Development of Biomarkers for Radiation Biodosimetry and Medical Countermeasures Research: Current Status, Utility, and Regulatory Pathways

Authors: Winters, Thomas A., Taliaferro, Lanyn P., and Satyamitra, Merriline M.

Source: Radiation Research, 197(5) : 554-558

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-21-00213.1

RADIATION RESEARCH **197**, 554–558 (2022)
0033-7587/22 $15.00

DOI: 10.1667/RADE-21-00213.1

COMMENTARY

Development of Biomarkers for Radiation Biodosimetry and Medical Countermeasures Research: Current Status, Utility, and Regulatory Pathways

Thomas A. Winters,[1] Lanyn P. Taliaferro and Merriline M. Satyamitra

Radiation and Nuclear Countermeasures Program (RNCP), Division of Allergy, Immunology and Transplantation (DAIT), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, Maryland

INTRODUCTION

Biomarkers are important indicators of biological processes in health or disease. Consequently, they play an important role in advancing development of radiation biodosimetry tools and in the development of medical countermeasures (MCMs) (1). They can aid in the assessment of radiation exposure level, as well as help determine the extent of radiation-induced injury, and/or MCM efficacy. In response to the threat of a radiological or nuclear incident, in 2004, the U.S. Department of Health and Human Services tasked the National Institute of Allergy and Infectious Diseases (NIAID) with identifying and funding early-to-late-stage MCM development to treat radiation-induced injuries. Although there are products approved to treat radiation-induced hematopoietic damage (Neupogen®, Neulasta®, Leukine®, Nplate®), there are still no qualified biomarkers of radiation injury, or approved biodosimetry tools, to assess the extent or severity of radiation exposure for triage or treatment purposes in the event of a radiation mass casualty incident.

Biomarkers are integral to research and development pathways for both biodosimetry and MCM advancement. For this reason, the NIAID Radiation and Nuclear Countermeasures Program (RNCP) sponsored a workshop in Bethesda, MD on June 1, 2020, titled ''Biomarkers in Radiation Biodosimetry and Medical Countermeasures.'' Speakers included academicians, representatives of industry, as well as members of U.S. Government (USG) agencies [NIAID, U.S. Food and Drug Administration (FDA), and the Biomedical Advanced Research Development Authority (BARDA)]. This commentary provides a brief overview of the information presented at the workshop and key points considered during discussions. A more comprehensive summary of the workshop presentations and discussions are presented in the full meeting report [(2) available at https://doi.org/10.1667/RADE-21-00157.1].

[1] Address for correspondence: DAIT, NIAID, NIH, 5601 Fishers Lane, Room 7A65, Bethesda, MD 20892; e-mail: twinters@mail.nih.gov.

BACKGROUND

Characteristics and Requirements for Radiation Biomarkers

Ideally, a successful biomarker goes through stages of early discovery, analytical validation, clinical validation, and qualification. Biomarkers in advanced development would need to demonstrate utility for clinical use and for drug development. If such characteristics can be confirmed, the biomarker may be appropriate to submit for regulatory consideration.

The potential broad applicability of biomarkers for clinical use and product development has led to a formalized joint effort by the FDA and NIH to define what constitutes the term ''biomarker'' and harmonize biomarker terminology. The outcome of this effort was the BEST Resource document, or Biomarkers EndpointS and other Tools (3). The BEST document established the definition of a biomarker as ''A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how a patient feels, functions, or survives.'' The NIH and the FDA have encouraged stakeholders to adopt BEST terms to harmonize terminology in the field.

However, because biomarker development is a dynamic field that is continuously incorporating new model systems and treatment settings for application to product development, clinical use, and policy interpretation, attempts have been made to further categorize biomarkers based on their use (diagnostic, monitoring, pharmacodynamic, predictive, prognostic, etc.). Radiation biomarkers described to date span the majority of these broad categories (4–7). The types of biomarkers that are being used for radiation approaches

within such categories, range from gene expression panels and other molecular markers such as miRNA expression, to cytogenetic, histologic, radiographic, physiological, and other ''omics'' approaches.

Although biomarkers are often considered surrogates of an outcome, a cautionary note was raised regarding such correlations between biomarkers and outcome, since many biomarkers ultimately are not validated as direct surrogates of an outcome. It is also good practice to consider regulatory concerns early in the process of biomarker development to ensure their optimal future use.

