Expanding the Utility of Leukine®, an FDA-Approved Treatment for Hematopoietic Acute Radiation Syndrome (H-ARS), as a Medical Countermeasure (MCM) to Counter Higher Priority and Emerging Chemical, Biological and Radio-Nuclear Threats

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Leukine (sargramostim / yeast-derived rhu GM-CSF)

Host-Based Therapeutic and Context-Dependent Immunomodulator

Current Key Leukine Development Programs

Pulmonary / Critical Care

Indication	Timing
COVID-19**	2022
Peripheral Artery Disease	2023
Pulmonary Alveolar Proteinosis (aPAP)*	2024
Sulfur Mustard Exposure*	2024
Hospitalized Influenza*	2026
High Risk Bacterial Threats*	2026
Adult Sepsis*	2027
Pediatric Sepsis (MODS)*	2027

Neurodegeneration

Indication	Timing
Amyotrophic Lateral Sclerosis	2025
Parkinson's Disease*	2026
Alzheimer's Disease	2026
Down Syndrome	2027

Immuno-Oncology

Indication	Timing
Refractory Melanoma	2025
Front-line Melanoma*	2026
NSC Lung Cancer	2026
Renal Cell Carcinoma	2027

MODS – Multi-Organ Dysfunction Syndrome

* Signifies approval opportunity
** Signifies EUA opportunity

Acute Radiation Syndrome: Efficacy/Mechanism Provide Rationale for Sulfur Mustard

Leukine Improves Survival by Accelerating Hematologic Recovery/Immune Response

60-Day Survival after Total Body Irradiation

	Clayton (6.55 Gy)	TSK-0143 (6.70 Gy)	LRRI 14-045 (6.80 Gy)	Clayton (7.13 Gy)	Zhong (7.13 Gy)	LRRI 1302 (7.30 Gy)
Control	42%	42%	29%	17%	15%	33%
Leukine 96 Hour					36%	
Leukine 72 Hour					27%	
Leukine 48 Hour	82%	67%	60%	69%	35%	
Leukine 24 Hour			49%			58%
NHP per arm	*36**	*12***	*35*	*18**	*44**	*12*

**Excludes animals euthanized for "ethical concerns"* ***Study duration was 45 days*

NHP Study 14-045 (105 Animals)

- **Primary cause of death: toxemia and/or septicemia**
- Hemorrhage and bacteria across tissues and necrosis of tissues associated with bacteria in decedents
- Hemorrhage from thrombocytopenia, but it was likely contributory rather than primary
- Survivors did not demonstrate gross or histologic evidence of a prior septicemic episode

"Clayton" NHP Study (108 Animals)

		Incidence of:				
	Mortality*	Sepsis	Hemorrhage	Tamponade	DIC	Infection
LD50						
Control	58%	53%	25%	14%	8%	3%
Leukine	18%	14%	17%	3%	3%	3%
LD70						
Control	83%	83%	50%	67%	6%	6%
Leukine	31%	22%	22%	0%	6%	0%

*3 animals euthanized for ethical concerns and 1 animal found dead from hematoma excluded

- **Primary cause of death: Sepsis (95% of 47* decedents)**
- Hemorrhage, cardiac and pulmonary issues co-factors in mortality
- Severe, widespread lymphatic system atrophy and GI damage; bone marrow hypocellularity in >90% of deceased control animals
- Terminal hematology consistent with sepsis and multi-organ damage from improper oxygenation
- 45 of the 47* deaths (96%) occurred between day 14 and 21

Sources: Doyle-Eisele M, Study 14-045 Final Report (2015); Clayton NP, et al, Rad Res 195, 191-199 (2021); Zhong Y, et al. Int J Radiat Biol. 2020:1-17; Sanofi communications; Final Study Report TSK-0143; Final Study Report TSK-0144, Final Study Report 1017-3493. Funding: Office of the Assistant Secretary for Preparedness and Response (ASPR), Biomedical Advanced Research and Development Authority (BARDA), Contract# HHSO1002013000051

