Myeloid Progenitors Mitigate Radiation Injury and Improve Intestinal Integrity after Whole-Body Irradiation

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ABSTRACT

We have demonstrated the efficacy of mouse myeloid progenitor cells (mMPC) as a promising radiation countermeasure with the potential to mitigate radiation injury across a broad range of lethal radiation doses in unmatched recipients even when transfused days after radiation exposure. Here we investigated how transfusion of mMPC mitigates death from supralethal doses of radiation known to cause death by gastrointestinal injury. CD2F1 mice were exposed to different doses of radiation and then transfused with mMPC intravenously after irradiation. Intestinal tissues were harvested at different times after irradiation and analyzed for tissue architecture, surviving crypts, villus height and number. We also monitored the effect of infused mMPC on bacterial translocation from gut to heart, spleen, and liver in irradiated mice by bacterial tissue cultures, and estimated endotoxin in serum samples. We observed that the infusion of mMPC significantly improved survival of mice receiving high doses of radiation, decreased the number of bacterial infection, and lowered endotoxin levels in serum. Histopathology of jejunum from irradiated and mMPC-transfused mice revealed significant mitigation of gastrointestinal tissue injury. In brief, results of this study further supports our contention that the transfusion of mMPC acts as a bridging therapy for gastrointestinal system recovery by improving intestinal structural integrity and inhibiting bacterial translocation in the gastrointestinal tract of lethally irradiated mice. This novel cell therapeutic approach consisting of the infusion of mMPC following acute radiation injury appears to be one of the most promising radiation countermeasures for acute radiation syndrome.

1.0 INTRODUCTION

The risk of exposure to ionizing radiation due to terrorist activities is widely thought to be increasing [1]. Although efforts to find suitable radiation countermeasures were initiated more than half a century ago, no safe and effective radiation countermeasure for acute radiation syndrome (ARS) has been approved by the United States Food and Drug Administration (FDA). Thus, there is a pressing need for both radioprotectant and radiomitigator therapeutics to address ARS, as recognized by civilian and military government agencies [2]. Major themes of countermeasure development have been free radical scavengers and stimulation of hematopoietic progenitors. Other therapeutic avenues are also being explored, such as enhancing DNA repair or blocking cell death pathways. Therapies utilizing cytokine treatment and supportive care including antibiotics and blood component transfusion have shown moderate success in animal models [3], prompting intensified research to identify a new generation of countermeasures.

The biological effects of radiation are strongly dependent upon the dose of radiation received [4, 5]. ARS developing from whole-body or partial-body irradiation can involve hematopoietic, gastrointestinal, and cerebrovascular components [6]. Cerebrovascular damage invariably leads to death within several days. In
contrast, mortality from hematopoietic and gastrointestinal syndromes occurs with lower frequency and more slowly (over weeks rather than days) and is more likely to be amenable to radiation countermeasures. There are a number of potential radiation countermeasures that are currently in different stages of development, falling broadly into two categories depending upon their primary mechanism of action: immunomodulators/cytokines/growth factors [7-12] and antioxidants/free-radical scavengers [13-15]. In large part, the focus on cytokines and growth factors has been based on their potential ability to act as radiomitigators enhancing recovery of the hematopoietic system from radiation damage, as demonstrated in multiple in vitro and in vivo studies [11, 12]. Some cytokines have received FDA approval for treatment of neutropenia and thrombocytopenia caused by anti-cancer radiotherapy and chemotherapy, and several others are under development [16-18]. The anti-radiation potential of antioxidants and free-radical scavengers derives from their ability to reduce levels of reactive oxygen species induced by radiation, thus decreasing DNA damage, lipid peroxidation and other types of chemical modification [14]. Efficacy of this class of agents is limited to prophylaxis of radiation injury.

