The clinical applications of Hematopoietic growth factor - GCSF

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ABSTRACT
Enormous number of Hematopoietic growth factors like CSF, GCSF, and GMCSF have been cloned, purified and produced in E.coli. Recombinant human GCSF is a Hematopoietic growth factor, extensively used in Cancer therapy to treat Neutropenia and to combat infections, immune suppression, anti-inflammatory responses, immunomodulation, stem cell transplantation, myocardial infarctions etc. This review discusses about G-CSF signaling, mechanisms of G-CSF-induced stem cell mobilization, and influence of G-CSF on T-cell function and dendritic cell activation. An attempt has been made to link the current issues about the biology of G-CSF with its clinical uses, both present and future.

It also depicts various applications of GCSF in Chemotherapy, radiation therapy and the use of GCSF in repair, regeneration of neuronal tissues etc. by Stem cells and progenitor cells.

Keywords: Hematopoietic growth factor, Granulocyte Colony Stimulating Factor (GCSF), neutropenia. Colony stimulating Factors (CSF3), Granulocyte Colony Stimulating factor Receptor( GCSFR), therapy, myeloablaive therapy, chemotherapy, cancer, neutrophils, stem cells, bone marrow.

1. INTRODUCTION
GCSF is a Hematopoietic growth factor, produced by a number of tissues that stimulate bone marrow to produce granulocytes and stem cells [3] and release them into the blood. It also stimulates the survival, proliferation, differentiation, and neutrophil precursors and maturation functions [4-6]. GCSF is also involved in the regulation of various signal transduction pathways like JAK, STAT, MAPK, P13K, and Akt [1-2, 7].

During past 10 years a major change has been observed in understanding the role of biological molecules in production and activation of blood cells [8-10]. It is evident that some of the molecules like granulocyte colony stimulating factors play vital role in granulopoiesis stimulate bone marrow to produce more WBC and enhance circulating neutrophils. GCSF has found its use for new therapies for cancer patients [11], for combating life threatening infections, healing and regeneration of tissues. With the recent advances in science and enthusiasm for the use of biological molecules like Colony stimulating factors, cytokines, biological response modifiers are used in cancer therapy. The recovery and enhancement of circulating blood cells is a new paradigm for molecular medicine.

The mature human glycoprotein-GCSF exists in two forms with 174 and 180 amino acid long protein which differs by the presence or absence of 3 amino acids, but the active form is 174 amino acids long protein with a free cysteine at 17th position, two intra molecular disulfide bonds between Cys36 - Cys42 and Cys64 – Cys74 [12-13] which are necessary for biological activity of GCSF [14-15]. GCSF is a Hematopoietic growth factor of myeloid lineage [16] that affects proliferation and differentiation of
progenitors of neutrophil [17-19] and granulocyte lineages. It is produced mainly by monocytes, macrophages, endothelial cells [6, 20-21], fibroblasts, astrocytes and a number of immune cells. In addition various carcinoma cells and myeloblastic leukemia cells constitutively express GCSF.

**Mechanism of action:**
GCSF produced in the bone marrow in response to cell stimuli, it binds to specific receptors called cytokine receptors with one trans membrane domain, intracellular signal transduction domain and homo-oligomerizes upon ligand binding. GCSF receptors are present on hematopoietic progenitors, monocytes, platelets, neurons, endothelial cells [20, 22-24] and small-cell lung cancer cells [25-26].

Activation of these receptors, upon binding of GCSF, followed by induction of signaling cascade like Janus kinase (JAK) / signal transducer and transcription activator pathways (STAT), Ras/ Mitogen activated protein (MAP) kinase and Phosphotidyl inositol 3-kinase (P13K) / Protein kinase B (pkb) / (Akt) pathways. These pathways have shown to induce cellular proliferation [27], anti-inflammatory processes and anti-apoptotic processes [28-33] these signaling pathways play a role in mobilizing stem cells and targeted to injured site especially to heart and brain. These investigations lead to the potential use of GCSF in bone marrow transplantsations, treating myocardial infarctions [34-38] and cerebral ischemia [1, 39].

GCSF-R involved in a range of malignancies due to the mutations in this receptor has been seen in several clinical implications like severe congenital neutropenia [40], myelodysplastic syndrome and acute myeloid leukemia [41].