Hematological Effects of Sulfur Mustard (HD) Exposure

Bone Marrow Suppression and Lymphatic Damage Drives Pancytopenia

Clinical management of mustard gas casualties

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170 HD Casualties evacuated to European hospitals during 1984-1986

Clinical files of 65 patients were analyzed

Hospitalization occurred between day 4 to 17 post–exposure

Leukopenia occurred in 29 of 65 HD casualties

9 of the other 65 HD casualties had **lymphopenia**

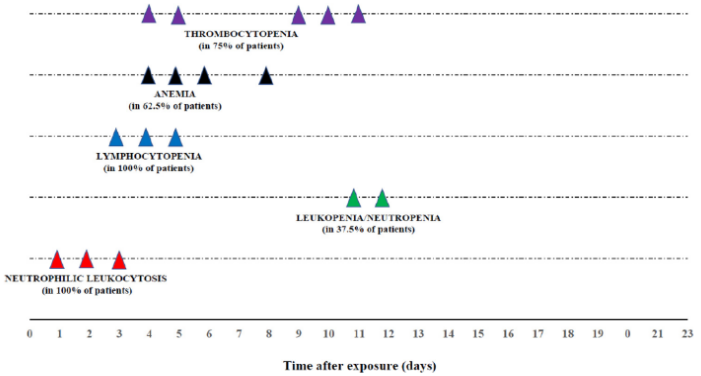
9 patients died – 7 of 9 had severe **Leukopenia** (WBC < 200/ml)

High frequency of septicemia

It is of interest that older observations report that leukocyte counts started to drop on the third to fourth day after exposure (Alexander, 1947) and that a minimum was obtained at 9 days (Krumbhaar, 1919; Krumbhaar and Krumbhaar, 1919). Alexander (1947) further states that cell counts of 100/μ1 or below were recorded and that all patients with extremely low leukocyte counts died. In our series also, all patients who had minimum counts of 200 cell/μl or less died.

"Mustard acts at different sites in the body as a radio-mimetic. The resulting symptoms are headache, nausea, vomiting, anorexia, epigastric pain, **leukopenia, thrombocytopenia and anemia**."

Onset of Pancytopenia after HD Exposure



Pancytopenia:	severity increases with exposure
Leukopenia:	**serious** at highest exposure
Lymphopenia:	**severe** in all patients
Neutropenia:	mild to none
Thrombocytopenia:	moderate at highest exposure
Anemia:	moderate at highest exposure

Myelosuppression and acute hematological complications of sulfur mustard exposure in victims of chemical terrorism

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University of Health Sciences, Dept. of Medical CBRN Defense, Etlik, 06010, Ankara, Turkey

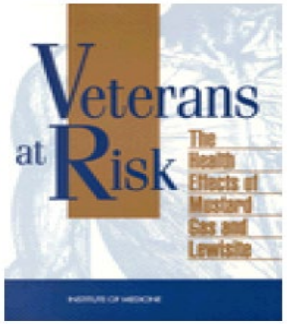
rhu GM-CSF Utility in Sulfur Mustard ("HD") Exposure

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH | Center for Health Security

Sulfur Mustard (Blister Agent)

Fact Sheet

There is no broadly recognized treatment algorithm for SM-exposed patients.[10] Medical management should prioritize rapid decontamination with copious amounts of soap and water, followed by supportive care that may include fluids, respiratory support, analgesia, infection prevention, and basic burn dressings. Larger blisters (> 2cm) may heal faster if lanced and debrided. Fluid from the blister does not pose a threat to healthcare workers.[11] Administration of sodium thiosulfate or GM-CSF has also been suggested.[11,14]



emea

The European Agency for the Evaluation of Medicinal Products
Pre-authorisation Evaluation of Medicines for Human Use

London, 25 April 2003
EMEA/CPMP/1255/03

EMEA/CPMP Guidance Document on the Use of Medicinal Products for the Treatment of Patients Exposed to Terrorist Attacks with Chemical Agents

Systemic toxicity

Severe bone marrow depression may require treatment with colony stimulating factors (e.g. granulocyte monocyte-colony stimulating factor GM-CSF). Advice from a consultant haematologist is considered essential.

