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Development of PLX-R18, a promising and novel countermeasure for radiation-associated injuries

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PLX-R18 is a novel cell-based product of *ex vivo* expanded adherent human-placenta-derived stromal cells. After intramuscular administration, these living cells are capable of secreting various cytokines that produce a therapeutic benefit. The endogenously secreted cytokines facilitate the recovery of hematopoietic progenitor cells and regenerate multiple blood lineage cells. Preclinical studies have demonstrated that PLX-R18 cells can prevent and also mitigate hematopoietic acute radiation syndrome in experimental animal models. This agent has an open US Food and Drug Administration investigational drug status for hematopoietic system-associated ARS (H-ARS). A phase I study using patients with bone marrow failure demonstrated the safety of the agent while promoting hematopoietic regeneration in humans.

Keywords: Acute radiation syndrome; ionizing radiation; medical countermeasures; placenta-derived stromal cells; PLX-R18; total-body irradiation

Introduction

Risks associated with nuclear and radiological accidents, military use of related weapons, and possible terrorist attacks have renewed interest in radiation medical countermeasures (MCMs) to treat unwanted radiation exposures.(p1),(p2) Exposure to acute, potentially lethal doses of ionizing radiation can result in acute radiation syndrome (ARS) with deleterious effects to various organs. The radiosensitive tissues of the body, such as the hematopoietic and gastrointestinal (GI) systems, are the most susceptible to exposure-related radiation injuries.(p3) Hematopoietic-system-associated ARS (H-ARS) is characterized by dose-dependent bone marrow depression, leading to life-threatening neutropenia and thrombocytopenia, and possible death resulting from opportunistic infections and uncontrolled bleeding, with subsequent, not uncommon, multi-organ failure.(p4) Neutropenia and thrombocytopenia begin at different days post-irradiation depending on the absorbed dose. Thrombocytopenia increases the risk of hemorrhage and poor wound healing, whereas neutropenia increases the risk of infections. Death caused by H-ARS that results in infection, excessive bleeding or multi-organ failure occurs within several weeks of high dose exposure.(p5),(p6)

Radiation-induced depletion of vital hematopoietic stem and progenitor cell (HSPC) pools is the ultimate cause of morbidity and mortality during H-ARS.(p7) HSPCs are specialized, self-renewing cells of the bone marrow that have the ability to differentiate into lineage-specific progenitors, giving rise to mature cells belonging to various blood cell types that have the ability to self-renew.(p8) Through these processes, stem cells are capable of sustaining lifelong hematopoiesis. Damage to these cells, for example by exposure to high doses of ionizing radiation, negatively impacts the body's ability to generate the blood cell

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lineages. The decline in HSPC numbers that occurs naturally over time, because of aging or damage to these cells at younger ages is most problematic. Survival following H-ARS is dependent on the recovery of the surviving stem and progenitor cells and, in turn, on the production of mature, functional neutrophils and platelets.

The development of MCMs is an important security issue for both individuals and the security of nations across the world.[p9] In any nuclear or radiological scenario of considerable scale, the number of exposed individuals will be large.[p10],[p11] In addition, in light of the increasing clinical use of radiotherapy for various medical conditions, there is also a need to develop preventive and mitigative options that can minimize collateral injury to normal tissues.[p12],[p13] Furthermore, radioprotective MCMs will also be needed for other applications, such as space travel.[p14] These options and medicinals generally are defined by the time of their administration in relation to the radiation exposure, being categorized as radioprotectors, radiomitigators, or therapeutics, but rarely as all three.[p2],[p15]

Ionizing radiation includes γ-rays, X-rays (electromagnetic radiation), and particulate radiation (neutron, electrons, protons, β-particles and α-particles). X-rays and γ-rays are of low linear energy transfer (LET), illustrated by issue tracks of relatively sparse ionizations. Neutrons and α-particles are of high LET, with a high density of ionizations per unit track. The free radicals produced by the radiolysis of the aqueous milieu in cells and tissues are largely the causative agent for the inflicted injuries. Free radicals (mostly hydroxy radicals) lead to indirect radiation injury, producing roughly 75% of the low LET damage.[p3],[p16],[p17] This damage may be prevented by free radical scavengers, but these scavengers are less efficient in preventing injuries arising from high-LET radiation. Variation in human response to injury may result largely from each individual's ability to detoxify free radicals through the actions of endogenous antioxidants and related enzymes.

