EXPERT OPINION

Biologics as countermeasures for acute radiation syndrome: where are we now?

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Despite significant scientific advances toward the development of a safe, non-toxic and effective radiation countermeasure for acute radiation syndrome (ARS) over the past six decades, no drug has been approved by the US FDA. Several biologics are currently under development as radiation countermeasures for ARS, of which three have received FDA Investigational New Drug (IND) status for clinical investigation. Presently, two of these agents, entolimod (CBLB502) and HemaMax (recombinant human IL-12) are progressing with large animal studies and clinical trials. Neupogen (G-CSF, filgrastim) has recently been recommended for approval by an FDA Advisory Committee. Filgrastim, GM-CSF (Leukine, sargramostim), and PEGylated G-CSF (Neulasta) have high potential and well-documented therapeutic effects in countering myelosuppression and may receive full licensing approval by the FDA in the future. The former two biologics are available in the US Strategic National Stockpile (SNS) for use in the event of nuclear or radiological emergency. The Emergency Use Authorization (EAU) application for entolimod may be filed soon with the FDA. Biologics are attractive agents that are progressing along the path for FDA approval, to fill the unmet need for ARS countermeasures.

Keywords: biologics, countermeasures, mice, nonhuman primates, radiation

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1. Introduction

Human acute radiation syndrome (ARS) follows intense, acute whole-body or significant partial-body radiation, of doses > 1 Gy, delivered at relatively high rates. Clinical components of ARS include the hematopoietic sub-syndrome (H-ARS, 2 – 6 Gy), gastrointestinal sub-syndrome (GIS; 6 – 8 Gy) and the cerebrovascular (> 8 Gy) sub-syndrome [1]. Dividing ARS into these 'sub-syndromes' oversimplifies the clinical reality of ARS as it often involves complex, concurrent and multi-organ dysfunctions. Cerebrovascular sub-syndrome is considered incurable, whereas H-ARS alone or in combination with GIS, are more likely to be amenable to countermeasures; therefore, the later two sub-syndromes are specific targets for the development of novel medical countermeasures (MCM). There are several biologics at different developmental stages to be considered as MCM for ARS (**Figure 1**, **Tables 1** and **2**) [2]. A brief description and current status of promising biologics are provided in this article.

2. Regulatory issues of biologics approval for use as ARS countermeasures

Biologics are evaluated for marketing by the FDA through the filing of a Biologic License Application (BLA), the equivalent to New Drug Application for other

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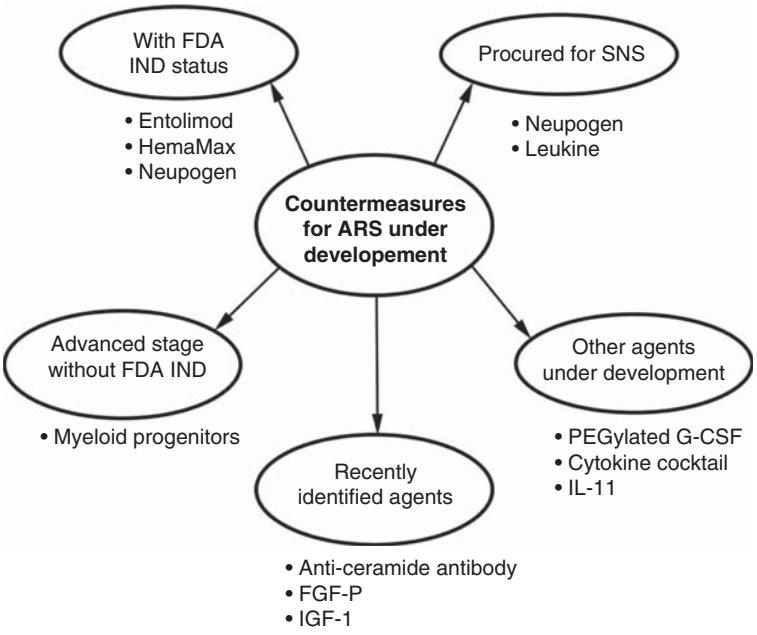

Figure 1. Schematic representation of the biological agents as radiation countermeasures under development. Currently, there are three agents with FDA IND status: entolimod, HemaMax and Neupogen. Neupogen and Leukine have been procured for SNS availability and are expected to obtain FDA EUA in the near future. PEGylated G-CSF is not currently stocked in the SNS but may also obtain FDA EUA approval once filgrastim is approved. Additional countermeasure candidates, at various developmental stages, are presented.

