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REVIEW PAPER

Progenitor Cells as a Bridging Therapy for Radiation Casualties

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ABSTRACT

Hematopoiesis is the process by which daughter cells of multipotential, self-renewing stem cells progress along differentiation pathways to become progressively more committed to specific lineages while losing their self-renewal capacity. Leucopenia and thrombocytopenia after ionising radiation exposure are due largely to injury to stem cells and progenitors in the hematopoietic tissue of the bone marrow; and in mice, the spleen. Recovery depends on the ability of the remaining stem cells and progenitors to proliferate and differentiate sufficiently to reconstitute the immune system before it is challenged by potential microorganisms and lethal infections occur. This mini review discusses various approaches to the mobilisation of progenitors and their utility as a bridging therapy for radiation casualties.

Keywords: Gamma-radiation, granulocyte-colony stimulating factor, hematopoietic progenitors, mice, myeloid progenitors, transfusion

1. INTRODUCTION

The detonation of a nuclear device through either military or terrorist action would lead to a mass-casualty scenario involving victims with varying degrees of exposure to ionising radiation.[1,2] Victims exposed to high doses of ionising radiation will present with the signs and symptoms of various degrees of acute radiation syndrome (ARS). ARS describes three distinct dose-dependent syndromes: hematopoietic, gastrointestinal (GIS), and the central nervous syndrome (CNS); CNS being the most severe. Prognosis for victims who have received a gastrointestinal or CNS dose, even with supportive care, is poor. Moderate exposure (2 to 4 Gy) will lead to the more medically manageable hematopoietic syndrome, characterised by depletion of hematopoietic stem cells (HSC) in the bone marrow[3].

Over the last 30 years, the expected outcome for accidental exposure to high doses of ionising radiation has been very poor[4]. For radiation-induced neutropenia, often observed in the accidental-exposure scenario, currently there is only one treatment protocol. There are two components to this protocol: aggressive supportive care, and the administration of granulopoietic cytokines as soon as possible. There is a substantial preclinical database demonstrating the effect of these growth factors in stimulating granulopoiesis and survival after lethal doses of radiation; however, the outcome is far from satisfactory. Various investigators have been evaluating combined regimens of different cytokines in experimental models[5].

Development of countermeasures to ionising radiation, both radioprotectants and mitigators, has been identified by the US Department of Homeland Security as the highest priority in preparation for a terrorist attacks[6]. Currently, there are no suitable countermeasures against nuclear/ radiological hazards for military or civilian personnel, other than the limited hazard of internalised radioisotopes. Any radiation exposure can significantly influence operations of our military, national guards, and first responders. The problem has become more acute in recent times given the unpredictable nature of attacks. The US Department of Defense, as indicated in a current "Defense Technical Objective" that lists developing medical countermeasures to radiation exposure as a top priority and cites both early and late occurring health effects as major concerns, has recognised this deficiency. The US Army Qualitative Research Requirements rank and prioritise these radiation exposure-associated health effects as being very important. Furthermore, the US Navy has identified "enhanced treatment regimens for radiation injuries" and protection against radiation injury as priority needs. These concerns reinforce the urgent need to develop an appropriate modality to sustain the war-fighting capabilities of our military. Strategically, first responders and medical providers should have an array of radiation countermeasures at their disposal[7]. With this in mind, considerable progress has been made in the development of radioprotectants[8,9]. There are several radiation countermeasures at different stages of development[10-14]. In fact, some potential candidates have been given investigational new drug (IND) status by the Food and Drug Administration (FDA). Due to the unpredictable nature of ionising radiation exposures, therapies may prove to

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be more useful to medical providers[9,15-17].

The human hematopoietic system is highly susceptible to radiation injury. During any ionising radiation exposure scenario, a majority of victims will be exposed to a sufficient dose to possibly impair but not entirely ablate hematopoietic (bone marrow) function. As such, these victims would likely recover from their injuries but would have faced a period of 30–60 days during which they would have difficulty fighting infections, uncontrolled bleeding and anemia[2]. Therefore, in order to keep these compromised individuals alive, substantial supportive care must be provided, at least to the point of partial recovery of their hematopoietic system.

Effective post-exposure treatments are critical as these serve to broaden the utility of first responders for the effective rescue and salvage of the more critically injured victims of a given radiological disaster. Acute ionising radiation injuries are managed by treating the developing symptoms with blood transfusions, electrolytes, and antibiotics. If the estimated exposure is substantial (e.g., 2 Gy or more) with markedly severe cytopenias (lymphopenia), it is recommended that cytokine therapy be commenced shortly after exposure[18,19]. For patients who do not respond to cytokine therapy, the transplantation of HSC remains an option[20,21].