The basic types of injury that arise from radiological/nuclear exposures are either external or internal in nature, with the latter being associated with contamination by radioactive materials (radionuclides) that are incorporated into the body. A few types of radiation syndrome are based on the time of symptom manifestation in relation to exposure, namely acute, delayed, late, and chronic.[p18] Higher doses of radiation generally result in more severe early effects (i.e., the so-called deterministic type of radiation-associated injuries). With prolonged survival, there is an increased risk of an array of late-arising (i.e., delayed) pathologies and related diseases. Late-arising pathologies may include cancer or organ fibrosis.

The clinical progression of and survival rates for ARS depend on the absorbed dose and its distribution.[p3] Clinical manifestations of ARS in humans comprise three main subsyndromes: H-ARS, gastrointestinal ARS (GI-ARS), and neurovascular ARS (NV-ARS).[p19] Selective MCMs can, both in principal and in practice, effectively target the H-ARS and GI-ARS subsyndromes of ARS and thus are high on the priority list for further research and development. The US Food and Drug Administration (FDA) has approved eleven MCMs for human use for H-ARS (Neupogen, Zarxio, Nypozi, Releuko, Neulasta, Udenyca, Stimufend, Ziextenzo, Fylnetra, Nplate and Leukine). Leukine has recently been approved in the European Union by the European Medicines Agency (EMA). All of these agents are approved as radiomitigators for use after exposure.[p20],[p21],[p22],[p23],[p24],[p25],[p26],[p27],[p28],[p29],[p30],[p31],[p32] Although there are a large number of potentially useful agents at various stages of drug development, no MCM has been approved specifically for use prior to unwanted (non-clinical) radiation exposure.[p2],[p33],[p34],[p35],[p36]

Pluri Inc. is developing PLacental eXpanded (PLX)-R18 as a radiation MCM for H-ARS under the FDA Animal Rule.[p37] PLX-R18 is a novel cell-based product, comprised of *ex vivo* expanded adherent human-placenta-derived stromal cells for parenteral administration.[p38],[p39],[p40],[p41],[p42] These cells are placental-derived mesenchymal-like adherent stromal cells of fetal origin. These cells express mesenchymal stromal cell surface markers and have limited reproductive capacity (undergoing approximately 60 population doublings before senescence) while maintaining stable karyotypes, and display several distinct differentiative and maturational pathways (giving rise to both osteocytes and adipocytes). PLX-R18 cells aid vital functions by secreting critically important cytokines that serve to support the hematopoietic system; these processes, in turn, act to reduce radiation exposure-induced lethality.[p40],[p43],[p44],[p45] The mechanism of PLX-R18 reparative action during H-ARS is thought to involve the secretion by these cells of multiple hematopoietic cytokines and growth factors within the microenvironment of lymphohematopietic tissues following irradiation. These soluble, signaling agents, which include stem cell factor (SCF), granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), interleukin 6 (IL-6), interleukin 8 (IL-8), and monocyte chemoattractant protein 1 (MCP-1), promote the differentiation, proliferation and maturation of hematopoietic progenitor cells and precursor cells.[p46]

A series of preclinical, animal-based safety and efficacy assessments of PLX-R18 has been conducted by Pluri. A toxicological study in unirradiated immunocompromised mice supports the safety of PLX-R18, with no adverse effect after treatment following repeated injections (Unpublished – Pluri Biotech Investigator brochure). Biodistribution studies, conducted in both immunocompromised, unirradiated mice and in immunocompetent, unirradiated and irradiated rhesus nonhuman primates (NHP), demonstrated that PLX-R18 cells remain locally at the intramuscular (*im*) injection site for a limited time, after which these cells are cleared from the body (Unpublished – Pluri Biotech Investigator brochure). A clinical phase I study of patients suffering from bone marrow failure, resulting from incomplete engraftment following bone marrow transplantation, demonstrated that PLX-R18 cell treatment is safe and well tolerated, and offers promising improvements in hematopoietic recovery profiles.[p41],[p47] Further, the efficacy of PLX-R18 in mitigating radiation-induced bone marrow failure and in enhancing survival has been demonstrated in a series of experiments using animal models; for example, PLX-R18 promoted the survival of lethally irradiated mice over a wide range of total-body radiation doses when administered either pre- or post-exposure.[p42],[p47],[p48] In brief, previous studies have shown beneficial medical effects of PLX-R18 in reducing radiation-induced H-ARS, when used either as a mitigator administered after exposure to ionizing radiation or as pro-

