in an antigen-specific or in a nonspecific manner; the latter implies that the immune system requires to be stimulated to restore the patient's immunocompetence. Immunomodulators are biological or nonbiological substances that can modify one or more components of the immunoregulatory network to achieve a specific antitumor immunity, such as inducing effector tumor-specific cytotoxic T lymphocytes (CTLs), activating macrophages and natural killer (NK) cells, and/or promoting the production of inflammation mediators [17–21].

Immunomodulators include adjuvants, vaccines, and immunoglobulins used to prevent or treat infectious diseases. They are characterized by their ability to activate cells of the innate immune system, mainly dendritic cells (DCs) and macrophages. Some examples of this type of agents are pathogen-associated molecular patterns (PAMPs) and molecules like squalene, aluminum salts, and peptides, which are often used as adjuvants in vaccines [21, 22].

1.2. Peptide-based therapies

Anticancer strategies based on peptides have several advantages over other chemotherapeutic approaches, like being nongenotoxic or possessing adjuvant properties; they also have a strong specificity, high affinity, good tissue penetration, and low toxicity with respect to small-molecule drugs and monoclonal antibodies [23–26]. Examples of anticancer peptides are (1) necrotic peptides (some of them are expressed in a wide diversity of species, including insects, fish, amphibians, and mammals, e.g., cecropins A and B found in mammals and various insects) [27]; (2) apoptotic peptides, cationic peptides known as host defense peptides (HDP) such as the bovine lactoferricin, magainin 2, hCAP109-135 (comprising the C-terminal

domain of human CAP18), and BMAP-28 from bovine myeloid cathelicidin [28–31]; (3) blocking peptides; (4) receptor-interacting peptides; (5) peptides that bind to cell-adhesion proteins; (6) protein kinase inhibitors; (7) protease inhibitors; (8) peptides with antiangiogenic properties; and (9) peptides with immunostimulatory activity [27].

With regard to receptor-interacting peptides, compounds like CpG, imiquimod, poly I:C (toll-like receptor (TLR) agonists), α -GalCer (glycolipid ligands), GM-CSF, IL-2, and IFN α/β have antitumoral activity, as well as adjuvant properties [32–34]. These compounds are capable of directly or indirectly enhancing APC functions and T effector activity. In this sense, some of the most employed immunotherapeutic agents in polytherapy induce the effector function of tumor microenvironment (TM)-associated T cells and macrophages [35–38]. For instance, CpG was a promising cancer immunotherapy adjuvant due to its capacity to induce a Th1 immune response and activate APCs through TLR9 signaling [35–38]; however, it failed to stimulate the immune response in clinical trials [39]. The identification of new adjuvants showing low toxicity and capable of stimulating a cellular Th1 response in humans would be a great advancement in the development of vaccines for infectious and noninfectious diseases such as cancer [40].

Unfortunately, several immunostimulators have failed to revert the immunosuppressive conditions in TM. For example, IL-2, IL-12, GM-CSF [41–44], and immunological adjuvants administered with highly immunogenic antigens like incomplete Freund's adjuvant, bacillus Calmette-Guerin [BCG], and MF59 have shown disappointing results [43, 44]. Moreover, these compounds have been associated with toxic effects [45–47].

2. Identification of the GK-1 peptide

Based on the nonspecific reactivity and immunopotentiator properties of GK-1, our group has been studying it as a promising adjuvant for cancer immunotherapy. This 18-amino acid peptide was first derived from the KETc7 protein, isolated from a *Taenia crassiceps* cysticercus cDNA library [48]; KETc7 is part of a broad family of proteins associated with membrane processes [49]. When searching for T cell epitopes *in silico*, GK-1 exhibited a strong association with MHC-I and, to a lesser extent, with MHC-II [49]. The immunomodulatory properties of GK-1 are associated with an efficient activation of cells involved in antigen presentation (such as DCs) by promoting the expression of the costimulatory molecules CD86 and MHC-II, as well as the secretion of soluble pro-inflammatory factors like IFN- γ , TNF- α , and CCL2 [50]. GK-1-treated DCs enhanced the proliferative response of antigen-specific CD4⁺ T cells both *in vivo* and *in vitro* [50]. GK-1 also induced the proliferation of CD8⁺ T cells and higher IFN- γ levels [51] even in the absence of adjuvant [52]. Considering that this peptide can promote APC function and enhance Th1 cell effector pathways, its capacity as an adjuvant of the influenza vaccine was evaluated. GK-1 increased the levels of specific IgG antibodies *in vivo*, before and after infection, in a murine model of influenza in elderly mice [53], favoring virus clearance after infection in both young and aged mice, which could be associated with an early infiltrate of mononuclear cells (lymphocytes and macrophages) to the lung parenchyma following the GK-1 peptide co-administration. Furthermore, lung histological examination showed better preserved alveolar spaces and less congested alveolar walls with respect to the vaccine-only animals [53].

3. GK-1 as an anticancer immunotherapy

In neoplasms, the host is often immunocompromised due to the presence of immunosuppressive cells and molecules in the TM, to prevent the removal of cancer cells [2]. This highlights the relevance of stimulating the host immune response against cancer antigens by administering immunoadjuvants along with chemotherapy, radiotherapy, or surgery [54]. In this regard, small peptides with a nonspecific immunostimulatory response like GK-1, long known to act as vaccine adjuvants, are potentially useful in cancer therapy. The antitumor effect of GK-1 has been studied in melanoma and breast cancer murine models.

3.1. GK-1 in a mouse melanoma model

Melanoma is the most malignant form of skin cancer, mainly affecting the Caucasian population [55, 56]. Until recently, systemic therapy for metastatic melanoma had been inefficient, with a 5-year survival rate for patients (<30%) [57, 58]. However, new therapies were recently approved to treat melanoma, such as pegylated-interferon- α 2b (IFN- α 2b) in the adjuvant setting; ipilimumab, an anti-CTLA4 monoclonal antibody, for metastatic disease; vemurafenib, an oral BRAF inhibitor indicated for patients with metastatic melanoma harboring BRAFV600 mutations, and more recently antibodies against PD-1 like pembrolizumab [59–61] and antibodies blocking PD-L1 pathways, as well as inhibitors of the mitogen-activated protein kinase (MAPK) pathway. Additionally, nonspecific immunomodulation by several cytokines (IL-2, IL-12, TNF- α , and IFN- γ) and TLR ligands [62–64] in addition to adoptive transfer approaches have been widely used [65]. For over a decade, DCs have also been used in immunotherapy against various types of cancer [66–68] as an alternative to chemotherapy, by vaccination with DCs loaded with tumor peptides (i.e., MAGE-AX [69–72] and/or with necrotic or apoptotic tumor cells to induce effector tumor-specific T cells [73, 74]). The efficacy of this immunotherapeutic approach was also evaluated against murine melanoma, using GK-1 as an immunostimulant.

