

# Use of G-CSF in Hematopoietic Stem Cell Donors

Victor M. Santana, MD

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## Outline

- Biologic effects of G-CSF
- Current indications
- Side effects profile
- Data on G-CSF and Risks
  - In vitro/In vivo studies
  - Patients with hematologic disorders and mutations in G-CSF receptor
  - Children with ALL
  - Studies in stem cell donors
    - Adults
    - Children
- Summary

## Biologic Effects of G-CSF

- Hematopoietic cytokine produced by monocytes, fibroblasts and endothelial cells
- Maintain normal steady state hematopoiesis by regulating production, differentiation, and functional activation of neutrophils
- Recombinant G-CSF at pharmacologic doses augments this response and stimulates development of committed and primitive stem cell progenitors and their release from marrow into peripheral blood (i.e., CD34+ progenitors, other subsets)
- Other effects: ↑ mononuclear cell procoagulant activity and thrombin generation, ↑ in other cytokines (IL-1ra, TNF receptors, etc.)
- Pharmacokinetics: short half life ~ 3.5 hours

## Current Indications

- To decrease the duration and severity of chemotherapy induced neutropenia in both adults and children.
- Guidelines for the use of CSFs are published by the American Society of Clinical Oncology (ASCO)
  - It recommends the use of CSFs as primary prophylaxis in adults, when the expected incidence of febrile neutropenia is greater than 40%
  - The guideline states that pediatric patients should be treated by the above recommendation (which is based on adult data), because pediatric data is too limited.

## Other Indications

- Congenital neutropenia of childhood and cyclical neutropenia
- Autologous peripheral blood stem cell donors (patients undergoing ASCT): adults and children
- Healthy adult peripheral blood stem cell (PBSC) and bone marrow donors for stem cell mobilization (granulocyte donation, allogeneic stem cell donors)
- Sepsis ? (off-label)

## Acute side effects associated with G-CSF administration

Common (>20 % rate)	Bone pain Headache Myalgia
Less Common (5-20% rate)	Nausea Vomiting Diarrhea Fatigue Redness at the injection site Insomnia
Rare (<5%)	Splenic rupture Acceleration of autoimmune disease Allergic reactions Vascular problems
Hypothetical	MDS and/or AML

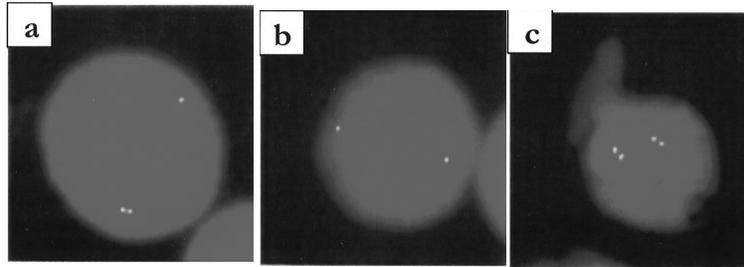
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## Allelic Replication

- Normal DNA replication during S-phase is highly correlated with gene expression
- Temporal order of allelic replication is important:
  - counterpart alleles replicate synchronously – biologically expressed genes
- Asynchronous replication: one allele is replicated earlier than the other one or there is monoallelic expression (i.e. silencing – imprinting, X-inactivation, allelic exclusion)
  - Common in regulation of T and B cell antigen specific receptors
  - Transition to the asynchronous mode of replication is a non-disease specific, cancer associated phenomenon (epigenetic)

## Asynchronous Allelic Replication



**FIGURE 1 – Fluorescence signals in PHA-stimulated lymphocytes** after FISH with AML1. (a) Cell with 1 singlet and 1 doublet (SD cell) representing S-phase cells where only 1 of the allelic sequences has replicated. (b) Cell with 2 singlets (SS cell) representing cells in which both alleles have not yet replicated. (c) Cell with 2 doublets (DD cell) representing cells in which both alleles have replicated.

