

**Lung cancer school
2022**

Immune evasion of G-CSF and GM-CSF in lung cancer

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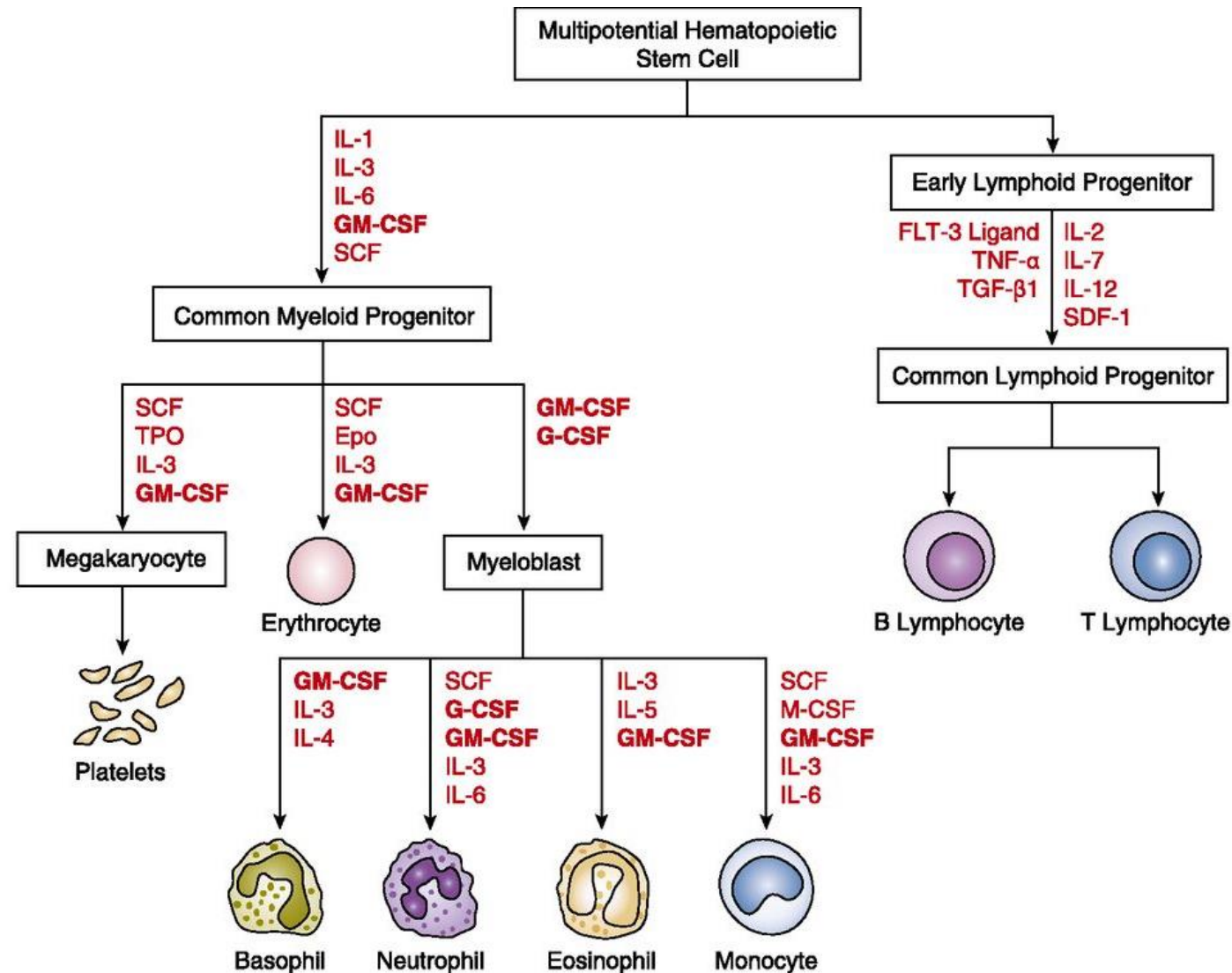
Key questions?

- **1. The effects of G-CSF/GM-CSF in lung cancer ?**
- **2. The sources of G-CSF/GM-CSF in lung cancer ?**
- **3. The mechanism of immune evasion of G-CSF/GM-CSF ?**
- **4. The clinical implication of G-CSF/GM-CSF in lung cancer ?**

Contents

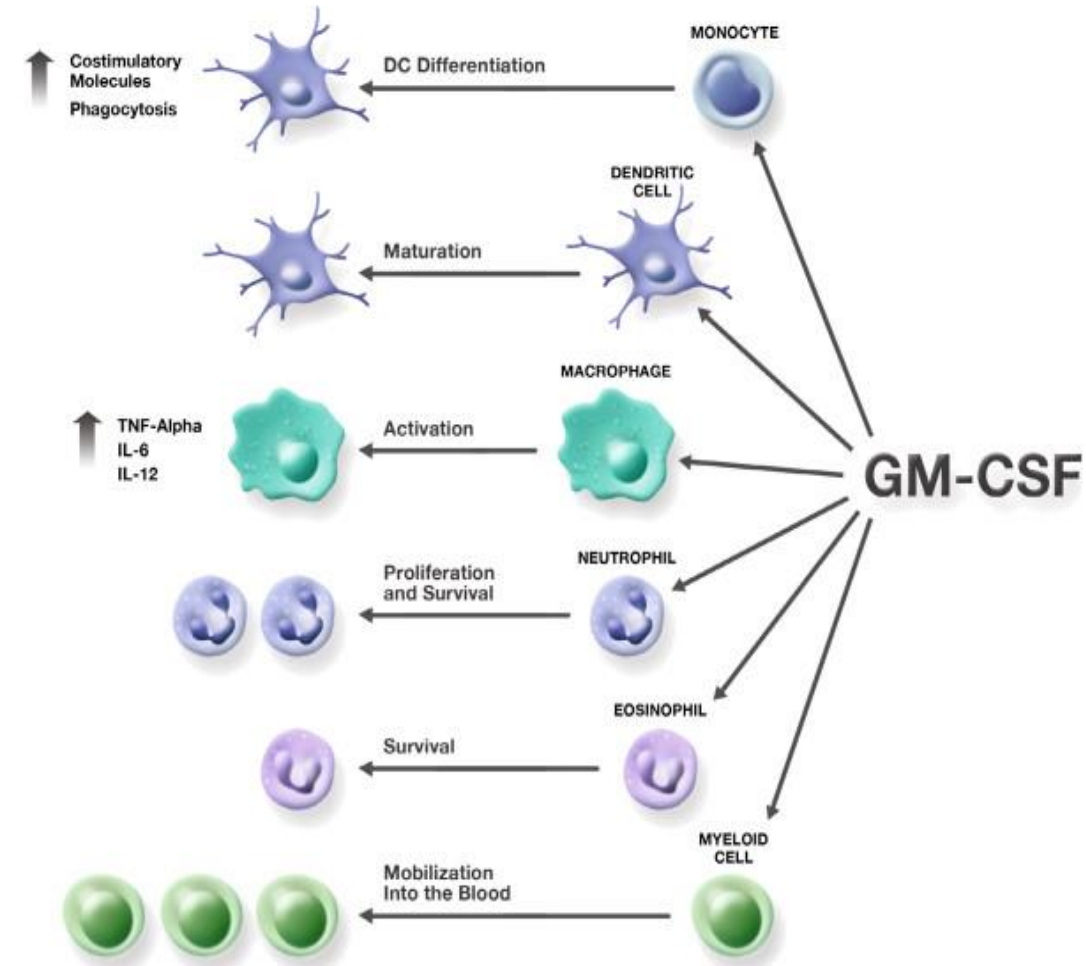
- **1. G-CSF/GM-CSF**
- 2. G-CSF/GM-CSF and neutrophil/MDSC
- 3. G-CSF/GM-CSF and PD-L1
- 4. Clinical implication of G-CSF and GM-CSF

The role of G-CSF and GM-CSF in hematopoiesis

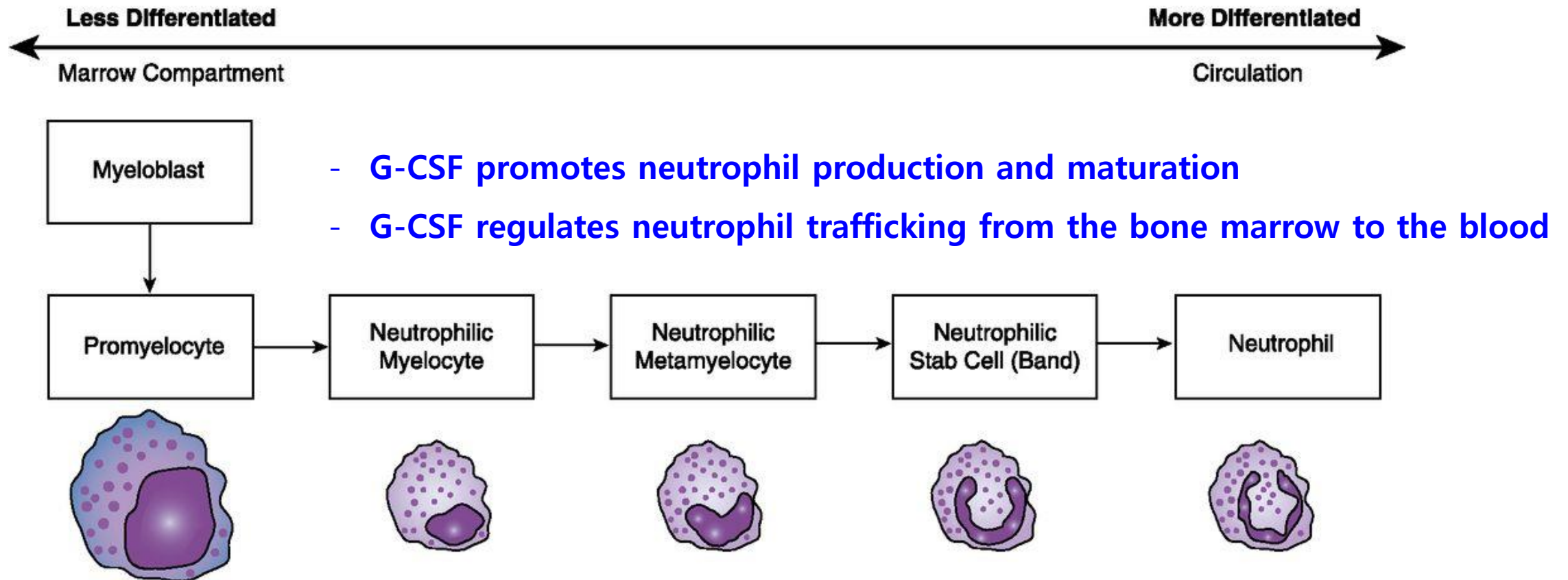


Discovery of GM-CSF and its biological functions

- **Signals for GM-CSF production**
: Inflammatory cytokines and innate immune activation
- **Production of GM-CSF**
: T cells, B cells, macrophages, mast cells, vascular endothelial cells and fibroblasts
- **Function of GM-CSF**
: Proliferation of bone marrow-derived macrophages and granulocytes



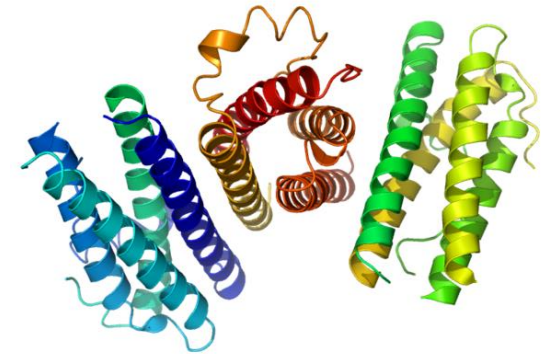
The role of G-CSF and GM-CSF in the differentiation of neutrophil



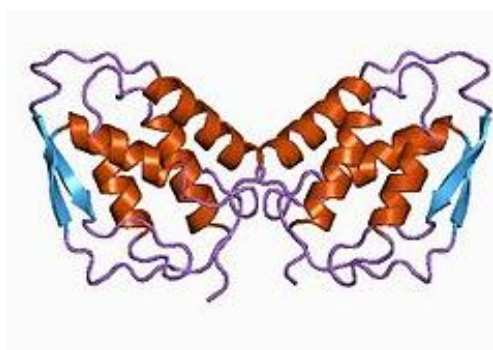
G-CSF and GM-CSF

	G-CSF	GM-CSF
Term	Granulocyte colony-stimulating factor CSF3	Granulocyte-macrophage colony-stimulating factor CSF2
Sources	Endothelium, macrophages, and several other immune cells Cancer cells	Macrophages, T cells, mast cells, natural killer cells, endothelial cells and fibroblasts Cancer cells
Function	<ul style="list-style-type: none"> - Stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream - Stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. -Immune evasion 	<ul style="list-style-type: none"> -Stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes. -Enhances neutrophil migration -Immune evasion
Drug	Filgrastim, lenograstim	Sargramostim and molgramostim