COMPREHENSIVE REVIEW

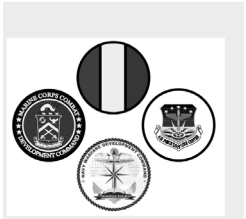
Mustard Gas or Sulfur Mustard: An Old Chemical Agent as a New Terrorist Threat

Monica Wattana, MD, BSc; Tareg Bey, MD, FACEP

Systemic

Oral antibiotics should be considered to sterilize the gastro intestinal tract and protect damaged intestinal mucosa in patients with cell counts <200 cells/mm^3. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) should be also considered to counteract severe sulfur mustard-induced leucopenia.[19]

ARMY, MARINE CORPS, NAVY, AIR FORCE



MULTISERVICE TACTICS, TECHNIQUES, AND PROCEDURES FOR TREATMENT OF CHEMICAL AGENT CASUALTIES AND CONVENTIONAL MILITARY CHEMICAL INJURIES

FM 4-02.285 (FM 8-285)
MCRP 4-11.1A
NTRP 4-02.22
AFTTP (I) 3-2.69

MILITARY MEDICINE, 158, 7:470, 1993

Granulocyte Colony Stimulating Factor (G-CSF) for Mustard-Induced Bone Marrow Suppression

*Barry R. Meisenberg, MD**
CAPT Anthony J. Melaragno, MC USN†
Rodney L. Monroy, MD‡

Leukine for HD Hematopoietic and Immune Effects

FDA Approved for Adults and Pediatrics/Already Held by SNS for ARS

- ✓ Human Clinical Safety - Completed
  - Demonstrated in over 500,000 patients
  - Safe for use in pediatric and elderly populations
- ✓ Manufacturing/CMC - Completed
  - Commercial Product Currently Held by SNS for H-ARS
  - Cost of treatment expected to be approximately $775 per patient
  - Stability: 48 months refrigerated; 12 months room temperature; 3 months accelerated[(1)]
- Animal Efficacy – In Progress / to be Completed under Contract
  - NHP model of hematopoietic effects of systemic HD exposure
  - Extensive data in ARS demonstrates Leukine efficacy in accelerating recovery from pancytopenia and provides dose and regimen
  - Clinical data demonstrating Leukine's ability accelerate hematopoietic recovery after treatment with mustard-derived chemotherapy (cyclophosphamide) supportive [(1)]
  - Proof-of-concept study in NHP should provide basis for Special Protocol Assessment with FDA to discuss design/requirements for expansion of the Leukine label under the "Animal Rule"

[(1)] Jones SE, Shottstaedt MW, Lewis DA, Kirby RL, Good RH, Mennel RG, George TK, Snyder DA, Watkins DL, Denham CA, Hayes FA, Rubin AS: Randomized Double-Blind Prospective Trial to Evaluate the Effects of Sargramostim Versus Placebo in a Moderate-Dose Fluorouracil, Doxorubicin, and Cyclophosphamide Adjuvant Chemotherapy Program for Stage II and III Breast Cancer (1996). Lazarus HM, Ragsdale CE, Gale RP, Lyman GH: Sargramostim (rhu GM-CSF) as a Cancer Therapy (Systematic Review) and an Immunomodulator. A Drug Before Its Time? 2021.