phylactic treatment initiated up to 24 h prior to radiation exposure.[p44],[p48],[p49]

Radioprotective and radiomitigative efficacy of PLX-R18 in murine models

The radioprotective and radiomitigative efficacies of the novel agent PLX-R18 have been well-investigated in acutely irradiated murine models, as well as in a pilot rhesus NHP model.[p44],[p48],[p49] Different doses of cells with different treatment schedules and different radiation doses have been used in these studies. Usually, a radiation dose of $LD_{50/30}$ (lethal dose 50% within 30 days) to $LD_{90/30}$ (lethal dose 90% within 30 days) is preferred in initial studies using murine models to evaluate the efficacy of any agent. Similarly, the administration of various numbers of cells has been investigated to identify the optimal treatment dose. PLX-R18 has been tested as both a radioprotector and a radiomitigator, and in both cases, two doses were needed for optimal efficacy. In the radioprotector strategy, a second dose was used after radiation exposure, and hence the agent was not used according to the strict definition of a radioprotector. For radiomitigation, both doses were used after exposure.

PLX-R18 efficacy in murine models

PLX-R18 was shown to reduce irradiation-induced lethality caused by a range of total-body radiation doses in C57BL/6 (normal radiation sensitivity) and C3H/HeNHsd (increased radiation sensitivity) mice.[p44],[p48] Studies showed a beneficial effect of PLX-R18 in reducing irradiation-induced H-ARS when used either as a mitigator, given after exposure, and/or as prophylactic treatment, given up to 24 h prior to exposure. PLX-R18 enhanced survival in these two mouse strains that have markedly different radiation sensitivities following widely ranging, potentially lethal radiation exposures (i.e., total-body radiation doses ranging from $LD_{50/30}$ to $LD_{90/30}$). From these lethality-based experiments, measures of the agent's overall radioprotectiveness (in terms of a survival benefit) were estimated; for example, the radiation dose reduction factor (DRF) was calculated to be 1.22 using C3H/HeNHsd mice. PLX-R18 has been shown to decrease mortality rates in mice significantly when given *im* at both 1 and 5 days after irradiation.[p44],[p48],[p49],[p50]

Safety in murine models

A toxicology study was conducted in nonobese diabetic (NOD)/-severe combined immunodeficiency (SCID) mice, which are immunocompromised, under good laboratory practice conditions. Following two *im* injections of 1 million cells of PLX-R18 per mouse (40 million cells/kg) one week apart, no adverse effects were noted (Pluri Biotech, unpublished observation). PLX-R18, administered in two doses at four days apart, has also been reported to be safe in unirradiated C57BL/6 mice.[p48] In this study, PLX-R18 was administered to naïve mice at an increased dose of 2 million cells/dose, with two doses given 4 days apart. Control animals received the carrier (PlasmaLyte) alone. Unirradiated mice in both the PLX-R18-test group and the PlasmaLyte-control group demonstrated low white blood cells, lymphocytes, and platelets when compared with the naïve mice on day 1.[p48] By day 5, the white blood cell counts were almost the same in all three groups. By day 7, counts in all groups were the same and no signs of acute toxicity were observed. Necropsy at the end of the study found no differences between the three groups. There was no difference between the various groups with respect to the renal and hepatic serum biochemistry panels. In brief, acute toxicity data suggested that PLX-R18 administered to mice as two doses of 2 million cells/dose given four days apart appeared to be safe.

Survival benefit afforded by PLX-R18 in murine models

PLX-R18 has been tested as both a radioprotector for prophylaxis and a radiomitigator for post-exposure use.