G-CSF



GM-CSF

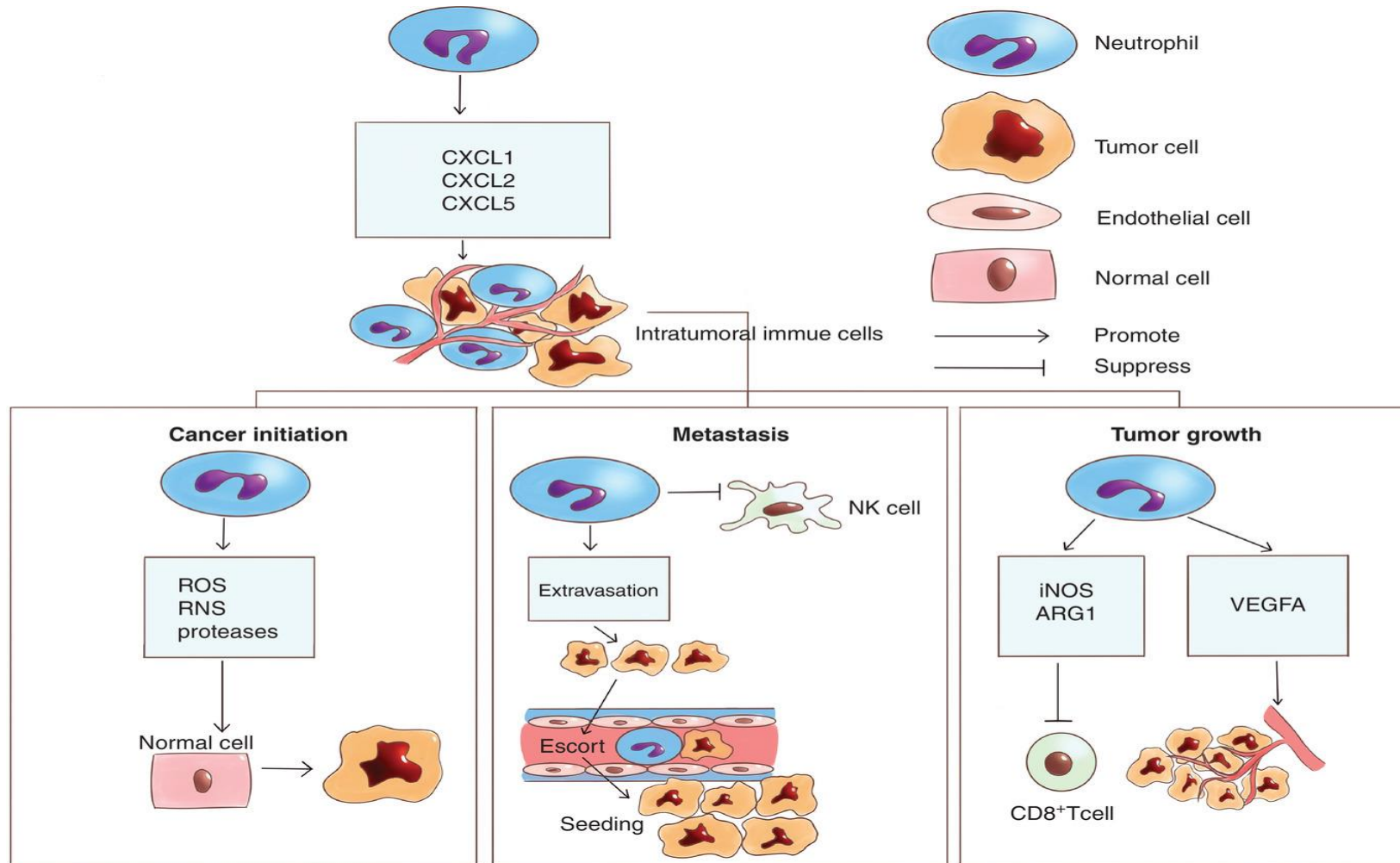


M-CSF = CSF1

Contents

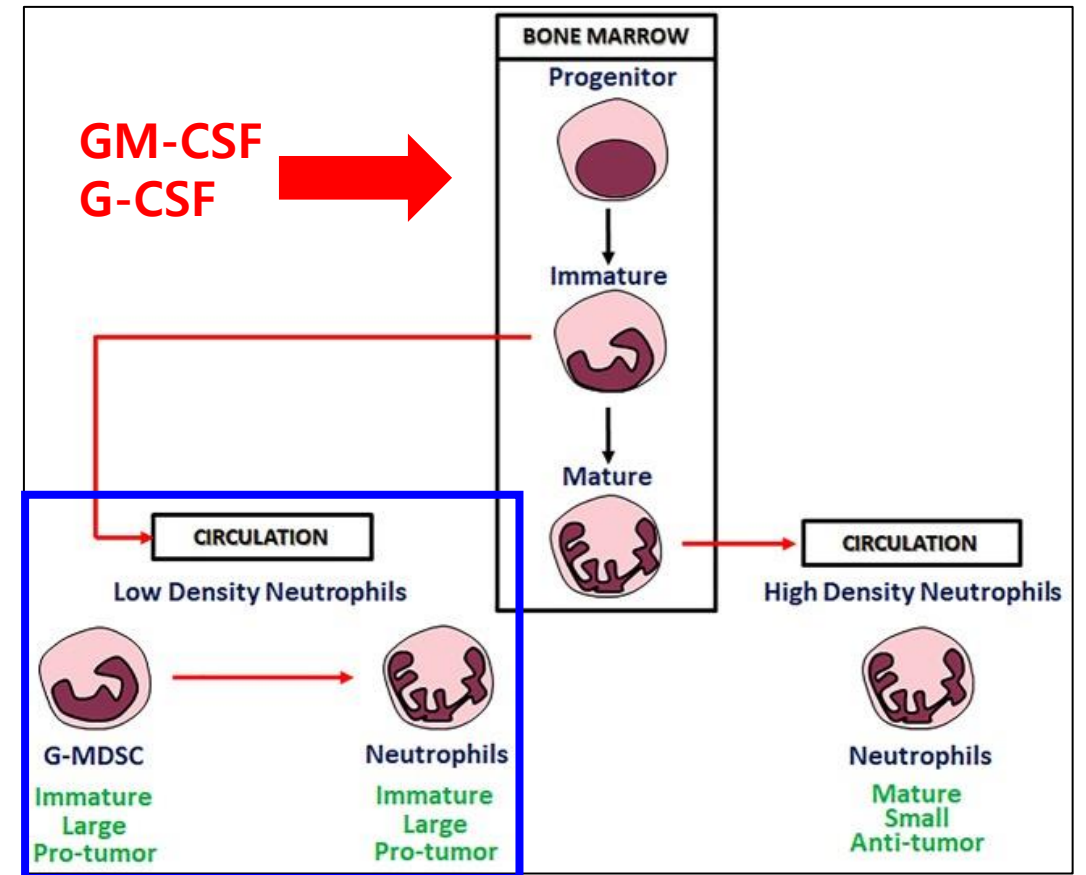
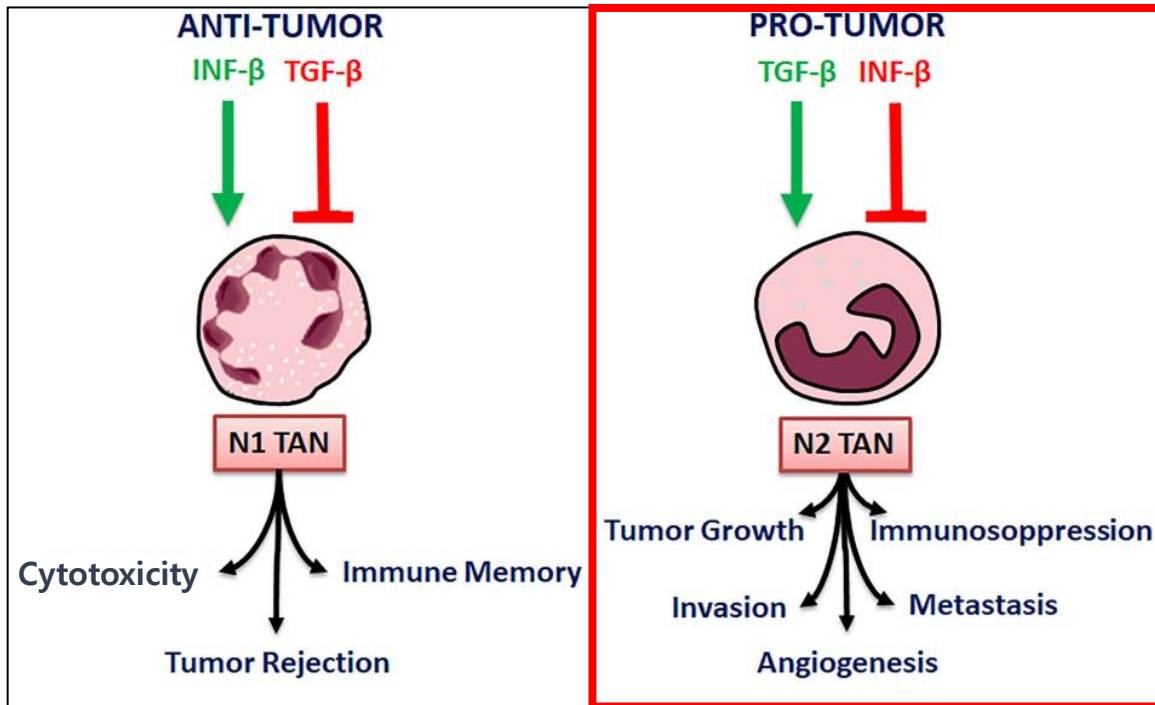
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The roles of neutrophil in cancer development, progression, and metastasis



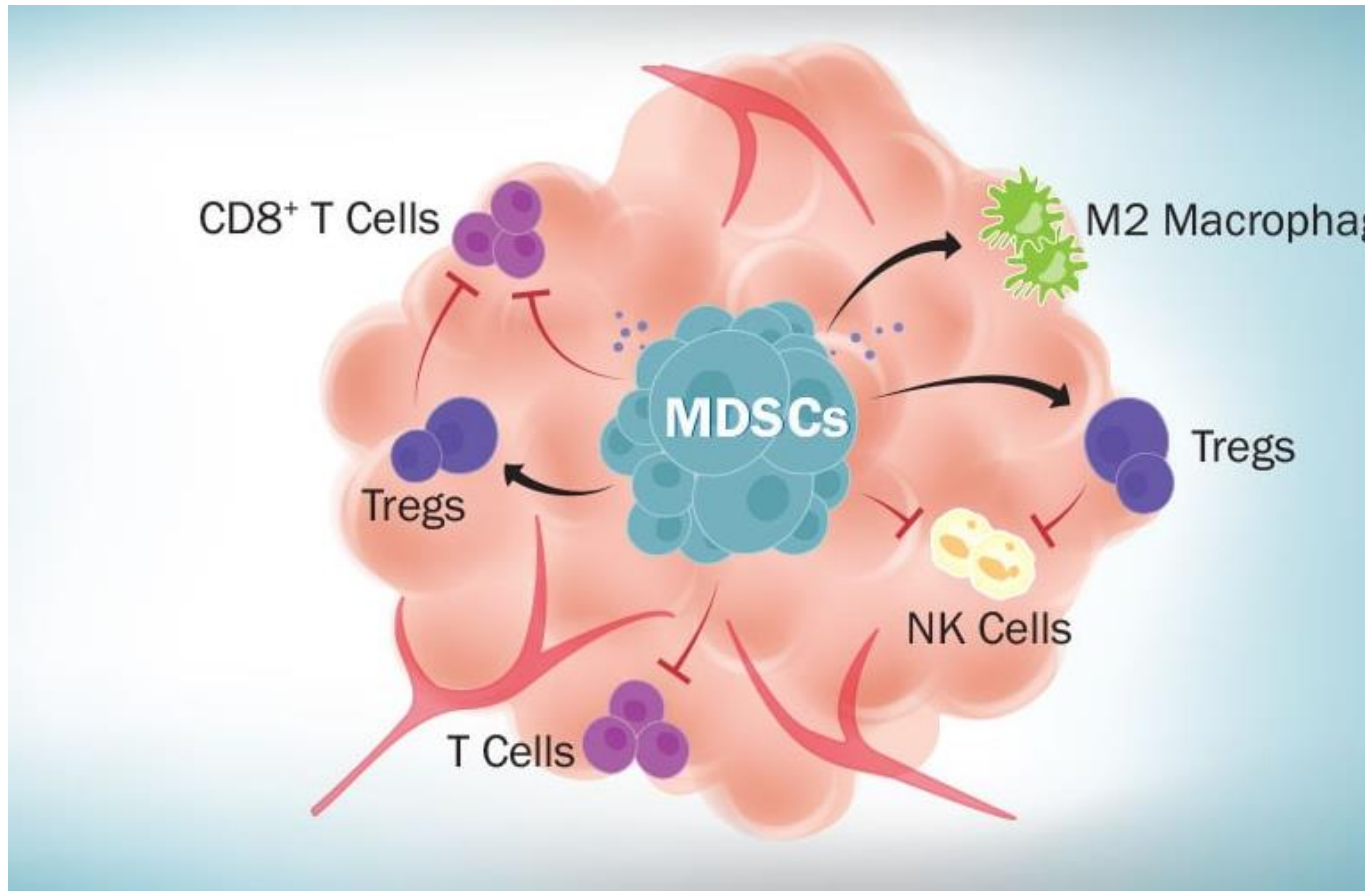
Tumor associated neutrophil

Tumor derived G-CSF can reprogram myeloid cell differentiation and generate immunosuppressive neutrophils.



Myeloid-derived suppressor Cells (MDSC)

Myeloid-derived suppressor Cells (MDSC)

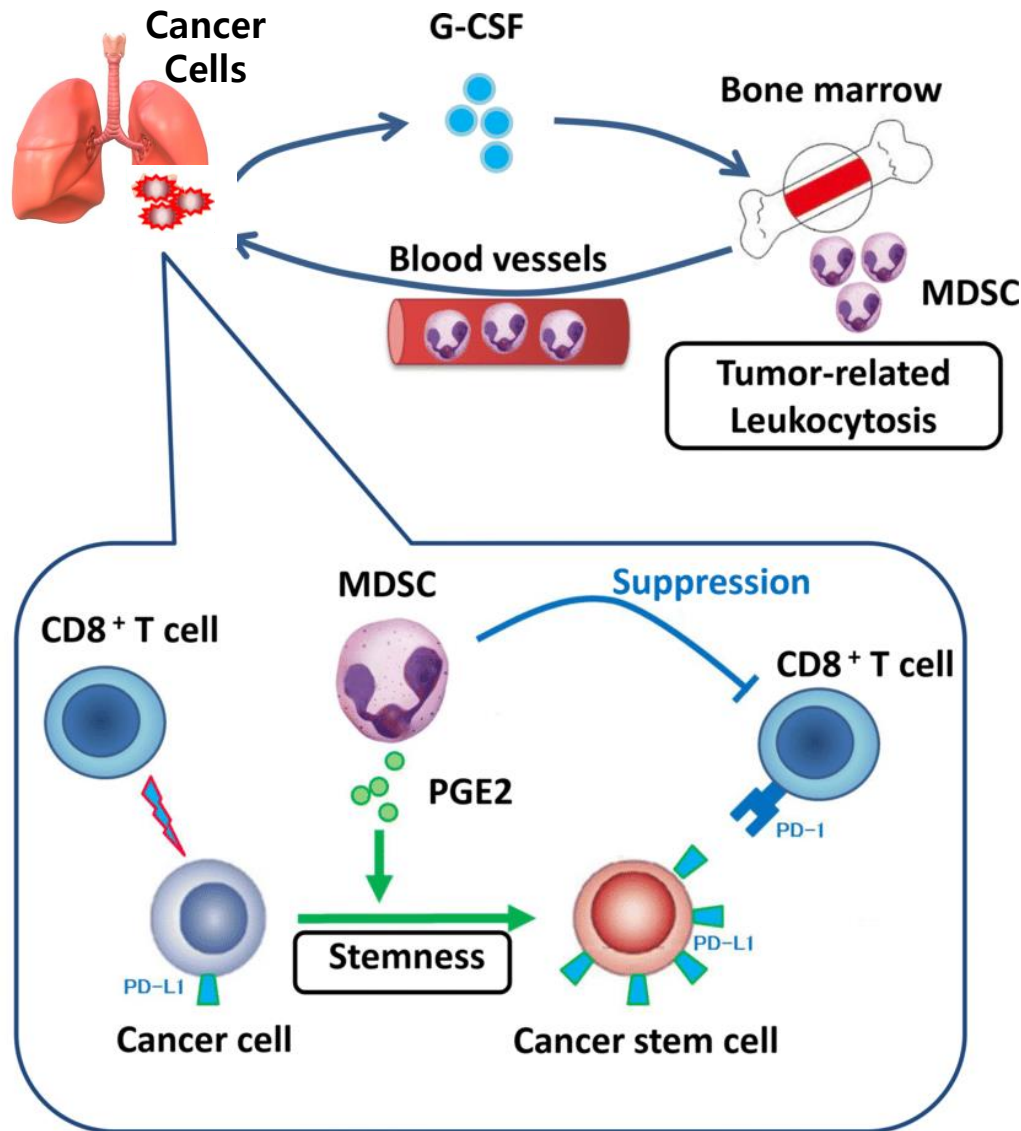


Under normal physiological conditions, MDSCs are not present in the circulation, but under pathological conditions, these cells accumulate.

GM-CSF and G-CSF promotes accumulation of MDSC.