Sulfur Mustard-Induced Myelosuppression NHP Model

- Cynomolgus macaques
- 1.0 mg/kg HD IV exposure
- 28-Day In-life
- Primary expected effects:
  - Leukopenia
  - Lymphopenia
  - Neutropenia
  - Monocytopenia
  - Eosinopenia
  - Thrombocytopenia
  - Bone marrow cellularity
  - Lymphatic system damage

Observed Myelosuppression (Nadir – Cells x $10^3/\mu L$)	Pilot #2+	Anderson	Meisenberg*	Sezigen	H-ARS
Leukocytes	0.81	~1.00	NA	0.56	0.22
Lymphocytes	0.17	0.46	0.35	0.15	0.17
Monocytes	0.03	NA	NA	NA	0.00
Neutrophils	0.59	0.14	0.12	0.37	0.02
Eosinophils	0.00	NA	NA	NA	0.00
Basophils	0.01	NA	NA	NA	0.00
Platelets	163	32	NA	20	6
Reticulocytes	9	NA	<1	NA	5
Erythrocytes	6.90*	4.25	NA	NA	3.48
HD Exposure Level (Effective Dose in mg/kg)					
HD Exposure	1.0	1.0	1.5	Unknown	NA
Species	CM	AGM	RM	Human	RM

**Nitrogen mustard (mechlorethamine) was used in this study; CM = cynomolgus macaques; AGM = African green monkey; RM = rhesus macaques; +Current Pilot data is through Day 9*

Sources: Personal Communication; Anderson D, Sulfur Mustard-Induced Neutropenia: Treatment with G-CSF, Military Medicine 2006; Meisenberg B, G-CSF for Mustard-Induced Bone Marrow Suppression, Military Medicine 1993; Sezigen S, Myelosuppression and acute hematological complications of sulfur mustard exposure in victims of chemical terrorism, Toxicological Letters 2020; Doyle-Eisele M, Study 14-045 Final Report (2015)

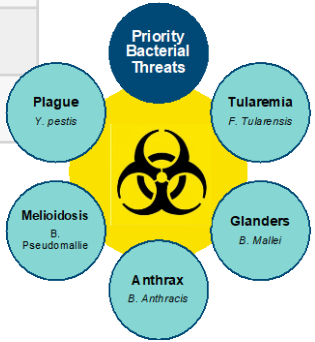
Leukine Sulfur Mustard-Induced Myelosuppression Program
Key Milestones

Program Element	Targeted Completion	Comments
Pilot Study Completion	Completed	Model confirmed
Proof-of-Concept Study	February 2023	Ready to initiate
Clinical Practice Guidelines	2023	Pending PoC results
Adequate & Well Controlled Study	Q4-23 / Q1-24	Pending FDA concurrence
sBLA	2024	

Funded, in part, by the US Department of Defense's (DoD) Joint Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense (JPEO-CBRND) under Agreement W911SR2290003

Leukine Potential as a Broad-Spectrum MCM against USG Priority Threats

Threat	Cellular Effects	System Effects	Acute Outcomes	Morbidity / Mortality
Chlorine	Mucosal damage Epithelial tissue damage	Immune dysregulation Hypoxia / pneumonitis	Opportunistic infection Acute Lung Injury	Persistent infection + sepsis / ARDS
Sulfur Mustard	Leukopenia Epithelial tissue damage	Bone marrow suppression Immune dysregulation	Opportunistic infection Acute Lung Injury	Systemic infection + sepsis / ARDS
Burns/Wounds	Blistering Epithelial tissue damage	Skin sloughing Barrier integrity loss	Opportunistic infection Poor wound healing	Systemic infection + sepsis
Acute Radiation Syndrome	Pancytopenia Epithelial tissue damage	Bone marrow suppression Immune dysregulation	Opportunistic infection Poor wound healing	Systemic infection + sepsis / ALI / ARDS
Priority Bacterial Threats	Immune dysregulation Epithelial tissue damage	Pneumonia Disruption of gut flora	Opportunistic infection Acute GI & lung Injury	Persistent infection + sepsis / ARDS
Influenza	Immune dysregulation Lung epithelial damage	Pneumonia Alveolar macrophage loss	Opportunistic infection Hypoxia	Systemic infection + sepsis / ALI / ARDS
SARS-CoV-2/Threat X	Immune dysregulation Lung epithelial damage	Alveolar macrophage loss Barrier integrity loss	Opportunistic infection Hypoxia	Systemic infection + sepsis / ALI / ARDS
Viral Hemorrhagic Fever	Immune dysregulation Epithelial damage	Alveolar macrophage loss Barrier integrity loss	Opportunistic infection Acute GI & Lung Injury	Systemic infection + sepsis / ARDS