Cellerant Therapeutics has developed culture conditions to produce large numbers of mouse myeloid progenitors (mMPC) from hematopoietic stem cells as a radiomitigator. mMPC primarily consist of lineage^{-}low-c-Kit^{-} myeloid progenitors expressing varying levels of CD11b^{+} and Gr1^{+} indicating a commitment to the myeloid lineage [12, 19]. In contrast to whole bone-marrow grafts, T and B cells are not present in detectable levels among mMPC and the risk of graft-versus-host disease (GVHD) is negligible. Earlier published in vivo studies suggest that allogeneic myeloid progenitors give rise to myeloid and erythroid cells that transiently engraft and functionally protect mice from death due to invasive fungal infections in major histocompatibility complex disparate recipient mouse strains [20, 21].

We demonstrate that mMPC are a promising radiation countermeasure with the potential to mitigate radiation injury in unmatched recipients across a broad range of lethal radiation doses even when transfusion is delayed seven days after radiation exposure [19]. With respect to efficacy, timing and practicality of administration, mMPC appear to be one of the most promising radiation countermeasures for ARS among all candidate therapeutics currently under development [12]. To understand the mechanism of radiomitigation by mMPC transfused into lethally irradiated mice, we investigated the effects of mMPC transfusion on the extent of gut injury by histopathology analysis. We also studied gut bacterial translocation in irradiated mice. Our results demonstrated that mMPC transfusion significantly mitigated radiation injury in vital gastrointestinal tissue, and inhibited bacterial translocation in mice exposed to high doses of 60Co γ-radiation when administered 2-24 h after radiation exposure.

2.0 MATERIALS AND METHODS

2.1 Mice

Male 6–8 week-old specific-pathogen-free (SPF) CD2F1 mice were purchased (Harlan, Indianapolis, IN, USA) and housed (8 per cage) in an air-conditioned facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International at the Armed Forces Radiobiology Research Institute. Upon arrival, mice were held in quarantine for 1 week. A microbiological examination of representative samples ensured the absence of Pseudomonas aeruginosa. Mice were provided certified rodent rations (Harlan Teklad Rodent Diet, Harlan Teklad, Madison, WI, USA), acidified water (HCl, pH = 2.5 - 2.8) ad libitum, sterilized bedding and housed in rooms with a 12-h light/dark cycle. The mouse holding room was maintained at 21 ± 2 °C with 10–15 hourly cycles of fresh air and a relative humidity of 50 ± 10%. Research was conducted according to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, U.S. National Academy of Sciences [22]. At the
time of experiment, mice were 8-9 wk old. Experimental mice were observed daily in the morning and any mice found sick/morbid, but not moribund, were allowed to continue in the experiment.

All mice used at Cellerant Therapeutics for preparing mMPC were SPF males and were received at 6-8 weeks of age, quarantined for at least one week and subjected to experimental procedures at 8-12 weeks of age. AKR/J and B6.Pl-Thy1.1 mice were procured from Jackson Laboratories, Bar Harbor, ME. FVB mice were procured from Charles River Laboratories, Hollister, CA, USA. Mice were housed in individually ventilated cages of up to five mice per cage on a 12 h light/dark cycle at 21 ± 2 ºC with 10–15 hourly cycles of fresh air and given sterilized bedding and acidified water. All animal procedures were performed according to a protocol approved by the Institutional Animal Care and Use Committee at Cellerant Therapeutics.

2.2 Irradiation

Mice were placed in ventilated acrylic boxes compartmentalized to accommodate eight mice per box and exposed to bilateral irradiation in the AFRRI cobalt-60 facility at a dose rate of 0.6 Gy/min to total midline doses indicated in experiments as described recently [23]. After irradiation, mice were returned to their cages and monitored. Sham-irradiated mice were treated in the same manner as irradiated animals except that the cobalt-60 rods were not raised from the pool of shielding water. Radiation dosimetry was based on the alanine/EPR (electron paramagnetic resonance) system [24, 25], currently accepted as one of the most accurate methods and used for intercomparison between national metrology institutions. The calibration curves used in dose measurements at AFRRI (spectrometer e-Scan, Burker Biospin, Inc., Germany) are based on standard alanine calibration sets purchased from The United States National Institute of Standards and Technology, Gaithersburg, MD, USA.