2. USE OF PROGENITORS IN RADIATION CASUALTIES

Weissman and colleagues first isolated a population of highly enriched mouse HSC through extensive phenotypic cell surface analysis and *in vitro* and *in vivo* functional assays for stem cell activity[22]. Additional investigations have shown that the HSC population is functionally heterogeneous, which comprised a lineage of cells demonstrating a continual loss of self-renewal. The long-term reconstituting HSC (LT-HSC) provide life-long hematopoiesis, ultimately giving rise to mature cells of all lineages. This is the only hematopoietic stem or progenitor cell that durably engrafts, and is the functional component in bone marrow transplantation.

Directly downstream of the LT-HSC in the hematopoietic maturation pathway are transiently reconstituting HSC with identical multilineage potential but which possess little or no self-renewal capacity[23]. These are termed short-term reconstituting HSC (ST-HSC) and multipotent progenitors (MPP). While these HSC provide only a transient repopulating ability, these are equally radioprotective when myeloablation is not complete[23].

The earliest branch points between the lymphoid and myelo-erythroid lineages are defined as the oligopotent progenitors; the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP). Like the HSC, both the CLP and CMP have been isolated through techniques that use prospective identification via cell surface markers, flow cytometry, and an array of functional assays[24,25].

From the CMP, the myelo-erythroid lineages are further defined by discrete progenitors: the granulocyte/macrophage progenitor (GMP) and the megakaryocyte/erythrocyte progenitor (MEP). None of these cells (the CLP, the CMP and their downstream progenitors–GMP, MEP) possesses extended self-renewal ability. However, progenitor cells are capable of partially restoring functional hematopoiesis for a limited period of time[26-28]. A combination of CMP/GMP isolated from the bone marrow has been shown to protect myeloablated mice from otherwise lethal doses of pathogenic fungus or bacteria in syngeneic and allogeneic mouse models[26,29,30]. Furthermore, purified populations of CMP or MEP but not GMP protect lethally irradiated congenic mice from death in a dose-dependent manner[28].

Mice receiving a minimal lethal dose of radiation typically die within 12-18 days from bone marrow failure. CMP and MEP purified from mouse bone marrow were found to protect these mice for this critical period post-irradiation[28]. All mice surviving for 30 days went on to survive longer than 6 months and possessed only host-derived hematopoiesis after 30 days. These findings illustrate that if a transient bridging therapy is delivered providing needed granulocytes, red blood cells, and platelets during the critical period of bone marrow failure, host HSC surviving radioablation may provide recovery as a result of functional hematopoiesis.

On the molecular level, much is being learned about signals that regulate self-renewal, proliferation, and differentiation of hematopoietic stem and progenitor cells[31,32]. Murine $CD117^+$ (or $cKit^+$) lineage negative cells are indeed multipotential and primitive in nature, with strong repopulating and self-renewing capacities, as are the equivalent $CD34^+$ lineage negative cells in humans, and are used to quantitate stem cell content in transfused blood/marrow volumes. Because of the complex protein and cellular interactions involved, identification of the preferred mobilisation method requires assessment of the qualities of cells mobilised with different agents.

3. MOBILISATION OF PROGENITORS BY GRANULOCYTE COLONY STIMULATING FACTOR

Colony-stimulating factors (CSF) are a family of glycoproteins that control the functional activity, survival, proliferation, and differentiation of myeloid hematopoietic cells[33] (Fig. 1). These cytokines are involved at various stages of the proliferation and differentiation processes, from the proliferation and survival of the pluripotent stem cells to the final differentiation and mobilisation of mature granulocytes and monocytes from the marrow to the blood. Commercially available forms of G-CSF include filgrastim, an *Escherichia coli*-derived recombinant protein, and lenograstim, a glycosylated form of G-CSF produced in Chinese hamster ovary cell lines[34].