Radioprotection

Administration of PLX-R18 both one day prior and three days after lethal irradiation (8 Gy) increased 30-day survival compared to administration of the vehicle PlasmaLyte ($p < 0.001$).[p48]

Hematological recovery

In the groups exposed to 8 Gy total-body radiation, white blood cells, neutrophils, platelets, and lymphocytes all declined, reaching nadirs shortly after irradiation. Administration of PLX-R18 (one day prior to and three days after irradiation) resulted in higher numbers of white blood cells, neutrophils, platelets, and lymphocytes in treated mice compared with mice given only the control, PlasmaLyte.[p48] In unirradiated mice, no significant differences in the blood counts of PlasmaLyte and PLX-R18 groups were noted over time, suggesting that the administration of PLX-R18 had no adverse effect. PLX-R18 provided radioprotection (evidenced by less severe nadirs in multiple cell lineages) and accelerated hematopoietic recovery following radiation exposure.[p48]

Radiomitigation

In order to determine the efficacy of delayed administration, a study was carried out in which the first dose of PLX-R18 was delivered at 24, 48 or 72 h post-irradiation, followed by a second dose at 5 days post-irradiation. PLX-R18 treatment in all three treatment groups conferred increased survival following irradiation with the highest impact on survival observed in the mice receiving doses on days 1 and 5 (Pluri Biotech, unpublished observation).[p50] All PLX-R18 treatment groups showed significant increases in white blood cells, red blood cells, and platelets compared to vehicle control.

Mechanistic studies in murine models

Blood plasma levels of erythropoietin increased following irradiation, but this response was marked in the PLX-R18-treated mice as compared to control, with pre-exposure baseline levels being restored by day 30 post-exposure in the PLX-R18-treated mice.[p48] The FMS-like tyrosine kinase 3 ligand (Flt-3L) showed improved recovery in the PLX-R18-treated group compared to vehicle control. Further, within the PLX-R18-treated group, levels of serum amyloid A (a sepsis marker) and procalcitonin (an inflammation marker) remained low throughout the study. Previous studies established that these plasma biomarkers exhibit dose- and time-dependent responses to radiation exposure and can function as early prognostic indicators of ARS severity. When combined with blood cell counts, these markers enable rapid assessment and triage of radiation casualties, particularly valuable for mass-casualty incidents where timely dose estimation is critical for treatment decisions.[p51],[p52] Levels of E-selectin

and sP-Selectin (vascular endothelial injury biomarkers) dropped in the irradiated groups, but recovery was observed in the PLX-R18 group. However, the cytokine response patterns that were induced following irradiation were somewhat in contrast to the patterns noted in a larger study that measured a broad panel of cytokines. In this larger study, irradiation tended to elicit elevated (not suppressed) responses, whereas treatment with the test agent, PLX-R18, appeared to have a modulating effect that was often dampening by nature, i.e., ameliorating the effect of irradiation.[p48] In addition, PLX-R18 has been demonstrated, by qRT-PCR assay, to abrogate the expression of several genes that are involved in autoimmune pathways.[p48]

The interplay between radiation exposure and genes that regulate apoptosis is well recognized.[p53],[p54],[p55] For example, AKT phosphorylation mediates pro-survival and anti-apoptotic events.[p50],[p56] pAKT levels were elevated 4 h after irradiation when the irradiated PlasmaLyte group was compared with the unirradiated PlasmaLyte group, whereas pAKT elevation in the PLX-R18-treated group was comparatively less, implicating the involvement of the AKT pathway in PLX-R18's ability to reduce radiation injury.[p48]

