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ARS, DEARE, AND MULTIPLE-ORGAN INJURY: A STRATEGIC AND TACTICAL APPROACH TO LINK RADIATION EFFECTS, ANIMAL MODELS, MEDICAL COUNTERMEASURES, AND BIOMARKER DEVELOPMENT TO PREDICT CLINICAL OUTCOME

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INTRODUCTION

Expanding the database

The Team of collaborators self-named as the Medical Countermeasures Against Radiological Threats (MCART) Consortium and other respected colleagues have provided a series of three issues in *Health Physics*, published in 2012 (volume 103, issue 4), 2014 (volume 106, issue 1), and 2015 (volume 109, issue 5). The issues focused on the continued development of a clinically relevant animal model research platform designed to adhere to the criteria of the US Food and Drug Administration (FDA) guidance document for product development under the FDA Animal Rule and the qualification process for drug development tools (US FDA 2014, 2015). The research effort is focused on the acute radiation syndrome (ARS), delayed effects of acute radiation exposure (DEARE), and multiple-organ injury (MOI) by developing a strategic and tactical approach to link radiation effects, animal models, medical countermeasures (MCMs), and biomarker development to predict clinical outcome.

Two successive issues of *Health Physics* have been devoted to a number of studies conducted in both small and large mouse, rat, and nonhuman primate (NHP) animal models. The first issue, herein, is devoted to studies that used the NHP while the second issue will focus on studies that utilized the mouse, rat, and NHP. There is a special section devoted to biomarkers: “Biomarkers: a multidisciplinary approach to predict clinical outcome in mouse and NHP models of MOI.”

The animal model research platform utilized (1) small-to-large animal species to include the mouse, rat, and NHP; (2) radiation facilities using 320-kVp x radiation (10–13 mA, 1.2–2.3-mm copper half-value layer [HVL]), ^{137}Cs gamma radiation, and 6 MV linac-derived photons; (3) dose rates ranging from 0.6 to 1.4 Gy min^{-1} d^{-1} from homogeneous, uniform exposure to total- or partial-body irradiation; (4) an established radiation physics core that ensured consistent and accurate dose delivery across all research sites; and (5)

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potentially lethal doses that were species, organ sequelae, and study dependent. Each site developed well-designed dose-response relationships (DRR) for mortality due to respective organ-based subsyndromes characteristic of ARS or DEARE.

The respective studies in these, the fourth and fifth MCART-dedicated issues of *Health Physics*, were conducted at five radiation research and commercial histopathology sites: the University of Maryland Schools of Medicine and Pharmacy, Indiana University School of Medicine, Shin Nippon Biomedical Laboratories (SNBL), the Medical College of Wisconsin, and Charles River Laboratories.

Dose- and time-dependent radiation-induced ARS, composed of the overt, acute hematopoietic ARS (H-ARS) and gastrointestinal ARS (GI-ARS) and associated MOI, provided the foundation for the concomitant, latent, and overt development of DEARE and MOI. The MCART team and colleagues have focused on (1) developing a more strategic and integrated organ-based approach to determine radiation effects to single and multiple organs characteristic of ARS and DEARE, (2) defining the efficacy of MCM administration in the context of MOI, and (3) developing biomarker paradigms that will predict clinical outcome within the context of use.

RATIONALE FOR A SHIFT IN FOCUS OF THE ANIMAL MODEL RESEARCH PLATFORM: ARS AND DEARE—ASKING BETTER QUESTIONS

Challenge: developing animal models

The animal model research platform was initially developed based on models of organ-specific injury characteristic of ARS, e.g., GI-ARS, H-ARS, and DEARE, as characterized primarily by radiation-induced lung injury (RILI) (Booth et al. 2012a and b; Chua et al. 2012, 2014; Farese et al. 2012a; Jackson et al. 2012, 2014; MacVittie et al. 2012a and b, 2014; Plett et al. 2012, 2015; Garofalo et al. 2014; de Faria et al. 2015). The basic models adhered to the US FDA definition as a specific combination of an animal species, challenge agent (acute ionizing radiation), and route of exposure (external radiation) that produces a disease process or pathological condition that in multiple important aspects corresponds to the human disease or condition of interest: ARS and DEARE (US FDA 2015). The models utilized total-body irradiation (TBI) and whole-thorax lung irradiation (WTLI) and supported a tactical approach to assess the efficacy of MCMs against organ-specific ARS and DEARE. The systematic, more strategic approach to link the dose- and time-dependent multiorgan sequelae of ARS and DEARE required a different set of questions relative to acute and delayed radiation effects and the challenges presented to define the relationship relative to MCM efficacy within three predominant species: the mouse, rat, and NHP.