2.3 Derivation of mouse myeloid progenitor cells (mMPC)

mMPC were generated separately from three MHC disparate mouse strains: AKR/J, FVB, and B6.Pl-Thy1.1 mice. HSC for mMPC derivation were isolated from the bone marrow of donor mice as described previously [26]. Monoclonal antibodies used for immunofluorescence staining for HSC isolation included PE-Cy7-conjugated CD117 (2B8), APC-conjugated Sca-1 (Ly6a/E), FITC-conjugated Thy1.1 (HIS51), and a lineage cocktail of PE-conjugated B220 (RA3-6B2), CD3 (145-2C11), CD4 (GK1.5), CD5 (53-7.3), CD8 (53-6.7), CD11b (M1/70), Gr-1 (RB6-8C5), and Ter119 (TER-119) (eBioscience, San Diego, CA). HSC were double sorted for purity as CD117+Thy-1.1lowSca-1+lin\textsuperscript{low} [27] using a 3-laser FACSAria (BD Biosciences, San Jose, CA). Sorted HSC from B6.Pl-Thy1.1, AKR, or FVB mice were plated in X-VIVO 15 media (Lonza, Walkersville, MD, USA) supplemented with recombinant mouse (rm) stem cell factor (rm SCF, Invitrogen, Carlsbad, CA), rm thrombopoietin (rm TPO) (Invitrogen), rm Fms like tyrosine kinase 3 ligand (Flt3L) (R&D Systems, Minneapolis, MN), Primocin (Invivogen, San Diego, CA), 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), and Glutamax (Invitrogen). On d 9 of culture, B6.Pl-Thy1.1 mMPC were harvested and cryopreserved. AKR and FVB mMPC were harvested and cryopreserved on d 10. mMPC from each strain were cryopreserved separately in X-VIVO 15 media containing 10% dimethyl sulfoxide (Protide Pharmaceuticals, Lake Zurich, IL, USA) and 25% FCS at 20 million cells/ml.

2.4 Transfusion of mMPC to mice

Mice were anesthetized in a ComPac\textsuperscript{5} anesthesia system (VetEquip Inc., Pleasanton, CA) with isoflurane (Abbott Laboratories, Chicago, IL, USA) aerosol used as the anesthetic agent. Anesthetized mice were transfused intravenously (retro-orbital sinus) with a 0.5 ml insulin syringe and 28 G needle. Each mouse received 100 µl of cell suspension containing the desired number of mMPC pooled in equal parts from all

STO-MP-HFM-223
three donor strains or vehicle. Mice were continuously monitored until regaining consciousness before transferring to cages.

2.5 Histopathology of jejunum

To evaluate the effect of mMPC transfusion on radiation-induced intestinal damage, mice were irradiated and transfused with mMPC or vehicle as described above. Mice were euthanized by CO₂ asphyxiation and jejunum was collected for histopathology. Samples were gently perfused with formalin, placed in a cassette and submerged in formalin for at least 12 h. Jejuna were immersion-fixed in a 20:1 volume of fixative (Z-FIX®, Anatech Ltd., Battle Creek, MI, USA) to tissue for at least 24 h and up to seven days [28]. Paraffin sections were used for immunohistochemistry examination. Cross sections of jejunum were cut with a manual rotary microtome at 4 μm [29]. The crypt microcolony survival assay was performed on hematoxylin and eosin (H&E) paraffin sections as described by Withers and Elkind [30]. The cross sections were observed under a Nikon Eclipse TS100 microscope (Nikon Inc. Melville, NY) equipped with the Retiga 2000R Q imaging camera (Surrey, BC, Canada). The circumference of a transverse cross-section of the intestine was used as a unit. The cross section tissue area was determined by subtracting the lumen area from total cross section area of jejunum. Crypts of Lieberkühn were considered viable if they contained at least 10 epithelial cells (either columnar enterocytes or goblet cells), a lumen and at least one Paneth cell. The number of surviving crypts was counted in each circumference. The total number of villi was scored in each circumference. Six circumferences were scored per mouse and 8 mice were used in each group. The six longest villi were measured in each circumference.