GK-1 has been reported to increase the mean survival and significantly delays tumor growth in a melanoma model with B16-F10 cells, showing more necrotic areas along with the presence of numerous neutrophils (**Figure 1**). Neutrophilia inside pulmonary blood vessels was also observed, without evidence of macroscopic or microscopic metastasis. In a melanoma lung metastatic model, GK-1 decreased lymphocyte count, while increased the number of neutrophils and decreased the serum levels of IFN- γ ; on the other hand, an increase in the levels of IFN- γ and IL-12 in the intratumor (lung metastases) environment, along with a decrease in IL-17, IL-4, IL-22, and IL-23 was also observed [75, 76]. The antitumor activities of IL-12 have been established in preclinical studies against various tumor cell lines; the increased concentration of the antitumor cytokine IL-12 found in primary tumors may enhance the damage to tumor cells, limiting the number of cancer cells detaching from the primary tumor [77–79]. Its antitumor activity is also mediated by the induction of IFN- γ [67, 78, 80], which upregulated the expression of MHC-I and -II by B16 cells *in vitro*, favoring a cytolytic response in MHC-I-restricted CTL (**Figure 1**) [81]. There is a consensus that the induction of a Th1 profile or the release of cytokines like IFN- γ and TNF- α by T cells is essential for an effective antitumor immune response in

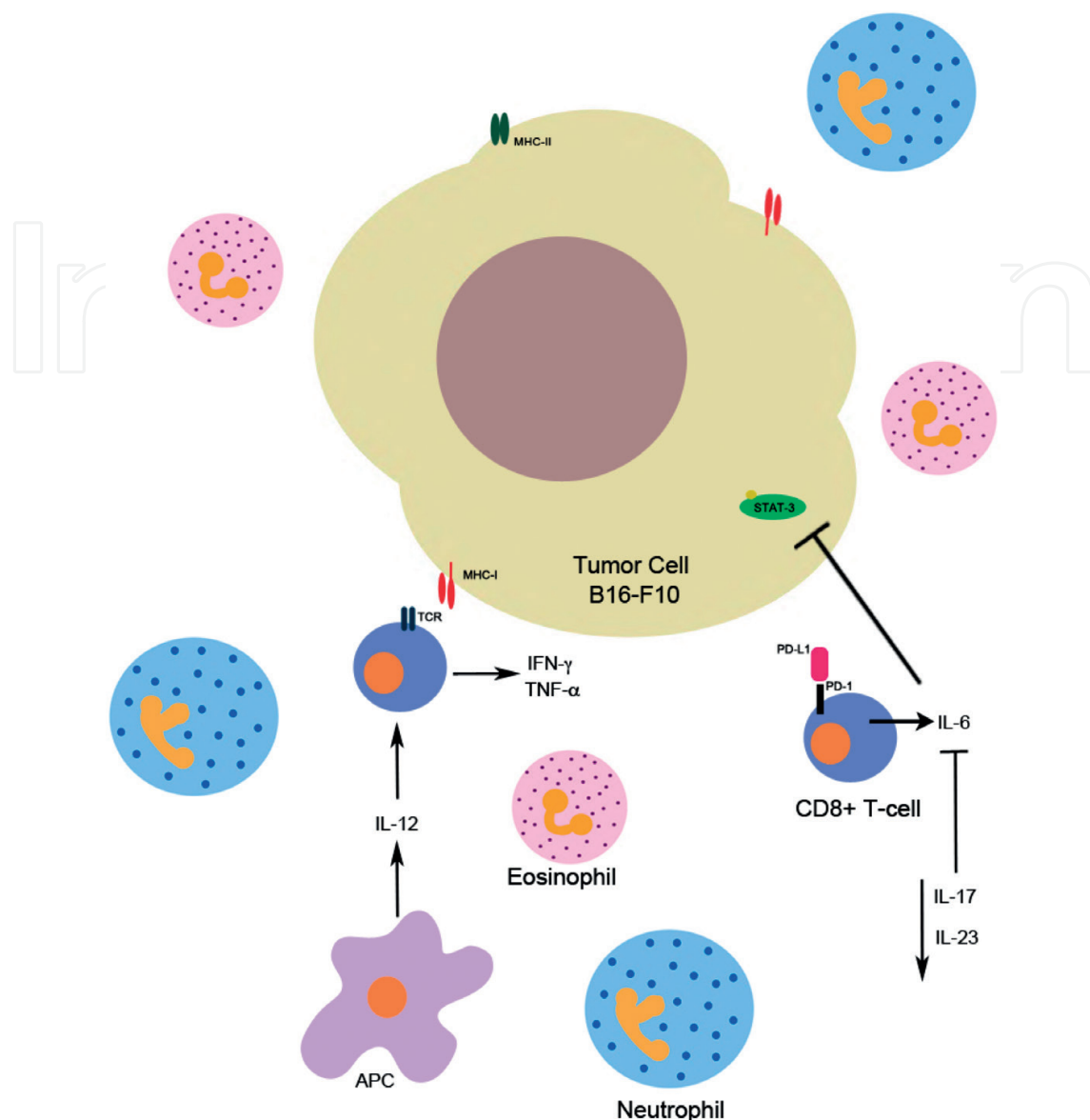


Figure 1. GK-1 in a preclinical mouse melanoma model. In a melanoma murine model with B16-F10 cells, GK-1 led to an increase in neutrophils with the increase of IFN- γ and IL-12 cytokines, along with a decrease in IL-17 and IL-23. On the other hand, the MAGE-AX/GK-1 treatment showed an increase in areas of cell death, characterized by eosinophilic regions and production of IFN- γ by CD8+ T-cells.

melanoma [82–84]. In fact, IFN- γ released from CTLs has been considered as a potent mediator of the antitumor response in bulky melanoma tumors [17, 85]. In contrast, IL-17 was proved to directly promote tumor growth and angiogenesis [86–88]. Indeed, it has been shown that IL-17 can promote tumor growth by a direct effect on IL-6 induction, which in turn activates STAT3 in both tumor and nontransformed cells in the TM [89]. Finally, IL-23 is an important molecular driver of Th17 cells in humans; IL-23 is increased in several tumors, and the expression of this cytokine antagonistically regulates local inflammatory responses in the TM, as well as the infiltration of epithelial lymphocytes [80]. Thus, the intratumoral subexpression of IL-17 and IL-23 in GK-1-treated mice could explain the reduced tumor progression (**Figure 1**).

Considering the capacity of GK-1 to enhance DC activation [50], BMDCs matured with TNF- α and stimulated with GK-1 and MAGE-AX were administered to tumor-bearing mice in the melanoma model with B16-F10 cells; the treatment with MAGE-AX/GK-1 increased survival rates, while mice receiving GK-1 alone had a smaller increase in survival. Moreover, the combination MAGE-AX/GK-1 significantly delayed tumor growth and increased cell death areas, characterized by eosinophilic regions within melanomas. Similarly, both GK-1 alone and MAGE-AX/GK-1 increased the production of IFN- γ -producing CD8 cells, while GK-1 increased the percentage of IL-10 producing CD8+ T-cells [90]. The effect of MAGE-AX/GK-1 could be associated with higher levels of CD8+ lymphocytes in peritumoral lymph nodes, which have been correlated with the survival of patients suffering from melanoma and other cancer types [67, 91, 92].

