Expanding the Use of an FDA-Approved Host-Directed Therapy for Known and Unknown Threats: Treating Pancytopenia and Immune Dysregulation Due to Acute Radiation or Sulfur Mustard (HD) Exposure with Leukine® [sargramostim/Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)]

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Abstract

There are no FDA-approved treatments for sulfur mustard (HD)-induced myelosuppression and associated immune dysregulation. Leukine (sargramostim) is FDA-approved to treat the myelosuppressive effects of acute radiation exposure (H-ARS) and is Technology Readiness Level (TRL) 7B for HD. Partner Therapeutics (PTx) is collaborating with the Joint Project Manager for Chemical, Biological, Radiological, and Nuclear Medical (JPM CBRN Medical) Countering Emerging Threats - Rapid Acquisition and Investigation of Drugs for Repurposing (CET RAIDR) program to expand sargramostim's utility to protect warfighters against known and unknown, chemical, biological, radiological, and nuclear (CBRN) threats. Studies are ongoing to generate data to inform DoD Clinical Practice Guidelines and potentially expand the H-ARS label to include treatment of HD-induced myelosuppression.

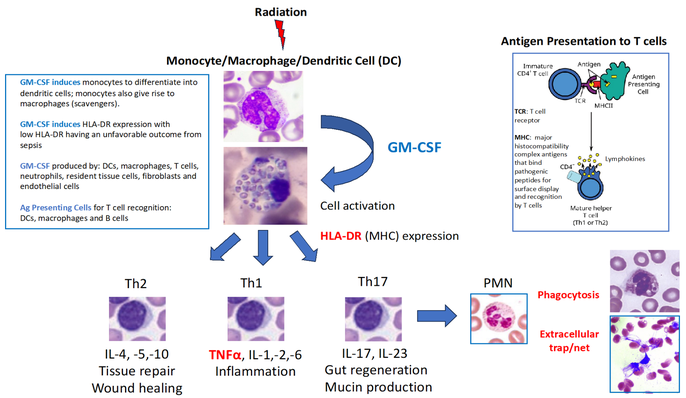
Radiation and Systemic HD Exposure

Exposure to high-dose HD or whole-body ionizing radiation induces dose-dependent damage to the bone marrow (BM), spleen, thymus, lymph nodes and blood cells, resulting in pancytopenia. Both radiation and HD exposure dysregulate host immune response and homeostasis, which culminates in immunosuppression that leads to bacterial, viral, and fungal infections and ultimately sepsis. Significant causes of death among patients with ARS and systemic HD exposures include infection, sepsis, uncontrollable bleeding, and severe acute anemia, all of which contribute to multi-organ dysfunction and failure (MOD/MOF) (Wolbarst 2010, Dainiak 2018, Willems 1989, Rafati-Rahimzadeh 2019).

Sargramostim (Leukine® / rhu GM-CSF)

Sargramostim (rhu GM-CSF) has been shown to play a critical role in host defense, immune homeostasis, and tissue repair. While classically known for promoting differentiation of bone marrow (BM) precursor cells, and stimulating proliferation, differentiation, and maturation of myeloid cells, GM-CSF also plays important roles in antigen presentation, T-cell activation, and myeloid cell survival and function. It also promotes tissue repair, wound healing, and resolution of inflammation. These effects have shown benefit in H-ARS and may have similar benefit in HD exposure. (Rosler 2016, Bernasconi 2010).

Figure 1: GM-CSF is a critical regulator of adaptive immune response



Sargramostim Improves Survival after Radiation Exposure

Sargramostim improved survival after radiation exposures between 655 and 730 cGy (Figure 2) when administered 24 to 96 hours after exposure (Figure 3). This improvement has been shown in three large, GLP studies (LBRI14-405, TSK-0144, 1017-3493) in an NHP-model of TBI with minimal supportive care (no transfusions or targeted antibiotics). Control decedents presented with marked to severe BM hypocellularity and severe atrophy of the spleen, thymus and lymph nodes, and sepsis was identified as a primary probable cause of death in 97% of control decedents (TSK-0144, 1017-3493).