2.6 Evaluation of gut bacterial translocation

Heart ventricular blood, liver, and spleen of mice were collected aseptically as described recently [31] and cultured on sheep blood agar, colistin-nalidixic acid in sheep-blood agar, and xylose-lysine-desoxycholate agar media. Single colonies of isolated microorganisms were observed for their characteristics including morphology and color. Pertinent characteristics were recorded. A portion of an isolated colony was subcultured to obtain a pure culture and the remaining portion of the same colony was used to prepare a Gram-stain. The Gram-stain characteristics for each isolate were observed under oil immersion at 1000X magnification and recorded (cell-wall structure, cellular shape, and cellular arrangement). The subcultures on SBA were incubated in 5% CO₂ at 35°C for 18–24 h and then observed to assure a pure culture. Pure cultures were identified by a Vitek-2 Compact automated system (bioMérieux, Inc., Durham, NC, USA) according to the manufacturer’s validated procedure.

2.7 Determination of bacterial endotoxin in serum

Concentration of bacterial endotoxin was determined in serum obtained from mMPC-treated and vehicle-treated mice by a kinetic turbidimetric method of the limulus amebocyte lysate assay (ENDOSAFE® KTA2™, Charles River Laboratories, Inc., Charleston, SC) [32].

2.8 Statistical analysis

Means with standard error or percentage were reported if applicable. Analysis of variance (ANOVA) was used to detect whether there was a significant difference among groups. If significant, then a pair-wise comparison by Tukey-Kramer was used to identify which groups were different from the others. For survival data, a log-rank test was used to compare survival curves. Fisher’s exact test was used to compare survival rates at the end of 30 d, with Bonferroni correction used to control type-I error if multiple comparisons
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were used. ANOVA was used to detect whether there were significant differences between groups. If significant, a Tukey’s post-hoc test was used to determine significant differences between particular groups. A significance level was set at 5% for each test. All statistical tests were two-sided with a 5% significance level. Statistical software, SPSS version 19, was used for statistical analyses.

3.0 RESULTS

3.1 mMPC mitigate death from a potentially lethal dose of $^{60}$Co γ-radiation in CD2F1 mice when administered days after irradiation

To investigate how long mMPC administration can be delayed after irradiation, CD2F1 mice (H-2$^b$) were irradiated using $^{60}$Co γ-radiation (LD$_{90/30}$ dose, 9.2 Gy) and transfused with mMPC pooled from AKR (H-2$^k$), B6.Pl-Thy1.1 (H-2$^b$), and FVB (H-2$^q$) mice at different times after irradiation. For each administration time point a control group of mice was irradiated with $^{60}$Co γ-radiation and received vehicle. The irradiated mice were monitored for survival over 30 d and survival curves were plotted. mMPC administration was delayed for 5, 6, and 7 days after irradiation. When administered 5 or 6 d after $^{60}$Co γ-radiation (LD$_{90/30}$ dose, 9.2 Gy), 88% of mice treated with 4 million mMPC survived compared to 13% in the vehicle control treatment groups (figure 1). Administration of 6 million mMPC 7 d after $^{60}$Co γ-irradiation (LD$_{90/30}$ dose) significantly mitigated death in CD2F1 mice compared to vehicle control group (56 % survivors in mMPC treated mice compared with 0% survival in vehicle control). When 12 million mMPC were administered, there was no additional benefit.
3.2 mMPC mitigated death from supralethal doses of radiation