Radiomitigative efficacy in an NHP model

A rhesus macaque study involving 62 animals was conducted with the objective of further characterizing the injury-mitigative effect of PLX-R18 (Table 1).[p49],[p50] NHPs were treated with three different *im* doses (4, 10 and 20 million cells/kg) of PLX-R18 at days 1 and 5 post-irradiation, or with vehicle. NHPs were exposed to total-body radiation at 6 Gy ($LD_{30/45}$) and were monitored for 45 days post-irradiation.[p49],[p50] Although this study was not powered to demonstrate a statistically significant survival difference, all three doses of PLX-R18 appeared to improve survival outcomes in irradiated NHPs relative to untreated controls.[p49],[p50] Despite the limitations presented by small sample size, there was a tendency towards earlier recovery of neutrophil, platelet, hemoglobin, and lymphocyte levels in irradiated groups that were treated with PLX-R18. In irradiated animals, PLX-R18 treatment was also associated with a trend towards a delay in reaching critical periods of blood cytopenias, which were generally less severe and less prolonged than those in animals that were not treated with PLX-R18.[p49] In unirradiated NHPs, no clinical changes were noticed following treatment with PLX-R18. In addition, in all of the treated animals (irradiated or unirradiated), PLX-R18 was cleared from the system (including the *im* injection site) by day 45 post-irradiation.

Safety and toxicity in phase I clinical studies

Pluri has completed a human phase I study (NCT03002519) in adult patients who had prolonged incomplete hematopoietic recovery following either allogeneic or autologous hematopoietic cell transplant to assess the safety of PLX-R18 (Table 2).[p41],[p43],[p46],[p57] The study was designed to include patients who were at least 3 months post-transplant, presenting with persistent thrombocytopenia (≤50,000/μL), and/or anemia (hemoglobin ≤8 g/dL), and/or neutropenia (absolute neutrophil ≤1,000/mm^3), who were otherwise stable and had no other observed cause of cytopenia, such as infection or graft rejection. A dose-escalation (1, 2 and 4 million cells/kg, administered at days 1 and 7) protocol was embedded into the study design to manage potential risks.[p41],[p43],[p46],[p57] Treatment with PLX-R18 was found to be safe and well-tolerated. This agent was clearly effective in some patients.[p43] The most frequently reported treatment emergent adverse effects were transient injection site reactions with mild to moderate severity, and without sequelae. Improvement was observed in the majority of the patients. Among the high-dose group, improvements in hemoglobin, absolute neutrophil count, and platelets were observed. On the basis of these and other data, the US FDA granted orphan drug designation to PLX-R18 cell therapy as a treatment for ARS in 2017.

Biodistribution

Studies were performed to assess the biodistribution of PLX-R18 in both mice (NOD-SCID of both sexes) and NHPs (immunocompetent unirradiated and irradiated). In the murine model, PLX-R18 that was injected *im* remained local to the injection site, and the quantities of PLX-R18 cells that were present fell over the time. At 13 weeks post-injection, very few cells remained in the bodies of the injected mice. In the NHPs, there was no detectable human-origin DNA either in the injection sites (the PLX-R18/vehicle was evenly divided and administered by *im* injection to the four lumbar dose areas or into both hind limbs) or in

TABLE 1

Details of a nonhuman primate (NHP) study of the ability of PLX-R18 to mitigate responses to two different radiation doses[p49]

Group	Number of NHP used	Males/females	Dose of PLX-R18 cells (million)	Radiation dose (Gy)	Numbers of surviving males/females	Survival rate
1	6	3M/3F	Control	Unirradiated	All	100%
2	6	3M/3F	4	Unirradiated	All	100%
3	6	3M/3F	10	Unirradiated	All	100%
4	6	3M/3F	20	Unirradiated	All	100%
5	6	3M/3F	Control	6.0 Gy	2 M/1F	50%
6	6	3M/3F	4	6.0 Gy	3 M/2F	83%
7	7	3M/4F	10	6.0 Gy	3 M/3F	86%
8	6	3M/3F	20	6.0 Gy	2 M/2F	67%
9	3	3M/0F	Control	2.93 Gy	All	100%
10	3	3M/0F	4	2.93 Gy	All	100%
11	3	3M/0F	10	2.93 Gy	All	100%
12	4	3M/1F	20	2.93 Gy	All	100%

TABLE 2

Safety profile of PLX-R18 in auto- and allo-hematopoetic cell transplant patients.