Vera-Aguilera et al. [79] hypothesized that a combined GK1/anti-PD-L1 therapy could synergize and maximize the individual antitumor effect and extend survival. An increased survival was observed in mice treated with GK1/anti-PD-L1, as well as in mice treated with GK-1 or PD-L1 alone. Animals treated with GK1/anti-PD-L1 had smaller tumor masses. Additionally, GK1/anti-PD-L1 decreased the serum levels of IL-4, IL-5, IL-6, and IL-10. The mechanism by which the combined GK1/anti-PD-L1 treatment improved survival rates remains to be determined; however, the expression of PD-1 on T cells has been proved to be upregulated by IL-6 through the signal transducer and activator of transcription 3 (STAT3) [93], a point of convergence for several oncogenic signaling pathways leading to the expression of immunosuppressing molecules [94]. Similarly, the expression of PD-L1 and PD-L2 is also upregulated by numerous mechanisms, including the production of IL-4 and GM-CSF [93]. All these findings point to a possible synergistic mechanism associated with the reversion from an exhausted phenotype.

3.2. GK-1 in a breast cancer model

Considering the evidence described above, it is now clear that changes in the microenvironment could induce an antitumor response against the primary tumor and reduce the metastatic disease, which could allow us to control cancer progression. In this regard, immunomodulators like GK-1 can be used as anticancer therapies. In 2017, GK-1 was evaluated in a murine model of invasive breast adenocarcinoma, which spontaneously metastasizes to the lungs, liver, brain, and bone, similarly to breast cancer in humans [95–98]. GK-1 was associated with an increased survival in 4T1 tumor-bearing mice and a reduction in the primary tumor volume rate, which was accompanied by an increase of tumor cell death areas with morphologic features associated with necrosis (pyknosis, karyorrhexis, and karyolysis) and apoptosis (apoptotic bodies) at the primary tumors. These findings, along with an increase in IL-12 concentration in the primary tumor, denote deep changes in the TM induced by GK-1 [98], which could involve the infiltration of TCD8+, NK, and NKT cells in the primary tumor [77, 99, 100] (**Figure 2**). As described in the previous section, IL-12 has been associated with antitumor and antiangiogenic activities [100, 101], due to its capacity of inducing the infiltration of TCD8+ cells within tumor tissues [100]. In fact, it has been reported that a combined treatment with tamoxifen and IL-12 enhanced tumor inhibition due to an increase in apoptosis, and reduced tumor growth in a 4T1 cancer murine model [100].

Those changes suppose a TM that could reduce the tumor growth rate, and the concomitant reduction of cancer cell egress by detachment from the primary tumor, which allows them to invade the stroma and break the basement membrane. These changes could explain the reduction of pulmonary metastasis associated with the GK-1 treatment [98]. Additionally, changes in lung microenvironment associated with the GK-1 treatment have been reported. In this sense, a reduction in the concentration of b-FGF, CCL-3, GM-CSF, CCL-2, TNF- α , and CXCL-9, along with an increased concentration of IL-6 has been found [98] (**Figure 2**). These changes could reduce metastasis development, possibly by inhibiting the proliferation of cells that are essential for the growth of secondary tumors, such as macrophage-associated metastasis (MAM) and MDSC [102, 103]. Considering these results, GK-1 could change the tumor microenvironment, inducing an active antitumoral immune response that could lead to a decrease in cancer burden.

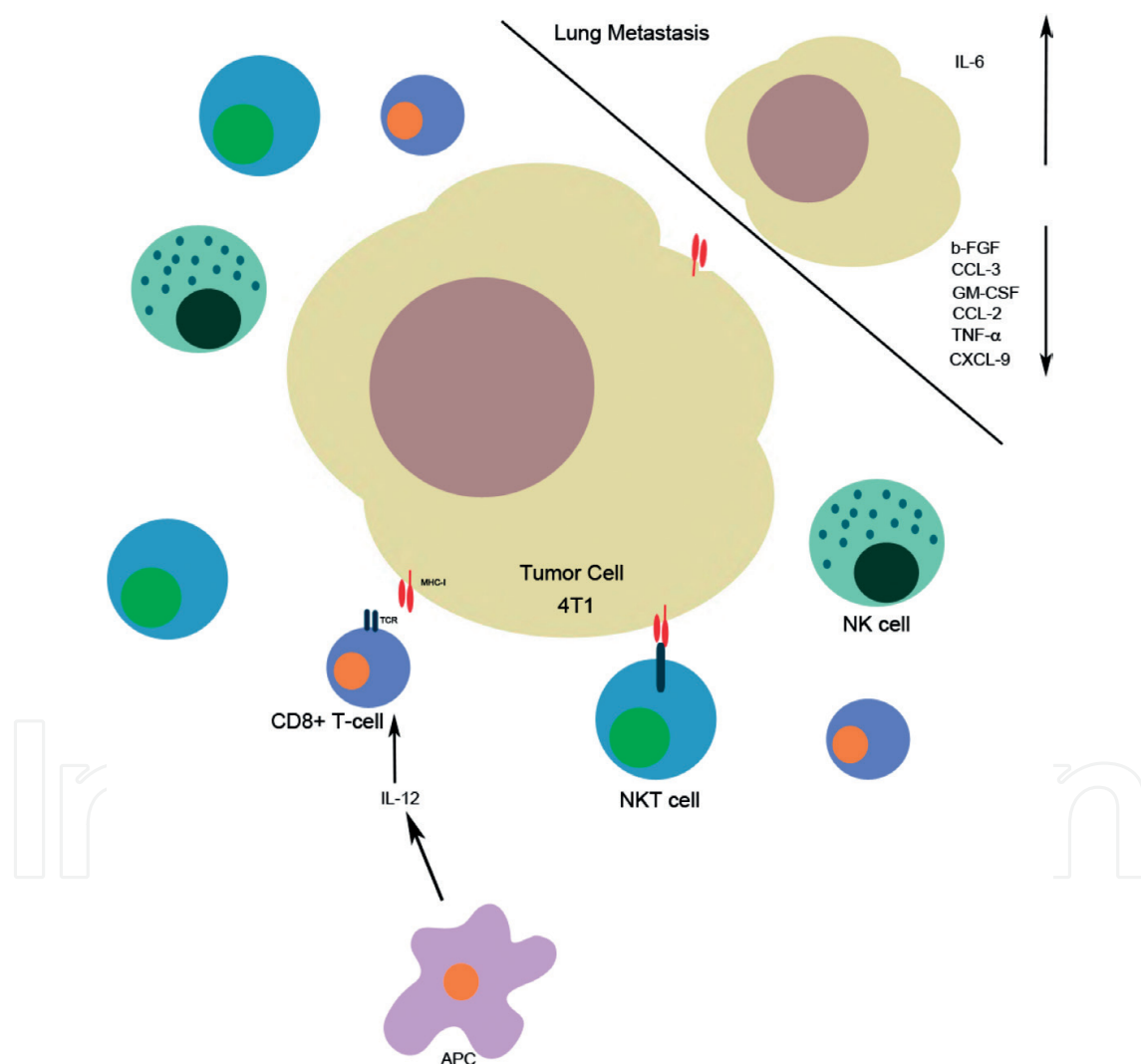


Figure 2. GK-1 in a breast cancer model. GK-1 was associated with an increased IL-12 concentration in the primary tumor, which could involve the infiltration of CD8+ T-cells, NK, and NKT cells. IL-12 is a cytokine produced principally by APC, such as monocytes, macrophages, and dendritic cells. This cytokine can induce specific CD8+ T-cells that are primed against tumor antigens and could serve as a tumor-specific CTL. Additionally, in the lungs, the GK-1-treatment induces a reduction in the concentration of b-FGF, CCL-3, GM-CSF, CCL-2, TNF- α , and CXCL-9, along with an increased concentration of IL-6, which correlates with a minor lung-metastatic burden.