**Applications of GCSF**
1. GCSF used in therapy since it stimulates the production of white blood cells (WBC), a recombinant form of GCSF used in oncology and hematology to treat certain cancer patients[11] to accelerate- recovery from neutropenia after chemotherapy[42].
2. Recombinant human GCSF used for the treatment of severe chronic neutropenia patients receiving myelosuppressive therapy [43], bone marrow transplants [15]. GCSF is also used in Stem cell or bone marrow transplantation in order to increase the number of hematopoietic stem cells.
3. GCSF is an important cytokine in regulating Immune defense against pathogenic bacterial infections. Recently GCSF is widely used in Cancer patients with Chemotherapy induced neutropenia and in the preparation of hematopoietic stem cells mobilization for transplantation before the initiation of myeloablative chemotherapy. In addition to this HSC donors receive GCSF for 5 days, their T cells produce more IL-4 and less IFN-g associated with a lower risk of acute GVHD.
4. Besides the function of Hematopoietic effect, it can also act as Neurotrophic factor, induce neurogenesis and to counteract apoptosis. These properties Plays a major role in the development of treatments of neurological diseases such as cerebral ischemia [33, 44].
5. GCSF decreases the cytotoxicity of NK cell activity by cytokine receptors and other signaling regulated pathways of P13K/Akt and ERK/MAPK and decrease the cytotoxicity related gene expression [45]. GCSF impairs NK cell cytotoxicity usually seen in Autoimmunity and transplant rejections [46]. After the GCSF administration for 5 consecutive days the number of WBCs, CD34+, and neutrophils are markedly increased might regulate the immunological network, activation of CD34+ cells, lymphocytes and granulocytes [45].
6. GCSF also plays a potent role in inducing the mobilization of hematopoietic stem cells (HSCs) from the bone marrow into the blood stream [47-48].
7. GCSF has an important role in defense against infection, inflammation and repair processes and also in maintenance of steady state hematopoiesis [49-51]. Recent studies reported that GCSF has regenerating and repairing function in the skeletal muscle regeneration therapy [52] and to reduce the hepatic damage [53].
8. Apart from this GCSF have special properties like tumorocidal activity, blast cell growth factor activity and in controlling the neuropathic pain [54].

**GCSF Production**

- Cells release GCSF in response to infection
- GCSF binds to receptors on Bone marrow progenitors
- Produce stem cells
- Stem cells start proliferating and giving rise to new cells.
- These enormous new cells forms colonies by continuous proliferation
- Functional activation of these new cells help in combating infection.

**Fig 1:** Depicts the GCSF production in response to infection and contributes to Immune defense.
Mechanism of Action of GCSF

Fig 2: GCSF binds to GCSF receptors, activates signaling pathways (JAK / STAT) and has specific functions in different cells.

Cancer and Neutropenia
Since 1990 the role of GCSF in clinical treatment has gone from strength to strength in raising white cell counts and protect from potentially lethal infections following high dose chemotherapy, radiation therapy and bone marrow transplantation.

The American Society of Clinical Oncology (ASCO) and recently several other organizations have renovated the clinical guidelines [55] for cancer therapy by using Colony stimulating factors/hematopoietic growth factor like GCSF. Since GCSF influence the proliferation, differentiation and maturation of neutrophils and increase the rate of neutrophil recovery following chemotherapy [56-57].

As per the European Society of Medical Oncology (ESMO) and the Infectious Diseases Society of America (IDSA) recommended broad spectrum antibiotics [58-59] for immediate treatment of neutropenia. Neutropenia is a hematological disorder characterized by an abnormal decrease in neutrophil count that is less than 500cells/mm3 (0.5 X 10 9/L) [60] which reflects a higher infection risk, usually seen in malignancies, bone marrow transplantation, suppression, chemotherapy, leukemia, lymphoma, multiple myeloma and extensive myelosuppressive therapy [61].