To investigate whether mMPC transfusion could be used to treat injuries due to supralethal doses of ionizing radiation causing gastrointestinal injury, three sets of mice were irradiated with 14.5, 15.0, and 15.5 Gy, respectively, and transfused with 5 million mMPC 24 h after irradiation. Three additional sets of mice receiving the same radiation exposures were transfused with vehicle. All mice were observed for 30 d post-irradiation. Results presented in figure 2 demonstrate that all mice transfused with mMPC receiving 14.5 and 15 Gy radiation survived and all mice given 15.5 Gy died by d 11 after irradiation. All mice in vehicle-transfused groups (14.5, 15 and 15.5 Gy) also died by d 11 after irradiation. These results suggest that a single transfusion of 5 million mMPC can mitigate radiation injury in mice exposed to $^{60}$Co $\gamma$-radiation as high as 15 Gy.

3.3 Effect of mMPC transfusion on radiation-induced intestinal damage

To assess the effect of mMPC transfusion on radiation-induced intestinal damage, 6 million mMPC or vehicle were transfused to recipient mice 2 h after irradiation with 13 Gy and jejunum (which is the most sensitive organ to enumerate effects of high doses of radiation) was analyzed by histopathology 4 and 8 days post irradiation (figure 3). Histological analyses indicated that the jejunal tissue from mMPC treated groups had improved normal histological structure and integrity compared to vehicle treated animals. A variety of histological assessments of intestinal damage were carried out, including evaluation of viable crypts of Lieberkühn, cross section tissue area, villus height, and villus number were selected as parameters to evaluate intestinal damage. The cross section tissue areas of jejunum in mMPC transfused mice were significantly higher compared to vehicle control mice on days 4 and 8 ($p < 0.001$). Mice receiving mMPC had a greater number of crypts compared to vehicle control on d 8 after irradiation ($p < 0.001$). Our results also demonstrate that mean villus height and circumference were higher in mMPC-transfused mice compared to vehicle control on days 4 and 8 ($p < 0.001$). These results demonstrate overall improved structural integrity of jejunum in mMPC treated mice.
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**Figure 3.** The effect of mMPC and vehicle transfusion on jejunum tissue recovery in CD2F1 mice exposed to $^{60}$Co $\gamma$-irradiation. CD2F1 male mice were irradiated with 13 Gy and transfused 2 h after irradiation with 6 million pooled, allogeneic mMPC or vehicle. Jejunum samples were collected 4 and 8 days after irradiation, and scored for mean tissue area, mean crypt number per circumference, mean villus height, and mean villus number per circumference. Upper two panels show representative photomicrographs of jejunal cross sections stained with hematoxylin and eosin (circumference at 40x, villus height at 100x). Lower panel shows the quantification of representative cross sections (*$p<0.05$).

