



Immune Evasion of G-CSF and GM-CSF in Lung Cancer

<https://doi.org/10.4046/trd.2023.0037>

ISSN: 1738-3536(Print)/

2005-6184(Online)

Tuberc Respir Dis 2024;87:22-30

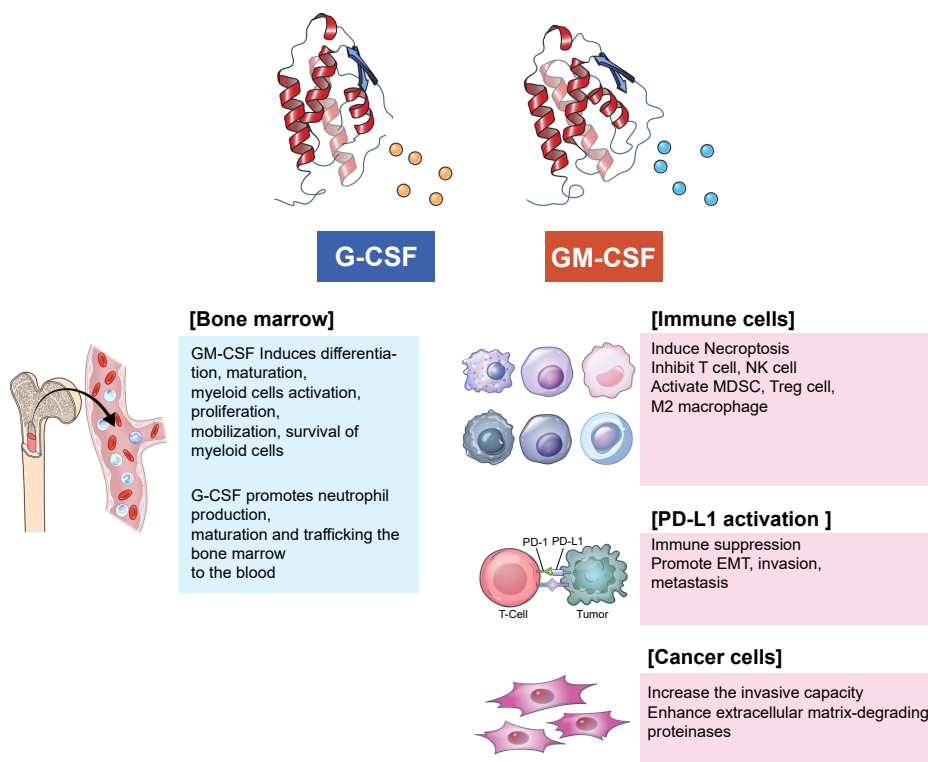
Yeonhee Park, M.D., Ph.D.¹  and Chaek Chung, M.D., Ph.D.^{2,3} 

¹Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Daejeon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Daejeon, ²Division of Pulmonology and Critical Care Medicine, Department of Internal Medicine, ³Infection Control Convergence Research Center, Chungnam National University College of Medicine, Daejeon, Republic of Korea



Copyright © 2024 The Korean Academy of Tuberculosis and Respiratory Diseases

Address for correspondence
Chaek Chung, M.D., Ph.D.
 Division of Pulmonology and Critical Care Medicine, Department of Internal Medicine, Chungnam National University College of Medicine, 282 Munhwa-ro, Jung-gu, Daejeon 35015, Republic of Korea
Phone 82-42-280-7147
E-mail universe7903@gmail.com
Received Mar. 23, 2023
Revised Jul. 20, 2023
Accepted Sep. 12, 2023
Published online Sep. 20, 2023



© It is identical to the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>).

Abstract

Tumor immune evasion is a complex process that involves various mechanisms, such as antigen recognition restriction, immune system suppression, and T cell exhaustion. The tumor microenvironment contains various immune cells involved in immune evasion. Recent studies have demonstrated that granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) induce immune evasion in lung cancer by modulating neutrophils and myeloid-derived suppressor cells. Here we describe the origin and function of G-CSF and GM-CSF, particularly their role in immune evasion in lung cancer. In addition, their effects on programmed death-ligand 1 expression and clinical implications are discussed.

Keywords: Granulocyte Colony-Stimulating Factor; Granulocyte-Macrophage Colony-Stimulating Factor; Neutrophils; Myeloid-Derived Suppressor Cell; Programmed Death-Ligand 1; Immune Evasion

Introduction

Immune evasion hinders the effective treatment of lung cancer with immune checkpoint inhibitors. Programmed death-ligand 1 (PD-L1) binds to programmed cell death-1 (PD-1) receptors on cytotoxic T cells to inhibit T cells and decrease cytokine production¹⁻³. In addition, neutrophils and myeloid-derived suppressor cells (MDSCs) inhibit immune responses within the tumor microenvironment. Recent studies have demonstrated that granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) induce immune evasion through various mechanisms⁴⁻¹⁰. Clinical studies have demonstrated that high levels of G-CSF and GM-CSF are associated with a poor prognosis¹¹⁻¹³. Moreover, exogenous GM-CSF induces PD-L1 expression and promotes tumor progression^{8,10,14,15}.

Here we describe the origin and functions of G-CSF and GM-CSF in lung cancer, focusing on their role in modulating neutrophils, MDSCs, and PD-L1 expression.