GCSF has been used in clinics to treat congenital, acquired and febrile neutropenia before or during the cytoreductive therapy [62]. GCSF is used to decrease the incidence of infection [63-64], as manifested by febrile neutropenia, patients with non-myeloid malignancies receiving myelosuppressive anti cancer drugs associated with incidence of severe neutropenia and the duration of fever. It can be managed by reducing the chemotherapy doses in order to reduce the myelosuppression but these also reduce the clinical effectiveness of chemo. GCSF effects mainly on reducing the time of neutrophil recovery in oncology patients receiving chemotherapy [65]. The mean time of neutrophil recovery is 5 days.

In Clinical studies GCSF currently given to shorten the duration of neutropenia following chemotherapy [66] induction in older adults of AML, for BMT failure to mobilize the stem cells for transplantation and for myeloid reconstitution in BMT (bone marrow transplantation).

GCSF has been used in combination therapy to treat advanced endometrial cancer, breast cancer patients [67-70], transitional cell carcinoma of the urothelium [71-72], and small cell lung carcinoma [73]

Recombinant human GCSF is used as primary prophylaxis in reducing the incidence of Febrile neutropenia [74] and chemotherapy induced neutropenia [75] in patients with myelosuppressive chemotherapy for various cancers (Breast cancer, colorectal cancer) lymphoma and solid tumors(lung, ovarian, breast). It is also given to prevent premature labor in severe neutropenia patients [76].

Bone marrow suppression
The bone marrow is the thick liquid present in the inner part of some bones and it produces white blood cells (WBCs), red blood cells (RBCs) and platelets.

Bone marrow suppression or myelosuppression is a common side effect of chemotherapy, characterized by reduced number of blood cell production [77]. The majority of chemotherapy drugs associated with myelosuppression, affecting the immune system is Azathioprine, Flurouracil, Oxalipatin, Irinotecan and Capecitabine. The patients will develop moderate to life threatening infections as well as bleeding.

Growth factor injections (GCSF) ,a natural chemical that boost the bone marrow performance is widely used to mobilize the bone marrow stem cells in leukemia patients [78] treated with bone marrow transplantation and chemotherapy induced neutropenia [79-80].

The blood cell count falling below the lowest count is called nadir, usually WBCs and platelets will reach nadir in 7-14 days of chemotherapy treatment because of their little life span. Whereas RBCs take few weeks (3-4 weeks) after chemotherapy to reach their nadir.

Doctors prescribe hematopoietic factors/colony stimulating factors to keep the WBC from falling too low so that chemotherapy can be given as scheduled. Normal human body produces hematopoietic or growth factors to prompt the bone marrow to make various blood cells.
Aplastic Anemia
Aplastic Anemia patients have impaired proliferation and differentiation of hematopoietic stem cells. Recombinant GCSF has been extensively evaluated clinically for transient increase of neutrophil count and is used in treatment and prophylaxis of infections in majority of Aplastic Anemia (AA) patients. It also used to alleviate anemia in aplastic anemia, CSF3 is essential for an emergency granulopoiesis [81-83], in response to invading bacterial pathogens and infections by enhancing multiple neutrophil functions [84].

Role of GCSF in central nervous system
In response to stimuli like hematopoietic growth factors, myeloblastic therapy and infection, there is an increase in the number of hematopoietic stem and progenitor cell (HSPCs) in the circulation [85]. GCSF has a potential role in protecting the myocardium. Stem cell mobilization with GCSF has a potential regenerative strategy for treating acute myocardial infarction [86-87]. The potential beneficial action GCSF cytokine attributed by inhibiting the apoptosis on injured myocardium [88] rather than the stem cell mobilization and differentiation from bone marrow into myocytes.

GCSF has a prominent role in central nervous system and of potential relevance to a number of neurological conditions [33]. Neurons have expressed GCSF and its receptor [89] in many regions of brain and are up regulated in experimental stroke. GCSF activates several neuroprotective pathways like mobilization of hematopoietic stem cells [90-91] neuronal differentiation, angiogenesis and anti inflammation [92-93] and act anti-apoptotically [94-95]. An optimal dose of GCSF will increase the CD-34+ cells [96-97] in peripheral blood it decreases infarct volumes [98] in vivo acute stroke models [99].