Hematopoietic stem and progenitor cells normally reside in the bone marrow but can be rapidly released into the peripheral blood in response to a wide variety of stimuli[35]. Mobilisation of stem cells can be so effective that a sufficient number of cells can be harvested for use in stem cell transplantation. In allogeneic stem cell transplantation, G-CSF-mobilised peripheral blood stem and progenitor cells

now are replacing marrow-derived hematopoietic stem and progenitors as a stem cell source[36]. Because of the ease with which blood stem cells can be obtained, and because of their potentially superior engraftment characteristics compared with marrow-derived stem cells, at present most stem-cell transplantations in human beings are performed using blood-derived grafts[37]. The main advantages of using peripheral blood progenitor cells in place of bone marrow in clinical transplantation protocols include the more rapid hematopoietic recovery of transplanted recipients, the ability to collect the hematopoietic graft less invasively, and the feasibility of harvesting the graft from patients with bone marrow fibrosis[38].

Previous studies have shown that the administration of hematopoietic growth factors, either alone or in combination with cytotoxic drugs, efficiently mobilises hematopoietic progenitors from bone marrow[39]. Although several growth factors are capable of inducing mobilisation of hematopoietic progenitors, G-CSF at present is one of the most efficient mobilising molecules used both in experimental models and clinical protocols[40]. The efficiency of G-CSF to mobilise bone marrow precursors and long-term repopulating cells initially was shown in early preclinical studies[41]. Administration of G-CSF stimulates hyperplasia within the bone marrow as well as a reduction in stromal cell-derived factor-1 (SDF-1; also called CXCL12) mRNA expression, and an increase in matrix metalloproteinase-9-dependent degradation of existing SDF-1[42-44]. The latter molecular events would serve to enhance the mobilisation process via a lessening of binding affinities of resident HSCs to the hematopoietically-inducive marrow stroma.

Overall, use of exogenous G-CSF is associated with various disadvantages, such as occasional severe toxic side effects; drug instability at environmental temperatures, requiring refrigeration throughout the supply chain; and high cost.

4. AMD3100 (PLERIXAFOR–MOZOBIL) AS AN AGENT FOR MOBILISING PROGENITORS

The ability of AMD3100 to increase the number of circulating white blood cells was first discovered during clinical trials as an anti-HIV drug candidate[45]. A bicyclam, AMD3100 is composed of two cyclam units linked by an aromatic linker. Commercially known as Plerixafor or Mozobil, AMD3100 is a powerful CXCR4 (a CXC chemokine receptor) antagonist that disrupts interactions between CXCR4 and SDF-1. AMD3100 chemokine antagonist interactions promote the migration of $CD34^+$ HSC from bone marrow into peripheral blood, where these can be collected for use in autologous hematopoietic stem cell transplant (HSCT) (Fig. 1). In combination with G-CSF, AMD3100 has been approved to mobilise HSC for autologous HSCT in patients with non-Hodgkin's lymphoma or multiple myeloma[36]. Plerixafor is a reversible, pure antagonist of CXCR4 that competes with SDF-1[46]. It disrupts the interactions between CXCR4 on $CD34^+$ HSC and SDF-1 on bone marrow stromal cells, essentially blocking the chemotactic actions of SDF-1[47]. This displacement of previously anchored $CD34^+$ HSC causes their release from stromal cells, allowing their subsequent migration from the bone marrow into the peripheral blood[48,49]. The ability of plerixafor to facilitate this movement was first observed in studies evaluating the agent's potential antiviral effects[50]. Plerixafor has not been found to interact with other chemokine receptors[49].

Although both G-CSF and plerixafor have the ability to mobilise HSC, a study of the mobilisation products of plerixafor has suggested that this agent acts on a more primitive subsets of $CD34^+$ HSC than does G-CSF. The need to better understand the hematopoietic repopulating ability of plerixafor has led to additional studies to characterise the quality of the cells being mobilised. The difference in the mechanisms by which G-CSF and plerixafor mobilise HSC also suggests that different subsets of HSC may be mobilised by each agent and a potential for mobilising a better quality of HSC exists when the two agents are used in combination compared with either agent alone[51]. The combination of G-CSF and plerixafor has been found to facilitate HSC mobilisation in patients with multiple myeloma, non-Hodgkin's lymphoma, and Hodgkin's lymphoma[51].

5. TOCOPHEROL SUCCINATE

Vitamin E is a generic term used for eight naturally occurring tocopherols and tocotrienols (α, β, γ, δ) as well as their derivatives[52]. These analogs are known as tocols; α-tocopherol has been the focus of research because it is the predominant form in human beings and animal tissues. In addition, it is by far the most bioactive form based on the rat fetal resorption test, which is the classical assay for vitamins. Recently, the authors investigated the radioprotective efficacy of α-tocopherol succinate (TS) and their results indicate that TS is effective in protecting mice when given 24 h before irradiation[53] (dose reduction factor 1.28). TS also modulates the expression of various antioxidant genes and helps in hematopoietic recovery[54]. The authors demonstrated that TS stimulates G-CSF production and showed that TS-mediated protection can be neutralised by administration of G-CSF antibody[53,55,56].