Origin and Function of G-CSF/GM-CSF

G-CSF and GM-CSF are essential growth factors for hematopoiesis. GM-CSF causes the proliferation and differentiation of myeloid cells, whereas G-CSF regulates neutrophil production, maturation, and mobilization. Vascular endothelial cells, fibroblasts, and immune cells, such as T cells, B cells, macrophages, natural killer cells, and mast cells, produce GM-CSF in response to inflammatory cytokines and the innate immune response¹⁶. In turn, GM-CSF affects myeloid cell differentiation, maturation, activation, proliferation, mobilization, and survival¹⁷. Conversely, G-CSF promotes the production, maturation, and migration of neutrophils from the bone marrow to the bloodstream¹⁸. Clinicians frequently use recombinant G-CSF and GM-CSF to increase the absolute neutrophil count in cancer patients who develop neutropenia following chemotherapy.

Immune Evasion in Lung Cancer by G-CSF/GM-CSF by Modulating Neutrophils, Macrophages and MDSCs

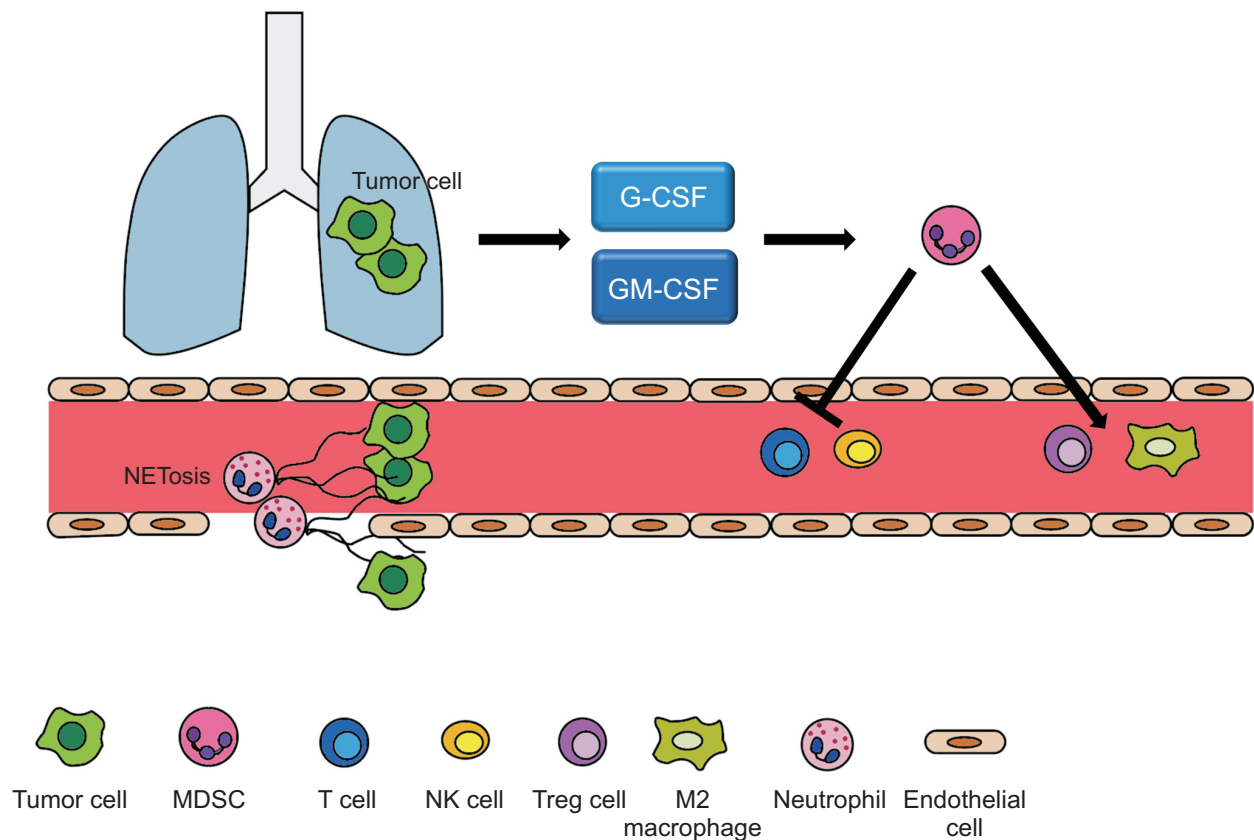
Lung cancer cells secrete G-CSF and GM-CSF to evade the immune system in response to oncogenic and immunologic signals¹⁹. Tumor-derived G-CSF reprograms myeloid differentiation and produces immunosuppressive neutrophils (Figure 1)^{20,21}. Neutrophils play a major role in innate immunity against bacterial infections.