MDSC causes **immunosuppression** in the tumor microenvironment which is a **major obstacle for cancer immunotherapy**

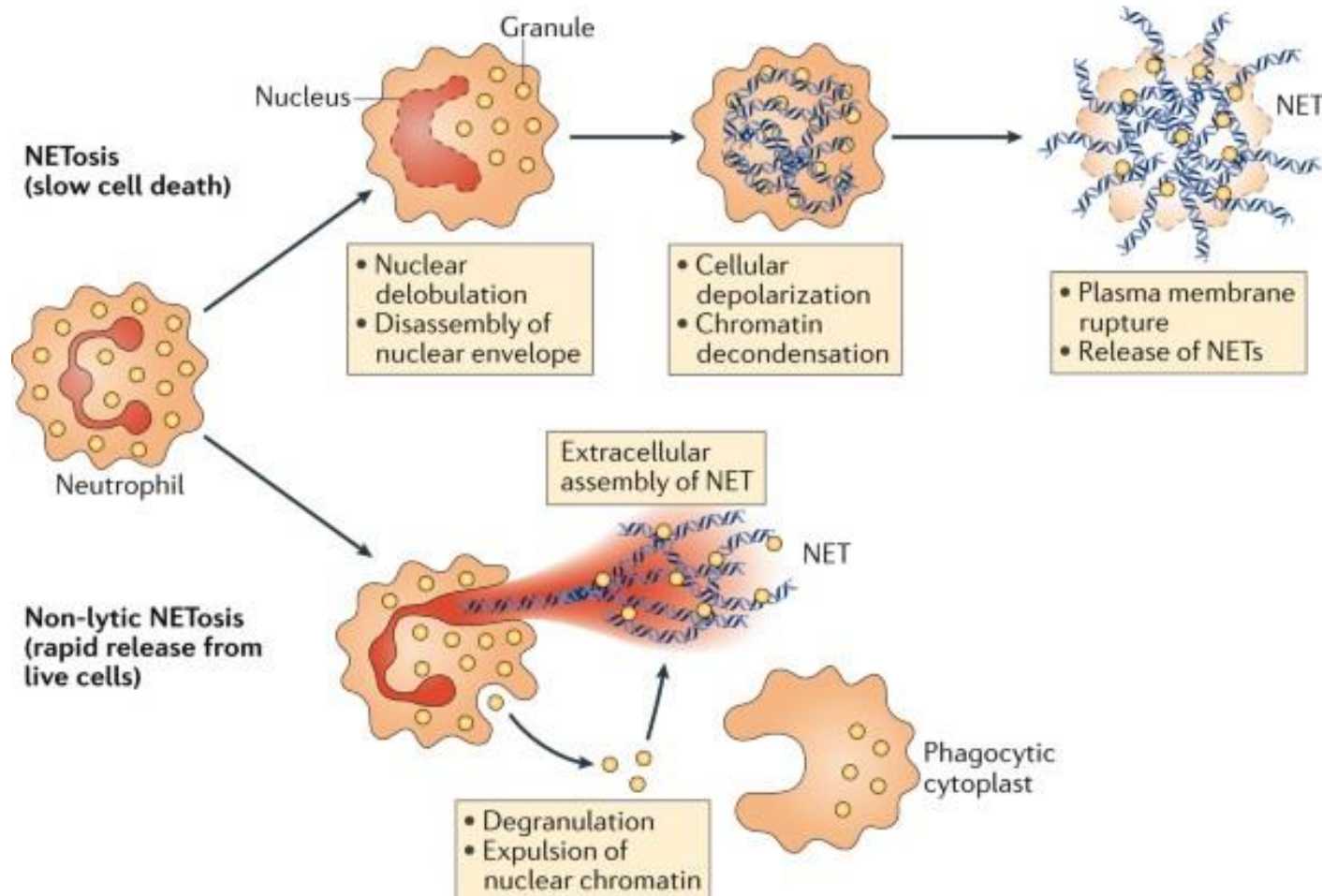
The role of G-CSF in tumorigenesis



**Myeloid-derived suppressor Cells (MDSC)
: immunosuppressive activities**

**Exogenous administration of G-CSF may be
responsible for the reduced tumor cell
chemosensitivity.**

Neutrophil extracellular traps (NETs)

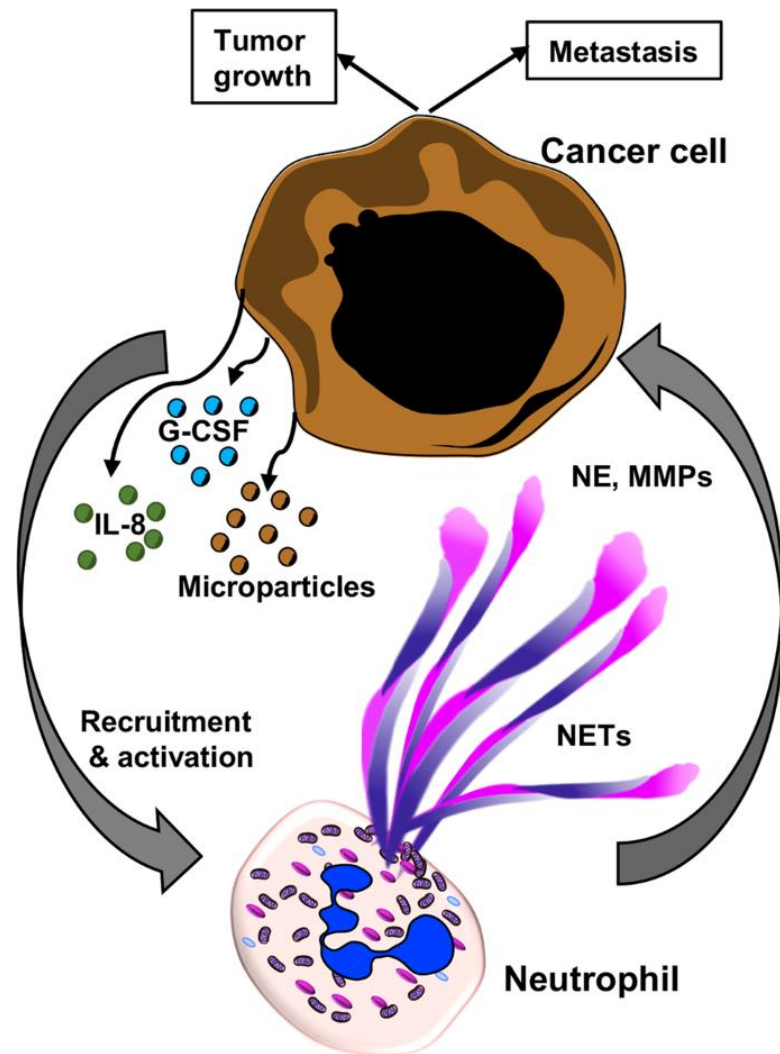


NETs : Web-like chromatin structure

Neutrophil extracellular traps (NETs) protect against infection, in particular by large pathogens.

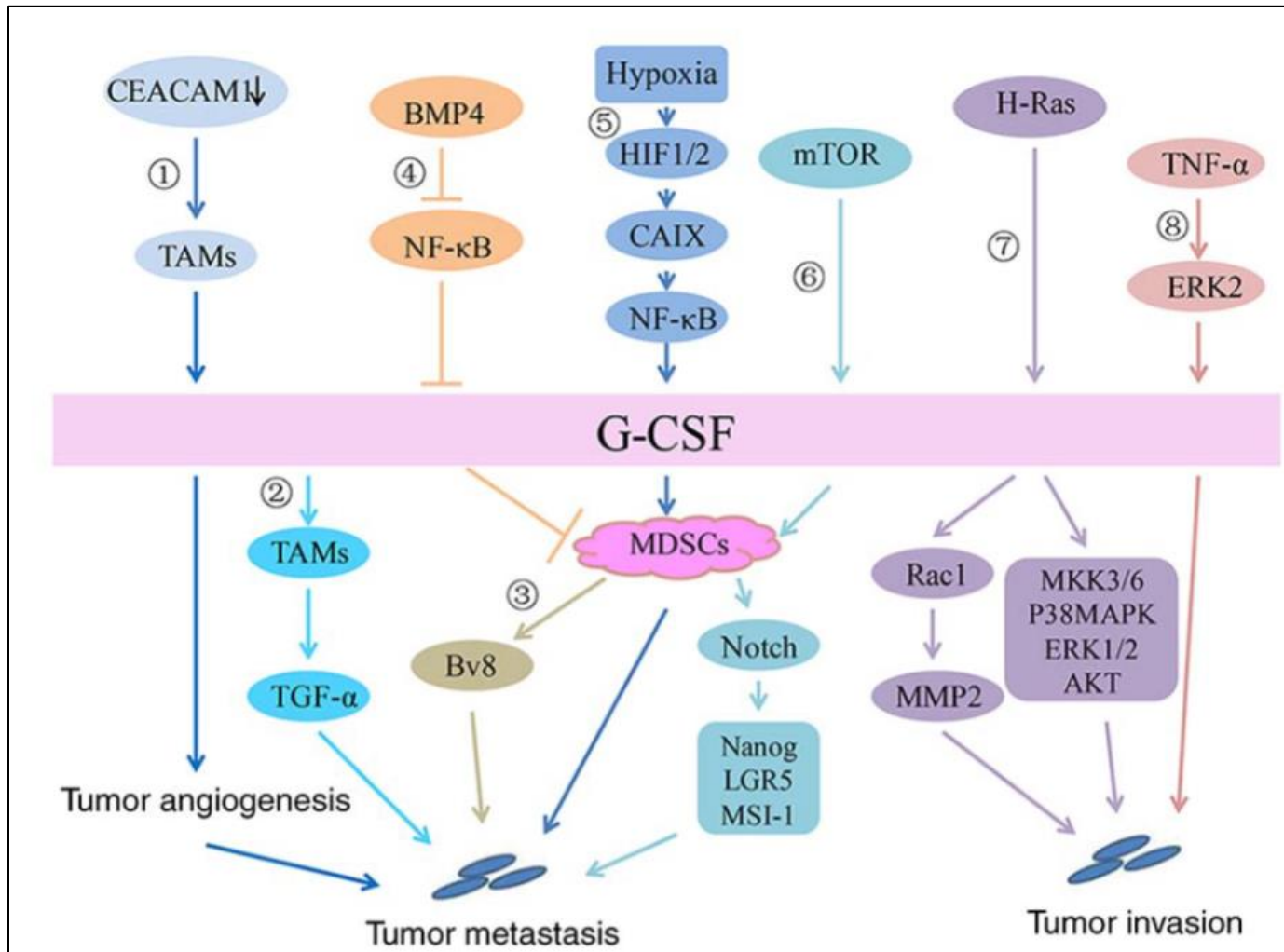
NETs can also occlude the vasculature by **promoting thrombosis** and obstruct important organ areas, **capture metastatic tumour**.

Interactions between tumor cells and neutrophils determines the outcome of tumor growth, progression, and metastasis.



NETs-associated granule proteins can contribute to tumor metastasis by releasing proteases such as **matrix metalloproteases (MMPs)** and **neutrophil elastase (NE)** that allows tumor cells to move out of the primary niche and to migrate to other organs

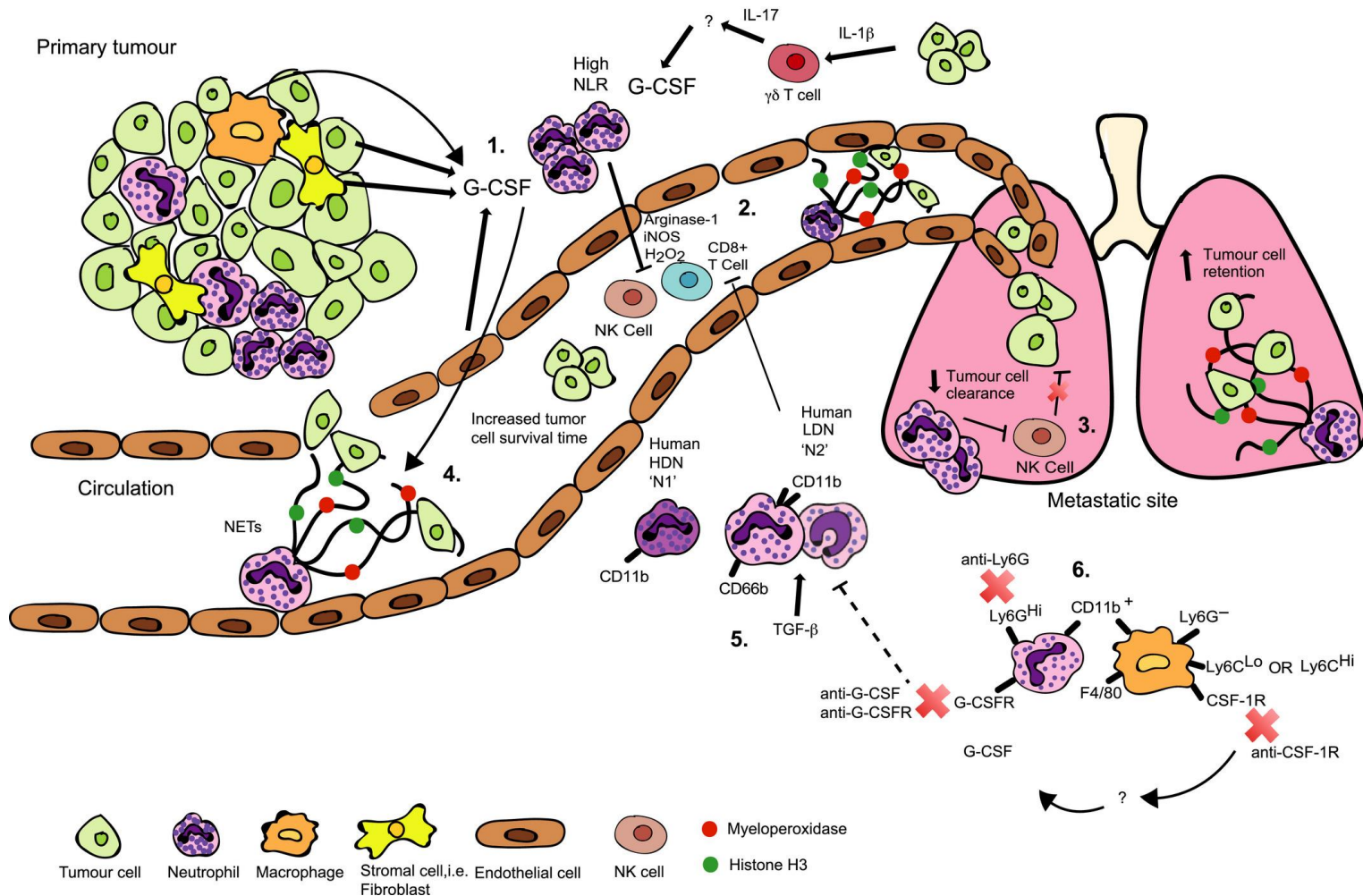
Signaling pathway of G-CSF in cancer



G-CSF is a promoter of tumor growth, which plays a role in **immunosuppression** by increasing **tumor angiogenesis** and mobilizing MDSCs

Secreted G-CSF not only has an **antiapoptotic effect** but also promotes the formation of tumors by **mobilizing MDSCs** to inhibit T cell activity and Bv8 secretion