Biomarkers in Radiation Biodosimetry

Gene expression profiles and miRNAs have been investigated for their potential to inform triage decisions, radiation dose reconstruction, and prognosis. Several gene expression studies were described that were designed to reconstruct radiation exposures doses (Gy) which could help to inform medical triage and treatment decisions (8). The goal of these studies was to investigate potential gene expression biomarkers from blood samples that could quantitatively reconstruct exposure doses ranging from 4.5 to 6 Gy. However, divergent intraspecies radiation-induced gene expression patterns observed between murine and nonhuman primate (NHP) models creates complex challenges for validating and translating this approach to humans (9, 10).

A further challenge to employing gene expression profiling to dose reconstruction is inter-individual variability. A variety of preexisting comorbidities (e.g., chronic inflammation, DNA repair defects, etc.) may act to confound gene expression profiles. However, investigations into the effects of confounders suggests that inclusion of appropriate samples representing such confounders in the training data used to develop classifier algorithms may have the potential to alleviate this problem (11, 12). Although gene expression profiling is a promising approach to radiation dose reconstruction, many gaps and challenges still exist or are under investigation. Further investigations on the effects of radiation dose and source, sex differences, age, preexisting conditions, medications, and stochastic variations among individuals are either needed or underway to help build robust models and classification systems.

In addition to reconstruction of the physical absorbed radiation dose, another potential application of gene expression profiling in biodosimetry is to predict the biological effect of the exposure. Many parameters can affect the overall biological effect of a given radiation dose, including the biological characteristics of the exposed individual, radiation quality, dose rate, exposure homogeneity [e.g., total-body irradiation (TBI) vs. partial-body irradiation (PBI)], and many others. One approach to address this issue has employed the Medical TREatment ProtocOLs (METREPOL) hematopoietic (H)-acute radiation syndrome (ARS) severity scoring system, which assigns a score of 0–4 based on published dose-exposure estimates in humans from radiation accidents. This scoring system can be used to differentiate ''concerned citizens'' at doses below 1.0 Gy (H0), from those who have received doses in the range of 1–6 Gy [for whom H-ARS mitigation interventions might need to be employed (H1-3)], and from those with exposures greater than 6 Gy (H4) (13). Gene expression profiling in a baboon model and correlation to the METREPOL H-ARS scoring system has shown some promise for establishing validated genes sets predictive of H-ARS severity scores in the range of H1-3 vs. genes sets predictive of H-ARS scores of H2-3 (14).

In a different approach to gene expression profiling, miRNAs have been evaluated as biomarkers with the potential to detect effects ranging from radiation-induced pancytopenia, to distinguishing between TBI and PBI, while also differentiating these effects from confounders. These experiments have led to the development of a 4 gene panel, with H-ARS severity score agreement ranging from 90–97%, and which may be applicable to high throughput screening using a point-of-care (POC) device (15, 16). While miRNA panels are promising biomarkers for radiation biodosimetry, many challenges remain to be met to develop their full potential as powerful and predictive biodosimeters.

Due to the complexity of developing biomarker assays for radiation biodosimetry use during a radiation mass casualty incident, the need for regulatory guidance early in the development process was emphasized at the meeting. The types of biodosimetry tools helpful for managing patients during a mass casualty scenario were considered, and included POC triage devices to differentiate patients exposed to <2 Gy from those exposed to >2 Gy, for whom additional clinical intervention may be required. Also, high-throughput (HT) assays to further stratify those exposed to >2 Gy would help to guide medical management of patients under potential scarce resource conditions. Radiation biodosimetry *in vitro* diagnostic devices (IVDs) that meet these needs might employ molecular, cytogenetic, and/or protein biomarker approaches (17). The FDA regulatory mechanisms for approval, authorization [Emergency Use Authorization (EUA)], or clearance of radiation biodosimetry devices are important parts of this process (2).

Biomarkers in Medical Countermeasure Development

Since experimental studies of acute, high-dose radiation injuries in humans is neither ethical nor feasible, the natural history of such injuries, along with the development of associated biomarkers and MCMs, are expected to be conducted according to the FDA Animal Rule (18). Consequently, well characterized animal models that are representative of human radiation responses are critical for the development and validation of radiation biomarkers and their application to the evaluation of radiation MCM efficacy. Candidate biomarkers are often initially identified

by HT screening employing many ''omics'', followed by defining a clear relationship between the biomarker and a clinical endpoint. Typically, clinical endpoints may include, histological analyses, functional assays, imaging analyses, clinical observations, and survival. In the case of gastrointestinal (GI)-ARS, TBI and PBI models [using either 2.5% (mouse) or 5% (NHP) bone marrow sparing] have been developed to simulate potential radiation exposures in humans (19).