In the bone marrow, myeloblasts differentiate into promyelocytes, neutrophilic myelocytes, neutrophilic metamyelocytes, neutrophil stab cells, and segmented neutrophils. Mature segmented neutrophils differentiate into small, high-density neutrophils with anti-tumor effects, whereas undifferentiated neutrophils are large, low-density neutrophils with pro-tumorous effects²². Immature neutrophils typically remain in the bone marrow under normal conditions. However, in cancer patients, stimulated low-density neutrophils migrate from the bone marrow to the blood. MDSCs are low-density neutrophils²³; their levels in the blood are increased by cancer-derived endogenous and exogenous G-CSF and GM-CSF²⁴. MDSCs consist of heterogeneous precursors of dendritic cells, macrophages, and granulocytes. Recent studies have demonstrated that 70% to 80% of MDSCs are polymorphonuclear cells, and 20% to 30% are monocytic cells²⁵. These cells cause immune suppression in the tumor microenvironment, presenting a significant barrier to cancer immunotherapy²⁶. They inhibit the CD8-positive cytotoxic T cells, thereby inhibiting the effects of immune checkpoint inhibitors. In addition, prostaglandin E2 derived from MDSCs can accumulate cancer stem cells and induce PD-L1 overexpression, resulting in immune suppression²⁷.

Tumor-associated macrophages (TAMs) are the key cells that create an immunosuppressive tumor microenvironment by producing cytokines, chemokines, growth factors, and triggering the release of inhibitory immune checkpoint proteins in T cells. TAMs can display very different and even opposing phenotypes, depending on the microenvironment in which they are embedded. Macrophages are divided into two main categories: classical activated M1 macrophages (major histocompatibility complex [MHC]-II+CD68+) and alternatively activated M2 macrophages (CD163+CD206+). M1 macrophages have an anti-tumor function, as they are responsible for killing tumor cells through tumor-killing molecules or antibody-dependent cell-mediated cytotoxicity. In contrast, M2 macrophages promote the proliferation and metastasis of tumor cells by expressing various growth factors such as vascular endothelial growth factor, transforming growth factor- β 1 (TGF- β 1), epithelial growth factor, and hepatocyte growth factor (HGF)^{7,28,29}.

In human triple-negative breast cancer, high G-CSF expression is significantly associated with CD163+ TAMs and decreased overall survival. *In vitro* and *in vivo* data using a panel of human breast cancer and mouse mammary tumor cell lines have demonstrated that tumor-derived GM-CSF is an important cytokine involved

Figure 1. Schematic diagram of immune evasion by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in lung cancer. Lung cancer cells secrete G-CSF and GM-CSF, which modulate myeloid differentiation, produce immunosuppressive neutrophils, and induce the accumulation of myeloid-derived suppressor cells (MDSCs) in circulation. In turn, MDSCs induce the production of Tregs and differentiation of M2 macrophages, reduce natural killer cell activity, and inhibit T cell proliferation and migration. In addition, neutrophil extracellular trap (NET)-associated granule proteins promote tumor metastasis by secreting neutrophil elastase and matrix metalloproteases. NK: natural killer.



in the activation of signal transducer and activator of transcription 5 (STAT5) signaling in macrophages³⁰.

There are two distinct types of neutrophils, N1 and N2 tumor-associated neutrophils (TANs). N1 TANs inhibit tumor growth and metastasis through antibody-dependent or direct cytotoxicity and activation of various innate and adaptive immune cells³¹⁻³³. Additionally, N1 TANs produce reactive oxygen species through enhanced nicotinamide adenine dinucleotide phosphate hydrogen oxidase activity, thereby exerting a cytotoxic effect on tumor cells³⁴. Conversely, N2 TANs promote tumor progression through multiple mechanisms. N2 TANs secrete several enzymes, such as myeloperoxidase, neutrophil elastase (NE), neutrophil collagenase (matrix metalloproteinase 8 [MMP8]), and MMP9, which promote extracellular matrix remodeling and angiogenesis, resulting in tumor proliferation and promote migration^{35,36}. Moreover, N2 TANs inhibit the function of effector T lymphocytes by recruiting Tregs

via CC motif chemokine ligand 17 (CCL17) secretion³⁷. TGF- β is known to be an important cytokine involved in the skewing of neutrophil differentiation toward N2 TANs^{33,38,39}, while treatment with TGF- β blockade and interferon- β showed a shift toward N1 TANs^{40,41}.

Neutrophils release an intracellular web-like chromatin structure called neutrophil extracellular traps (NETs), which induce an immune response against infections, particularly by large pathogens⁴². These are released through plasma membrane rupture or expel nuclear chromatin without plasma membrane rupture. NET-associated granule proteins promote tumor metastasis by releasing NE and matrix metalloproteases. These proteases cause cancer cells to undergo metastasis⁴³.

Tumor-derived G-CSF/GM-CSF induces activation and proliferation of TANs. G-CSF secreted from cancer cells induced TANs to form NETs³⁵. Hepatocellular carcinoma-derived GM-CSF stimulated cancer cell migration and invasion by enabling TANs to produce HGF

and activating the HGF/c-Met axis⁴⁴. Bv8 may function to modulate or amplify neutrophil mobilization stimulated by G-CSF, through paracrine or autocrine mechanisms⁴⁵. Tumor-derived G-CSF activates a myeloid differentiation program, resulting in increased immunosuppressive neutrophils that possess T cell-suppressive, and retinoblastoma protein (Rb1)^{low} phenotype in an oncogene-driven murine breast cancer model²⁰.

In addition, G-CSF causes tumor growth, metastasis, and chemotherapy resistance⁴⁶⁻⁴⁹. Several studies have shown that a high G-CSF level is associated with thrombosis and a poor prognosis⁵⁰.