The authors hypothesised that TS mobilises progenitor cells into the peripheral circulation. Therefore, they evaluated the efficacy of whole blood obtained from TS-treated mice for protection against γ-irradiation to compare with blood obtained from G-CSF-treated mice. All mice, that were irradiated but received no transfusion, died. Survival was significantly higher in the groups where mice received blood either from TS- or G-CSF-treated donors[57].

The authors hypothesised that the early progenitors mobilised by TS could be used to treat injuries due to ionising radiation. Therefore, they evaluated the efficacy of peripheral blood mononuclear cells (PBMC) obtained from TS-injected mice for protection against γ-radiation exposure. A significant survival benefit was afforded to groups that received from TS donors either 0.5 million or 2 million cells at 2 h or 24 h post-irradiation compared to untreated irradiated mice[57]. When both TS and AMD3100

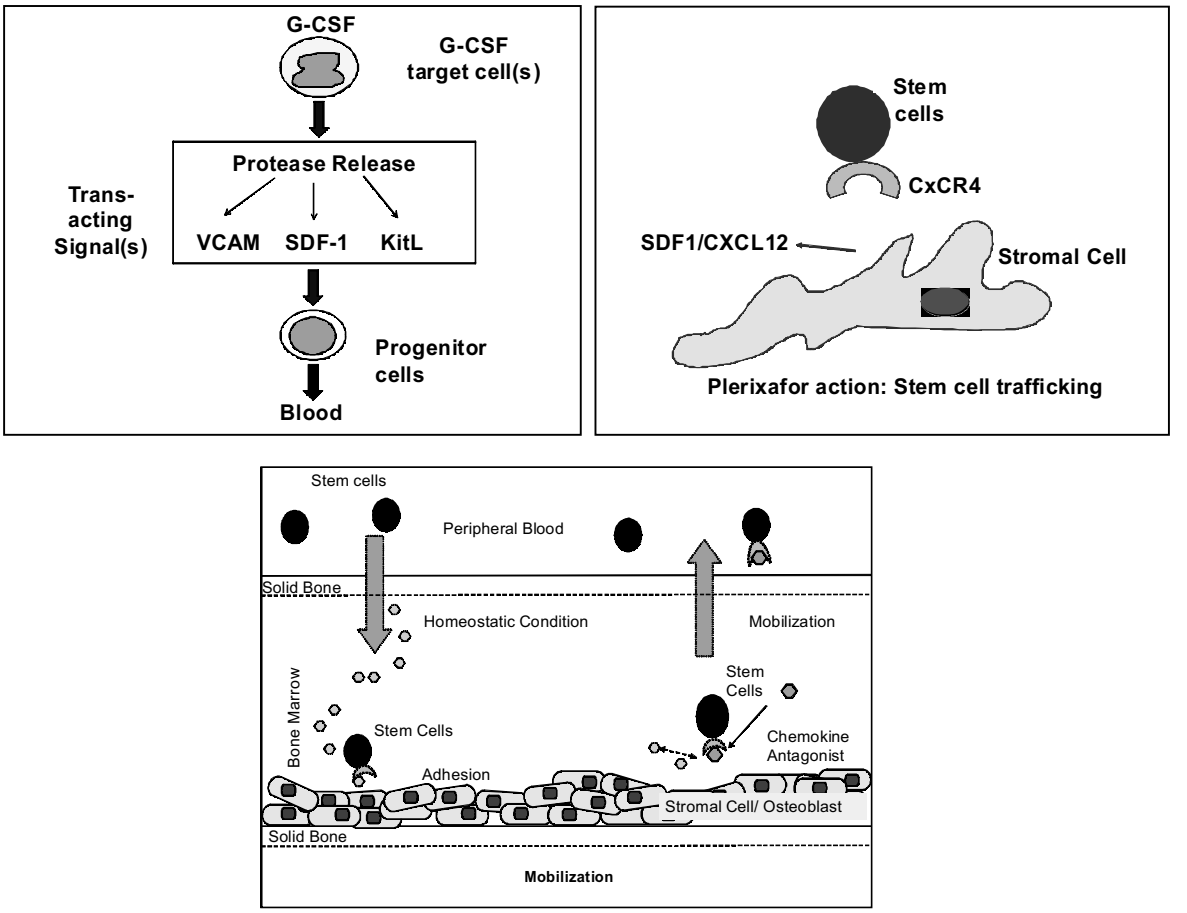

Figure 1. Diagrammatic representation of progenitor mobilisation by different mobilising agents such as G-CSF and AMD3100.