Use of liquid chromatography tandem mass spectrometry (LC-MS/MS) metabolomic analysis of NHP plasma and jejunal tissues has permitted identification of differential metabolites for irradiated vs. naïve animals. Parallel studies conducted in both NHP and mouse models have identified metabolites that were broadly responsive in both species across a variety of irradiation scenarios, suggesting their potential cross-species utility and possible translation to humans (20). In particular, citrulline was a strong candidate biomarker of GI radiation injury in the small intestine, with decreases in its circulating levels reported as a biomarker of GI damage in humans, with plasma values similar to those in NHPs (21–23).

Studies to investigate biomarkers predictive of radiation-induced lung injury in a rat model of whole thorax lung irradiation were also described. These injuries typically manifest much later than acute GI-ARS and H-ARS radiation injuries. In a rat model, lung complications were characterized by vascular regression, increased breathing rates, decreased perfusion and increased vascular permeability. Three clinically translatable, predicative methodologies for lung injury were identified that permitted prediction of 60-day survival with ~88.5% accuracy.

Delayed effects of acute radiation exposure (DEARE) are characterized by progressive and irreversible symptoms that can lead to organ damage of the kidney, lungs, cardiovascular, and central nervous system, and late problems in the GI tract. Since DEARE can manifest months to years postirradiation in ARS survivors, metabolomic approaches were presented as a means to identify early-onset, predictive biomarkers for individual organ systems. These studies employed a PBI mouse model to promote long-term survival through ARS and development of DEARE. Untargeted metabolomic and lipidomic analyses of urine and plasma samples were used to construct a DEARE prediction model. Many urine metabolites were found to vary longitudinally after irradiation, with a variety of kinetics and substantial sex differences. Early-observed metabolites were identified that correlated with later cardiac collagen deposition and functional changes. Studies of metabolites associated with DEARE in other organs are planned, as are further validation studies in NHPs.

Finally, the exciting potential of extracellular vesicles (EVs) as a novel class of radiation biomarkers was also highlighted. The utility of EVs as predictive biomarkers for severe radiotherapy complications was described, and their potential involvement as markers for a range of diseases with relevance to radiation exposure was discussed.

In support of developing biomarkers to assist drug and device development, the FDA has established a biomarker qualification process to reduce uncertainties and accelerate regulatory decisions during drug development (24). Sponsors seeking a biomarker qualification should address unmet drug development needs, as well as outline the biomarker's potential benefits and risks. The regulatory pathway involved in biomarker qualification is also an important process for FDA qualification of animal models.

Biomarker Translation from Models to Clinical Applications

Biomarkers can have key roles in drug development, including as secondary and safety endpoints, guides to supportive care, to establish mechanism(s) of action, and as a means of bridging an effective animal dose to a human dose. Many radiation-induced biomarkers have been characterized; however, for the purposes of marketing approval the FDA is most amenable to accepting biomarkers that are closely linked to clinical outcomes. For example, the FDA has accepted neutrophil and platelet changes as biomarkers reflecting radiation-induced myelosuppression, as well as for MCM efficacy in counteracting this effect. Other clinical markers of functional outcomes may also be acceptable; however, indirect biomarkers of radiation exposure and biological effects have yet to gain acceptance due to insufficient direct correlations to outcomes. In the context of drug development tools for radiation MCMs, biomarkers from samples collected noninvasively have been used as secondary, pharmacodynamic endpoints to indicate mechanism of injury and recovery and support dose selection. In the case of the four MCMs the FDA has approved to treat H-ARS, mitigation of radiation-induced myelosuppression was supported by animal models demonstrating enhanced survival and improved neutrophil and/or platelet counts as biomarkers of efficacy (25–27). Furthermore, NHP studies have been accepted by the FDA to predict an effective MCM dose in human patients (28).

Based on the successful use of these biomarkers for approval of MCMs for H-ARS indications, investigators addressing gaps for MCMs against GI-ARS and DEARE are advised to consider pathophysiologically-appropriate biomarkers in clinically relevant natural history animal models, with quantifiable endpoints for efficacy outcomes (e.g., survival, major morbidity). In addition to citrulline mentioned above, biomarkers of GI-ARS might include measures of intestinal and mucosal barrier function, or enterocyte measures (e.g., villus atrophy, crypt apoptosis, etc.) (29, 30). Whereas for DEARE, biomarkers such as indicators of pulmonary function, and pneumonitis and fibrosis assessments would likely be important. In summary, investigators should consider established roles for

biomarkers, such as proof of concept, dose ranging, secondary efficacy pharmacology, mechanism of action, and effective animal to human dose extrapolation, when initiating radiation MCM efficacy animal studies.