First indication of protective effect on cultured neurons against glutamate induced cell death. [1-2]. GCSF stimulates the brain neuronal stem cell differentiation and improves the long term recovery in more chronic stroke models. Thus GCSF is considered as a novel neurotrophic factor and is considered as attractive model for the treatment of neurodegenerative conditions. GCSF plays an important role in neuroprotection [100-101] and neurogenesis relevant to ischemia [33,102].

GCSF has been shown to promote structural and functional regeneration of the central nervous system in strokes patient. Neural growth factor like GCSF has been used to counteract neutropenia and mobilize hematopoietic stem cells from bone marrow in stem cell transplantation [33]. Animal strokes data showed that GCSF passes the intact blood brain barrier, acts on neurons for recovery. GCSF not only counteracts cell death but also has the potential to enhance neuroplasticity which leads to functional recovery at long intervals of stroke [1,33,103-105]. Recent study showed that GCSF treatment in elderly chronic stroke patients with concomitant vascular disease is safe and is reasonably well tolerated [106].

Apart from the hematopoietic recovery, neuronal death inhibition GCSF also induces the repair and regeneration of new neuronal tissues like spinal cord and brain by the mobilization of stem cells from bone marrow to the injured tissues [99].

Regulation of Immune system by GCSF
Immunosuppression
Immunosuppression means that reduces the activation or efficacy of immune system. Suppressing the excessive immune activation usually seen in the bowel walls of Crohn’s disease. Crohn’s disease (CD) is characterized as an immune deficiency disorder, also known as chronic inflammatory, granulomatous bowl disease. A variety of defects in Apoptosis [107] and T-cell regulation, which results in the modification in immune tolerance and predisposes the intestinal inflammation. Intestinal homeostasis can be maintained by special cells like T-regulatory cells have tolerance to microbial and dietary antigens. Treg cell defect [108-109] is observed in chronic intestinal inflammation. In this case GCSF plays a very important role, acts as an anti-inflammatory agent which could enhance the intestinal innate immune system by modulating the cellular proliferation, differentiation, angiogenesis, inflammation and serves as messengers between various systems including intestine, enteric nerves and immunity.

Clinically GCSF has been used in treating fistula in a CD patient which could act by enhancing the activity of subpopulations of T-regulatory cells [110] and reduces the T-cell activation [111] and gastro intestinal symptoms have been improved in CD [112-114].

Indirectly GCSF also acts on CD patients with neutropenia and septic complications [111] occurred due to intake of drugs like azathioprine/immunosuppressant drugs. GCSF also has a profound role in immuno regulatory effects in adoptive immunity by mediating anti inflammatory reactions accompanied by TH2 cell differentiation and promoting T cell tolerance [91]. These findings have highlighted the impact of GCSF even on Autoimmunity [115].

Several reports demonstrated that GCSF exerts immuno regulatory properties by expanding monocytes and macrophages and promote anti inflammatory processes. These hypotheses help in therapeutic potential in autoimmune diseases like experimental auto immune encephalomyelitis (EAE) [116], human demyelinating
disease-multiple sclerosis. It is mediated by the activation of inflammatory Th1 cells. Immune prevention of EAE with GCSF have been achieved by eliciting the regulatory T cells which release anti-inflammatory cytokines. GCSF not only prevents the development of EAE but it also protects from the onset of disease by exerting its remarkable, long lived protective effects on the clinical course of EAE and the progression of the disease is inhibited in the central nervous system (CNS).

Emerging evidence provided by experimental data for treating autoimmune type1 diabetes is a significant achievement which is a T cell-mediated disease in NOD mouse. Recombinant human GCSF has been used to treat Felty’s syndrome (FS), chronic T-cell lymphocytosis (CTL), rheumatic disease (RD), severe chronic neutropenia and Auto immune neutropenia, is a rare condition mostly associated with auto immune diseases, clonal lympho proliferative disorders and seen in young women. Auto immune neutropenia may be a cell or antibody mediated destruction of granulocytes and their precursors due to the inhibitory CD8+ T-cells present in the marrow spaces.

**GCSF role in Liver damage and Antiviral therapy**
Mobilization of CD34+ cells improves the survival rate in Acute –on-Chronic Liver Failure (ACLF), Alcoholic hepatitis and liver cirrhosis patients using GCSF. This condition is seen in chronic liver disease and is manifested by Jaundice, coagulopathy and results in multi organ failure.