The role of G-CSF in tumorigenesis

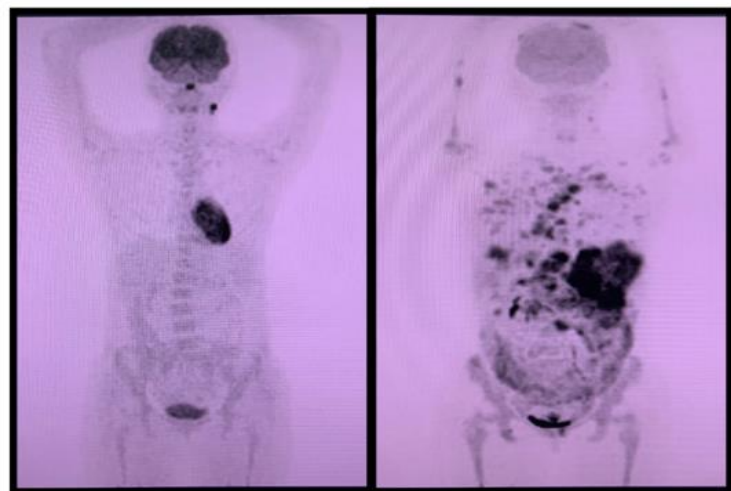
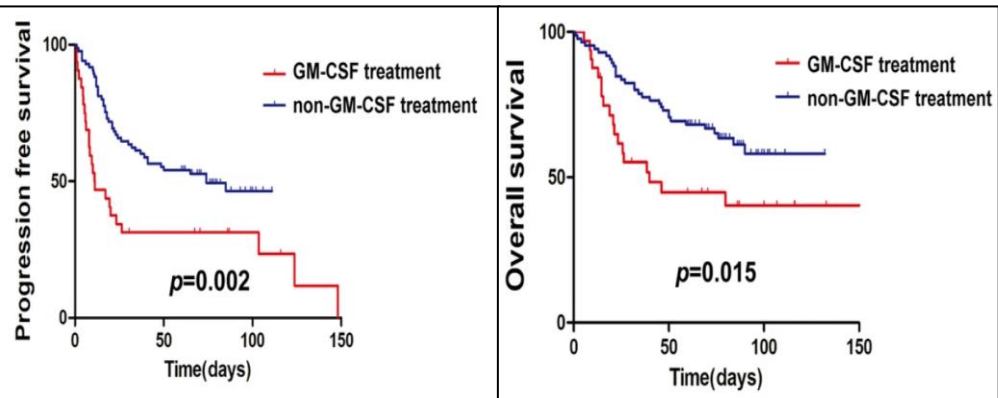


- **G-CSF promotes tumor growth, metastasis and chemotherapy resistance, inhibits tumor cell apoptosis, induces angiogenesis, participates in cancer-associated thrombosis, and is associated with a poor clinical prognosis**

Contents

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GM-CSF mediates immune evasion via upregulation of PD-L1 expression in extranodal natural killer/T cell lymphoma

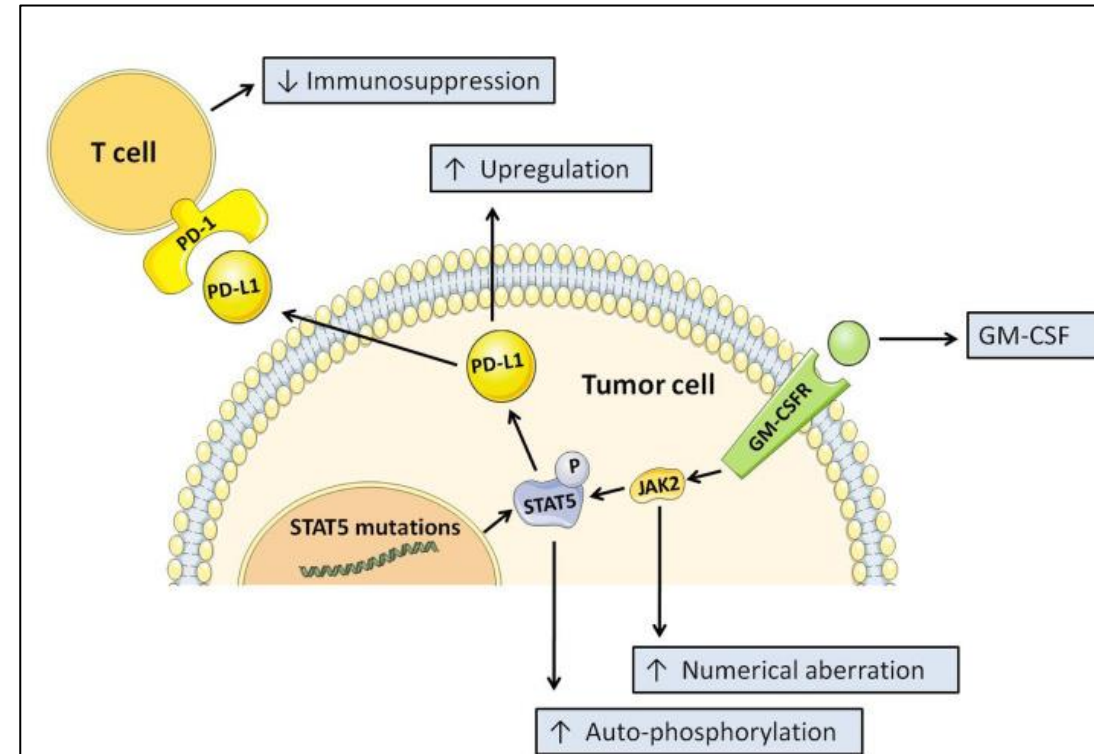
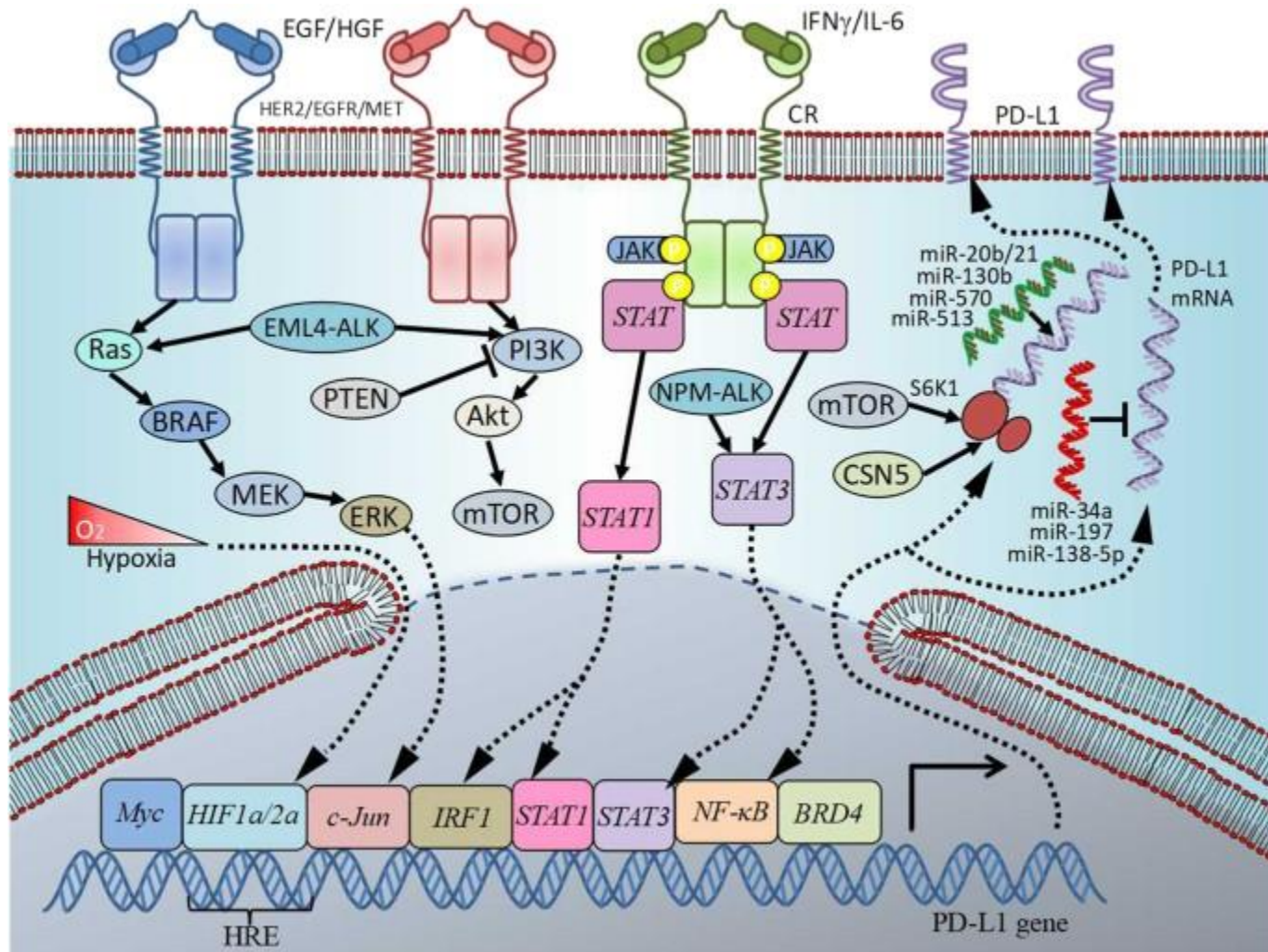


Baseline

After GM-CSF treatment

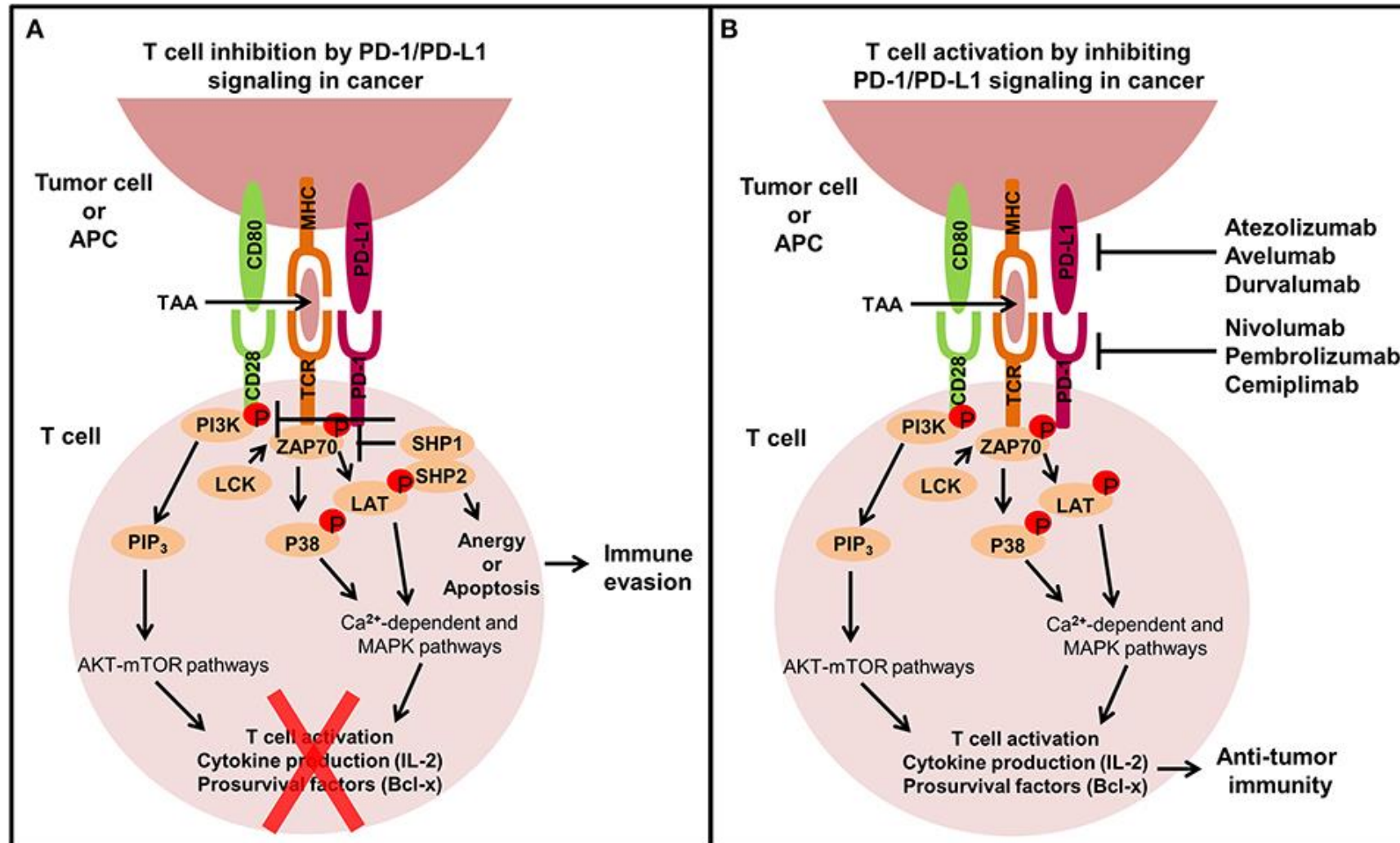
- We found that some of the extranodal natural killer/T cell lymphoma (ENKTL) patients with **the treatment of hGM-CSF rapidly experienced disease progression**
- The **PD-L1 expression** at mRNA and protein levels was significantly increased in ENKTL cells treated with **GM-CSF**.
- These findings demonstrate that **GM-CSF potentially triggers the loss of tumor immune surveillance** in ENKTL patients and promotes disease progression, which is associated with **STAT5 mutations and JAK2 hyperphosphorylation** and then **upregulates the expression of PD-L1**.

GM-CSF mediates immune evasion via upregulation of PD-L1 expression in extranodal natural killer/T cell lymphoma

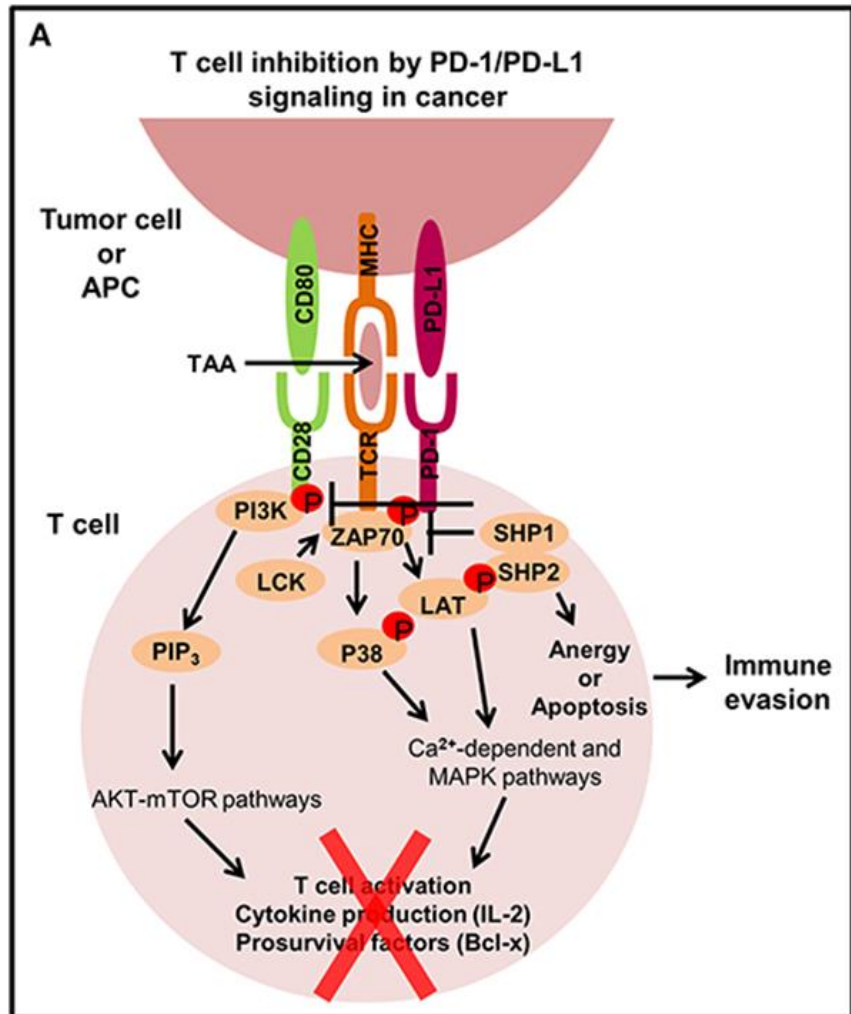


Mol Cancer . 2021 May 29;20(1):80.