4. Discussion

The ability of the GK-1 peptide to increase survival, significantly to delay tumor growth, and to reduce metastasis is discussed in this review. Considering that the immune system plays a crucial role in the outcome of cancer, orchestrating the response that may lead either to the control or dissemination of tumors [8, 78, 104], understanding the mechanisms that underlie the efficient response to the peptide is imperative.

It has been reported that the production of pro-inflammatory cytokines both by tumor and surrounding cells, along with the production of growth factors and chemokines, can promote the development of neoplasia by facilitating carcinogenesis programs, inducing a sustained cellular proliferative rate, inhibiting apoptosis and stimulating angiogenesis [105, 106]. As described above, GK-1 therapy contributed to decrease the levels of IL-4, IL-10, b-FGF, and GM-CSF; these chemoattractants, along with hypoxia, promote macrophage shift from a M1 to a M2 phenotype. M2-like tumor-associated macrophages (TAM) stimulate immunosuppression and increase blood vessel density, favoring angiogenesis. In a breast cancer model, lower CCL2 and CCL3 levels in the lungs of mice treated with GK-1 could be decreasing the migration of inflammatory monocytes such as MAM and MDSC, which promote metastasis [8, 13, 102, 107]. These changes in the microenvironment seem to contribute to control tumor burden and metastasis.

On the other hand, M1-like macrophages can contribute to tumor regression by recruiting cytotoxic CD8⁺ T (CTL) and NK cells [108–110]. In this regard, IL-12 induction by APCs could be contributing to the increase in the proliferation of CD8⁺ and CD4⁺ lymphocytes and the induction of a Th1 response, as previously reported [51, 52, 111]. Several studies have suggested a correlation of higher density levels of cytotoxic (CTL) and memory T lymphocytes (CD3⁺ CD45RO⁺) infiltrated in the primary tumor with increased survival rates of patients with different types of neoplasms [91, 112–116].

According to recent findings, the GK-1 peptide can induce a M1 phenotype and promote the efficient activation of DCs, which could be leading to the maintenance of an effector response against tumor growth, capable of counteracting the immunosuppressive response due to T cell exhaustion or DC dysfunction.

5. Conclusions

Considering the possible mechanisms of action of GK-1 and the information available, we propose that this peptide can decrease tumor growth and metastasis by changing the tumor microenvironment. GK-1 appears to reactivate the immune system affected by the tumor-associated suppressive microenvironment, thereby allowing immune cells to become activated. Although more studies focusing on the anticancer effect of GK-1 are required, this research gives new evidence on the possible clinical uses of GK-1 beyond its well-established adjuvant effect.

These results have also provided us with the rationale to evaluate the effectiveness of the GK-1 immunotherapy to revert the exhaustion of peripheral T-cells in several types of cancer.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

Abbreviations

APC	antigen-presenting cell
CCL-22	chemokine ligand-22
CTL	cytotoxic T lymphocytes
CTLA-4	cytotoxic T-lymphocyte antigen 4
DC	dendritic cell
HDP	host defense peptides
LAG-3	lymphocyte-activation gene 3
MDSC	myeloid-derived suppressor cells
NK	natural killer cells
PAMPs	pathogen-associated molecular patterns
PD-1	programmed cell death 1
PD-L1	programmed death-ligand 1
Treg	regulatory T cell
STAT3	signal transducer and activator of transcription 3
TM	tumor microenvironment
VEGF	vascular endothelial growth factor
VEGFR-2	vascular endothelial growth factor receptor 2

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References

- [1] Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 2004;**21**(2):137-148. DOI: 10.1016/j.immuni.2004.07.017
- [2] Swann JB, Smyth MJ. Immune surveillance of tumors. *The Journal of Clinical Investigation*. 2007;**117**(5):1137-1146. DOI: 10.1172/JCI31405
- [3] Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology*. 2007;**121**(1):1-14. DOI: 10.1111/j.1365-2567.2007.02587.x
- [4] Vesely MD, Schreiber RD. Cancer immunoediting: Antigens, mechanisms, and implications to cancer immunotherapy. *Annals of the New York Academy of Sciences*. 2013; **1284**:1-5. DOI: 10.1111/nyas.12105
- [5] Khodadoust MS, Alizadeh AA. Tumor antigen discovery through translation of the cancer genome. *Immunologic Research*. 2014;**58**(2-3):292-299. DOI: 10.1007/s12026-014-8505-4
- [6] Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nature Reviews. Immunology*. 2006;**6**(4):295-307. DOI: 10.1038/nri1806
- [7] Kim PS, Ahmed R. Features of responding T cells in cancer and chronic infection. *Current Opinion in Immunology*. 2010;**22**:223-230. DOI: 10.1016/j.coi.2010.02.005
- [8] Bennaceur K, Chapman J, Brikci-Nigassa L, Sanhadji K, Touraine JL, Portoukalian J. Dendritic cells dysfunction in tumour environment. *Cancer Letters*. 2008;**272**(2):186-196. DOI: 10.1016/j.canlet.2008.05.017
- [9] Li Q, Li Q, Chen J, Liu Y, Zhao X, Tan B, Ai J, Zhang Z, Song J, Shan B. Prevalence of Th17 and Treg cells in gastric cancer patients and its correlation with clinical parameters. *Oncology Reports*. 2013;**30**(3):1215-1222. DOI: 10.3892/or.2013.2570
- [10] Jie HB, Schuler PJ, Lee SC, Srivastava RM, Argiris A, Ferrone S, Whiteside TL, Ferris RL. CTLA-4⁺ regulatory T cells increased in cetuximab-treated head and neck cancer patients suppress NK cell cytotoxicity and correlate with poor prognosis. *Cancer Research*. 2015;**75**(11):2200-2210. DOI: 10.1158/0008-5472.CAN-14-2788