ARS: Acute radiation syndrome; EUA: Emergency Use Authorization; FGF: Fibroblast growth factor; IND: Investigational new drug; SNS: Strategic National Stockpile.

agents. The Public Health Services Act authorizes the FDA to regulate biologics. The agents discussed here qualify for consideration by the FDA under BLA for approval.

Since conventional human clinical efficacy trials for ARS MCM are not possible due to ethical reasons, these trials are substituted with Animal Efficacy Rule, a very stringent and possibly more difficult FDA approval pathway. The criteria required to move through this approval process include: well-characterized animal model(s) that is predictive of human response, a good understanding of the mechanism of action of radiation injury and that of the MCM, study end point focused on prevention of mortality or major morbidity, as well as the good understanding of pharmacodynamics so that the effective human MCM doses can be determined. This pathway relies heavily on a large animal model for preclinical safety and efficacy studies [3].

The majority of agents at advanced stages of development have received FDA 'fast track' and 'orphan drug' statuses [2]. The FDA fast track approval process is designed to facilitate development and expedite the review and approval processes for new treatments of serious or life-threatening conditions. Radiation MCM for ARS are considered emergency need drugs.

The US Emergency Use Authorization (EUA) program, established by Project Bioshield, is a critical tool for the medical and public health communities. It permits the FDA to approve the emergency, off-label use of products approved for other indications or the use of drugs, devices and medical products that currently have no prior approval, clearance or licensing by FDA. It is applicable to both civilian and military use, and it fills the need for timely medical treatment in emergency situations. The Strategic National Stockpile (SNS) program ensures that such agents are appropriately prepositioned so that they are readily available and easily accessible for state and local public health agency distribution in the event of a national emergency.

3. Biologics at advanced stages of development as radiation countermeasures

As stated above, there are several biologics under development as radiation MCM for ARS (**Tables 1** and **2**, **Figure 1**). Neupogen and two others, entolimod and HemaMax, have considerable efficacy and safety profiles and have received FDA Investigational New Drug status for clinical investigation. Mechanistic studies have suggested that the various countermeasures for ARS have different modes of action (**Figure 2**).

3.1 CSF

CSF have high potential and well-documented therapeutic efficacy in countering myelosuppression and may receive full licensing approval from the FDA in the future. Filgrastim,

Table 1. Biologics with US FDA IND/procured for SNS/close to FDA approval.

Countermeasures	Mode of action	Efficacy in animal model of radiation injury	Clinical remarks	Remarks	Ref.
Entolimod/CBLB502/ truncated flagellin	NF-κB activator, stimulates G-CSF and IL-6, immunomodulator, free radical scavenger	Radioprotective and radiomitigative efficacy in murine and NHP models	Safe	IND, Cleveland BioLabs, Inc. preparing pre-EAU application	[6-8]
HemaMax/NMIL12-1/ rhuIL-12	T-cell-activating factor, promotes Th1 maturation, possibly NF-κB activator	Effective in mice and NHPs, promotes hematopoietic and GI recovery as a protector and mitigator	Safe, well tolerated	IND, appears promising	[9,10]
Neupogen/filgrastim/ G-CSF	Stimulates proliferation, differentiation, maturation and function of neutrophils	Effective in murine, canines, swine (minipigs) and NHP models as a mitigator	Approved for other indications, used off-label for radiation-accident victims	IND, available in SNS, procured under PAHPRA	[4,5,20]
Leukine/sargramostim/ GM-CSF	Promotes differentiation, maturation and activation of granulocytes, monocytes and macrophages	Effective in murine, canines and NHP models of radiation injury as a mitigator	Approved for other indications, used off-label for radiation-accident victims	Available in SNS, procured under PAHPRA	[4,5]
Neulasta/PEGylated filgrastim/PEGylated G-CSF	Stimulates proliferation, differentiation, maturation and function of neutrophils	Effective in murine and NHP models, better mitigating efficacy than filgrastim in NHP survival study	Safe, longer half-life than filgrastim, used off-label for radiation-accident victims	Slow-acting, needs fewer injections, NHP efficacy study data submitted to study sponsor for FDA approval	[4,5,19]