were administered, they observed very high HSC mobilisation in peripheral blood. Their results demonstrate enhancement of lin$^-$Sca-1 (Ly-6A/E), c-Kit (CD117) as well as double positive cells (Sca-1, c-Kit) in response to TS/AMD3100 administration[56]. HSC mobilisation by TS was comparable to that achieved by G-CSF.

Compared to G-CSF and other agents used for mobilising progenitors (AMD3100–Mozobil/plerixafor), TS is a stable, inexpensive, and well-tolerated product that induces endogenous G-CSF. The authors believe that TS is highly efficient and will outperform exogenously administered G-CSF in the clinic, in terms of mobilising high quality, regenerative marrow progenitors into the blood for subsequent collection, transfusion, and effective therapy of various disease states associated with acute immunosuppression. The authors conclude that TS as an effective HSC mobiliser that might well offer a number of very practical, very significant advantages (i.e., less expense, more stable, simpler to administer, etc.) over the conventionally used recombinant growth factor, recombinant human G-cSF: however, advanced preclinical studies using large animal studies, with subsequent clinical trials using volunteers will be needed prior to making any claims of TS's potential clinical utility.

6. CONCLUSIONS

Victims of a terrorist attack presenting with the hematopoietic syndrome resulting from exposure to excessive levels of ionising radiation will succumb to sepsis if not adequately treated. Survival probability is increased substantially if the victim's immune system is allowed to recover before sepsis sets in.

Preclinical development of a new bridging therapy that will allow the victim's immune system to recover from damage caused by ionising radiation has been reported. TS has been found, using a well-defined, preclinical murine model of acute ionising radiation injury, to be a well-tolerated and promising radiation countermeasure. TS is inexpensive and induces high levels of G-CSF in circulation within 24 h of subcutaneous administration, leading to mobilisation of marrow progenitors into peripheral blood. Transfusion of frozen and stored whole blood, or selectively enriched progenitorial cell fractions from TS-treated donor mice, into lethally ionising radiation-exposed recipients can provide enhanced hematopoietic repair and recovery and, in turn, extend survival. Further preclinical work and refinements, as well as subsequent clinical translation of the TS-blood progenitor mobilisation and autologous blood infusion protocol, might provide a simpler, improved protocol in clinical management of individuals suffering from high ionising radiation dose-mediated ARS.

Recently, other investigators have initiated such studies. One group has developed a cellular therapy that contains

human progenitor cells. This group (Cellerant Therapeutics, San Carlos, CA) has been awarded a $153 million grant from the Biomedical Advanced Research and Development Authority (BARDA) to develop CLT-008 as a cellular therapy for treating the hematopoietic syndrome observed after acute exposure to ionising radiation[58]. Upon transfusion of CLT-008 into the irradiated victim, the progenitor cells will have the ability to mature into granulocytes, platelets, and erythrocytes.