	Total *N* = 21	PLX-R18 1 million cells/kg, *N* = 3	PLX-R18 2 million cells/kg, *N* = 6	PLX-R18 4 million cells/kg, *N* = 12
Demographics				
Age in years, mean (SD)	55.9 (11.9)	49.0 (5.6)	57.0 (3.8)	57.0 (15.1)
Male, *n* (%)	12 (57.1)	2 (66.7)	2 (33.3)	8 (66.7)
BMI, kg/m^2, mean (SD)	29.1 (6.6)	33.5 (7.5)	26.0 (5.1)	29.6 (6.7)
Baseline blood counts				
PLT, $10^3/\mu L$, median (min, max)	30.5 (4.0, 83.25)	24 (4.0, 24.5)	21.8 (12.0, 46.0)	36.3 (11.0, 83.25)
PLT < 10 × $10^3/\mu L$, *n* (%)	1 (4.8)	1 (33.3)	0 (0.0)	0 (0.0)
PLT < 20 × $10^3/\mu L$, *n* (%)	5 (23.8)	1 (33.3)	2 (33.3)	2 (16.7)
HGB, g/dL, median (min, max)	8.8 (6.3, 11.8)	8.7 (8.5, 8.9)	8.3 (6.5, 11.4)	9.1 (6.3, 11.8)
ANC, cells × $10^3/\mu L$, median (min, max)	1.3 (0.2, 3.2)	2.1 (0.9, 3.2)	1.2 (0.8, 1.6)	1.2 (0.2, 1.9)
ANC < 0.5 × $10^3/\mu L$, *n* (%)	3 (14.3)	0 (0.0)	0 (0.0)	3 (25.0)
ANC < 1 × $10^3/\mu L$, *n* (%)	7 (33.3)	1 (33.3)	1 (16.7)	5 (41.7)
Lymphocytes, cells × $10^3/\mu L$, median (min, max)	0.7 (0.1, 2.3)	0.4 (0.3, 1.4)	0.5 (0.1, 1.4)	0.7 (0.3, 2.3)
Disease history: primary diagnosis				
Acute lymphoblastic leukemia (ALL), *n* (%)	7 (33.3)	1 (33.3)	3 (50.0)	3 (25.0)
Acute myelogenous leukemia (AML), *n* (%)	3 (14.3)	1 (33.3)	0 (0.0)	2 (16.7)
Multiple myeloma, *n* (%)	2 (9.5)	0 (0.0)	0 (0.0)	2 (16.7)
Myelodysplastic syndrome (MDS), *n* (%)	2 (9.5)	0 (0.0)	1 (16.7)	1 (8.3)
Non-Hodgkin lymphoma (NHL), *n* (%)	2 (9.5)	1 (33.3)	0 (0.0)	1 (8.3)
Other (malignant), *n* (%)	5 (23.8)	0 (0.0)	2 (33.3)	3 (25.0)
HCT type and cell source				
Allogeneic, *n* (%)	19 (90.5)	2 (66.7)	6 (100.0)	11 (91.7)
Bone marrow, *n* (%)	6 (28.6)	1 (33.3)	1 (16.7)	4 (33.3)
Peripheral blood, *n* (%)	13 (61.9)	1 (33.3)	5 (83.3)	7 (58.3)
Umbilical cord, *n* (%)	2 (9.5)	1 (33.3)	0 (0.0)	1 (8.3)
Adverse events				
Patients with any TEAE, *n* (%)	21 (100.0)	3 (100.0)	6 (100.0)	12 (100.0)
Patients with a serious TEAEs, *n* (%)	15 (71.4)	3 (100.0)	4 (66.7)	8 (66.7)
Patients with TEAEs with fatal outcome, *n* (%)	4 (19.0)	0	2 (33.3)	2 (16.7)
Patients with TEAEs who were related to donor, *n* (%)	16 (76.2)	3 (100.0)	2 (33.3)	11 (91.7)
Patients with TEAEs who were not related to donor, *n* (%)	5 (23.8)	0	4 (66.7)	1 (8.3)

ANC, Absolute neutrophil count; BMI, Body mass index; HCT Hematopoietic cell transplantation; HGB Hemoglobin; PLT, Platelets; SD, Standard deviation; TEAE, Treatment emergent adverse event. Information gathered from McGuirk *et al.*.[(p41)]

un-injected tissues/organs (non-injection site muscle, liver left and right lobes, spleen, inguinal lymph nodes and iliac lymph nodes) on day 45 post-irradiation (<20 copies/μg genomic DNA, which was the lower limit of quantification).[(p43),(p46),(p48)] These results suggest that PLX-R18 cells remain local at the site of injection and are present within the body for a limited period, after which they are cleared from the body entirely.