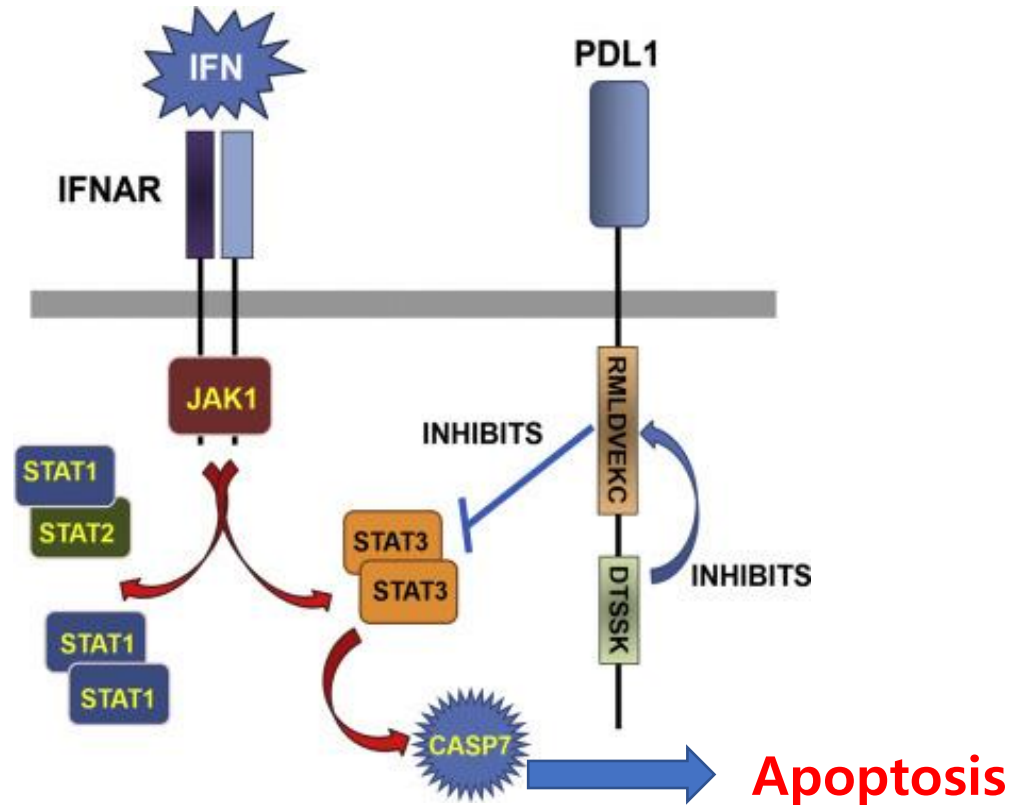
The extrinsic function of the PD-1/PD-L1 signaling axis in cancer



The intrinsic function of the PD-1/PD-L1 signaling axis in cancer



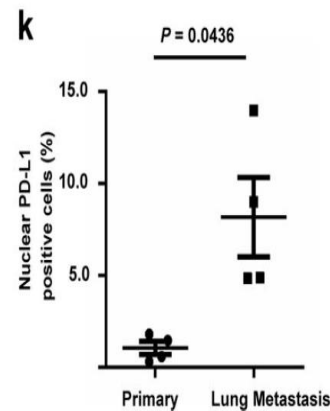
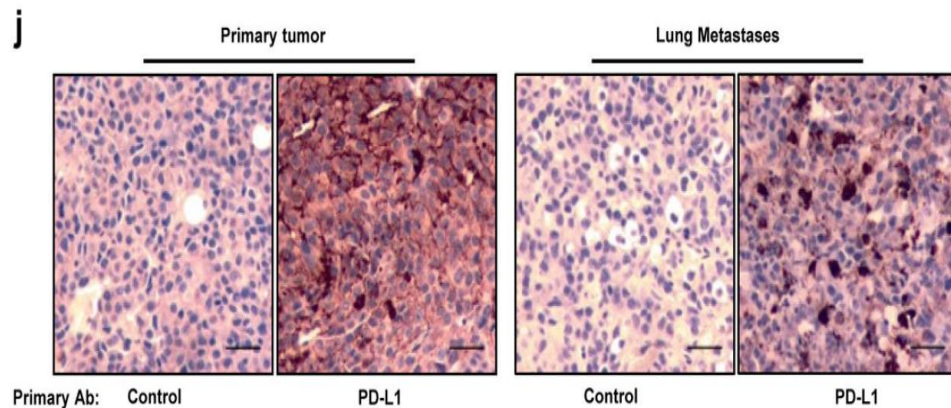
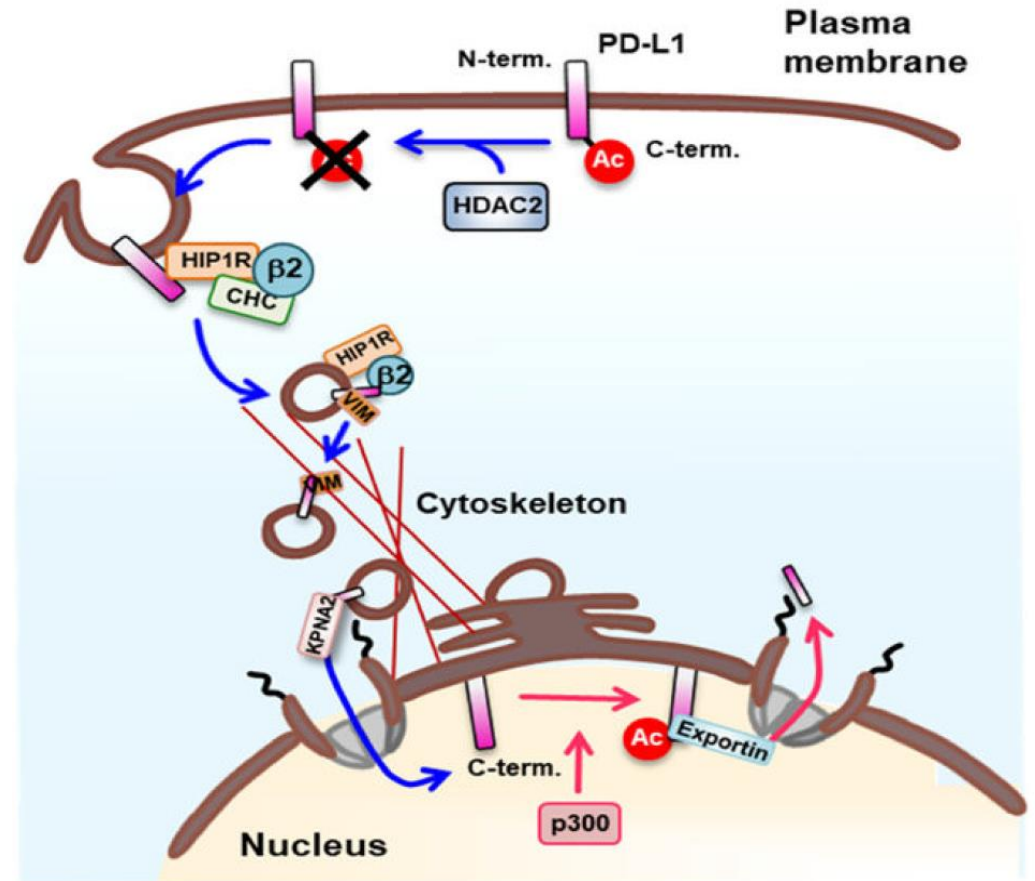
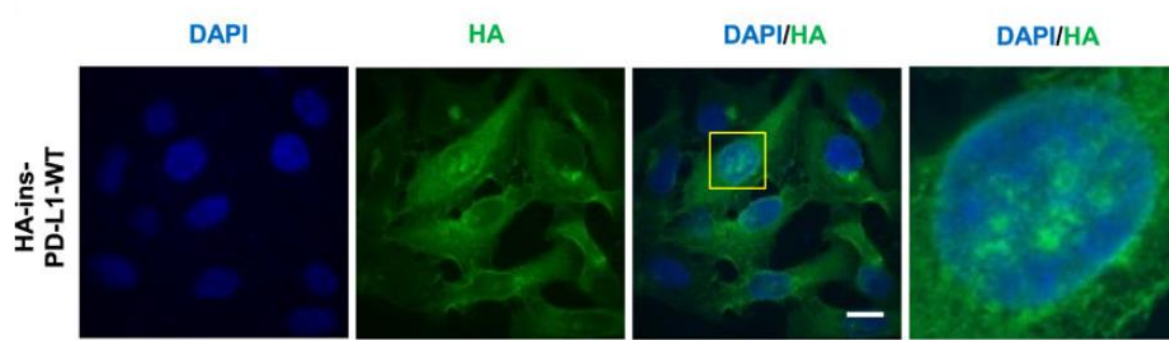
The intrinsic function of the PD-1/PD-L1 signaling axis in cancer



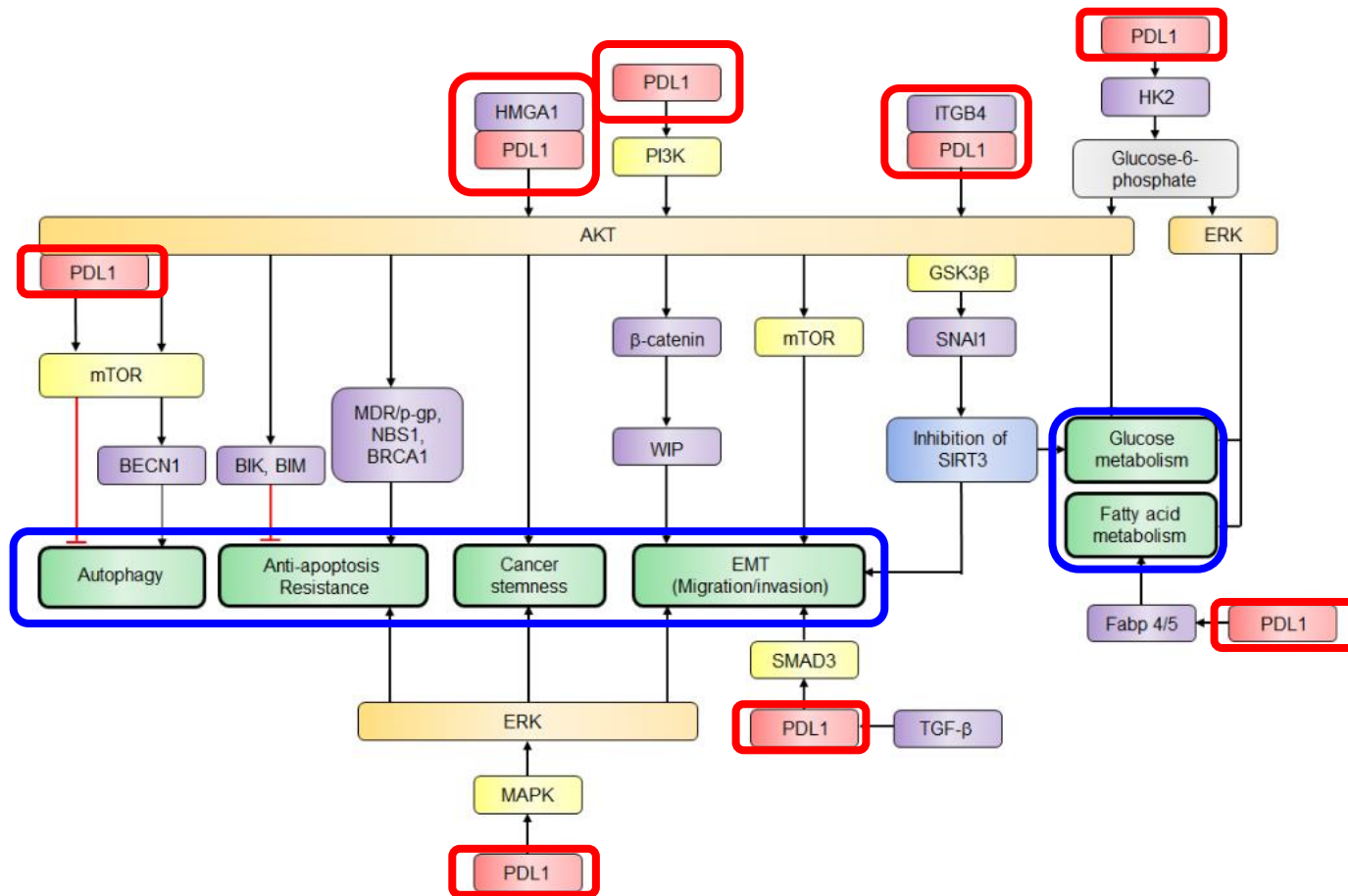
RMLDVEKC motif was required to inhibit IFN-mediated cytotoxicity toward tumor cells via directly **preventing STAT3 phosphorylation and caspase-mediated apoptosis.**

→ PD-L1 promotes survival of cancer cells.

Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy

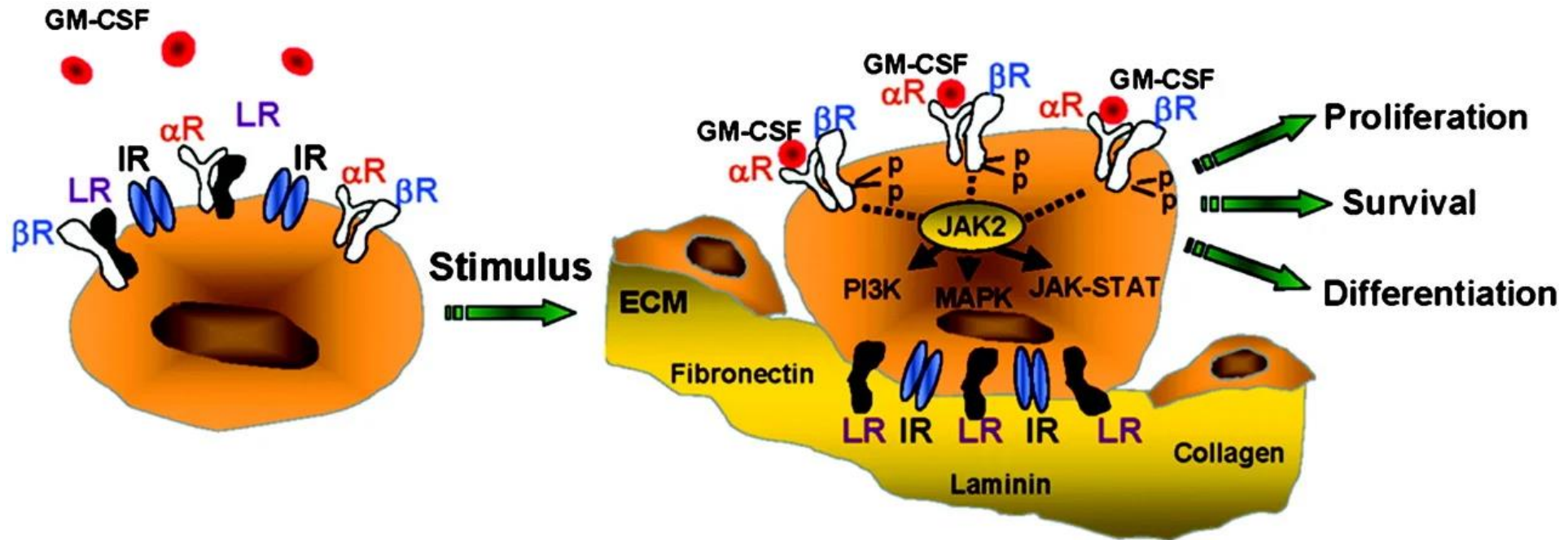


Protumoral activity of cancer-intrinsic PD-L1



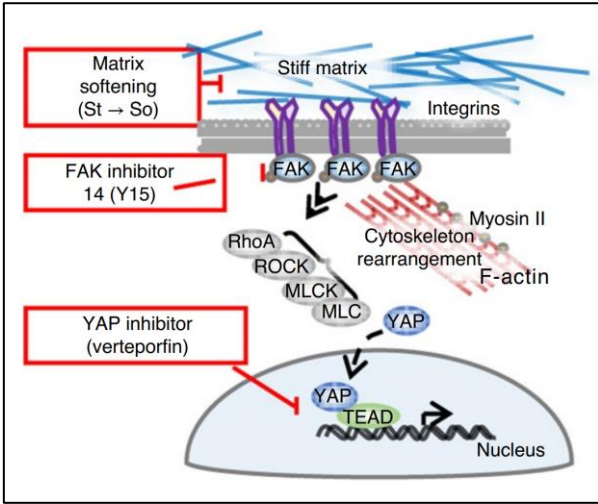
Non-immunological functions of PD-L1 in the regulation of cancer intrinsic activities including **mesenchymal transition, glucose and lipid metabolism, stemness, and autophagy.**