### Granulocyte colony-stimulating factor generates epigenetic and genetic alterations in lymphocytes of normal volunteer donors of stem cells

*Experimental Hematology. 2004, 32: 122–130*

- 18 healthy allogeneic stem cell donors, treated with G-CSF @ 10 mcg/kg x 5 days
- Lymphocytes:
  - ↑epigenetic asynchronous allelic replication; transient phenomenon lasting about 140 days
  - genetic alterations (aneuploidy), persistent in some donors
- Implications:
  - unmasking of mutated recessive genes
  - vulnerability to a “second-hit”

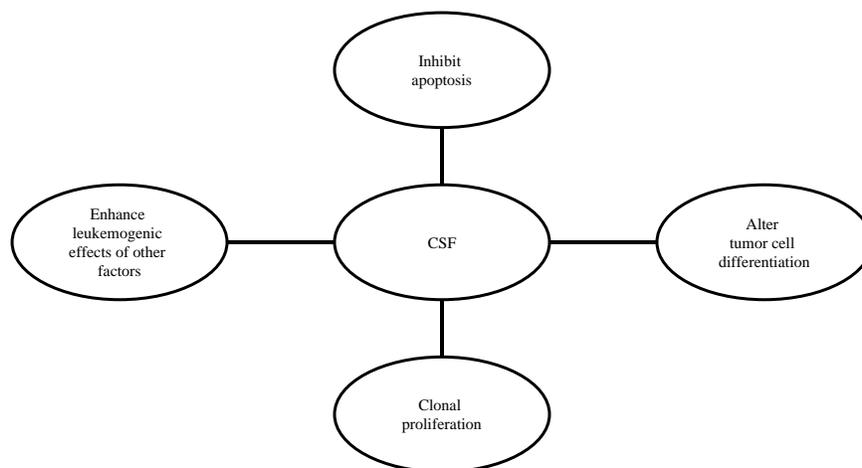
## Other Observations: Changes in gene expression pattern

Acta Haematol 2007; 117, 68-73

Leukemia 2005; 19, 1088-1091

- Adult donors treated with G-CSF x 4 days
- Affymetrix gene array studies: up regulation of key genes involved in hematopoiesis (GM-CSF receptor, macrophage receptor gene, CD34 antigen); down regulation of others
- Normalization over 6 months
- Interpretation:
  - transient de-differentiation
  - signature print of rare mobilized stem cells

## Proposed Mechanism for Leukemogenesis



## CSFs in Children: Leukemogenesis?

Rosenberg et al. *Blood*. 2006;107(12):4628-4635

- 403 patients with congenital neutropenias
  - Severe congenital neutropenia = 374
  - Shwachman-Diamond Syndrome = 29
- Results (incidence of secondary AML/MDS):
  - Cumulative incidence after 10 yrs G-CSF treatment = 21%
  - Cumulative incidence after 12 yrs G-CSF treatment = 36%
  - Cause-specific hazard increases 2.9% per year after 6+ years of G-CSF therapy
  - G-CSF dose correlated with increased risk of secondary AML/MDS
    - » 2.5 fold higher in patients receiving  $\geq 6$  mcg/kg/day vs. patients on  $< 6$  mcg/kg/day
- Prolonged G-CSF treatment in patients with congenital neutropenias increases risk of secondary AML/MDS

## CSFs in Children: Leukemogenesis?

Relling et al. *Blood*. 2003;101(10):3862-3867

- 412 acute lymphoblastic leukemia patients enrolled on TXIII A&B between 1991-1998
- Identical therapy with respect to topoisomerase-II inhibitors and alkylating agents
- TXIIIA: randomized to receive G-CSF (10 mcg/kg) or placebo x 15 days for neutrophil recovery post-remission induction
- TXIIIB: G-CSF used at prescriber's discretion for severe/prolonged neutropenia
- Results:
  - 20 pts developed t-ML (16 AML; 3 MDS; 1 CML)
  - Incidence higher in patients who received G-CSF vs. those who received placebo
    - » 11% G-CSF group vs. 2.7% placebo group (p=0.019)
- Increased incidence of secondary t-ML and MDS in pediatric ALL patients treated with CSF