Challenges and requirements of translating new radiation biomarkers into radiation dosimetry devices capable of being deployed in response to a radiological or nuclear mass casualty incident were addressed by representatives of BARDA. Topics considered included the regulatory framework for obtaining FDA pre-EUA and/or 510k filing, Good Manufacturing Practice (GMP), establishing a network of quick response laboratories, supply chain and first responder logistics, and reliability and ease of biodosimeter result interpretation. The many hurdles and challenges to realizing these goals were discussed, and areas of success in achieving some were illustrated, using the BARDA-supported DxTerity platform as an example.

CONCLUSION

Given the wide range of variables that may affect radiation biomarker performance and utility, careful consideration must be given to the characteristics and role a biomarker is expected to fill during the initial stages of radiation biomarker or biodosimeter development. Individual variations among patients/animals used for a model, conditions of radiation exposure (TBI vs. PBI), radiation quality, dose rate, comorbidities and other confounders can all affect biomarker levels and timing. Consequently, while important, reconstruction of physical exposure dose may not be sufficient to adequately reflect outcomes of radiation exposure. Rather, biomarkers and biodosimetry systems that are predictive of clinical outcomes regardless of exposure dose may be of greatest utility for patient management in response to a radiation mass casualty incident.

Many preclinical radiation biomarkers have been identified and characterized in animal model systems, and their usefulness within these systems should not be discounted; however, radiation biomarker and biodosimeter translation to human applications must also consider regulatory requirements for approval early in the development process. As of November 2021, no radiation-specific biomarkers have been qualified, nor have any biodosimetry devices been cleared by the FDA, although advancements in the field may make this possible in the near future. Linking molecular biomarker technologies to clinical outcomes may enlarge the range of radiation biomarker characteristics acceptable to the FDA for qualification/approval. Such an advancement would provide a powerful expansion of applications for novel biomarkers to evaluate radiation biodosimetry, expand triage options, enhance patient management, accelerate radiation MCM development, and improve clinical outcomes during and following a radiation public health emergency.

ACKNOWLEDGMENTS

Many thanks to RNCP/NIAID colleagues David Cassatt, Carmen Rios, Andrea DiCarlo, and Olivia Molinar-Inglis for their critical review of the manuscript.

Received: October 29, 2021; accepted: January 5, 2022; published online: February 7, 2022

REFERENCES

1. Robb MA, McInnes PM, Califf RM. Biomarkers and surrogate endpoints: developing common terminology and definitions. JAMA. 2016; 315(11):1107-8.
2. Satyamitra MM, DiCarlo AL, Hollingsworth BA, Winters TA, Taliaferro LP. Development of Biomarkers for Radiation Biodosimetry and Medical Countermeasures Research: Current Status, Utility, and Regulatory pathways. Radiat Res. 2022; 197:000-000.
3. FDA-NIH Biomarker Working Group. In: Food and Drug Administration (US), National Institutes of Health (US), editors. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD)2016-.
4. Abbatt J. Cytogenetic indicators of radiation (and other) damage calibration- present and future practical applications. Biochemical Indicators of Radiation INjury in Man. Vienna: International Atomic Energy Agency (IAEA); 1971.
5. Biju PG, Garg S, Wang W, Choudhry MA, Kovacs EJ, Fink LM, et al. Procalcitonin as a predictive biomarker for total body irradiation-induced bacterial load and lethality in mice. Shock. 2012; 38(2):170-6.
6. Kunwar A, Haston CK. Basal levels of glutathione peroxidase correlate with onset of radiation induced lung disease in inbred mouse strains. Am J Physiol Lung Cell Mol Physiol. 2014; 307(8):L597-604.
7. Rump A, Becker B, Eder S, Lamkowski A, Abend M, Port M. Medical management of victims contaminated with radionuclides after a ''dirty bomb'' attack. Mil Med Res. 2018; 5(1):27.
8. Ghandhi SA, Shuryak I, Morton SR, Amundson SA, Brenner DJ. New approaches for quantitative reconstruction of radiation dose in human blood cells. Sci Rep. 2019; 9(1):18441.
9. Ghandhi SA, Smilenov L, Shuryak I, Pujol-Canadell M, Amundson SA. Discordant gene responses to radiation in humans and mice and the role of hematopoietically humanized mice in the search for radiation biomarkers. Sci Rep. 2019; 9(1):19434.
10. Park JG, Paul S, Briones N, Zeng J, Gillis K, Wallstrom G, et al. Developing human radiation biodosimetry models: Testing cross-species conversion approaches using an ex vivo model system. Radiat Res. 2017; 187(6):708-21.
11. Mukherjee S, Laiakis EC, Fornace AJ, Amundson SA. Impact of inflammatory signaling on radiation biodosimetry: mouse model of inflammatory bowel disease. BMC Genomics. 2019; 20(1):329.
12. Rudqvist N, Laiakis EC, Ghandhi SA, Kumar S, Knotts JD, Chowdhury M, et al. Global gene expression response in mouse models of DNA repair deficiency after gamma irradiation. Radiat Res. 2018; 189(4):337-44.
13. Fliedner T.M. FI, Beyrer K, British Institute of Radiology, editors. Medical management of radiation accident—manual on the acute radiation syndrome (METREPOL European Commission concerted action). British Institute of Radiology; Oxford 2001. p. 1–66; compendium p. C1–C21.
14. Port M, Herodin F, Valente M, Drouet M, Lamkowski A, Majewski M, et al. First generation gene expression signature for early prediction of late occurring hematological acute radiation syndrome in baboons. Radiat Res. 2016; 186(1):39-54.
15. Ostheim P, Haupt J, Herodin F, Valente M, Drouet M, Majewski M, et al. miRNA expression patterns differ by total- or partial-body radiation exposure in baboons. Radiat Res. 2019; 192(6):579-88.
16. Port M, Ostheim P, Majewski M, Voss T, Haupt J, Lamkowski A,