The function of GM-CSF on extracellular matrix

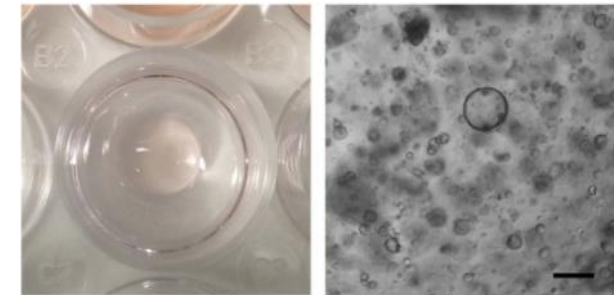
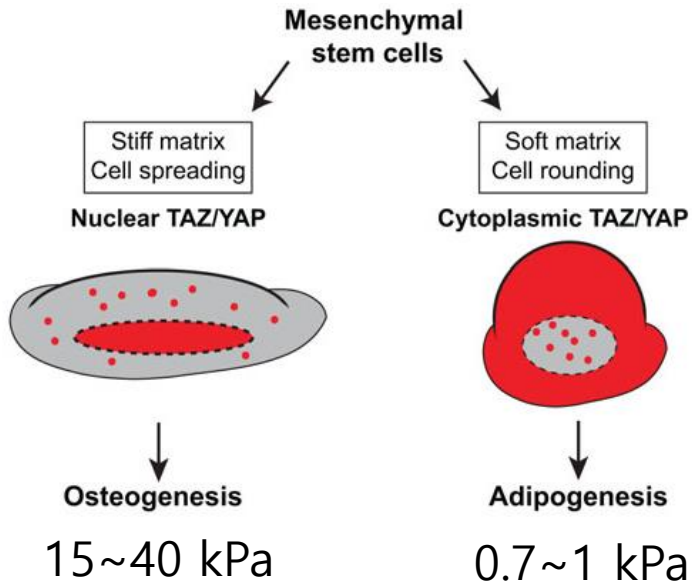


- GM-CSF may also have an important role **in regulating the extracellular matrix by modulating the metabolism of vascular collagens.**
- It can promote the proliferation and migration of vascular endothelial cells, thus contributing to angiogenic processes.

Matrix stiffness regulates the activity of PD-L1 (through mechano-sensor YAP)

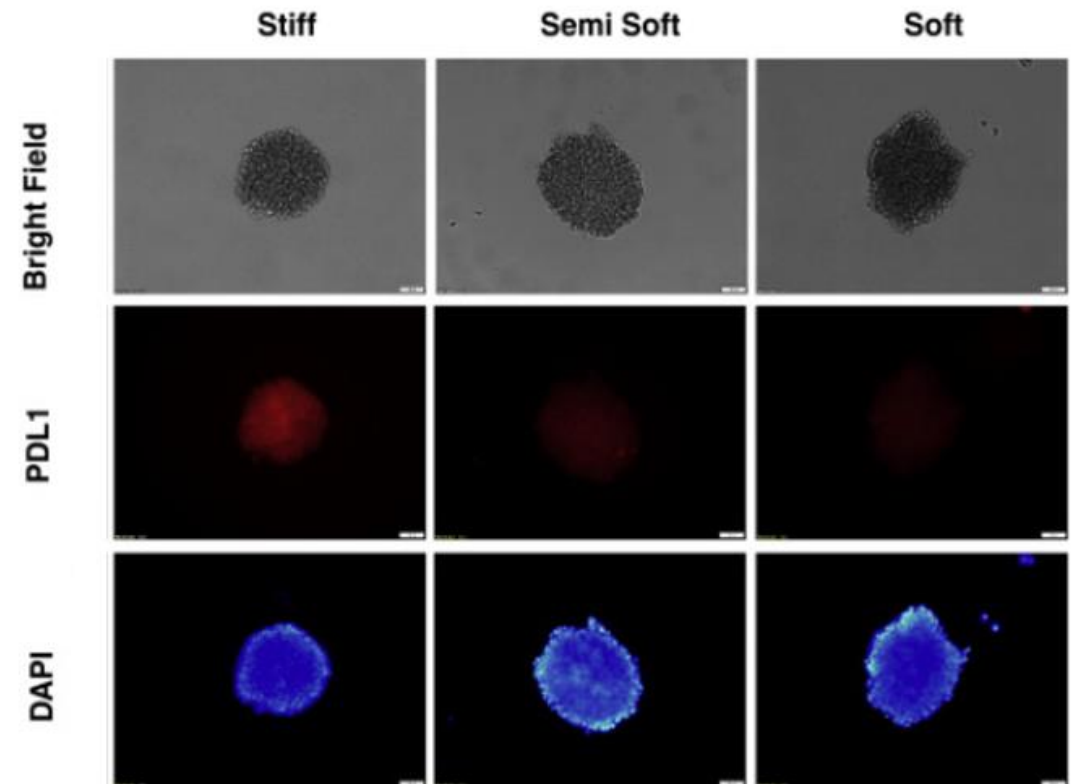
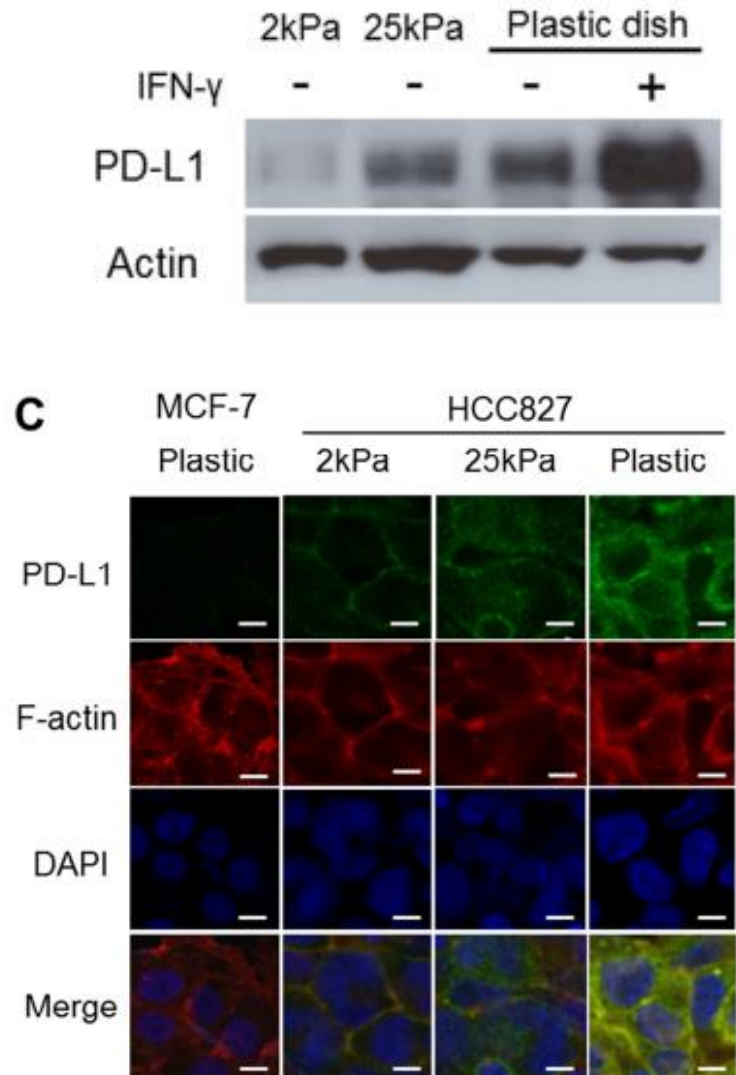


Liver cirrhosis : 3~12 kPa
Breast tumor : 2.4~4.8 kPa



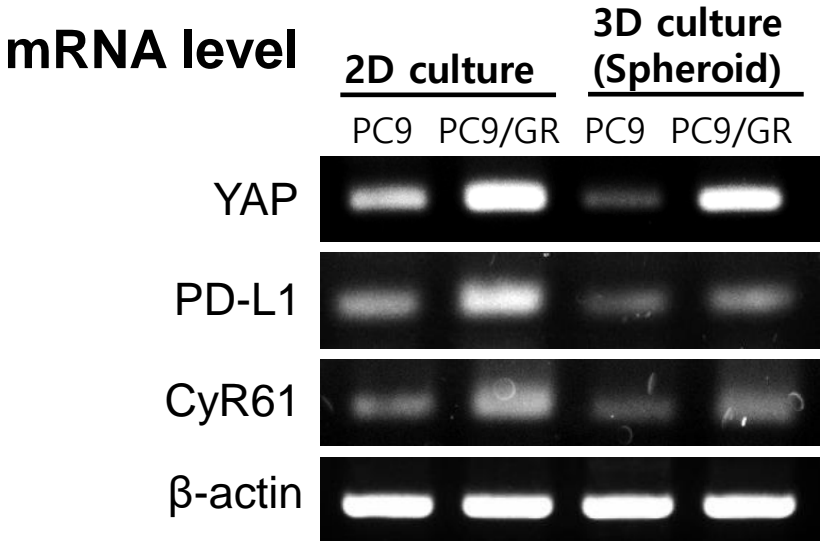
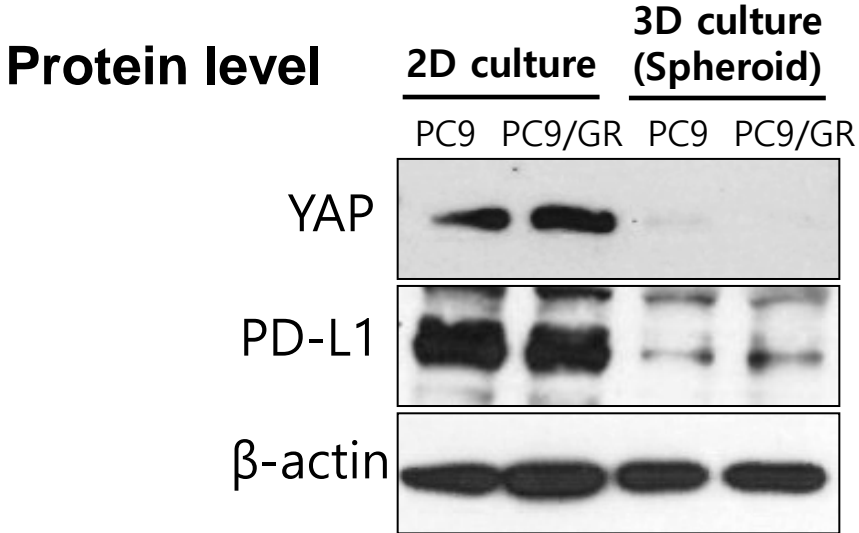
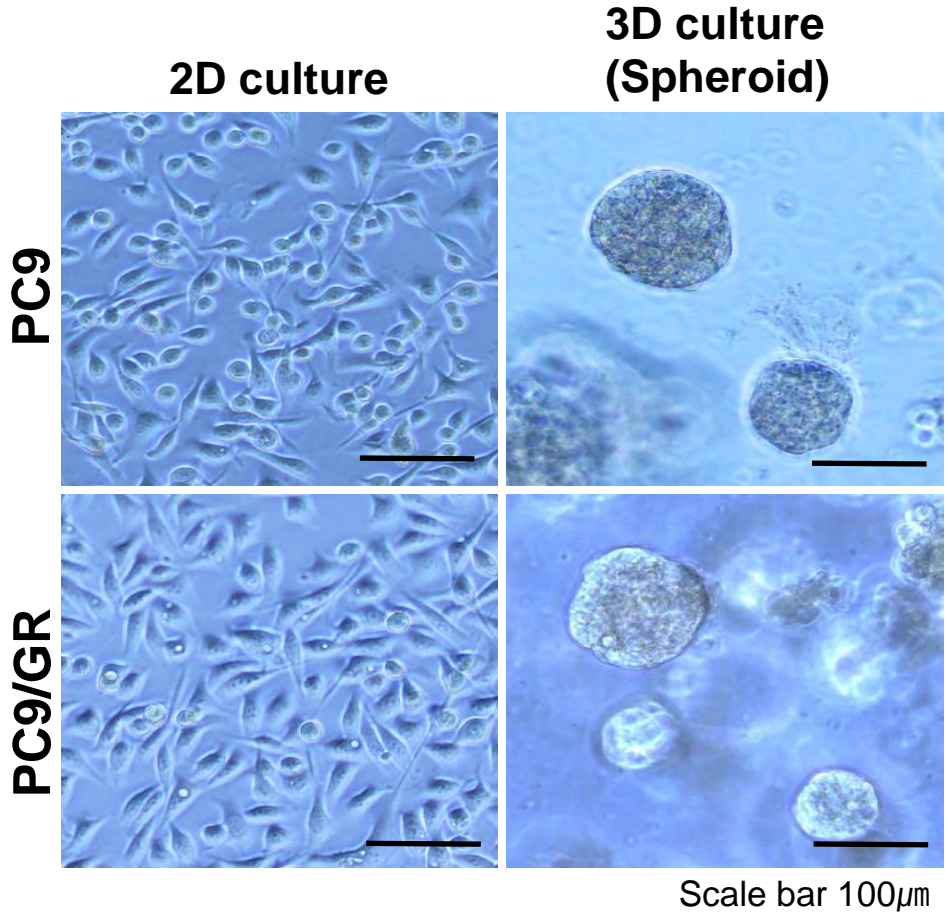
Matrigel: 0.02~0.3 kPa

Regulation of PD-L1 expression by matrix stiffness in lung cancer cells

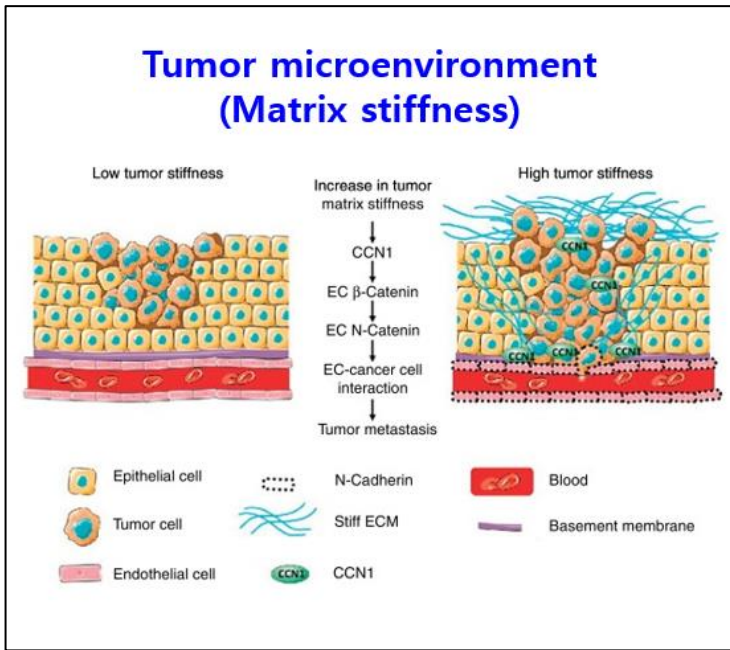


Ayako et al. Biochem Biophys Res Commun, 2018
Biochim Biophys Acta Mol Cell Res 2019 Dec;1866(12):118526