There is no proper treatment other than the Liver transplantation, which is the only definitive therapy for patients with ACLF, but there is lack awareness in the management of patient, availability of donors, limited experience and expensive. So there an exciting opportunity for GCSF for hepatic tissue repair and regeneration by mobilization and differentiation of blood derived stem cells into multiple lineages.

Hepatocytes, intra hepatic stem cells, bone marrow derived stem cells play a major role in regeneration of damaged liver tissue by its proliferative action. GCSF therapy can reduce the development of sepsis and multi organ failure and improves the patient survival in ACLF patients. Which has showed schematically (Fig: 3).

**GCSF ROLE IN LIVER DAMAGE**
**Antiviral Therapy:**
Hepatitis C is one of the leading chronic liver diseases seen globally; Hepatitis C Virus (HCV) infection can progress to liver cirrhosis and hepato cellular carcinoma and is the leading cause for liver transplants in US. Popular treatments now days for HCV are Peg interferon and Ribavirin. In HCV treatment major goal is viral clearance, thereby reducing the risk for liver cirrhosis and hepatocellular carcinoma and improves the patient quality of life. Viral eradication is characterized by the presence of HCV RNA from the serum, is termed as a Sustained Virological Response (SVR).

Enormous improvements for Hepatitis C treatments have been seen from past two decades. A combination of antiviral drugs came into picture in spite of these advancements hematological side effects also seen like anemia, neutropenia and thrombocytopenia. These were commonly seen in combination of antiviral therapy with pegylated (PEG)-interferon alfa and ribavirin.

Recent advancement in science and attempts has maximized the adherence towards the HCV treatment using Hematopoietic growth factors (GCSF) without altering the dose adjustments to treat side effects. GCSF is effective in raising Absolute Neutrophil Count (ANC) when interferon doses are giving in HCV therapy. Since defective synthesis of endogenous GCSF during HCV treatment with Combination therapy contributes to Neutropenia.

**Role of GCSF in Neuropathic pain**
Neuropathic pain arises from a direct consequence of lesion or disease with the involvement of peripheral and central nervous system (CNS). Neuropathic pain involves the interaction between leukocyte derived opioid peptides and their receptors on peripheral sensory neurons. Studies showed that GCSF can increase the number of opioid receptors.
contained polymorphonuclear cells and significantly relieve the pain.

GCSF is a potential mediator of cytokines, chemokines and CD34+ adhesion molecules and has a direct or indirect role in controlling the pain. GCSF can stimulate bone marrow to produce more polymorphonuclear cells; these are a kind of granulocytes which secretes opioid peptides [140]. GCSF therapy has proven to be innovative strategy for neuropathic pain treatment.

GCSF produces an analgesic effect by two major biological and molecular functions.

The exogenous single GCSF dose can increase the circulating WBC, PMNs [141] and plays an important role in peripheral analgesia by producing opioids.

GCSF has direct effect on CD34+ cells to increase the anti-inflammatory [142] cytokine expression [143] by cytokine modulation leads to analgesic effect.

It not only alleviates neuropathic pain but also repairs the injured sites/lesion of infection, ischemia, tumor growth or an auto immune process.

**Conclusion**

GCSF has found tremendous applications in various therapeutic conditions viz., cancer therapy, myeloablative therapy, Neutropenia, bone marrow suppression, stem cell transplantation and in immuno regulatory disorder like Crohn’s disease, EAE, autoimmune neutropenia etc. GCSF is also used in central nervous system disorders like cerebral ischemia and stroke, myocardial infarctions and liver failure conditions, owing to its repair and regenerating functions. GCSF is used in liver damage for regeneration of hepatic tissue and further improvement of neutrophil dysfunction and for prevention of multi organ failure. Apart from this GCSF is used to relieve neuropathic pain by opioid producing polymorphonuclear cells (PMNs) which have analgesic effect.

GCSF can modulate autoimmune processes in autoimmune neutropenia but it has not been proven. The potential for flare up of rheumatic disease means that judicious use of growth factors like GCSF; it can be seen in Felty’s syndrome (FD) and Systemic Lupus Erythematosus (SLE). GCSF have promising applications in neural disorders like stroke by its neuroprotective effects.

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