Figure 2: 60-Day Survival 655 to 730 cGy

	Radiation Exposure (cGy)				
	655	670	680	713	730
Control	42% (36)	42% (12)	29% (35)	17% (18)	33% (12)
Leukine (24 Hour)			49% (35)		58% (12)
Leukine (48 Hour)	78% (36)	67% (12)	60% (35)	61% (18)	

Source: LBRI14-405, TSK-0144, 1017-3493

Figure 3: Treatment Benefit up to 96-Hours Post -Exposure (60-Day Survival)

	Control	Leukine 48 hours	Leukine 72 hours	Leukine 96 hours	Leukine 120 hours
% Survival	9%	32%	25%	32%	16%

Source: 1017-3493

Sargramostim Effect on Radiation-Induced Sepsis

In the primary study (TSK-0144) that supported FDA approval for H-ARS, treatment with sargramostim reduced the incidence of sepsis from 63% to 17% (Figure 4) and reduced the frequency and severity of BM hypocellularity (Figure 5) and lymphatic system atrophy (Figure 6) as well as the duration of severe lymphopenia (Figure 7). Absolute lymphocyte count (ALC) nadir with treatment occurred earlier and at a higher level. ALC became severe (<0.50 x10^9/L) by Day 2 and absolute monocyte count (AMC) became severe (<0.10 x10^9/L) by Day 4. ALC and AMC remained suppressed until death for 97% and 94% of decedents (TSK-0144, 1017-3493).

Figure 4: Reduction in Radiation-Induced Sepsis

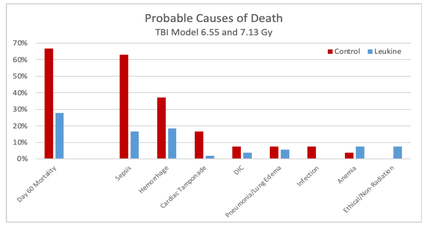


Figure 5: Reduced BM Hypocellularity

Bone Marrow Hypocellularity: Femur & Sternum (Decedents) 655 cGy				
	Leukine Males	Control Males	Leukine Females	Control Females
Severe	1	7	5	11
Marked	0	1	0	0

Bone Marrow Hypocellularity: Femur & Sternum (Decedents) 713 cGy				
	Leukine Males	Control Males	Leukine Females	Control Females
Severe	2	5	2	7
Marked	0	1	1	1

Figure 6: Reduced Thymic/Splenic Atrophy

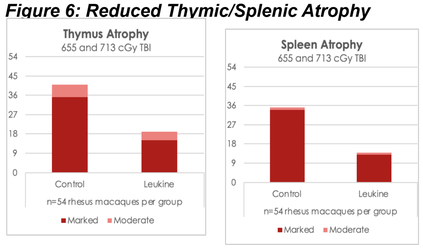
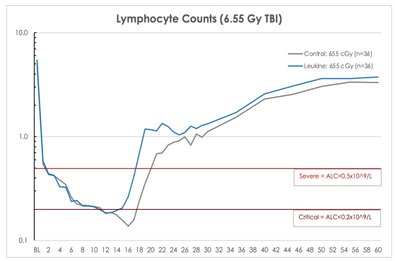


Figure 7: Enhanced Lymphocyte Recovery



Development of NHP Model of Systemic HD Exposure

Under the CET RAIDR program, PTx and Lovelace Biomedical Research Institute (LBRI) developed an NHP model of systemic HD exposure in the cynomolgus macaques (CMIVLD50 model). This model recapitulates the clinical course of systemic toxicity in humans after dermal and inhalational exposures. In humans, initial toxicity is localized to target tissues (eyes, skin, lung). However, HD is rapidly absorbed into the circulation leading to systemic toxicity. HD-induced damage to the BM, lymphoid tissues, and GI tract is due to its high reactivity with rapidly proliferating cells. The resultant leukopenia and lymphopenia lead to infection, sepsis and, in some cases, death. (Sezigen 2020; Willems 1989)