et al. Rapid high-throughput diagnostic triage after a mass radiation exposure event using early gene expression changes. Radiat Res. 2019; 192(2):208-18.

17. U.S. Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH). Radiation Biodosimetry Medical Countermeasure Devices - Guidance for Industry and Food and Drug Administration Staff. FDA-2014-D-2065. Silver Spring, MD: Office of Communication; April 2016.

18. U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER) Product development under the animal rule. Guidance for industry. FDA-2009-D-0007. Silver Spring, MD: Office of Communication; 2015.

19. MacVittie TJ, Bennett A, Booth C, Garofalo M, Tudor G, Ward A, et al. The prolonged gastrointestinal syndrome in rhesus macaques: the relationship between gastrointestinal, hematopoietic, and delayed multi-organ sequelae following acute, potentially lethal, partial-body irradiation. Health Phys. 2012; 103(4):427-53.

20. Jones JW, Tudor G, Bennett A, Farese AM, Moroni M, Booth C, et al. Development and validation of a LC-MS/MS assay for quantitation of plasma citrulline for application to animal models of the acute radiation syndrome across multiple species. Anal Bioanal Chem. 2014; 406(19):4663-75.

21. Crenn P, Messing B, Cynober L. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. Clin Nutr. 2008; 27(3):328-39.

22. Lutgens L, Lambin P. Biomarkers for radiation-induced small bowel epithelial damage: an emerging role for plasma Citrulline. World J Gastroenterol. 2007; 13(22):3033-42.

23. Rabier D, Kamoun P. Metabolism of citrulline in man. Amino Acids. 1995; 9(4):299-316.

24. U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Draft Guidance - Biomarker Qualification: Evidentiary Framework Guidance for Industry and FDA Staff. FDA-2018-D-4267. Silver Spring, MD: Office of Communication; 2018.

25. Beveridge RA, Miller JA, Kales AN, Binder RA, Robert NJ, Harvey JH, et al. A comparison of efficacy of sargramostim (yeast-derived RhuGM-CSF) and filgrastim (bacteria-derived RhuG-CSF) in the therapeutic setting of chemotherapy-induced myelosuppression. Cancer Invest. 1998; 16(6):366-73.

26. Bunin DI, Bakke J, Green CE, Javitz HS, Fielden M, Chang PY. Romiplostim (Nplate®) as an effective radiation countermeasure to improve survival and platelet recovery in mice. Int J Radiat Biol. 2020; 96(1):145-54.

27. Clayton N, Khan-Malek R, Dangler C, Zhang D, Ascah A, Gains M, et al. Sargramostim (rhu GM-CSF) improves survival of non-human primates with severe bone marrow suppression after acute, high-dose, whole body irradiation. Radiat Res. 2020; 195.

28. Krzyzanski W, Sutjandra L, Perez-Ruixo JJ, Sloey B, Chow AT, Wang YM. Pharmacokinetic and pharmacodynamic modeling of romiplostim in animals. Pharm Res. 2013; 30(3):655-69.

29. Booth C, Tudor G, Tudor J, Katz BP, MacVittie TJ. Acute gastrointestinal syndrome in high-dose irradiated mice. Health Phys. 2012; 103(4):383-99.

30. Bujold K, Hauer-Jensen M, Donini O, Rumage A, Hartman D, Hendrickson HP, et al. Citrulline as a biomarker for gastrointestinal-acute radiation syndrome: species differences and experimental condition effects. Radiat Res. 2016; 186(1):71-8.