Effects of G-CSF/GM-CSF on PD-L1

Some patients develop remarkable progression of extra-nodal natural killer/T cell lymphoma (ENKTL) after GM-CSF treatment. GM-CSF treatment significantly increases the expression of PD-L1 mRNA and protein in ENKTL cells. Because PD-L1 has immunosuppressive functions, GM-CSF treatment results in the loss of tumor immune surveillance in ENKTL patients, accelerating cancer cell progression. GM-CSF stimulates PD-L1 expression through the Janus kinase 2 (JAK2)-STAT5 signaling pathway in ENKTL¹⁰.

PD-L1 expression is regulated by multiple signaling pathways, such as MYC, Kirsten rat sarcoma virus (KRAS), STAT3, JUN, phosphatase and tensin homolog (PTEN), and epidermal growth factor receptor (EGFR), which are responsible for the constitutive expression of PD-L1⁵¹. In addition, interferon- γ , interleukin-6, interleukin-27, tumor necrosis factor- α , and epidermal growth factor induce PD-L1 expression⁵². The interaction between PD-L1 and PD-1 activates downstream signaling pathways, such as phosphoinositide-3-kinase (PI3K), Linker for activation of T cells (LAT), and Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2), in T cells, which inhibits T cell activation and cytokine production⁵³. Immune checkpoint inhibitors, including pembrolizumab, nivolumab, and atezolizumab, reactivate cytotoxic T cells and overcome immune evasion. In a recent *in vitro* study using lung adenocarcinoma cell lines and human monocyte-derived macrophages, it was suggested that cancer cell-derived GM-CSF induces PD-L1 overexpression on TAMs through the STAT3 pathway (Figure 2)⁵⁴.

Recent studies have demonstrated that PD-L1 has intrinsic functions independent of PD-1⁵⁵⁻⁵⁷; it promotes cancer stemness, epithelial-mesenchymal transition, and drug resistance in cancer cells. PD-L1 contains RMLDVEKC and DTSSK motifs in the cytoplasmic domain, which inhibit interferon-mediated cytotoxicity in

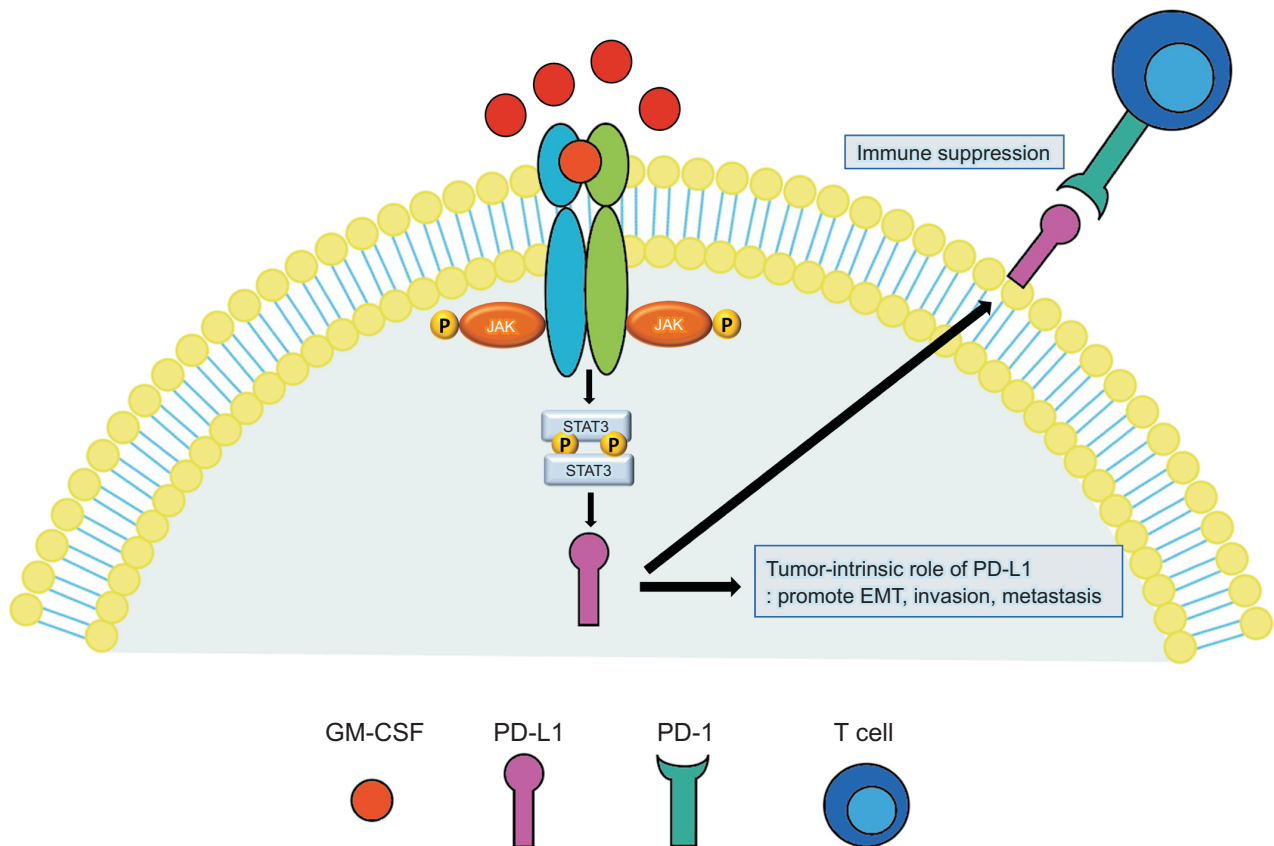
cancer cells by preventing STAT3 activation and subsequent caspase-7-mediated apoptosis⁵⁵. A recent study demonstrated that PD-L1 was translocated to the nucleus after deacetylation⁵⁸. Nuclear PD-L1 modulates immune response gene expression and consequently decreases the effects of immune checkpoint inhibitors. Therefore, the prevention PD-L1 translocation is a novel strategy for improving the outcomes of immunotherapy in cancer. Because G-CSF enhances PD-L1 expression in cancer, G-CSF blockade may be used as a novel adjuvant treatment with immunotherapy. In the results of a preclinical study using a murine Lewis lung carcinoma cell line, it was shown that blocking GM-CSF can significantly inhibit tumor development. Although anti-GM-CSF therapy did not affect PD-L1 expression in tumor tissues, it suppressed the infiltration and maturation of TAMs and increased T cell infiltration. Although further studies are still required, cancer-derived GM-CSF may be a promising target for anti-cancer therapy⁵⁴.