GI: Gastrointestinal; IND: Investigational new drug; NHP: Nonhuman primate; PAHPRA: Pandemic and All Hazards Preparedness Reauthorization Act; rhuIL-12: Recombinant human IL-12; SNS: Strategic National Stockpile.

sargramostim and PEGylated filgrastim have already been used off-label for treating radiation accident victims [4]. Currently, there are four recombinant leukocyte growth factors with BLA for related indications: BLA 103353 (Neupogen), BLA 125031 (Neulasta), BLA 103362 (Leukine) and BLA 125294 (TBO-filgrastim) [4]. G-CSF/filgrastim has completed a good laboratory practice compliant study in a nonhuman primate (NHP) model. The efficacy of these agents in various animal models has been recently reviewed [4]. The use of growth factors to treat radiation-exposed victims has been rationalized based on three facts: i) a large clinical database documenting consistent efficacy in mitigating chemotherapy-induced myelosuppression and that associated with stem-cell transplant conditioning regimens as well as consistent safety profile; ii) enhanced recovery from radiation-induced myelosuppression in four animal species and improved survival in sublethal and lethally irradiated animal models; and iii) demonstration of effective granulopoietic activity in a number of radiation-accident victims. Additionally, the American Society of Clinical Oncology extended their recommendation for use of recombinant human G-CSF (rhu G-CSF) and PEGylated rhu G-CSF to treat patients exposed to therapeutic doses of total-body radiation.

Radiation-accident reports show that CSFs have been used to treat the victims of 16 radiological and nuclear accidents with observed benefits [4]. In three accidents CSFs were used within 48 h of accidents (Tokai-Mura, Soreq and Nesvizh), but in others CSF administration was initiated weeks after the incidence. The limited and anecdotal clinical data available regarding these growth factors validate their biological response; however, the variable and delayed manner in which these agents were administered makes the CSF's role in recovery difficult to determine concisely.

During a recently conducted FDA meeting, members overwhelmingly voted (17:1) to support that filgrastim will produce clinical benefits to humans who have been exposed to radiation with doses capable of inducing myelosuppression [5]. The one committee member, who voted 'No', concluded that those who survive a radiological or nuclear incident will most likely not have received a radiation dose high enough to produce myelosuppressive effects and therefore would not benefit G-CSF administration.

3.2 Entolimod

Entolimod is a truncated derivative of the *Salmonella* bacteria flagellin. Its pharmacological action is based on its binding to Toll-like receptor 5 and the activation of NF-κB signaling [6]. Studies conducted with entolimod using rodent and NHP (good laboratory practice) models suggest that it will be a highly promising treatment for lethally irradiated humans,

Table 2. Promising biologics under development as radiation countermeasures for ARS.

Countermeasures	Mode of action	Efficacy in animal model of radiation injury	Clinical remarks	Remarks	Ref.
Myeloid progenitors	Bridging therapy, stimulates myeloid, erythroid and dendritic cell development	Effective against supralethal doses and efficacious when administered as late as 7 days post-irradiation in mice	CLT-008 (cells of human origin), appears to be safe	CLT-008 under Phase I trial in patients undergoing transplant for hematological malignancies	[11,12]
Anti-ceramide antibody	Reduces apoptosis, increases crypt survival and GI recovery	Efficacious in GIS of murine model	Not used in clinical study	Effective against supralethal doses of radiation causing GIS	[13]
FGF-2/FGF-P	Reduces apoptosis, increases cell proliferation and crypt survival	Effective as radioprotector and radiomitigator in murine model	FGF-2 has been reported as safe	Also holds promise for thermal burns, ischemic wound healing, tissue engineering, and stem-cell regeneration	[14]
IGF-1	Decreases apoptosis and promotes hematopoietic progenitor cell survival	Accelerates hematopoietic recovery in mice	Safe	Mitigates radiation-induced H-ARS through protecting hematopoietic stem and progenitor cells	[15]
Cytokines	Tissue protective, anti-apoptotic, anti-inflammatory	Most of the cytokines have been investigated in murine and NHP models, also used as 4F and SFT3 cytokine cocktails	Overall safe with some side effects	Majority of these cytokines have been used off-label for treating radiological accident victims	[16-18]