Adapted from ASPR/BARDA presentation on Anti-microbial resistance – Fall 2021

Leukine in Critical Care: Immunoparalysis

Leukine helps overcome immunoparalysis and systemic inflammation by normalizing innate immune function and restoring proper monocyte function

- Improves innate immune cell function and numbers
- Immunoparalysis seen in >40% of severe sepsis patients

POC data summary:

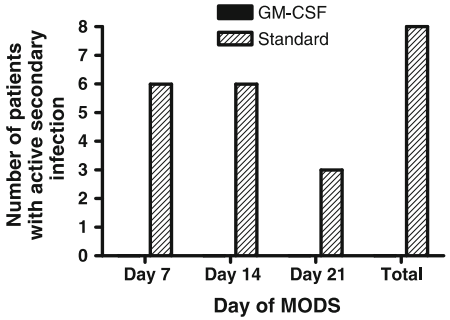
Survival outcomes from clinical trials of rhu GM-CSF in sepsis (RR <1 favors use of rhu GM-CSF)

	Risk ratio (95% CI)
Meisel 2009	0.75 (0.19, 2.91)
Orozco 2006	1.07 (0.16, 7.10)
Paine 2012	0.76 90.38, 1.52)
Pinder 2018	0.82 (0.28, 2.45)
Presneill 2002	0.80 (0.14, 4.49)
Rosenbloom 2005	0.56 (0.19, 1.61)
TOTAL	0.74 (0.47, 1.16)

Development plan includes two phase 3 clinical studies:

- PRECISE Pediatric Sepsis with MODS study enrolling 1,000 patients who will be sorted by TNF-α response level; 400 patients expected to be enrolled in the GRACE-2 sub-study comparing Leukine treatment to SoC
- 3,758 patient Sep-TIC study sorting patients by immuno-paralysis state w/ 1300 expected in Leukine vs. SoC arm

Immunoparalysis and Nosocomial Infection in Children with Multiple Organ Dysfunction



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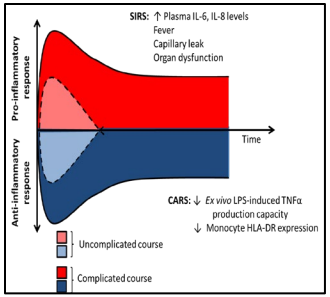
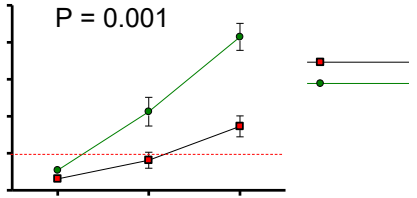


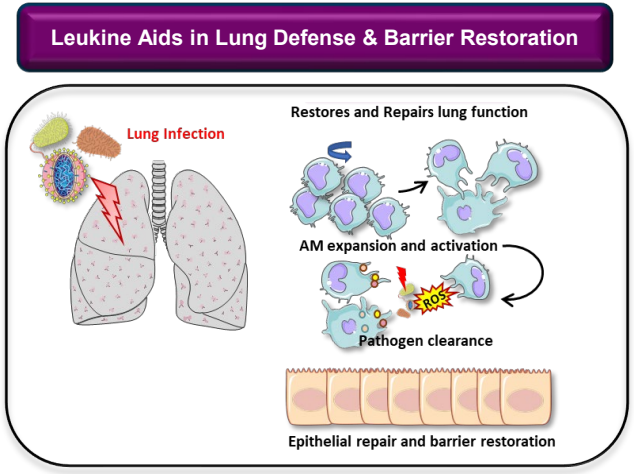


- There were no nosocomial infections in the rhu GM-CSF treated group (n=7)
- ***All*** patients in the standard therapy group developed secondary infection (n-8); P value <0.05

Hall M, et al., Immunoparalysis in Pediatric Critical Care; Pediatr Clin North Am. 2017 October ; 64(5): 1089–1102.
Hall, MJ, et. al., Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome, Intensive Care Medicine, 2011.