Conclusions

Although efforts to develop MCMs for acute radiation exposure-related injuries were initiated more than six decades ago, to date, only four agents and their seven biosimilars have been approved by the US FDA for treatment of H-ARS.[(p31),(p32)] All of these agents are defined as radiomitigators for H-ARS, and no agent has been approved for GI-ARS, for the delayed effects of acute radiation exposure, or for late cognitive dysfunctions and other quality-of-life issues following lower and survivable radiation doses. Furthermore, no radioprotector has been approved specifically for prophylaxis of ARS. In addition, these approved agents have some limitations, including the need for repeated injections (except Nplate), side effects, and the use of blood products. Thus, there is an urgent need to continue to develop additional agents for ARS and all of its sub-syndromes.

The positive features of the novel agent PLX-R18 are that it does not require human leukocyte antigen matching, is produced on a cGMP industrial scale and can easily be stored, distributed and administered *im*. After injection, the PLX-R18 cells remain at the injection site, do not migrate to other locations, do not differentiate, and are cleared from the body within several weeks. While in the body, these cells induce a systemic effect through multi-factorial secretion of pro-hematopoietic cytokines. The efficacy of PLX-R18 to mitigate H-ARS has been demonstrated using the well-established preclinical models of mice and NHP. These studies have provided evidence of both decreased mortality of acutely irradiated animals and significantly improved blood profiles in these animals following

treatment with PLX-R18. The stimulation of multiple hematopoietic lineages, coupled with increased rates of survival, were clearly demonstrated in animals following the administration of test agent, PLX-R18. By contrast, and in general support of the agent's favorable safety profile, no change was noted either in blood cell counts/differentials or in survival when treated, irradiated animals were compared with treated, unirradiated animals. Furthermore, this good safety profile was supported and fully documented following the completion of a Phase I clinical study on the safety of PLX-R18 within patients who had incomplete hematopoietic recovery following hematopoietic cell transplantation.[(p46)]

Despite these promising characteristics, implementation of PLX-R18 in mass casualty settings would require consideration of practical limitations, including specialized storage requirements, thawing protocols, and trained personnel for administration. These factors may limit the immediate deployment of PLX-R18 in the field compared more than would be the case for conventional therapeutics. Nevertheless, PLX-R18 has the significant therapeutic potential, as evidenced by comprehensive preclinical and clinical studies that have shown a unique multifactorial mechanism that stimulates all three blood cell lineages. These findings support continued development efforts to address the operational considerations.

The studies presented in this review all used total-body gamma-irradiation, but additional studies with partial-body, linear accelerator X-ray, and mixed field (neutron plus gamma) irradiation are planned. It will be important to investigate the efficacy of PLX-R18 against low as well as high linear energy transfer radiation. Similarly, investigations into its efficacy against partial-body in addition to total-body exposure will be important.

In summary, PLX-R18 has proven to be both efficacious and safe in medically managing ARS. The efficacy of PLX-R18, both as a radioprotector and as a radiomitigator, has been demonstrated by a series of animal studies in both mice and NHP. We believe that this rather unique medical product represents a very promising new class of MCM for ARS, specifically in terms of its efficiency to block or mitigate the adverse effects of acute, potentially fatal, radiation injuries in experimental animals and possibly in humans. We have little doubt that with further advanced clinical work, PLX-R18 will find its way into the medical pharmacological 'toolbox' for the safe and effective treatment of ARS in humans. In addition to ARS, other traditional clinical uses for PLX-R18 may include aplastic anemia, bone marrow failure, and myelofibrosis.

CRediT authorship contribution statement

Vijay K. Singh: Writing – review & editing, Writing – original draft, Conceptualization. **Arthur Machlenkin:** Writing – review & editing, Writing – original draft. **Thomas M. Seed:** Writing – review & editing, Writing – original draft, Conceptualization.

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Declaration of competing interest

AM is an employee and stockholder of Pluri Biotech Ltd. The other authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes current employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Data availability

No data was used for the research described in the article.

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