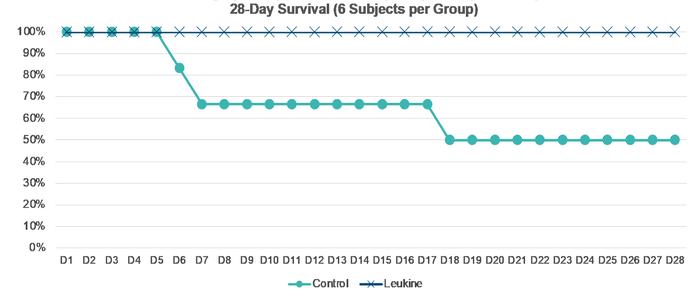
NHP Model of Systemic HD Exposure

In the CMIVLD50 model, animals display signs of soft or liquid stool consistent with diarrhea, reduced appetite, and anorexia as early as the third day after exposure. This is accompanied by a decline in white blood cells. Lymphocytes are the first cell population to decline. Lymphopenia is observed at 24-hours post-exposure, which reaches severe lymphopenia (<0.5 x 10^9/L) by Day 3 after exposure and nadir by Day 7. A decline in monocytes and neutrophils follows reaching severe levels by Day 5 and remaining severe through Day 11 to 14. Mortality is generally observed between Day 5 and 21. Among animals expiring prior to the study endpoint, microscopic exam revealed decreased cellularity of sternal and femoral marrow and prominent abnormalities in the intestines, including loss of crypts and epithelial denudation. Decreased cellularity in the spleen, thymus, lymph nodes and gut-associated lymphoid tissue (GALT) was also observed (LBRI122-106B,C,D).

Evaluation of Sargramostim to Treat HD Myelosuppression

A Proof-of-Concept (PoC) study (LBRI22-106B) was conducted in the CMIVLD50 model with an intravenous (IV) HD exposure of 0.75 mg/kg ($LD50_{28}$) to investigate the potential benefit of sargramostim in mitigating HD-induced mortality and injury for 28 days. Twelve male and female NHPs were divided equally into two groups and received either sargramostim (6.1 µg/kg) or sterile water for injection (control) subcutaneously once daily for 14 days beginning on Day 1 post HD exposure. Endpoints for this study included morbidity/mortality, body weights, hematology, clinical chemistry, pathology, and histopathology.

Figure 8: Increased Survival after HD Exposure



Day 28 control group survival was 3 of 6 (50%), compared to 6 of 6 (100%) for the sargramostim group (Figure 8). ALC decreased markedly in all animals on Day 1 and remained below baseline. Sargramostim treatment resulted in earlier and higher ALC nadir and reduced duration of moderate and severe lymphopenia. Severe leukopenia was observed on Day 7 with nadir on Day 7 for the sargramostim group and Day 9 for the control group. Observed systemic toxic effects were similar to descriptions of human HD exposures. Results from a confirmatory study are expected by the end of 2024.

Conclusions

In conclusion, BM and lymphatic damage, and immune suppression are strongly associated with mortality after radiation and HD exposure. In a PoC study, sargramostim treatment increased survival and generated signals of benefit on key measures of HD systemic toxicity in an NHP model generating effects similar to descriptions of human HD exposures.

Expanding CPGs and Leukine's label to include HD exposure will enhance warfighter protection by addressing the primary driver of prolonged hospitalization and mortality for a threat with no approved treatments.

References available, contact: John.McManus@partnertx.com

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COI: JM, IJ & SA are employed by and have stock options for Partner Therapeutics, Inc. (PTx). MD-E is employed by LBRI, the primary subcontractor for PTx's HD sulfur mustard contract. ILJ and MH are paid consultants for PTx.