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REFERENCES

1. Benjamin, G.C.; McGeary, M. & McCutchen, S. R. *In* Assessing Medical Preparedness to Respond to a Terrorist Nuclear Event: Workshop Report. 2009.
2. Singh, V.K. & Seed, T. M. Radiation Effects. *In* Physician's guide to terrorist attack, edited by M. J. Roy. Humana Press, Totowa, 2003. pp. 339-62.
3. Koenig, K. L.; Goans, R. E.; Hatchett, R. J.; Mettler, F. A., Jr.; Schumacher, T. A.; Noji, E. K. & Jarrett, D. G. Medical treatment of radiological casualties: Current Concepts. *Ann. Emerg. Med.*, *2005,* **45**(6), 643-52.
4. Meineke, V.; van Beuningen, D.; Sohns, T. & Fliedner, T. M. Medical management principles for radiation accidents. *Military Medicine*, 2003*,* **168**(3), 219-22.
5. Dainiak, N.; Waselenko, J. K.; Armitage, J. O.; MacVittie, T. J. & Farese, A. M. The hematologist and radiation casualties. *Hematology Am. Soc. Hematol. Educ. Prog.*, 2003, 473-96.
6. Pellmar, T.C. & Rockwell, S. Priority list of research areas for radiological nuclear threat countermeasures. *Radiation Research*, 2005*,* **163**(1), 115-23.
7. Seed, T.M. Radiation protectants: Current status and future prospects. *Health Physics*, 2005*,* **89**(5), 531-45.
8. Augustine, A.D.; Gondre-Lewis, T.; McBride, W.; Miller, L.; Pellmar, T.C. & Rockwell, S. Animal models for radiation injury, protection and therapy. *Radiation Research*, 2005*,* **164**(1), 100-109.
9. Hosseinimehr, S.J. Trends in the development of radioprotective agents. *Drug Discov. Today*, 2007*,* **12**(19-20), 794-805.
10. Ghosh, S.P.; Kulkarni, S.; Hieber, K.; Toles, R.; Romanyukha, L.; Kao, T.C.; Hauer-Jensen, M. & Kumar, K.S. Gamma-tocotrienol, a tocol antioxidant as a potent radioprotector. *Int. J. Radiat. Biol.*, 2009*,* **85**(7), 598-606.
11. Landauer, M.R.; Srinivasan, V. & Seed, T.M. Genistein treatment protects mice from ionising radiation injury. *J. Appl. Toxicol.*, *2003,* **23**(6), 379-85.
12. Whitnall, M.H.; Elliott, T.B.; Harding, R.A.; Inal, C.E.; Landauer, M. R.; Wilhelmsen, C. L.; McKinney, L.; Miner, V. L.; Jackson III, W. E.; Loria, R. M.; Ledney, G. D. & Seed, T. M. Androstenediol stimulates myelopoiesis and enhances resistance to infection in gamma-irradiated mice. *Int. J. Immunopharmacol*., 2000*,* **22**(1), 1-14.
13. Burdelya, L.G.; Krivokrysenko, V.I.; Tallant, T.C.; Strom, E.; Gleiberman, A. S.; Gupta, D.; Kurnasov, O.V.; Fort, F. L.; Osterman, A. L.; Didonato, J. A.; Feinstein, E. & Gudkov, A. V. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*, *2008,* **320**(5873), 226-30.
14. Srinivasan, V.; Doctrow, S.; Singh, V.K. & Whitnall, M.H. Evaluation of EUK-189, a synthetic superoxide dismutase/catalase mimetic as a radiation countermeasure. Immunopharmacol. *Immunotoxicology*, 2008*,* **30**(2), 271-90.
15. DiCarlo, A.L.; Hatchett, R.J.; Kaminski, J.M.; Ledney, G.D.; Pellmar, T.C.; Okunieff, P. & Ramakrishnan, N. Medical countermeasures for radiation combined injury: Radiation with burn, blast, trauma and/or sepsis. Report of an NIAID Workshop, March 26-27, 2007. *Radiation Research*, 2008, **169**(6), 712-21.
16. Weiss, J.F. & Landauer, M.R. History and development of radiation-protective agents. *Int. J. Radiat. Biol.*, 2009, **85**(7), 539-73.
17. Xiao, M. & Whitnall, M.H. Pharmacological countermeasures for the acute radiation syndrome. *Curr. Mol. Pharmacol.*, 2009, **2**, 122-33.
18. Herodin, F.; Grenier, N. & Drouet, M. Revisiting therapeutic strategies in radiation casualties. *Exp. Hematol.*, 2007, **35**(4 Suppl 1), 28-33.
19. Herodin, F. & Drouet, M. Myeloprotection following cytotoxic damage: the sooner the better. *Exp. Hematol.*, 2008, **36**(7), 769-70; author reply 71-2.
20. Herodin, F.; Mayol, J.F.; Mourcin, F. & Drouet, M. Which place for stem cell therapy in the treatment of acute radiation syndrome? *Folia Histochem. Cytobiol.*, 2005*,* **43**(4), 223-7.
21. Radiation Injury Treatment Network. http://www.ritn.net, 2010. (Accessed on 5 October 2010)
22. Spangrude, G.J.; Heimfeld, S. & Weissman, I.L. Purification and characterisation of mouse hematopoietic stem cells. *Science*, 1988*,* **241**(4861), 58-62.
23. Morrison, S.J.; Wandycz, A. M.; Hemmati, H. D.; Wright, D. E. & Weissman, I. L. Identification of a lineage of multipotent hematopoietic progenitors. *Development*, 1997*,* **124**(10), 1929-39.
24. Kondo, M.; Weissman, I.L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell*, 1997*,* **91**(5), 661-72.
25. Akashi, K.; Traver, D.; Miyamoto, T. & Weissman, I.L. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*, 2000, **404**(6774), 193-7.
26. BitMansour, A.; Burns, S.M.; Traver, D.; Akashi, K.; Contag, C.H.; Weissman, I.L. & Brown, J.M. Myeloid progenitors protect against invasive aspergillosis and Pseudomonas aeruginosa infection following