## Studies in Healthy Donors: Long-term follow up data on donors who received G-CSF

- Anderlini et al. (MD Anderson)
  - Peripheral blood donors: n=281
  - Follow-up 3.3 years (median)
  - Cases of HM: none
- NMDP Registry

	<u>Peripheral Blood Donors</u>	<u>Marrow Donors</u>
Number	4015	1160
Follow-up	Up to 9 years	Up to 3 years
Cases of HM	None	None

## Studies in Healthy Donors: Long-term follow up data on donors who received G-CSF

- Japanese Registry
  - Peripheral blood donors: n=3264
  - Follow-up time: not available
  - Cases of HM: 1 AML (sibling with MM)
- German Bone Marrow Donor Center

	<u>Peripheral Blood Donors</u>	<u>Marrow Donors</u>
Number	7236	3713
Follow-up	5 years (18,000 observation yrs)	Periodic contact
Cases of HM	1 HD	1 CLL, 1 AML

## Studies in Healthy Donors: Long-term follow up data on donors who received G-CSF

- Cavallaro et al. *Bone Marrow Transplantation*. 2000, 25:85-89
  - Peripheral blood donors: n=101
  - Follow-up 43 months (range, 35-73)
  - Cases of HM: none
    - 1 case lymphadenopathy, 1 breast Ca, 1 prostate Ca

## Studies in Healthy Donors

- Conclusion: Low rates of hematologic malignancies
- Caveats:
  - Retrospective reporting
  - Relatively short periods of follow-up
  - Under-reporting
- In cases of HM: Sibling-effect (shared genetic susceptibility)

## Studies in Healthy Children: Spanish Cooperative Group

de la Rubia et al. *Transfusion*. 2001, 41:201-205.

- 61 donors < 18 years of age
  - median 14 yrs. (range, 1-17)
- G-CSF dose 10-15 mcg/kg/day x 5 days (4-6)
- Common side effects: bone pain (90.5%), headache (21.5%)
- Overall mild symptoms, managed with minor analgesics; no discontinuation secondary to toxicity
- Few donors with follow-up at 4 years: 9 of 61 (< 15%)

## Studies in Healthy Children: Japanese Studies

Kawano et al. *Cancer Research*. 1999, 59:3321-3324.

- 19 donors < 18 years of age
  - Median 6 yrs. (range, 2-16)
- G-CSF dose 10 mcg/kg/day x 5 days
- No side-effects in donors < 10 years; older donors commonly had mild headaches, general fatigue, treated with acetaminophen p.r.n.
- No follow-up data

Watanabe et al. *BBMT*. 2002, 8:26-31.

- 57 donors < 18 years of age
  - Median 8 yrs. (range, 9 mo 24 yrs)
- G-CSF dose 7.5-10 mcg/kg/day x 5-6 days
- Older donors commonly had bone pain (17.5%), mild headaches (5.3%), treated with NSAID p.r.n.
- Follow-up data: in 40 of 56 donors at a median follow up 25 months (range, 6-56 months) --- normal blood counts and medical examination

## Studies in Healthy Children: Pediatric Blood and Marrow Transplantation Consortium (USA)

Pulsipher et al. *Bone Marrow Transplantation*. 2005, 35:361-367.

- 201 donors < 18 years of age
  - median 11.8 yrs. (range, 18 months – 17 yrs)
- G-CSF dose 10 mcg/kg/day x 4 days
- Common side effects: bone pain and myalgias 11-15%
- 21 of 197 treated with a minor analgesic; 1 older child required oral narcotic
- No long term follow-up data reported

## In Summary

- G-CSF in normal healthy adults and children:
  - Acute, mild side effects are common
  - In vitro/In vivo studies suggest genomic changes that appear to be transient and present at very low levels --- clinical significance is unknown
  - Adult studies do not suggest increased risk of HM
  - Lack of long-term follow up data in children