- [11] Santoiemma PP, Powell DJ Jr. Tumor infiltrating lymphocytes in ovarian cancer. *Cancer Biology & Therapy*. 2015;**16**(6):807-820. DOI: 10.1080/15384047.2015.1040960
- [12] Chen J, Chen Z. The effect of immune microenvironment on the progression and prognosis of colorectal cancer. *Medical Oncology*. 2014;**31**(8):82. DOI: 10.1007/s12032-014-0082-9
- [13] Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nature Immunology*. 2018;**19**(2):108-119. DOI: 10.1038/s41590-017-0022-x
- [14] Mlecnik B, Tosolini M, Charoentong P, Kirilovsky A, Bindea G, Berger A, Camus M, Gillard M, Bruneval P, Fridman WH, Pagès F, Trajanoski Z, Galon J. Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. *Gastroenterology*. 2010;**138**(4):1429-1440. DOI: 10.1053/j.gastro.2009.10.057
- [15] Angell H, Galon J. From the immune contexture to the Immunoscore: The role of prognostic and predictive immune markers in cancer. *Current Opinion in Immunology*. 2013;**25**(2):261-267. DOI: 10.1016/j.coi.2013.03.004
- [16] Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: Prognostic, predictive, and mechanistic signatures. *Immunity*. 2013;**39**(1):11-26. DOI: 10.1016/j.immuni.2013.07.008
- [17] Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science*. 2002;**298**(5594):850-854. DOI: 10.1126/science.1076514
- [18] Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: Moving beyond current vaccines. *Nature Medicine*. 2004;**10**(9):909-915. DOI: 10.1038/nm1100
- [19] Ishizuka M, Kawatsu M, Yamashita T, Ueno M, Takeuchi T. Low molecular weight immunomodulators produced by microorganisms. *International Journal of Immunopharmacology*. 1995;**17**(2):133-139. DOI: 10.1016/0192-0561(94)00086-4
- [20] Fauci AS, Rosenberg SA, Sherwin SA, Dinarello CA, Longo DL, Lane HC. Immunomodulators in clinical medicine. NIH conference. *Annals of Internal Medicine*. 1987;**106**(3):421-433. DOI: 10.7326/0003-4819-106-3-421
- [21] Bellanti J. Immunology IV: Clinical applications in health and disease. *World Allergy Organization Journal*. 2012;**5**(8):94. DOI: 10.1097/WOX.0b013e3182641db0
- [22] Pichichero ME. Improving vaccine delivery using novel adjuvant systems. *Human Vaccines*. 2008;**4**(4):262-270
- [23] Janin YL. Peptides with anticancer use or potential. *Amino Acids*. 2003;**25**(1):1-40. DOI: 10.1007/s00726-002-0349-x
- [24] Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. *Expert Opinion on Investigational Drugs*. 2006;**15**(8):933-946. DOI: 10.1517/13543784.15.8.933

- [25] Rüegg C, Hasmim M, Lejeune FJ, Alghisi GC. Antiangiogenic peptides and proteins: From experimental tools to clinical drugs. *Biochimica et Biophysica Acta*. 2006;**1765**(2):155-177. DOI: 10.1016/j.bbcan.2005.09.003
- [26] Kaplan N, Morpurgo N, Linial M. Novel families of toxin-like peptides in insects and mammals: A computational approach. *Journal of Molecular Biology*. 2007;**369**(2):553-566. DOI: 10.1016/j.jmb.2007.02.106
- [27] Bhutia SK, Maiti TK. Targeting tumors with peptides from natural sources. *Trends in Biotechnology*. 2008;**26**(4):210-217. DOI: 10.1016/j.tibtech.2008.01.002
- [28] Risso A, Braidot E, Sordano MC, Vianello A, Macrì F, Skerlavaj B, Zanetti M, Gennaro R, Bernardi P. BMAP-28, an antibiotic peptide of innate immunity, induces cell death through opening of the mitochondrial permeability transition pore. *Molecular and Cellular Biology*. 2002;**22**(6):1926-1935. DOI: 10.1128/MCB.22.6.1926-1935.2002
- [29] Okumura K, Itoh A, Isogai E, Hirose K, Hosokawa Y, Abiko Y, Shibata T, Hirata M, Isogai H. C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells. *Cancer Letters*. 2004;**212**(2):185-194. DOI: 10.1016/j.canlet.2004.04.006
- [30] Lehmann J, Retz M, Sidhu SS, Suttman H, Sell M, Paulsen F, Harder J, Unteregger G, Stöckle M. Antitumor activity of the antimicrobial peptide magainin II against bladder cancer cell lines. *European Urology*. 2006;**50**(1):141-147. DOI: 10.1016/j.eururo.2005.12.043
- [31] Mader JS, Richardson A, Salsman J, Top D, de Antueno R, Duncan R, Hoskin DW. Bovine lactoferricin causes apoptosis in Jurkat T-leukemia cells by sequential permeabilization of the cell membrane and targeting of mitochondria. *Experimental Cell Research*. 2007;**313**(12):2634-2650. DOI: 10.1016/j.yexcr.2007.05.015
- [32] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double stranded RNA and activation of NF-kappaB by toll-like receptor 3. *Nature*. 2001;**413**(6857):732-738. DOI: 10.1038/35099560
- [33] Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annual Review of Immunology*. 2002;**20**:709-760. DOI: 10.1146/annurev.immunol.20.100301.064842
- [34] Netea MG, van der Graaf C, Van der Meer JW, Kullberg BJ. Toll-like receptors and the host defense against microbial pathogens: Bringing specificity to the innate-immune system. *Journal of Leukocyte Biology*. 2004;**75**(5):749-755. DOI: 10.1189/jlb.1103543
- [35] Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. *The Journal of Experimental Medicine*. 1997;**186**(10):1623-1631. DOI: 10.1084/jem.186.10.1623
- [36] Weiner GJ, Liu HM, Wooldridge JE, Dahle CE, Krieg AM. Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**(20):10833-10837. DOI: 10.1073/pnas.94.20.10833

- [37] Krug A, Towarowski A, Britsch S, Rothenfusser S, Hornung V, Bals R, Giese T, Engelmann H, Endres S, Krieg AM, Hartmann G. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *European Journal of Immunology*. 2001;**31**(10):3026-3037. DOI: 10.1002/1521-4141(2001010)31:10<#60;3026::AID-IMMU3026>#62;3.0.CO;2-H
- [38] Krieg AM. Therapeutic potential of toll-like receptor 9 activation. *Nature Reviews. Drug Discovery*. 2006;**5**(6):471-484. DOI: 10.1038/nrd2059
- [39] Schmidt C. Clinical setbacks for toll-like receptor 9 agonists in cancer. *Nature Biotechnology*. 2007;**25**(8):825-826. DOI: 10.1038/nbt0807-825
- [40] Mocellin S, Pilati P, Nitti D. Peptide-based anticancer vaccines: Recent advances and future perspectives. *Current Medicinal Chemistry*. 2009;**16**(36):4779-4796. DOI: 10.2174/092986709789909648
- [41] Borrello I, Pardoll D. GM-CSF-based cellular vaccines: A review of the clinical experience. *Cytokine & Growth Factor Reviews*. 2002;**13**(2):185-193. DOI: 10.1016/S1359-6101(01)00034-X
- [42] Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine & Growth Factor Reviews*. 2002;**13**(2):155-168. DOI: 10.1016/S1359-6101(01)00032-6
- [43] Mocellin S, Mandruzzato S, Bronte V, Lise M, Nitti D. Part I: Vaccines for solid tumours. *The Lancet Oncology*. 2004;**5**(11):681-689. DOI: 10.1016/S1470-2045(04)01610-9
- [44] Choudhury A, Mosolits S, Kokhaei P, Hansson L, Palma M, Mellstedt H. Clinical results of vaccine therapy for cancer: Learning from history for improving the future. *Advances in Cancer Research*. 2006;**95**:147-202. DOI: 10.1016/S0065-230X(06)95005-2
- [45] Ribas A, Amarnani SN, Buga GM, Butterfield LH, Disette VB, McBride WH, Glaspy JA, Ignarro LJ, Economou JS. Immunosuppressive effects of interleukin-12 coexpression in melanoma antigen gene-modified dendritic cell vaccines. *Cancer Gene Therapy*. 2002;**9**(11):875-883. DOI: 10.1038/sj.cgt.7700512
- [46] Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Research*. 2004;**64**(17):6337-6343. DOI: 10.1158/0008-5472.CAN-04-0757
- [47] Parmiani G, Castelli C, Pilla L, Santinami M, Colombo MP, Rivoltini L. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Annals of Oncology*. 2007;**18**(2):226-232. DOI: 10.1093/annonc/mdl158
- [48] Gevorkian G, Manoutcharian K, Larralde C, Hernandez M, Almagro JC, Viveros M, Sotelo J, Garcia E, Sciutto E. Immunodominant synthetic peptides of *Taenia crassiceps* in murine and human cysticercosis. *Immunology Letters*. 1996;**49**(3):185-189. DOI: 10.1016/0165-2478(96)02503-5