Acute Respiratory Distress Syndrome

- Leukine demonstrated improvement in lung function in two phase 2 trials in hospitalized COVID-19 patients with acute hypoxia
- SARPAC, an 81 patient randomized controlled multi-center study in Belgium demonstrated a significant (p = .014) improvement in patients that had at least a 33% improvement in oxygenation as measured by P(A-a)O2 ratio vs. control (SoC) at day six
- iLeukPulm, a 122 patient randomized, controlled multicenter study in the US demonstrated a significant improvement (p = .0333) in oxygenation as measured by P(A-a)O2 ratio vs. control (SoC) at day six
- Across both studies Leukine was shown to be safe with no tolerability issues via inhaled (mesh nebulizer) administration
- Patients on the Leukine arm also experienced a reduction in pro-inflammatory cytokines; increases in HLA-DR+ CD38+ effector memory CD8 T cells; switched memory B cell formation; and improvement in key marker of fibrosis (KL-6) in the lung



SARPAC Primary Endpoint	Leukine+SoC	SoC
n	35	38
33% change baseline n (%)	19 (54.3)	10 (26.3)
	P Value = 0.0147	

iLeukPulm Co-Primary Endpoint	Leukine+SoC	SOC
n	63	33
P(A-a)O2 Change from Baseline	-102.3 (19.4)	-30.5 (26.9)
	P Value = 0.033	

Sources: Huang F-H, et al. Am J Resp Care. 2011; Sever-Chroneos Z, et al. Antiviral Res. 2011; Subramaniam R, et al. JID. 2014; Cakarova L, et al. AM J Resp Crit Care Med. 2009; Paine R, et al. AM J Physiology. 2003. Bart Lambrecht, MD, PhD - Ghent University Hospital; Data on File.
Note: AM, alveolar macrophage; ROS, reactive oxygen species.

Leukine – a Broad Spectrum Medical Countermeasure

Program	Status	Partner
Hematopoietic Acute Radiation Syndrome	FDA Approved	BARDA
COVID-19 hospitalized patients (ARDS)	End of Phase II	DoD JPEO-CBRND
COVID-19 non-hospitalized patients	In Phase II/III	DoD JPEO-CBRND
Sulfur Mustard Exposure	NHP Study	DoD JPM-CBRNM RAIDR
Sepsis-Induced Immunoparalysis	In Phase II/III	NIH (US) and NIHR (UK)
Radiation Combined Injury	Murine Studies	NIH and AFFRI
Pneumonic Plague	NHP Studies	Pending
Ebola Airway Inflammatory Response	NHP Studies	Pending
Acute Respiratory Distress Syndrome	Phase II	Pending
Influenza (Acute Lung Injury)	Phase II/III	Pending
Wound Healing	Phase II	Planned
Trauma / Traumatic Brain Injury	Phase II	Planned
Mycotoxin / Fungal Infection	Phase II	Planned
Antimicrobial Resistant Bacterial Infection	Phase II	Planned

Acknowledgements

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- Studies supporting Leukine's FDA-approval as a MCM to treat Acute Radiation Syndrome were funded by the Office of the Assistant Secretary for Preparedness and Response (ASPR), Biomedical Advanced Research and Development Authority (BARDA), under contract number HHSO1002013000051.
- Melanie Doyle-Eisele, PhD, Waylon Weber, PhD, Danielle Adney, PhD, DVM, and Andrew Nelson, DVM of Lovelace Biomedical Research Institute (LBRI) contributed to the design of the planned sulfur mustard gas studies.
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Conflicts: *Ila Joshi and John McManus are PTx employees and have received salary and stock options from the Company; Melanie Doyle-Eisele and Danielle Adney are employees of LBRI, and LBRI is the primary subcontractor for PTx's HD sulfur mustard contract.*