### 3.4 Effect of mMPC transfusion on the translocation of intestinal bacteria in irradiated mice

The effects of mMPC transfusion on intestinal mucosal integrity were analyzed by evaluating the translocation of gut bacteria to various organs. Mice were irradiated with 13 Gy and transfused with 6 million mMPC or vehicle 24 h later. Heart blood, liver, and spleen samples of mMPC- or vehicle-transfused mice were collected aseptically on d 9, 11, and 14 post-irradiation, and cultured on appropriate media. Table 1 shows all bacterial species identified in samples from mMPC-transfused and vehicle-control groups at each time point after irradiation. No bacteria were isolated from mMPC-transfused mice at any time point (total of 18 mice). In contrast, at least two bacterial species were isolated from each of six vehicle-control mice on d 9,
which demonstrate polymicrobial sepsis in all six vehicle-control mice. Both Gram-positive and Gram-negative bacterial species were isolated from tissues in five of the six control mice and two Gram-positive species were isolated from one mouse. A total of eight species of bacteria were isolated from the six mice. Six species were Gram-positive, *Staphylococcus aureus* (2 mice), *Streptococcus uberis* (1 mouse), *Streptococcus mitis/Streptococcus oralis* (1 mouse), *Streptococcus sanguinis* (1 mouse), *Staphylococcus haemolyticus* (1 mouse), and *Enterococcus durans/Enterococcus hirae* (1 mouse); and two species were Gram-negative, *Escherichia coli* (3 mice) and *Sphingobacterium thalpophilum* (2 mice). It is noteworthy that *E. coli* and either *S. aureus* or *S. haemolyticus* appeared in combination in three mice and *S. thalpophilum* and either *S. sanguinis* or *E. durans/E. hirae* appeared together in two mice. These cases demonstrate incidence of polymicrobial infection of both Gram-positive and Gram-negative bacteria. Isolated bacterial species were detected in either heart blood, liver, or spleen from each mouse. No vehicle-control mice survived for sampling on days 11 and 14. On day 9, the concentration of bacterial endotoxin in the serum of eight mMPC-treated mice was <0.5 endotoxin unit (EU)/ml, whereas concentrations in serum of five vehicle-treated mice ranged between 131.8 and 13100.0 EU/ml. These findings correlate with the isolation of bacterial species in vehicle-treated and mMPC-treated mice. That is, bacteria and endotoxin were detected in vehicle-treated but not in mMPC-treated mice. As stated above, no vehicle-treated mice survived for collection of serum samples on days 11 and 14.

Table 1. Effect of mMPC transfusion on the translocation of gut bacteria to heart, spleen, and liver in mice exposed to $^{60}$Co $\gamma$–radiation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice with bacterial growth on d 9 post-irradiation</th>
<th>Days after irradiation</th>
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<tr>
<td></td>
<td></td>
<td>9</td>
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<tr>
<td>Vehicle</td>
<td>6/6 Escherichia coli (3) Staphylococcus aureus (2) Streptococcus uberis (1) Streptococcus mitis/Streptococcus oralis (1) Sphingobacterium thalpophilum (2) Streptococcus sanguinis (1) Staphylococcus haemolyticus (1) Enterococcus durans/Enterococcus hirae (1)</td>
<td>No survivors</td>
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<tr>
<td>mMPC</td>
<td>0/6 No bacteria isolated</td>
<td>No bacteria isolated</td>
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4.0 DISCUSSION

The potential for nuclear incidents is expected to grow in coming years, and the need to develop countermeasures for radiation victims is pressing. The radiation-induced gastrointestinal syndrome is a major lethal radiation injury that might occur after a nuclear accident or radiation terrorism (dirty bomb).
gastrointestinal syndrome involves destruction of crypt/villus units, loss of mucosal integrity, and infection by resident enterobacterial flora, characterized clinically as anorexia, vomiting, diarrhea, dehydration, systemic infection, and in extreme cases, septic shock and death [33-35]. There are no radiation countermeasures (protectors, mitigators or therapeutics) against radiation-induced gastrointestinal syndrome lethality for first responders, military personnel, or other workers entering a contaminated area. Pathophysiology of this syndrome requires depletion of stem cell clonogens within the crypts of Lieberkühn, necessary for post-injury regeneration of gut epithelium. Infusion of cryopreservable, culture-derived mMPC pooled from MHC-disparate donors are effective in preventing death across a wide range of high radiation doses in unmatched recipient mice. We were able to significantly mitigate radiation injury in mice and delay administration of mMPC as late as 7 d after irradiation to rescue mice exposed to hematopoietic dose of radiation (9.2 Gy, LD90/30) [19]. The ability to delay the administration of any radiation countermeasure is critical to allow for dosing of radiation victims in an emergency situation. We have previously used tocopherol succinate to mobilize progenitors and used mobilized progenitors as a radiomitigator to protect 60Co γ-irradiated mice when administered as late as 48 h after irradiation [36, 37] and were able to rescue mice against 11 Gy radiation exposure. With mMPC, we demonstrate that mice can be rescued from radiation exposure as high as 15 Gy (LD90/10). The present study confirms our previous report that mMPC transfusion after irradiation ameliorates radiation injury and reduces lethality across a broad range of radiation doses. Further, the ability to cryopreserve mMPC prior to use and administration without matching donor and recipient makes them logistically more feasible compared to bone marrow transplant. mMPC can be stockpiled and readily made available on demand in case of an emergency. Currently there is no report of any radiation countermeasure under development, which can be administered as late as this mMPC product to a wide range of people and still confer significant mitigation of ARS [11, 12].