- [49] Bobes RJ, Navarrete-Perea J, Ochoa-Leyva A, Anaya VH, Hernández M, Cervantes-Torres J, Estrada K, Sánchez-Lopez F, Soberón X, Rosas G, Nunes CM, García-Varela M, Sotelo-Mundo RR, López-Zavala AA, Gevorkian G, Acero G, Laclette JP, Fragoso G, Sciutto E. Experimental and theoretical approaches to investigate the immunogenicity of *Taenia solium*-derived KE7 antigen. *Infection and Immunity*. 2017;**85**(12):e00395-e00317. DOI: 10.1128/IAI.00395-17
- [50] Segura-Velázquez R, Fragoso G, Sciutto E, Sarukhan A. Towards identification of the mechanisms of action of parasite-derived peptide GK1 on the immunogenicity of an influenza vaccine. *Clinical and Vaccine Immunology*. 2009;**16**(9):1338-1343. DOI: 10.1128/CVI.00106-09
- [51] Toledo A, Larralde C, Fragoso G, Gevorkian G, Manoutcharian K, Hernández M, Acero G, Rosas G, López-Casillas F, Garfias CK, Vázquez R, Terrazas I, Sciutto E. Towards a *Taenia solium* cysticercosis vaccine: An epitope shared by *Taenia crassiceps* and *Taenia solium* protects mice against experimental cysticercosis. *Infection and Immunity*. 1999;**67**(5):2522-2530
- [52] Fragoso G, Esquivel-Guadarrama F, Santana MA, Bobes RJ, Hernández B, Cervantes J, Segura R, Goldbaum FA, Sciutto E, Rosas G. Heterologous prime-boost oral immunization with GK-1 peptide from *Taenia crassiceps* cysticerci induces protective immunity. *Clinical and Vaccine Immunology*. 2011;**18**(7):1067-1076. DOI: 10.1128/CVI.05030-11
- [53] Segura-Velázquez R, Pérez-Torres A, Rosas G, Toledo A, Restelli M, Acosta E, Corral R, Rosetti F, Fragoso G, Grinstein S, Sciutto E. A novel synthetic adjuvant effectively enhances the immunogenicity of the influenza vaccine. *Vaccine*. 2006;**24**(8):1073-1080. DOI: 10.1016/j.vaccine.2005.09.014
- [54] Ocaña MC, Martínez-Poveda B, Quesada AR, Medina MÁ. Metabolism within the tumor microenvironment and its implication on cancer progression: An ongoing therapeutic target. *Medicinal Research Reviews*. 2018. DOI: 10.1002/med.21511
- [55] Brown CK, Kirkwood JM. Medical management of melanoma. *The Surgical Clinics of North America*. 2003;**83**(2):283-322, viii. DOI: 10.1016/S0039-6109(02)00187-1
- [56] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*. 2018;**68**(1):7-30. DOI: 10.3322/caac.21442
- [57] Balch CM, Soong SJ, Atkins MB, Buzaid AC, Cascinelli N, Coit DG, Fleming ID, Gershenwald JE, Houghton A Jr, Kirkwood JM, McMasters KM, Mihm MF, Morton DL, Reintgen DS, Ross MI, Sober A, Thompson JA, Thompson JF. An evidence-based staging system for cutaneous melanoma. *CA: A Cancer Journal for Clinicians*. 2004;**54**(3):131-149. DOI: 10.3322/canjclin.54.3.131
- [58] Miao Y, Hylarides M, Fisher DR, Shelton T, Moore H, Wester DW, Fritzberg AR, Winkelmann CT, Hoffman T, Quinn TP. Melanoma therapy via peptide-targeted {alpha}-radiation. *Clinical Cancer Research*. 2005;**11**(15):5616-5621. DOI: 10.1158/1078-0432.CCR-05-0619

- [59] Eggermont AM, Suci S, Santinami M, Testori A, Kruit WH, Marsden J, Punt CJ, Salès F, Gore M, Mackie R, Kusic Z, Dummer R, Hauschild A, Musat E, Spatz A, Keilholz U, EORTC melanoma group. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: Final results of EORTC 18991, a randomised phase III trial. *Lancet*. 2008;**372**(9633):117-126. DOI: 10.1016/S0140-6736(08)61033-8
- [60] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England Journal of Medicine*. 2010;**363**(8):711-723. DOI: 10.1056/NEJMoa1003466
- [61] Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, GA MA, BRIM-3 study group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *The New England Journal of Medicine*. 2011;**364**(26):2507-2516. DOI: 10.1056/NEJMoa1103782
- [62] Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews. Cancer*. 2004;**4**(1):11-22. DOI: 10.1038/nrc1252
- [63] Adams S. Toll-like receptor agonists in cancer therapy. *Immunotherapy*. 2009;**1**(6): 949-964. DOI: 10.2217/imt.09.70
- [64] Gnjjatic S, Sawhney NB, Bhardwaj N. Toll-like receptor agonists: Are they good adjuvants? *Cancer Journal*. 2010;**16**(4):382-391. DOI: 10.1097/PPO.0b013e3181eaca65
- [65] Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: A clinical path to effective cancer immunotherapy. *Nature Reviews. Cancer*. 2008;**8**(4):299-308. DOI: 10.1038/nrc2355
- [66] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998;**392**(6673):245-252. DOI: 10.1038/32588
- [67] Palucka AK, Ueno H, Connolly J, Kerneis-Norvell F, Blanck JP, Johnston DA, Fay J, Banchereau J. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. *Journal of Immunotherapy*. 2006;**29**(5):545-557. DOI: 10.1097/01.cji.0000211309.90621.8b
- [68] Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nature Reviews. Cancer*. 2012;**12**(4):265-277. DOI: 10.1038/nrc3258
- [69] Mackensen A, Herbst B, Chen JL, Köhler G, Noppen C, Herr W, Spagnoli GC, Cerundolo V, Lindemann A. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. *International Journal of Cancer*. 2000;**86**(3):385-392. DOI: 10.1002/(SICI)1097-0215(20000501)86:3<385::AID-IJC13>3.0.CO;2-T