We are unable to cite all relevant references because of limitation of total number of references for this article.
ARS: Acute radiation syndrome; FGF: Fibroblast growth factor; FGF-P: Fibroblast growth factor-derived peptide; GI: Gastrointestinal; GIS: Gastrointestinal sub-syndrome; NHP: Nonhuman primate.

due to its ability to efficiently ameliorate H-ARS and GIS, as well as having an extended therapeutic time window after radiation exposure [6-8]. Entolimod is currently in clinical development; a human safety study indicated that it was well tolerated and the biomarker results correspond to data from animal models. Cleveland BioLabs, Inc. (Buffalo, NY, USA) is preparing a pre-EUA application for entolimod.

3.3 HemaMax

HemaMax is recombinant human IL-12 (rhuIL-12) cytokine and has been shown to increase mice survival when a single dose was administered, either 24 h before or within 1 h after total-body irradiation. Currently, rhuIL-12 is being developed as a radiomitigator by Neumedicines Inc. (Pasadena, CA, USA) under the name HemaMax. Allometrically equivalent doses of mouse and human HemaMax had similar pharmacokinetics and significantly increased mouse and NHP survival, when administered 24 h post-irradiation, even when no antibiotics, fluids or blood products were administered [9]. To demonstrate the safety of HemaMax, Neumedicines conducted a Phase Ib study where healthy volunteers were administered a single dose of HemaMax that is predicted to be the effective dose for treating H-ARS, based on NHP data; this trial suggests rhuIL-12 to be safe and well tolerated. Phase II equivalent data (randomized, double-blinded, good laboratory practice) showed that single administration of rhuIL-12 to NHPs significantly increased survival and reduced radiation-induced hematopoietic toxicity when administered 24 h post-irradiation. Administration of rhuIL-12 promotes multilineage hematopoietic recovery, immune functions and possibly, GI functions. Additionally, there is a report of successful interspecies dose conversion. Neumedicines is developing rhuIL-12 for the treatment of H-ARS for BLA submission to the FDA under the Animal Efficacy Rule [10].

3.4 Additional potential biologics as countermeasures against ARS

Several additional biologics have been identified as potential countermeasures and have shown promise in murine and NHP models of ARS. Some of these agents have already been used off-label in radiological accident victims. Hopefully, in the future, they will be fully developed agents to combat radiation injury. Some of these potential agents are myeloid progenitors [11,12], anti-ceramide antibody [13], fibroblast growth factor-2 (FGF-2), its derived peptide (FGF-P) [14], IGF-1 [15] and various cytokines (**Table 2**) [16-18].