Miyazawa et al.⁵⁹ showed that PD-L1 expression is affected by matrix stiffness in lung cancer and that it is much higher in a stiff than a soft matrix. Although the exact mechanism by which matrix stiffness affects PD-L1 expression remains unknown, mechanosensitive Yes-associated proteins may be involved, as they regulate PD-L1 transcription. Some studies have shown that GM-CSF regulates the extracellular matrix by controlling the metabolism of vascular collagens⁶⁰. It also stimulates the proliferation and migration of vascular endothelial cells⁶¹. Therefore, GM-CSF can cause immune evasion by altering PD-L1 expression and extracellular matrix stiffness.

Clinical Uses of G-CSF and GM-CSF

G-CSF/GM-CSF has been widely used as supportive care for patients receiving chemotherapy. The use of G-CSF/GM-CSF has shown to reduce the incidence of chemotherapy-associated neutropenia by 37% and decrease the duration and severity of neutropenia in patients undergoing cancer chemotherapy⁶². However, recent reports have indicated that overexpression of G-CSF/GM-CSF is associated with poor prognosis in various types of cancers. In a clinical study, G-CSF level was significantly higher in patients who died than in those who survived gastric cancer⁶³. In addition, a high G-CSF level was negatively correlated with overall survival. Furthermore, the G-CSF level was significantly higher in a mammary cancer model than in controls, and G-CSF blockade slowed tumor growth. Tumor-derived G-CSF increases tumor growth through MD-

Figure 2. Illustration of the immune evasion mechanism by which granulocyte-macrophage colony-stimulating factor (GM-CSF) increases programmed death-ligand 1 (PD-L1) expression on macrophage through the Janus kinase/signal transducer and activator of transcription (JAK-STAT3) signaling pathway in lung adenocarcinoma. PD-L1 upregulation causes immune suppression by inhibiting T cell activation in a programmed cell death-1 (PD-1)-dependent manner. In addition, PD-L1 promotes cancer cell proliferation, metastasis, and epithelial-mesenchymal transition. This figure highlights the potential therapeutic use of the JAK-STAT3-PD-L1 axis in lung adenocarcinoma. EMT: epithelial-mesenchymal transition.



SC-related immune reactions²⁴. Serum levels of GM-CSF in patients with glioblastoma are more than two-fold higher than in healthy controls⁶⁴. Patients with non-small cell lung cancer have higher GM-CSF levels compared to healthy controls. Therefore, GM-CSF is a potential diagnostic marker for non-small cell lung cancer⁶⁵. G-CSF and GM-CSF upregulate the invasive capacity of human lung cancer cells by enhancing the production of extracellular matrix-degrading proteinases¹⁹. A clinical study of non-small cell lung cancer patients demonstrated that higher GM-CSF levels are positively correlated with poor prognosis⁶⁶.

The ongoing clinical trials of G-CSF/GM-CSF in lung cancer are described in Table 1. Apart from one study using pegylated G-CSF, most studies utilized recombinant human GM-CSF, such as sargramostim. Moreover, these studies primarily investigate the use of G-CSF/GM-CSF in combination with other drugs for

supportive care purposes, rather than confirming their therapeutic effects.

Interestingly, an *in vivo* study using a breast cancer model demonstrated that long-term exposure to high levels of G-CSF increased metastasis, while short-term G-CSF administration in conjunction with cytotoxic chemotherapy did not lead to increased metastasis⁶⁷. Therefore, caution should be exercised in the use of recombinant G-CSF/GM-CSF as it has the potential to promote tumor progression and metastasis¹⁷.

Conclusion

Tumor-derived or recombinant G-CSF and GM-CSF have diverse roles in the tumor microenvironment. They disrupt granulopoiesis and neutrophil maturation and increase TANs, particularly pro-tumor N2 neutrophils, which promote tumor growth, invasion, and metastasis.