- [70] Schuler-Thurner B, Schultz ES, Berger TG, Weinlich G, Ebner S, Woerl P, Bender A, Feuerstein B, Fritsch PO, Romani N, Schuler G. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. *The Journal of Experimental Medicine*. 2002;**195**(10):1279-1288. DOI: 10.1084/jem.20012100
- [71] Slingluff CL Jr, Petroni GR, Yamshchikov GV, Barnd DL, Eastham S, Galavotti H, Patterson JW, Deacon DH, Hibbitts S, Teates D, Neese PY, Grosh WW, Chianese-Bullock KA, Woodson EM, Wiernasz CJ, Merrill P, Gibson J, Ross M, Engelhard VH. Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. *Journal of Clinical Oncology*. 2003;**21**(21):4016-4026. DOI: 10.1200/JCO.2003.10.005
- [72] Trepiaakas R, Berntsen A, Hadrup SR, Bjørn J, Geertsen PF, Straten PT, Andersen MH, Pedersen AE, Soleimani A, Lorentzen T, Johansen JS, Svane IM. Vaccination with autologous dendritic cells pulsed with multiple tumor antigens for treatment of patients with malignant melanoma: Results from a phase I/II trial. *Cytotherapy*. 2010;**12**(6):721-734. DOI: 10.3109/14653241003774045
- [73] Fields RC, Shimizu K, Mulé JJ. Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;**95**(16):9482-9487. DOI: 10.1073/pnas.95.16.9482
- [74] Cornforth AN, Fowler AW, Carbonell DJ, Fan E, Dillman RO. Characterization of interferon- γ -treated melanoma tumor cells for use in dendritic cell-based immunotherapy. *Cancer Biotherapy & Radiopharmaceuticals*. 2011;**26**(3):345-351. DOI: 10.1089/cbr.2011.0959
- [75] Pérez-Torres A, Vera-Aguilera J, Hernaiz-Leonardo JC, Moreno-Aguilera E, Monteverde-Suarez D, Vera-Aguilera C, Estrada-Bárcenas D. The synthetic parasite-derived peptide GK1 increases survival in a preclinical mouse melanoma model. *Cancer Biotherapy & Radiopharmaceuticals*. 2013;**28**(9):682-690. DOI: 10.1089/cbr.2012.1438
- [76] Pérez-Torres A, Vera-Aguilera J, Sahaza JH, Vera-Aguilera C, Moreno-Aguilera E, Pulido-Camarillo E, Nuñez-Ochoa L, Jeganathan P. Hematological effects, serum, and pulmonary cytokine profiles in a melanoma mouse model treated with GK1. *Cancer Biotherapy & Radiopharmaceuticals*. 2015;**30**(6):247-254. DOI: 10.1089/cbr.2015.1835
- [77] Smyth MJ, Taniguchi M, Street SE. The anti-tumor activity of IL-12: Mechanisms of innate immunity that are model and dose dependent. *Journal of Immunology*. 2000;**165**(5):2665-2670
- [78] Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. *Nature Reviews. Immunology*. 2006;**6**(11):836-848. DOI: 10.1038/nri1961 Review
- [79] Vera-Aguilera J, Perez-Torres A, Beltran D, Villanueva-Ramos C, Wachtel M, Moreno-Aguilera E, Vera-Aguilera C, Ventolini G, Martínez-Zaguilán R, Sennoune SR. Novel

treatment of melanoma: Combined parasite-derived peptide GK-1 and anti-programmed death ligand 1 therapy. *Cancer Biotherapy & Radiopharmaceuticals*. 2017;**32**(2):49-56. DOI: 10.1089/cbr.2016.2123

- [80] Teng MW, Andrews DM, McLaughlin N, von Scheidt B, Ngiow SF, Möller A, Hill GR, Iwakura Y, Oft M, Smyth MJ. IL-23 suppresses innate immune response independently of IL-17A during carcinogenesis and metastasis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**(18):8328-8333. DOI: 10.1073/pnas.1003251107
- [81] Böhm W, Thoma S, Leithäuser F, Möller P, Schirmbeck R, Reimann J. T cell-mediated, IFN-gamma-facilitated rejection of murine B16 melanomas. *Journal of Immunology*. 1998;**161**(2):897-908
- [82] Ossendorp F, Toes RE, Offringa R, van der Burg SH, Melief CJ. Importance of CD4(+) T helper cell responses in tumor immunity. *Immunology Letters*. 2000;**74**(1):75-79. DOI: 10.1016/S0165-2478(00)00252-2
- [83] Brassard DL, Grace MJ, Bordens RW. Interferon-alpha as an immunotherapeutic protein. *Journal of Leukocyte Biology*. 2002;**71**(4):565-581. DOI: 10.1189/jlb.71.4.565
- [84] Knutson KL, Disis ML. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunology, Immunotherapy*. 2005;**54**(8):721-728. DOI: 10.1007/s00262-004-0653-2
- [85] Palmer DC, Chan CC, Gattinoni L, Wrzesinski C, Paulos CM, Hinrichs CS, Powell DJ Jr, Klebanoff CA, Finkelstein SE, Fariss RN, Yu Z, Nussenblatt RB, Rosenberg SA, Restifo NP. Effective tumor treatment targeting a melanoma/melanocyte-associated antigen triggers severe ocular autoimmunity. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;**105**(23):8061-8066. DOI: 10.1073/pnas.0710929105
- [86] Nam JS, Terabe M, Kang MJ, Chae H, Voong N, Yang YA, Laurence A, Michalowska A, Mamura M, Lonning S, Berzofsky JA, Wakefield LM. Transforming growth factor beta subverts the immune system into directly promoting tumor growth through interleukin-17. *Cancer Research*. 2008;**68**(10):3915-3923. DOI: 10.1158/0008-5472.CAN-08-0206
- [87] Zou W, Restifo NP. T(H)17 cells in tumour immunity and immunotherapy. *Nature Reviews. Immunology*. 2010;**10**(4):248-256. DOI: 10.1038/nri2742
- [88] Xiao M, Wang C, Zhang J, Li Z, Zhao X, Qin Z. IFNgamma promotes papilloma development by up-regulating Th17-associated inflammation. *Cancer Research*. 2009;**69**(5):2010-2017. DOI: 10.1158/0008-5472.CAN-08-3479
- [89] Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *The Journal of Experimental Medicine*. 2009;**206**(7):1457-1464. DOI: 10.1084/jem.20090207
- [90] Piñón-Zárate G, Herrera-Enríquez MÁ, Hernández-Téllez B, Jarquín-Yáñez K, Castell-Rodríguez AE. GK-1 improves the immune response induced by bone marrow dendritic cells loaded with MAGE-AX in mice with melanoma. *Journal of Immunology Research*. 2014;**2014**:158980. DOI: 10.1155/2014/158980