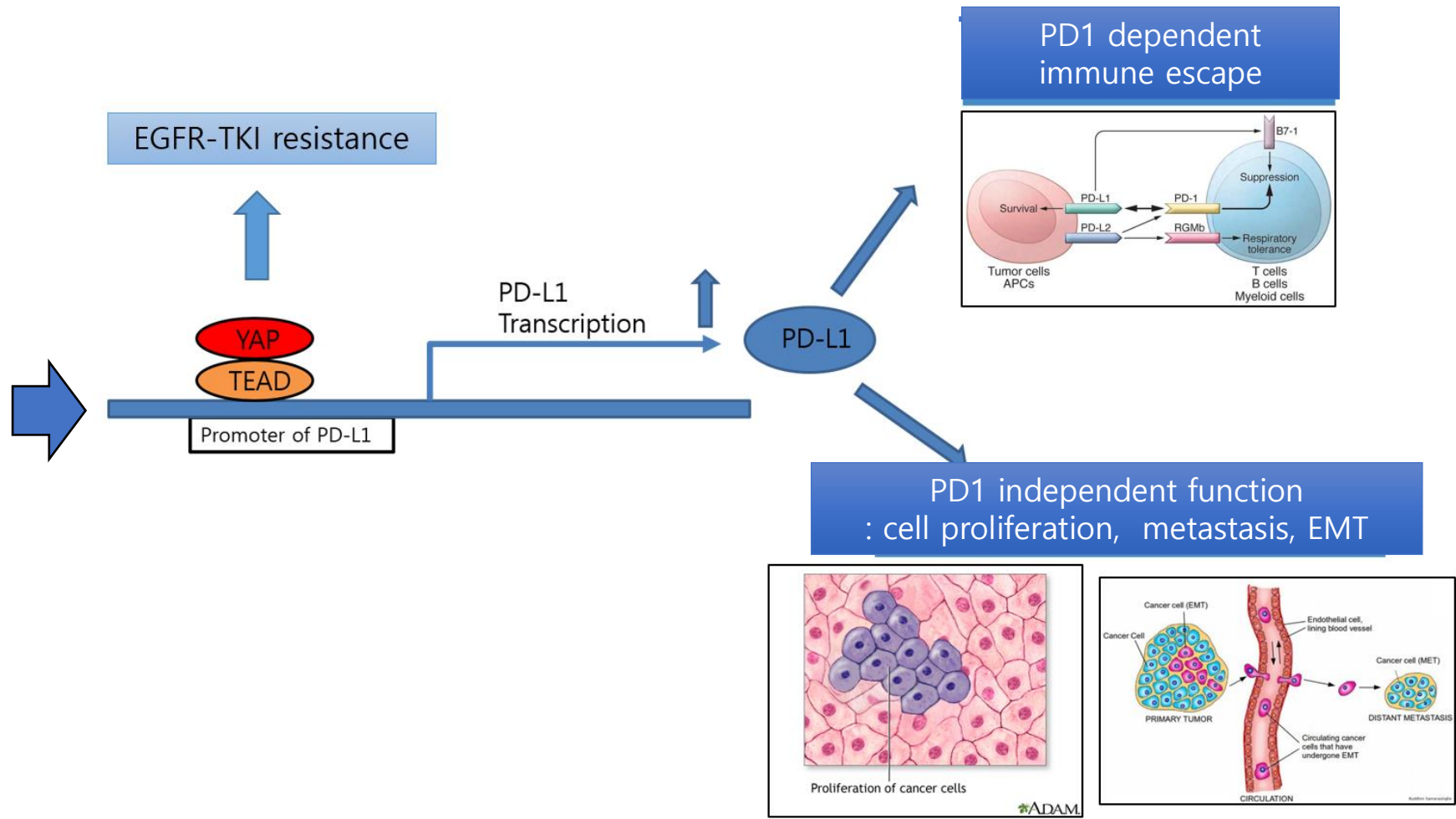
Matrix stiffness regulates the expression of YAP and PD-L1 in lung cancer



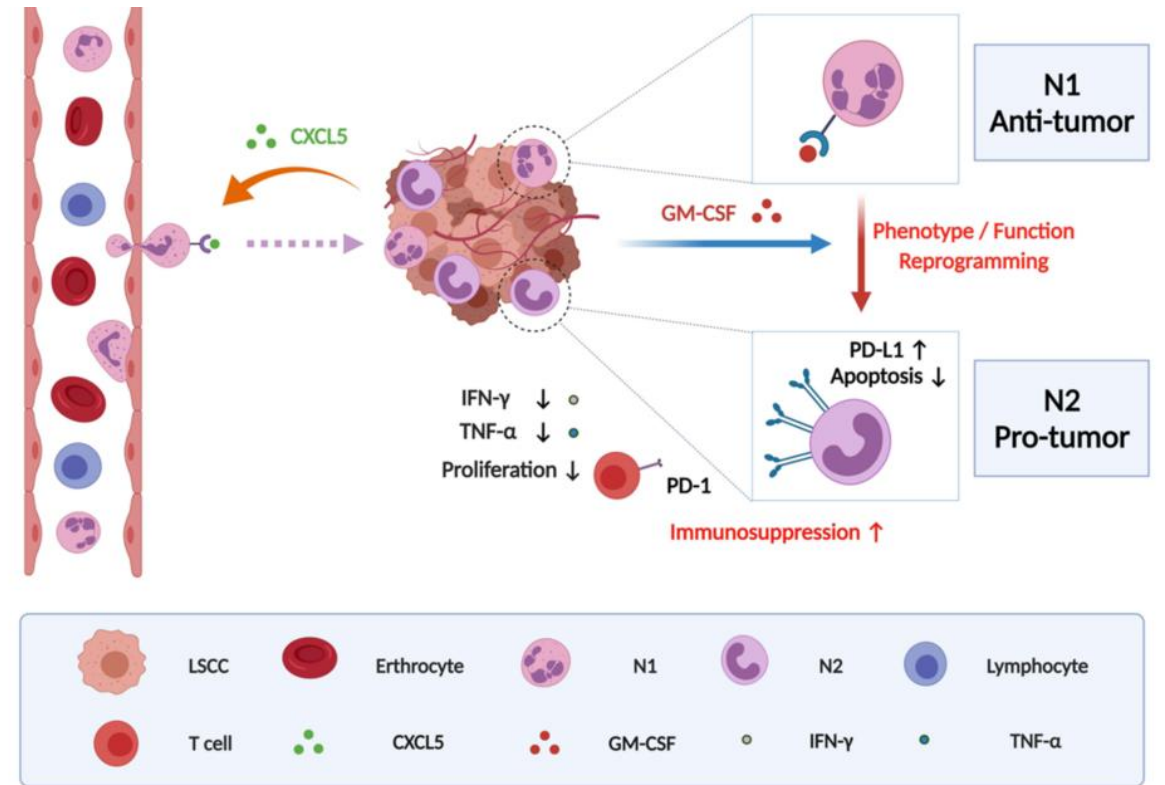
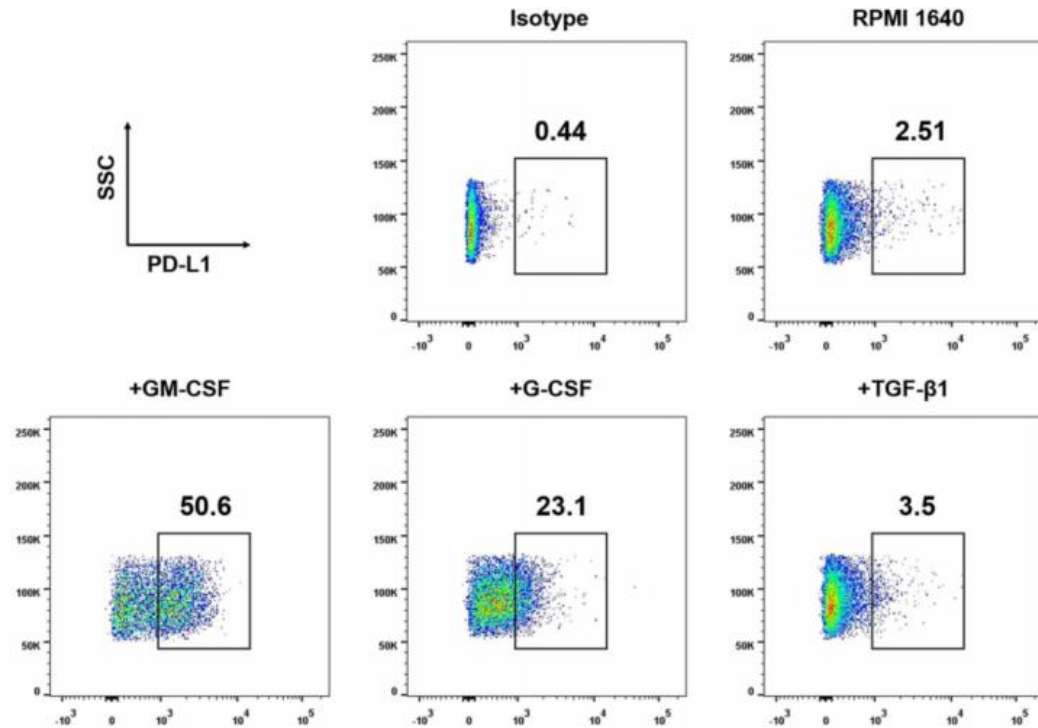
Mechano-sensor YAP directly regulates the expression of PD-L1 transcripts in lung adenocarcinoma



G-CSF/GM-CSF



Tumor-Infiltrating PD-L1+ Neutrophils Induced by GM-CSF Suppress T Cell Function in Laryngeal Squamous Cell Carcinoma and Predict Unfavorable Prognosis



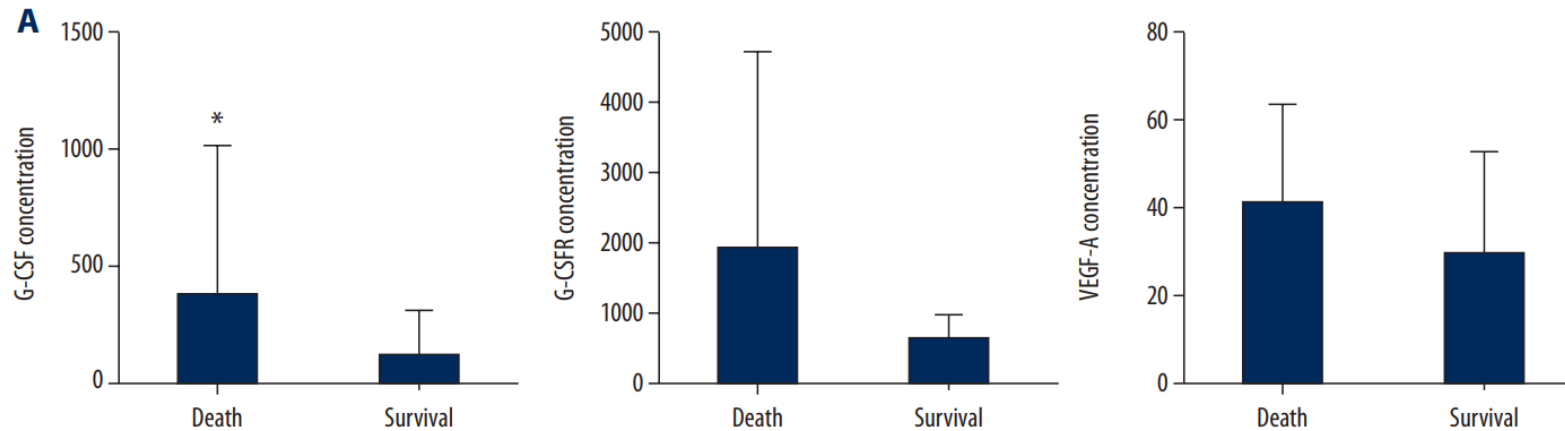
Cytokine **GM-CSF** most significantly **upregulated** the level of immunosuppressive molecule **PD-L1** on neutrophils

Contents

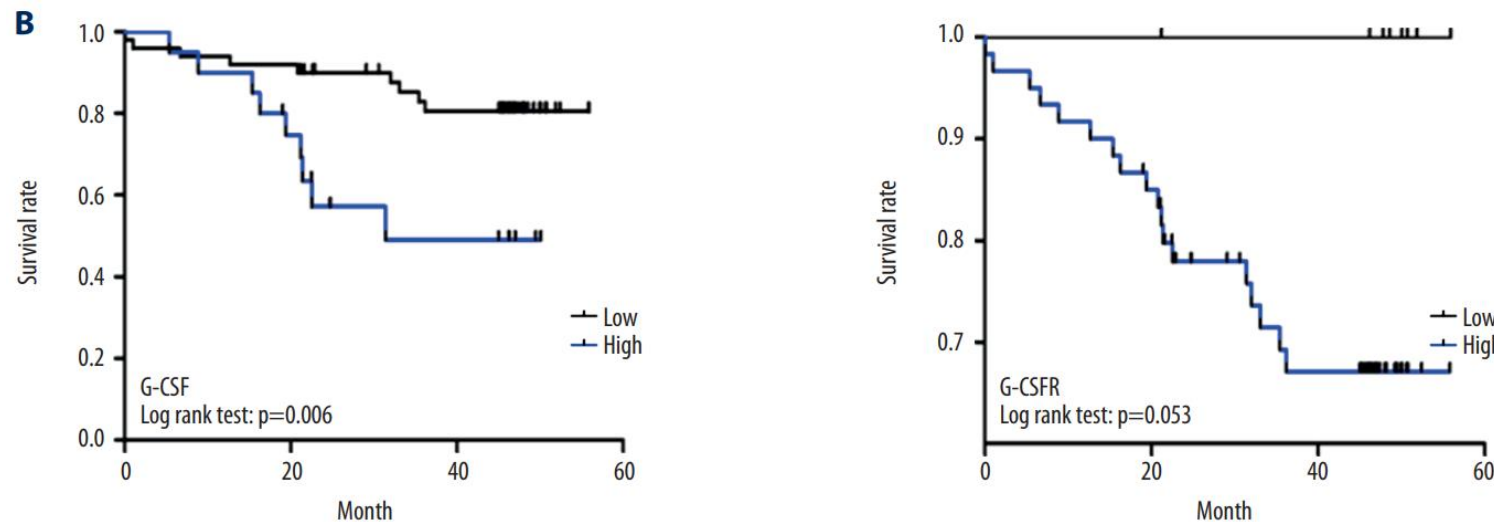
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G-CSF/G-CSFR correlation with cancer patient survival