Table 1. Ongoing clinical trials of G-CSF/GM-CSF in lung cancer

Study title	Stage	Experimental arm	Control arm	Enrollment	Primary outcome	Phase (status)	Registry number
A Study of Sargramostim Plus Pembrolizumab with or Without Pemetrexed in Patients with Advance Non-small Cell Lung Cancer After Completion of Chemotherapy	NSCLC Stage IV	Sargramostim plus maintenance pembrolizumab with pemetrexed	Sargramostim plus maintenance pembrolizumab without pemetrexed	NA	PFS and OS	2 (Recruiting)	NCT04856176
A Multiple Antigen Vaccine (STEMVAC) for the Treatment of Patients with Stage IV Non-Squamous Non-Small Cell Lung Cancer	Lung cancer Stage IV	STEMVAC, sargramostim	Sargramostim	40	Change from baseline percentage of CD8+ TIL in patients between the two arms	2 (Recruiting)	NCT05242965
SBRT in Combination with Sintilimab and GM-CSF for the Treatment of Advanced NSCLC	NSCLC Stage IV	SBRT + sintilimab + GM-CSF single arm		NA	Overall objective response rate	2 (Recruiting)	NCT04106180
Efficacy and Safety of Anti-PD-1/PD-L1 Treatment +/- UV1 Vaccination in Patients With Non-small Cell Lung Cancer	NSCLC Stage III, IV	Anti-PD-1/PD-L1 treatment + UV1 vaccination (and sargramostim)	Anti-PD-1/PD-L1 treatment	138	PFS	2 (Recruiting)	NCT05344209
Assess Efficacy & Safety of Selumetinib in Combination With Docetaxel in Patients Receiving 2nd Line Treatment for v-Ki-ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) Positive NSCLC	NSCLC Stage IIIb-IV	Selumetinib + Docetaxel + Pegylated G-CSF	Placebo + Docetaxel + Pegylated G-CSF	510	PFS	3 (Active, not recruiting)	NCT01933932

G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; NSCLC: non-small cell lung cancer; NA: not available; PFS: progression-free survival; OS: overall survival; TIL: tumor-infiltrating lymphocyte; SBRT: stereotactic body radiation therapy; PD-1: programmed cell death-1; PD-L1: programmed death-ligand 1.

Furthermore, they mobilize immunosuppressive MDSCs into circulation, where MDSCs suppress natural killer and cytotoxic T cells. GM-CSF stimulates PD-L1 expression, which has immunological and PD-1-independent intrinsic functions. Because high G-CSF and GM-CSF levels are associated with poor prognosis in cancer patients, recombinant G-CSF and GM-CSF should be used cautiously.

Authors' Contributions

Conceptualization: Chung C. Writing - original draft preparation: all authors. Writing - review and editing: Park YH. Approval of final manuscript: all authors.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT) (No. NRF-2017R1A5A2015385, NRF-2022R1A2C2010148) and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Korea (grant number: HR20C0025).

References

1. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* 2012;24:207-12.
2. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol* 2008;8:467-77.
3. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;8:793-800.
4. Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, et al. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* 2012; 21:822-35.
5. Bharadwaj U, Li M, Zhang R, Chen C, Yao Q. Elevated interleukin-6 and G-CSF in human pancreatic cancer cell conditioned medium suppress dendritic cell differentiation and activation. *Cancer Res* 2007;67:5479-88.
6. Tang D, Zhang D, Heng Y, Zhu XK, Lin HQ, Zhou J, et al. Tumor-infiltrating PD-L1+ neutrophils induced by GM-CSF suppress T cell function in laryngeal squamous cell carcinoma and predict unfavorable prognosis. *J Inflamm Res* 2022;15:1079-97.
7. He K, Liu X, Hoffman RD, Shi RZ, Lv GY, Gao JL. G-CSF/GM-CSF-induced hematopoietic dysregulation in the progression of solid tumors. *FEBS Open Bio* 2022;12: 1268-85.
8. Zhang X, Xu W. Neutrophils diminish T-cell immunity to foster gastric cancer progression: the role of GM-CSF/PD-L1/PD-1 signalling pathway. *Gut* 2017;66:1878-80.
9. Dolcetti L, Peranzoni E, Ugel S, Marigo I, Fernandez Gomez A, Mesa C, et al. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. *Eur J Immunol* 2010;40:22-35.
10. Rong QX, Wang F, Guo ZX, Hu Y, An SN, Luo M, et al. GM-CSF mediates immune evasion via upregulation of PD-L1 expression in extranodal natural killer/T cell lymphoma. *Mol Cancer* 2021;20:80.
11. Gutschalk CM, Herold-Mende CC, Fusenig NE, Mueller MM. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor promote malignant growth of cells from head and neck squamous cell carcinomas in vivo. *Cancer Res* 2006;66:8026-36.
12. Katsumata N, Eguchi K, Fukuda M, Yamamoto N, Ohe Y, Oshita F, et al. Serum levels of cytokines in patients with untreated primary lung cancer. *Clin Cancer Res* 1996;2: 553-9.
13. Braun B, Lange M, Oeckler R, Mueller MM. Expression of G-CSF and GM-CSF in human meningiomas correlates with increased tumor proliferation and vascularization. *J Neurooncol* 2004;68:131-40.
14. Thorn M, Guha P, Cunetta M, Espat NJ, Miller G, Jung-hans RP, et al. Tumor-associated GM-CSF overexpression induces immunoinhibitory molecules via STAT3 in myeloid-suppressor cells infiltrating liver metastases. *Cancer Gene Ther* 2016;23:188-98.
15. Wang TT, Zhao YL, Peng LS, Chen N, Chen W, Lv YP, et al. Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. *Gut* 2017;66:1900-11.
16. Mehta HM, Malandra M, Corey SJ. G-CSF and GM-CSF in neutropenia. *J Immunol* 2015;195:1341-9.
17. Hong IS. Stimulatory versus suppressive effects of GM-CSF on tumor progression in multiple cancer types. *Exp Mol Med* 2016;48:e242.
18. Eyles JL, Hickey MJ, Norman MU, Croker BA, Roberts AW, Drake SF, et al. A key role for G-CSF-induced neutrophil production and trafficking during inflammatory arthritis. *Blood* 2008;112:5193-201.
19. Pei XH, Nakanishi Y, Takayama K, Bai F, Hara N. Granu-