Our histopathology data demonstrate that transfusion of mMPC into irradiated mice mitigates radiation-induced gastrointestinal injury. There was significant recovery in mMPC recipients compared to vehicle control. We have observed similar recovery of radiation-induced gastrointestinal injury when either tocopherol- or tocopherol succinate-mobilized progenitors were administered to mice [31, 37]. Tocopherol succinate was used as a radioprotector (24 h prior to irradiation) and tocopherol succinate-mobilized progenitors were used 24 or 48 h after irradiation as a mitigator. Gamma-tocotrienol has also been shown to improve post-irradiation survival and intestinal radiation injury [38, 39]. Gamma-tocotrienol was used as a radioprotector (24 h before irradiation). In this study with mMPC, we have used a much higher dose of radiation (13 Gy, LD50/10) and observed significant protection of gastrointestinal tissue. Our results show significantly increased tissue area, numbers of crypt, villus number and height in jejunal samples of mMPC transfused mice compared to vehicle control mice.

Radiation is known to cause bacterial translocation from the gastrointestinal tract to different organs [40, 41], which is considered to be a clinically important event during radiation-induced gastrointestinal injury [42, 43]. Our results presented in table 1 suggest the prevention of bacterial translocation and polymicrobial infections in mMPC-transfused mice compared to recipients of vehicle. We have earlier reported that tocopherol succinate treatment inhibits bacterial translocation in 60Co γ-irradiated CD2F1 mice [31]. The protective role of α-tocopherol on intestinal mucosa in mice and rats has also been reported by others [44, 45]. These results support our current findings.

Our results demonstrate that pooled, allogeneic mMPC prevent death from lethal radiation known to cause death through hematopoietic and gastrointestinal injury. Administration of mMPC can be delayed as late as 7 d after irradiation to mitigate radiation injury. The effect of mMPC on the gastrointestinal tract presented here point to a function of mMPC and their progeny outside the hematopoietic system at supra-lethal radiation...
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doses. With respect to efficacy across a broad range of radiation doses, as well as timing and practicality of administration, mMPC appear to be one of the most promising radiation countermeasures for ARS among all candidate therapeutics currently under development. Further studies will be needed to understand the mechanism by which this benefit is mediated.

5.0 CONCLUSION

There are a number of major advantages that make mMPC ideal for the treatment of patients/casualties with ARS: a) myeloid progenitor cells can be cryopreserved and stored without compromise in function, b) treatment can be delayed to provide survival benefit, and c) myeloid progenitors provide radiomitigation in unmatched irradiated recipients without signs of rejection or graft-versus-host disease. These characteristics make myeloid progenitors a prime candidate as a bridging therapy for acute radiation victims that can be administered in the field with minimal infrastructure requirements. With further preclinical development in large animals (nonhuman primate, canine or minipig), we may be able to provide an appropriate protocol in the near future for the clinical management of individuals suffering from exposure to high doses of ionizing radiation. Cellerant Therapeutics has developed a culture system to expand CD34+ enriched human HSC in vitro and to direct their differentiation into human myeloid progenitor cells. These hMPC (human myelod progenitor cells, CLT-008) are currently in two Phase 1 clinical trials in patients undergoing cord blood transplants and in patients receiving high dose chemotherapy for the treatment of hematological malignancies.

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