Stomach cancer



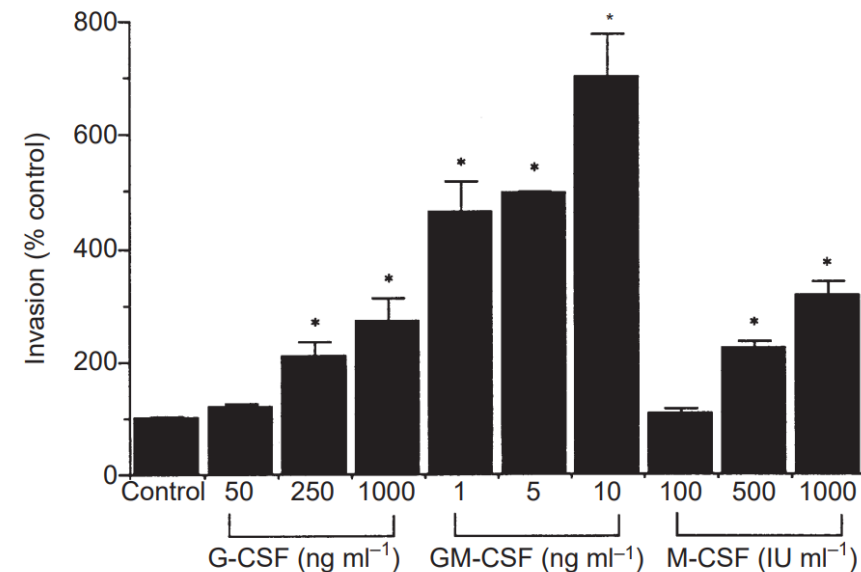
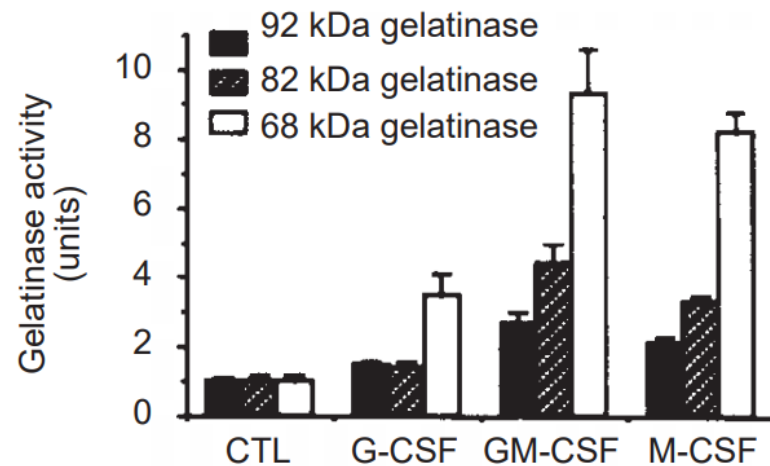
The G-CSF concentration was significantly higher in patients who had died than in those who had survived.



High G-CSF expression was associated with poor OS

Stimulatory effects of GM-CSF on tumor progression

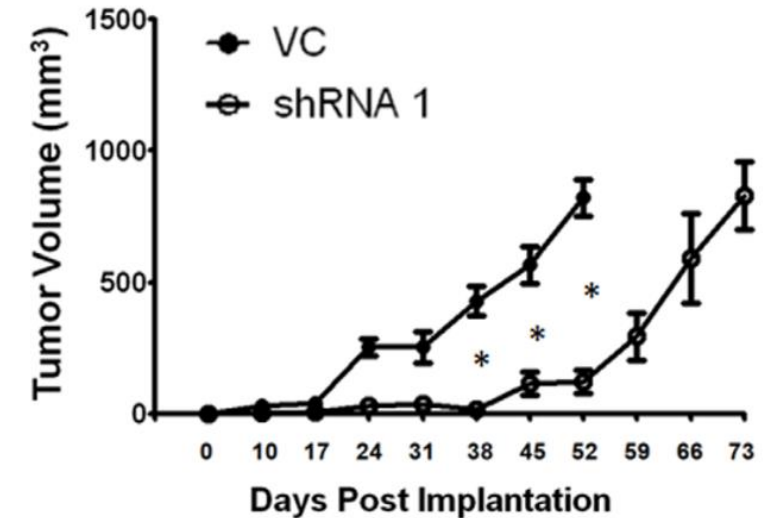
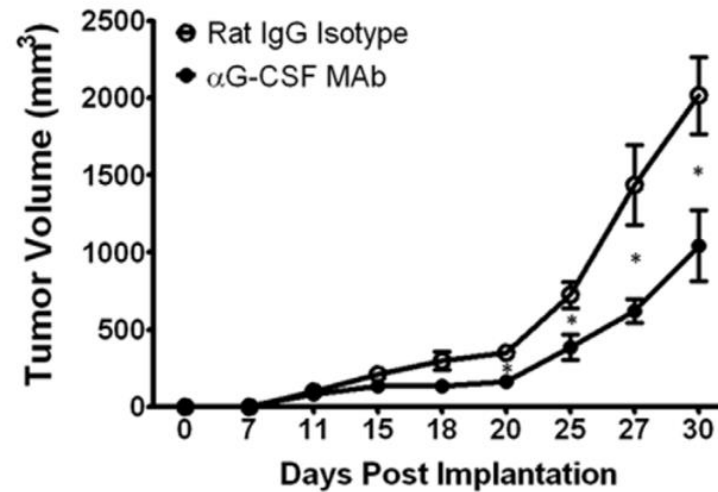
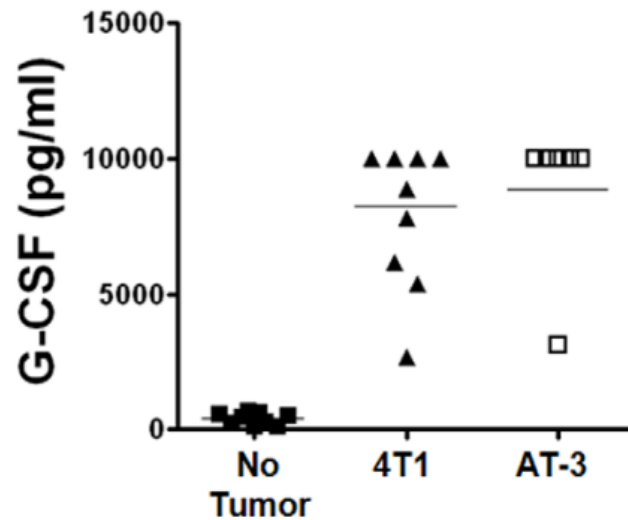
- Granulocyte, granulocyte–macrophage, and macrophage colony-stimulating factors can **stimulate the invasive capacity of human lung cancer cells**



Enhanced production of extracellular matrix-degrading proteinases

G-CSF blockade slows tumor growth

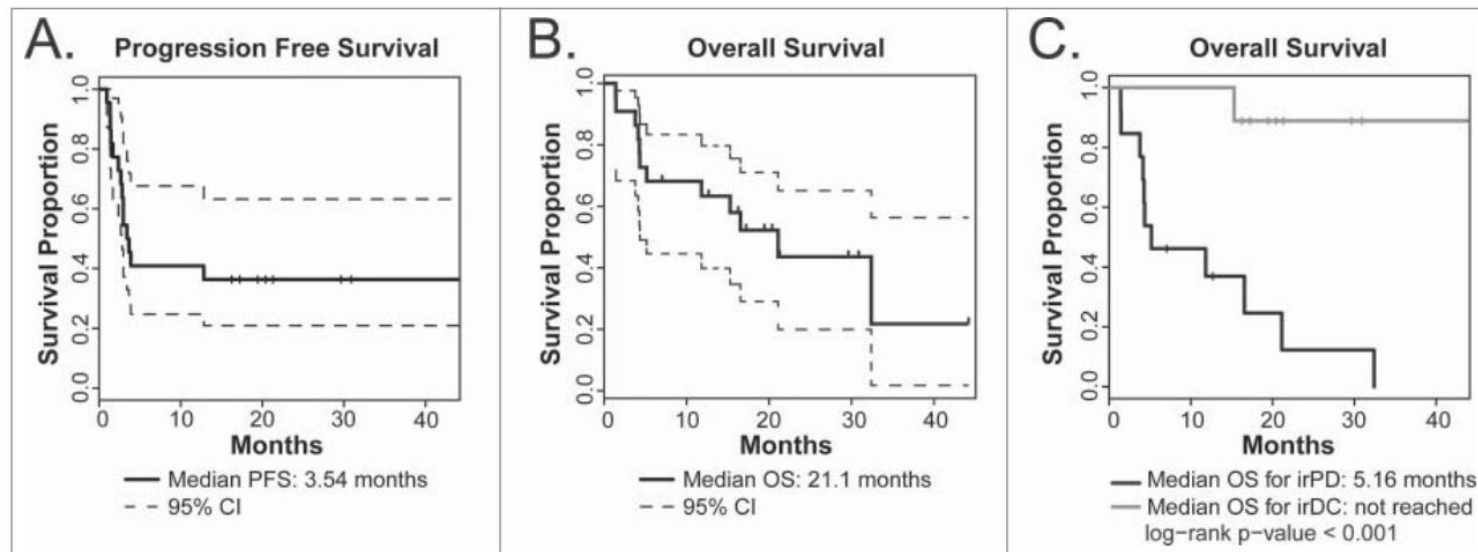
Mammary cancer model



Tumor-derived G-CSF enhances tumor growth through granulocytic MDSC-dependent mechanisms

ORIGINAL RESEARCH

GM-CSF and ipilimumab therapy in metastatic melanoma: Clinical outcomes and immunologic responses



- The immune response disease control (irDC) rate at 24 weeks was 41% and the overall response rate (ORR) was 32%.
- The median progression free-survival (PFS) was 3.5 mo and the median overall survival (OS) was 21.1 mo.
- 41% of the patients experienced Grade 3 to 4 adverse events.

A Study of Sargramostim Plus Pembrolizumab With or Without Pemetrexed in Patients With Advance Non-small Cell Lung Cancer After Completion of Chemoimmunotherapy

[Recruitment Status](#) ⓘ : Recruiting

[First Posted](#) ⓘ : April 23, 2021

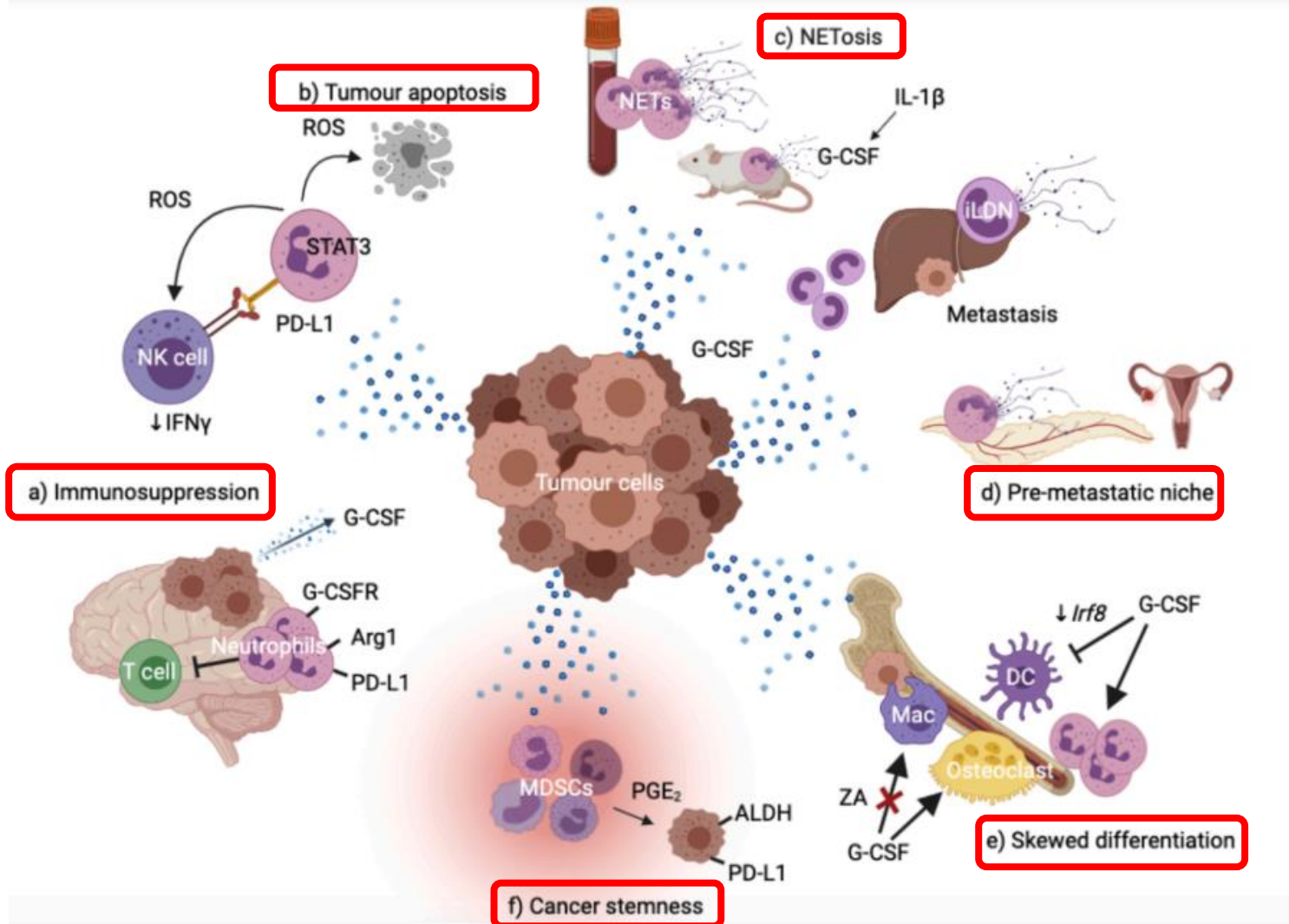
[Last Update Posted](#) ⓘ : January 21, 2022

- There are both pre-clinical and clinical evidence **supporting the combination of granulocyte macrophage colony stimulating factor (GM-CSF) with immunotherapy.**
- GM-CSF is a hematopoietic growth factor that triggers proliferation and differentiation of hematopoietic progenitor cells, mainly neutrophils, monocytes/macrophages and myeloid-derived dendritic cells,

GM-CSF in lung cancer

- The leukocytosis can be caused by an unregulated production of hematological growth factors, including CSFs, IL-1, IL-6 and tumor necrosis factor- α .
- **Increased serum levels of GM-CSF are considered markers for adverse clinical outcomes**, especially in patients with non-small-cell lung carcinomas.
- Consistently, GM-CSF expression positively correlates with the tumorigenicity and spontaneous metastatic capability of human carcinomas, and **the enhanced invasive ability of human lung cancer cells can be accompanied by an increased expression of extracellular matrix-degrading enzymes.**
- Therefore, because **GM-CSF may profoundly influence tumor progression and metastasis**, caution is warranted when using recombinant GM-CSF as an adjuvant therapy for patients with lung cancer.

Versatile roles of tumor-derived G-CSF in the tumour microenvironment



- Granulopoiesis and neutrophil maturation
- Mobilize MDSC into circulation
- Immune suppression
- Stimulates the expression of PD-L1
- Proliferation and survival of cancer cell
- Extracellular matrix formation

Take Home message

- **The sources of G-CSF/GM-CSF in lung cancer ?**

- Macrophages, T cells, mast cells, NK cells, endothelial cells and fibroblasts, **Cancer cell**

- **The mechanism of immune evasion of G-CSF/GM-CSF ?**

- Activates Neutrophil (N2 TAN) and MDSC (Myeloid-derived suppressor cell)

- Upregulates the expression of PD-L1 which has intrinsic and immunologic functions

- **The clinical implication of G-CSF/GM-CSF in lung cancer**

- High G-CSF expression was associated with poor OS in lung cancer

- GM-CSF may profoundly influence tumor progression and metastasis

경청해 주셔서 감사합니다.