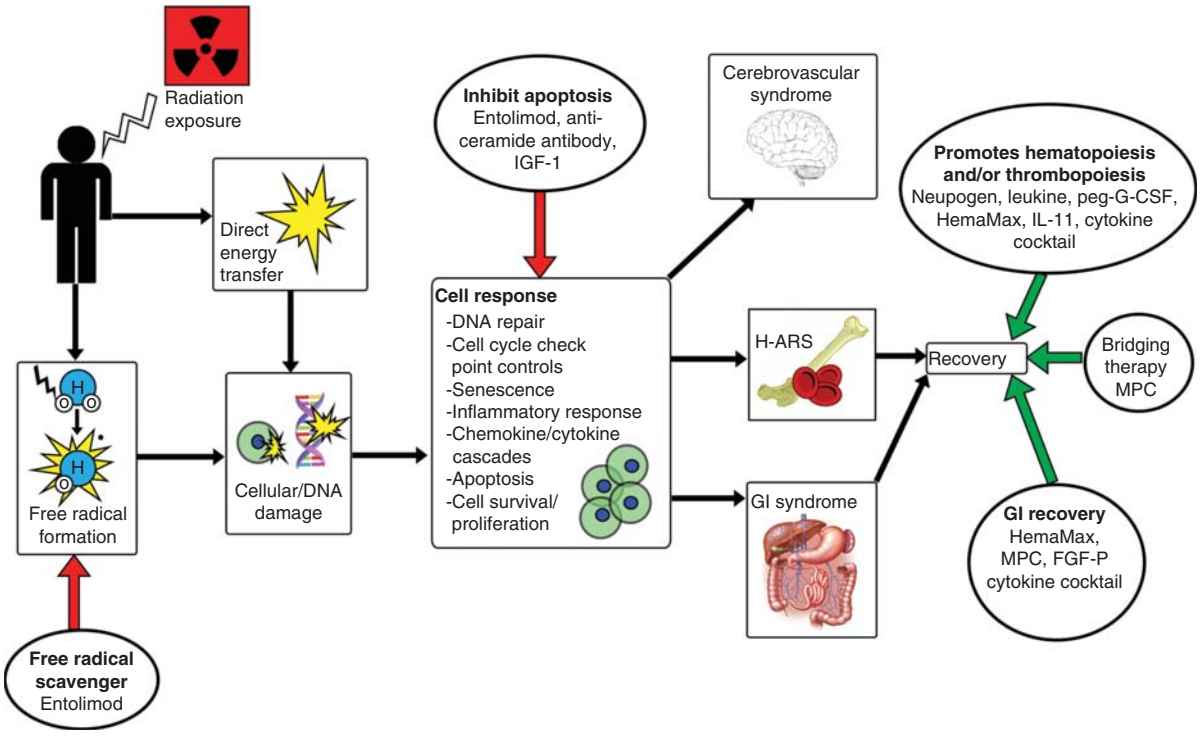

Figure 2. Simplified representation of systemic biological effects due to radiation exposure, with promising biologics intervening at various steps. Radiation induces free radical formation, DNA damage and apoptosis, which can then lead to ARS or death. Various biologics are able to minimize the damaging effects of irradiation through different mechanisms of action.
ARS: Acute radiation syndrome; FGF-P: Fibroblast growth factor-derived peptide; MPC: Myeloid progenitor cells.

4. Expert opinion

Since no FDA-approved ARS MCM exits, there is an urgent need to develop such agents. Based on studies with large numbers of NHPs, entolimod appears to be a promising radiation countermeasure for H-ARS as well as for GIS [7]. Entolimod's existing efficacy, safety data and animal-to-human dose conversion are enough to proceed with a pre-EUA application to reduce the risk of death following radiation exposure [7].

Independent of the FDA's approval and licensing process, the US federal government has procured filgrastim and sargramostim to be stockpiled in the SNS under the Pandemic and All-Hazards Preparedness Reauthorization Act. Filgrastim and PEGylated filgrastim have demonstrated efficacy in recently conducted NHP studies, and these data have been submitted to the study sponsor for submission to the FDA for approval [5,19,20]. In a recent study, G-CSF failed to demonstrate efficacy in the NHP model; however, this discrepancy may be due to the lack of supportive care [10]. The Center for Disease Control currently holds both investigational new drug and EUA applications with the FDA for the use of Neupogen/G-CSF in the event of a nuclear or radiological incident.

PEGylated filgrastim has demonstrated better efficacy than filgrastim in NHPs [19]. However, the stability, the implementation plans in the radiological nuclear incident scenario and potential side effects are not fully understood and are of concern [4,21]. PEGylated filgrastim has been suggested as an alternative to filgrastim.

Recent developments with HemaMax indicate that this agent is 'en route' to regulatory approval [9,10]. A single injection of HemaMax, without supportive care, significantly improved survival and promoted multilineage hematopoietic recovery in an NHP model of H-ARS. Initial published observations with myeloid progenitors appeared encouraging.

Each drugs' sponsor have made clinical progress; these drugs are moving forward to fill the need for MCM that has increased over the past decade due to increased terrorist threats. In our opinion, the above biologics hold the most promise in the future due to their limited side effects and would be safe and effective agents when approved.

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Declaration of interest

The 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 employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royalties.

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