- [91] Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer*. 1996;**77**(7):1303-1310. DOI: 10.1002/(SICI)1097-0142(19960401)77:7<1303::AID-CNCR12>3.0.CO;2-5
- [92] Barnes TA, Amir E. HYPE or HOPE: The prognostic value of infiltrating immune cells in cancer. *British Journal of Cancer*. 2017;**117**(4):451-460. DOI: 10.1038/bjc.2017.220
- [93] Chinai JM, Janakiram M, Chen F, Chen W, Kaplan M, Zang X. New immunotherapies targeting the PD-1 pathway. *Trends in Pharmacological Sciences*. 2015;**36**(9):587-595. DOI: 10.1016/j.tips.2015.06.005 Review
- [94] Jensen TO, Schmidt H, Møller HJ, Donskov F, Høyer M, Sjoegren P, Christensen IJ, Steiniche T. Intratumoral neutrophils and plasmacytoid dendritic cells indicate poor prognosis and are associated with pSTAT3 expression in AJCC stage I/II melanoma. *Cancer*. 2012;**118**(9):2476-2485. DOI: 10.1002/cncr.26511
- [95] Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Research*. 1992;**52**(6):1399-1405
- [96] Pulaski BA, Terman DS, Khan S, Muller E, Ostrand-Rosenberg S. Cooperativity of *Staphylococcal aureus* enterotoxin B superantigen, major histocompatibility complex class II, and CD80 for immunotherapy of advanced spontaneous metastases in a clinically relevant postoperative mouse breast cancer model. *Cancer Research*. 2000;**60**(10):2710-2715
- [97] Mi Z, Guo H, Wai PY, Gao C, Wei J, Kuo PC. Differential osteopontin expression in phenotypically distinct subclones of murine breast cancer cells mediates metastatic behavior. *The Journal of Biological Chemistry*. 2004;**279**(45):46659-46667. DOI: 10.1074/jbc.M407952200
- [98] Torres-García D, Pérez-Torres A, Manoutcharian K, Orbe U, Servín-Blanco R, Fragoso G, Sciutto E. GK-1 peptide reduces tumor growth, decreases metastatic burden, and increases survival in a murine breast cancer model. *Vaccine*. 2017;**35**(42):5653-5661. DOI: 10.1016/j.vaccine.2017.08.060
- [99] Brunda MJ, Luistro L, Warriar RR, Wright RB, Hubbard BR, Murphy M, Wolf SF, Gately MK. Antitumor and antimetastatic activity of interleukin 12 against murine tumors. *The Journal of Experimental Medicine*. 1993;**178**(4):1223-1230
- [100] Yang SX, Wei WS, Ouyan QW, Jiang QH, Zou YF, Qu W, Tu JH, Zhou ZB, Ding HL, Xie CW, Lei QM, Zhong CR. Interleukin-12 activated CD8+ T cells induces apoptosis in breast cancer cells and reduces tumor growth. *Biomedicine & Pharmacotherapy*. 2016;**84**:1466-1471. DOI: 10.1016/j.biopha.2016.10.046
- [101] Shi X, Cao S, Mitsuhashi M, Xiang Z, Ma X. Genome-wide analysis of molecular changes in IL-12-induced control of mammary carcinoma via IFN-gamma-independent mechanisms. *Journal of Immunology*. 2004;**172**(7):4111-4122

- [102] Kitamura T, Qian BZ, Soong D, Cassetta L, Noy R, Sugano G, Kato Y, Li J, Pollard JW. CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. *The Journal of Experimental Medicine*. 2015;**212**(7):1043-1059. DOI: 10.1084/jem.20141836
- [103] Gargett T, Christo SN, Hercus TR, Abbas N, Singhal N, Lopez AF, Brown MP. GM-CSF signalling blockade and chemotherapeutic agents act in concert to inhibit the function of myeloid-derived suppressor cells in vitro. *Clinical & Translational Immunology*. 2016;**5**(12):e119. DOI: 10.1038/cti.2016.80
- [104] Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors in cancer therapy: A focus on T-regulatory cells. *Immunology and Cell Biology*. 2018;**96**(1):21-33. DOI: 10.1111/imcb.1003
- [105] Lorusso G, Rüegg C. The tumor microenvironment and its contribution to tumor evolution toward metastasis. *Histochemistry and Cell Biology*. 2008;**130**(6):1091-1103. DOI: 10.1007/s00418-008-0530-8
- [106] Wolczyk D, Zaremba-Czogalla M, Hryniewicz-Jankowska A, Tabola R, Grabowski K, Sikorski AF, Augoff K. TNF- α promotes breast cancer cell migration and enhances the concentration of membrane-associated proteases in lipid rafts. *Cellular Oncology (Dordrecht)*. 2016;**39**(4):353-363. DOI: 10.1007/s13402-016-0280-x
- [107] Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA, Pollard JW. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature*. 2011;**475**(7355):222-225. DOI: 10.1038/nature10138
- [108] Turner N, Grose R. Fibroblast growth factor signalling: From development to cancer. *Nature Reviews. Cancer*. 2010;**10**(2):116-129. DOI: 10.1038/nrc2780
- [109] Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. *The Journal of Experimental Medicine*. 2015;**212**(4):435-445. DOI: 10.1084/jem.20150295
- [110] Jarosz-Biej M, Kamińska N, Matuszczak S, Cichoń T, Pamuła-Piłat J, Czapla J, Smolarczyk R, Skwarzyńska D, Kulik K, Szala S. M1-like macrophages change tumor blood vessels and microenvironment in murine melanoma. *PLoS One*. 2018;**13**(1):e0191012. DOI: 10.1371/journal.pone.0191012
- [111] Díaz MA, Villalobos N, de Aluja A, Rosas G, Gómez-Conde E, Hernández P, Larralde C, Sciotto E, Fragoso G. Th1 and Th2 indices of the immune response in pigs vaccinated against *Taenia solium* cysticercosis suggest various host immune strategies against the parasite. *Veterinary Immunology and Immunopathology*. 2003;**93**(3-4):81-90. DOI: 10.1016/S0165-2427(03)00071-0
- [112] Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England Journal of Medicine*. 2003;**348**(3):203-213. DOI: 10.1056/NEJMoa020177

- [113] Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, Kato K, Kurokawa T, Suzuoki M, Nakakubo Y, Hiraoka K, Itoh T, Morikawa T, Okushiba S, Kondo S, Katoh H. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas*. 2004;**28**(1):e26-e23. DOI: 10.1097/00006676-200401000-00023
- [114] Oldford SA, Robb JD, Codner D, Gadag V, Watson PH, Drover S. Tumor cell expression of HLA-DM associates with a Th1 profile and predicts improved survival in breast carcinoma patients. *International Immunology*. 2006;**18**(11):1591-1602. DOI: 10.1093/intimm/dxl092
- [115] Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, Rabbe N, Laurans L, Tartour E, de Chaisemartin L, Lebecque S, Fridman WH, Cadranet J. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *Journal of Clinical Oncology*. 2008;**26**(27):4410-4417. DOI: 10.1200/JCO.2007.15.0284
- [116] Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, Ellis IO, Green AR. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *Journal of Clinical Oncology*. 2011;**29**(15):1949-1955. DOI: 10.1200/JCO.2010.30.5037