hematopoietic stem cell transplantation. *Blood*, 2002, **100**(13), 4660-7.
27. Arber, C.; BitMansour, A.; Sparer, T. E.; Higgins, J.P.; Mocarski, E.S.; Weissman, I.L.; Shizuru, J.A. & Brown, J.M. Common lymphoid progenitors rapidly engraft and protect against lethal murine cytomegalovirus infection after hematopoietic stem cell transplantation. *Blood*, 2003, **102**(2), 421-8.
28. Na Nakorn, T.; Traver, D.; Weissman, I.L. & Akashi, K. Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J. Clin. Invest.*, 2002, **109**(12), 1579-85.
29. BitMansour, A.; Cao, T.; Chao, S.; Shashidhar, S. & Brown, J. M. Y. Single infusion of myeloid progenitors reduces death from *Aspergillus fumigatus* following chemotherapy-induced neutropenia. *Blood*, 2008, **105**(9), 3535-37.
30. Arber, C.; Bitmansour, A.; Shashidhar, S.; Wang, S.; Tseng, B. & Brown, J. M. Protection against lethal Aspergillus fumigatus infection in mice by allogeneic myeloid progenitors is not major histocompatibility complex restricted. *J. Infect. Dis*., 2005, **192**(9), 1666-71.
31. Arai, F.; Hirao, A.; Ohmura, M.; Sato, H.; Matsuoka, S.; Takubo, K.; Ito, K.; Koh, G. Y. & Suda, T. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell*, 2004, **118**(2), 149-61.
32. Rattis, F. M.; Voermans, C. & Reya, T. Wnt signaling in the stem cell niche. *Curr. Opin. Hematol.*, 2004, **11**(2), 88-94.
33. Beekman, R. & Touw, I. P. G-CSF and its receptor in myeloid malignancy. *Blood*, 2010, **115**(25), 5131-6.
34. Singh, V. K. & Yadav, V. S. Role of cytokines and growth factors in radioprotection. *Exp. Mol. Pathol.*, 2005, **78**(2), 156-69.
35. Shpall, E. J. The utilisation of cytokines in stem cell mobilisation strategies. *Bone Marrow Transplant.*, 1999, **23 Suppl 2**, S13-9.
36. Leung, A. Y. & Kwong, Y. L. Haematopoietic stem cell transplantation: current concepts and novel therapeutic strategies. *Br. Med. Bull.*, 2010, **93**, 85-103.
37. Thomas, E. D. A history of haemopoietic cell transplantation. *Br. J. Haematol.*, 1999, **105**(2), 330-9.
38. Bensinger, W.; Singer, J.; Appelbaum, F.; Lilleby, K.; Longin, K.; Rowley, S.; Clarke, E.; Clift, R.; Hansen, J.; Shields, T. & et al. Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. *Blood*, 1993, **81**(11), 3158-63.
39. Gianni, A.M.; Bregni, M.; Siena, S.; Villa, S.; Sciorelli, G.A.; Ravagnani, F.; Pellegris, G. & Bonadonna, G. Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy? *Hematol. Oncol.*, 1989, **7**(2), 139-48.
40. Pusic, I. & DiPersio, J.F. The use of growth factors in hematopoietic stem cell transplantation. *Curr. Pharm. Des.*, 2008, **14**(20), 1950-61.
41. Molineux, G.; Pojda, Z. & Dexter, T.M. A comparison of hematopoiesis in normal and splenectomised mice treated with granulocyte colony-stimulating factor. *Blood*, 1990, **75**(3), 563-9.
42. Jin, P.; Wang, E.; Ren, J.; Childs, R.; Shin, J.W.; Khuu, H.; Marincola, F.M. & Stroncek, D. F. Differentiation of two types of mobilised peripheral blood stem cells by microRNA and cDNA expression analysis. *J. Transl. Med.*, 2008, **6**, 39.
43. Petit, I.; Szyper-Kravitz, M.; Nagler, A.; Lahav, M.; Peled, A.; Habler, L.; Ponomaryov, T.; Taichman, R. S.; Arenzana-Seisdedos, F.; Fujii, N.; Sandbank, J.; Zipori, D. & Lapidot, T. G-CSF induces stem cell mobilisation by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol*, 2002, **3**(7), 687-94.
44. Lapidot, T.; Dar, A. & Kollet, O. How do stem cells find their way home? Blood, *2005,* **106**(6), 1901-10.
45. De Clercq, E. The AMD3100 story: the path to the discovery of a stem cell mobiliser (Mozobil). *Biochem. Pharmacol.*, 2009, **77**(11), 1655-64.
46. Uy, G.L.; Rettig, M.P. & Cashen, A.F. Plerixafor, a CXCR4 antagonist for the mobilisation of hematopoietic stem cells. *Expert Opin. Biol. Ther.*, *2008,* **8**(11), 1797-804.
47. Gupta, S.K.; Pillarisetti, K.; Thomas, R. A. & Aiyar, N. Pharmacological evidence for complex and multiple site interaction of CXCR4 with SDF-1alpha: implications for development of selective CXCR4 antagonists. *Immunol. Lett.*, 2001, **78**(1), 29-34.
48. Jin, F.; Zhai, Q.; Qiu, L.; Meng, H.; Zou, D.; Wang, Y.; Li, Q.; Yu, Z.; Han, J. & Zhou, B. Degradation of BM SDF-1 by MMP-9: the role in G-CSF-induced hematopoietic stem/progenitor cell mobilisation. *Bone Marrow Transplant*, 2008, **42**(9), 581-8.
49. Hatse, S.; Princen, K.; Bridger, G.; De Clercq, E. & Schols, D. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. *FEBS Letters*, 2002, **527**(1-3), 255-62.
50. Hendrix, C.W.; Collier, A.C.; Lederman, M.M.; Schols, D.; Pollard, R.B.; Brown, S.; Jackson, J.B.; Coombs, R.W.; Glesby, M.J.; Flexner, C.W.; Bridger, G.J.; Badel, K.; MacFarland, R.T.; Henson, G.W. & Calandra, G. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J. Acquir. Immune. Defic. Syndr.*, 2004, **37**(2), 1253-62.
51. Steinberg, M. & Silva, M. Plerixafor: A chemokine receptor-4 antagonist for mobilisation of hematopoietic stem cells for transplantation after high-dose chemotherapy for non-Hodgkin's lymphoma or multiple myeloma. *Clin. Ther.*, 2010, **32**(5), 821-43.
52. Packer, L. Protective role of vitamin E in biological