- lyocyte, granulocyte-macrophage, and macrophage colony-stimulating factors can stimulate the invasive capacity of human lung cancer cells. *Br J Cancer* 1999;79:40-6.
20. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci U S A* 2015; 112:E566-75.
 21. Masucci MT, Minopoli M, Carriero MV. Tumor associated neutrophils: their role in tumorigenesis, metastasis, prognosis and therapy. *Front Oncol* 2019;9:1146.
 22. Kuijpers T. Structure and composition of neutrophils, eosinophils, and basophils. In: Kaushansky K, Prchal JT, Burns LJ, Lichtman MA, Levi M, Linch DC, editors. *Williams hematology*. 10th ed. New York: McGraw-Hill Education; 2021 [cited 2023 Sep 22]. Available from: <https://accessmedicine.mhmedical.com/content.aspx?bookid=2962§ionid=252530906>.
 23. Silvestre-Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood* 2016;127:2173-81.
 24. Waight JD, Hu Q, Miller A, Liu S, Abrams SI. Tumor-derived G-CSF facilitates neoplastic growth through a granulocytic myeloid-derived suppressor cell-dependent mechanism. *PLoS One* 2011;6:e27690.
 25. Youn JI, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J Leukoc Biol* 2012;91:167-81.
 26. Tavazoie MF, Pollack I, Tanqueco R, Ostendorf BN, Reis BS, Gonsalves FC, et al. LXR/ApoE activation restricts innate immune suppression in cancer. *Cell* 2018;172:825-40.
 27. Komura N, Mabuchi S, Shimura K, Yokoi E, Kozasa K, Kuroda H, et al. The role of myeloid-derived suppressor cells in increasing cancer stem-like cells and promoting PD-L1 expression in epithelial ovarian cancer. *Cancer Immunol Immunother* 2020;69:2477-99.
 28. Lin Y, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J Hematol Oncol* 2019;12:76.
 29. Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity. *Front Immunol* 2020;11: 583084.
 30. Jesser EA, Brady NJ, Huggins DN, Witschen PM, O'Connor CH, Schwertfeger KL. STAT5 is activated in macrophages by breast cancer cell-derived factors and regulates macrophage function in the tumor microenvironment. *Breast Cancer Res* 2021;23:104.
 31. Matlung HL, Babes L, Zhao XW, van Houdt M, Treffers LW, van Rees DJ, et al. Neutrophils kill antibody-opsonized cancer cells by trogoptosis. *Cell Rep* 2018;23: 3946-59.
 32. Beauvillain C, Delneste Y, Scotet M, Peres A, Gascan H, Guermonprez P, et al. Neutrophils efficiently cross-prime naive T cells in vivo. *Blood* 2007;110:2965-73.
 33. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 2009;16:183-94.
 34. Gershkovitz M, Caspi Y, Fainsod-Levi T, Katz B, Michaeli J, Khawaled S, et al. TRPM2 mediates neutrophil killing of disseminated tumor cells. *Cancer Res* 2018;78:2680-90.
 35. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. *Sci Transl Med* 2016;8:361ra138.
 36. Das A, Monteiro M, Barai A, Kumar S, Sen S. MMP proteolytic activity regulates cancer invasiveness by modulating integrins. *Sci Rep* 2017;7:14219.
 37. Mishalian I, Bayuh R, Eruslanov E, Michaeli J, Levy L, Zolotarov L, et al. Neutrophils recruit regulatory T-cells into tumors via secretion of CCL17: a new mechanism of impaired antitumor immunity. *Int J Cancer* 2014;135:1178-86.
 38. Shen L, Smith JM, Shen Z, Eriksson M, Sentman C, Wira CR. Inhibition of human neutrophil degranulation by transforming growth factor-beta1. *Clin Exp Immunol* 2007;149:155-61.
 39. Saha S, Biswas SK. Tumor-associated neutrophils show phenotypic and functional divergence in human lung cancer. *Cancer Cell* 2016;30:11-3.
 40. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J Clin Invest* 2010;120:1151-64.
 41. Andzinski L, Kasnitz N, Stahnke S, Wu CF, Gereke M, von Kockritz-Blickwede M, et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int J Cancer* 2016;138:1982-93.
 42. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-5.
 43. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nat Rev Immunol* 2018;18:134-47.
 44. He M, Peng A, Huang XZ, Shi DC, Wang JC, Zhao Q, et al. Peritumoral stromal neutrophils are essential for c-Met-elicited metastasis in human hepatocellular carcinoma. *Oncoimmunology* 2016;5:e1219828.
 45. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* 2007;450:825-31.
 46. Phan VT, Wu X, Cheng JH, Sheng RX, Chung AS, Zhuang G, et al. Oncogenic RAS pathway activation promotes