systems. *Am. J. Clin. Nutr.*, 1991, **53**(4 Suppl), 1050S-55S.
53. Singh, V. K.; Brown, D. S. & T.C., K. Tocopherol succinate: A promising radiation countermeasure. *Int. Immunopharmacol*, 2009, **9**, 1423-30.
54. Singh, V. K.; Parekh, I. K.; Brown, D. S.; Kao, T. C. & Mog, S. R. Tocopherol succinate: Modulation of antioxidant enzymes and oncogene expression, and hematopoietic recovery. *Intl. J. Radiat. Oncolo. Biol. Phy.*, 2010, (in press).
55. Singh, V. K.; Shafran, R. L.; Jackson, W. E., III; Seed, T. M. & Kumar, K. S. Induction of cytokines by radioprotective tocopherol analogs. *Exp. Mol. Pathol.*, 2006, **81**(1), 55-61.
56. Singh, V. K.; Brown, D. S. & Kao, T. Alpha-tocopherol succinate protects mice from gamma-radiation by induction of granulocyte-colony stimulating factor. *Int. J. Radiat. Biol.*, 2010, **86**, 12-21.
57. Singh, V. K.; Brown, D.; Kao, T. C. & Seed, T. M. Preclinical development of a bridging therapy for radiation casualties. *Exp. Hematol.*, 2010, **38**, 61-70.
58. Yoon, J. Cellerant Therapeutics awarded $153 million contract by Biomedical Advanced Research and Development uthority (BARDA) to develop treatment for acute radiation syndrome (ARS). http://www.cellerant.com/pr_090110.html. 2010.

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