- resistance to anti-VEGF therapy through G-CSF-induced neutrophil recruitment. *Proc Natl Acad Sci U S A* 2013; 110:6079-84.
47. Yokoi E, Mabuchi S, Komura N, Shimura K, Kuroda H, Kozasa K, et al. The role of myeloid-derived suppressor cells in endometrial cancer displaying systemic inflammatory response: clinical and preclinical investigations. *Oncoimmunology* 2019;8:e1662708.
 48. Sasano T, Mabuchi S, Kozasa K, Kuroda H, Kawano M, Takahashi R, et al. The highly metastatic nature of uterine cervical/endometrial cancer displaying tumor-related leukocytosis: clinical and preclinical investigations. *Clin Cancer Res* 2018;24:4018-29.
 49. Kawano M, Mabuchi S, Matsumoto Y, Sasano T, Takahashi R, Kuroda H, et al. The significance of G-CSF expression and myeloid-derived suppressor cells in the chemoresistance of uterine cervical cancer. *Sci Rep* 2015;5:18217.
 50. Mouchemore KA, Anderson RL, Hamilton JA. Neutrophils, G-CSF and their contribution to breast cancer metastasis. *FEBS J* 2018;285:665-79.
 51. Shen X, Zhang L, Li J, Li Y, Wang Y, Xu ZX. Recent findings in the regulation of programmed death ligand 1 expression. *Front Immunol* 2019;10:1337.
 52. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer* 2019;18:10.
 53. Arasanz H, Gato-Canas M, Zuazo M, Ibanez-Vea M, Breckpot K, Kochan G, et al. PD1 signal transduction pathways in T cells. *Oncotarget* 2017;8:51936-45.
 54. Shinchi Y, Ishizuka S, Komohara Y, Matsubara E, Mito R, Pan C, et al. The expression of PD-1 ligand 1 on macrophages and its clinical impacts and mechanisms in lung adenocarcinoma. *Cancer Immunol Immunother* 2022;71: 2645-61.
 55. Gato-Canas M, Zuazo M, Arasanz H, Ibanez-Vea M, Lorenzo L, Fernandez-Hinojal G, et al. PDL1 signals through conserved sequence motifs to overcome interferon-mediated cytotoxicity. *Cell Rep* 2017;20:1818-29.
 56. Gupta HB, Clark CA, Yuan B, Sareddy G, Pandeswara S, Padron AS, et al. Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. *Signal Transduct Target Ther* 2016;1: 16030.
 57. Clark CA, Gupta HB, Sareddy G, Pandeswara S, Lao S, Yuan B, et al. Tumor-intrinsic PD-L1 signals regulate cell growth, pathogenesis, and autophagy in ovarian cancer and melanoma. *Cancer Res* 2016;76:6964-74.
 58. Gao Y, Nihira NT, Bu X, Chu C, Zhang J, Kolodziejczyk A, et al. Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy. *Nat Cell Biol* 2020;22:1064-75.
 59. Miyazawa A, Ito S, Asano S, Tanaka I, Sato M, Kondo M, et al. Regulation of PD-L1 expression by matrix stiffness in lung cancer cells. *Biochem Biophys Res Commun* 2018;495:2344-9.
 60. Plenz G, Reichenberg S, Koenig C, Rauterberg J, Deng MC, Baba HA, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) modulates the expression of type VIII collagen mRNA in vascular smooth muscle cells and both are codistributed during atherosclerosis. *Arterioscler Thromb Vasc Biol* 1999;19:1658-68.
 61. Gaynor RB. A role for extracellular matrix binding receptors in regulating hematopoietic growth factor signaling. *Proc Natl Acad Sci U S A* 2003;100:13737-8.
 62. Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Tabbara I, et al. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. *N Engl J Med* 1991;325:164-70.
 63. Fan Z, Li Y, Zhao Q, Fan L, Tan B, Zuo J, et al. Highly expressed granulocyte colony-stimulating factor (G-CSF) and granulocyte colony-stimulating factor receptor (G-CSFR) in human gastric cancer leads to poor survival. *Med Sci Monit* 2018;24:1701-11.
 64. Albuлесcu R, Codrici E, Popescu ID, Mihai S, Necula LG, Petrescu D, et al. Cytokine patterns in brain tumour progression. *Mediators Inflamm* 2013;2013:979748.
 65. Mroczyk B, Szmitkowski M, Niklinski J. Stem cell factor and granulocyte-macrophage-colony stimulating factor as candidates for tumour markers for non-small-cell lung cancer. *Clin Chem Lab Med* 1999;37:959-62.
 66. Stathopoulos GP, Armakolas A, Tranga T, Marinou H, Stathopoulos J, Chandrinou H. Granulocyte colony-stimulating factor expression as a prognostic biomarker in non-small cell lung cancer. *Oncol Rep* 2011;25:1541-4.
 67. Kowanzetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci U S A* 2010;107:21248-55.