Challenge: species differences, ARS and DEARE, dose-response relationships, and context of use

Dose-response relationships—The published database using animal models to assess the value of both tactical and strategic approaches to radiation effects and MCM efficacy provides a relatively organized view of species-, organ-, -time-, and dose-dependent ARS and DEARE MOI relative to established radiation exposure protocols. The comparative

value of multispecies data sets is dependent on the established DRR from well-characterized models. The DRR provides the relative dose-dependent values, the slope, and LD_{50} for comparison of the equivalent biological effect, e.g., organ-specific sequelae and MOI. The predominant species used in MCM studies (mouse, rat, and NHP) are characterized by variable LD_{50} values for GI- and H-ARS, radiation-induced lung injury, and MOI. The radiation effects on mice and rats are further differentiated by strain, sex, and age (Rauchwerger 1972; Down 1986; Jackson et al. 2014; Dabjan et al. 2016; Groves et al. 2018). In this regard, the NHP model has benefited from the almost exclusive and extensive database established for young, male, rhesus macaques (Bertho et al. 2005; Herodin et al. 2005; MacVittie et al. 2012a and b; Garofalo et al. 2014; MacVittie et al. 2015; Thrall et al. 2019). Thrall et al. (2019) have recently established an equivalent model, $LD_{50/180}$, slope, and pathophysiology of WTLI-induced lung injury in female macaques.

Model constraints relative to species—With respect to species, an established DRR within species, strain, sex, and age necessitates control of other factors critical for a well-characterized model; e.g., radiation physics, housing and husbandry conditions, Institutional Animal and Care Use Committee criteria for euthanasia, MCM route and schedule of administration, and medical management. Knowledge of these conditions permits more relevant extrapolation to the NHP and human databases. The Orschell and Booth teams have demonstrated the effects of medical management on the DRR for the H-ARS and GI-ARS organ sequelae for respective mouse strains (Booth et al. 2012b; Plett et al. 2012, 2015). The route and schedule of MCM administration, e.g., oral or intravenous for long durations, will cause considerable design problems for small-animal models. Stress to the animal may result in increased morbidity and mortality and shift the DRR. Small animals also limit the use of medical management that will be used in NHP models and will be considered as the standard of treatment for humans relative to the context of use. Medical management shifts the DRR to the right and will be the standard of care relative to context of use and enhanced MCM efficacy (Taketa 1962; MacVittie et al. 1991, 2005; Booth et al. 2012b; Plett et al. 2012, 2015; Yu et al. 2015).

The bottom line is that well-characterized mouse, rat, or NHP models that have established DRRs, relative to the context of use, i.e., (1) characterize the animal; (2) ensure consistent radiation physics and prescribed dose delivery; (3) have clearly defined primary, secondary, and tertiary end points; and (4) have established natural history over the study duration to include latency, incidence, severity, and progression of organ-specific sequelae and trigger points for pathophysiology and intervention and/or treatment, will provide a comparative small- and large-animal database to support MCM development predictive of human radiation effects and treatment.

Model development: partial-body irradiation with bone marrow sparing for the mouse, rat, and NHP—Linking the concomitant evolution of MOI throughout ARS and DEARE required an approach using high-threshold doses of partial-body irradiation (PBI) with minimal bone marrow (PBI/BM) sparing. The models permitted analysis of ARS, acute kidney injury (AKI), and delayed MOI to include prolonged immune suppression and GI damage, chronic kidney injury (CKI), and heart and lung injury in animals that had survived

the dose-dependent lethal effects of acute GI- and H-ARS as a result of marginal marrow sparing (approximate 5% or 2.5%) (Booth et al. 2012a, 2015; Chua et al. 2012; MacVittie et al. 2012a, 2014; de Faria et al. 2015; Medhora et al. 2015; Fish et al. 2016; Cohen et al. 2017).

Murine models of PBI/BM sparing—Booth and colleagues defined a mouse model of PBI with degrees of BM sparing to further characterize the acute DRR and long-term functional damage and recovery to intestinal clonogenic cells and the GI system (Booth et al. 2012a and b, 2015). Fish et al. (2016) established a comparable model of PBI/BM sparing using leg-out BM-sparing in the WAG/RijCmer rat strain that enabled definition of concurrent ARS and DEARE, e.g., GI-ARS, H-ARS, and lung, kidney, and cardiac injury (Fish et al. 2016). The respective research teams established DRR and longitudinal Kaplan-Meier analysis for mortality and MOI over the time course for DEARE. Additionally, the WTLI model was modified to use an exposure protocol that incorporated a dose of TBI below the threshold for inducing H-ARS plus a subsequent top-up dose to the whole thorax region (Williams et al. 2010). The top-up dose range could be used to develop an equivalent DRR in the 10–15 Gy range for lung injury in the prominent rodent models of WTLI given noted strain differences in the time course and biology associated with pneumonitis, pleural effusion, fibrosis, and mortality while promoting a more realistic exposure of survivable TBI in the nuclear scenario. The PBI/BM-sparing in the mouse, rat, and NHP models permit analysis of the concomitant dose- and time-dependent evolution of acute and delayed sequelae. The predictive validity of the models depends on relating respective dose- and time-dependent sequelae to relevant clinical and accidental nuclear exposures.

Murine H-ARS survivors: MOI at <10-Gy TBI—The established radiation exposure model for H-ARS, e.g., TBI in mouse models, has been extended to investigate the prolonged, residual hematopoietic stem cell (HSC) injury and delayed MOI and survival of cohorts that survived the high-lethal effects of H-ARS (Chua et al. 2012, 2014; Garofalo et al. 2014; de Faria et al. 2015; Medhora et al. 2015; Unthank et al. 2015). This more strategic approach defined marked impairment of HSC function to produce multilineage reconstitution and the development of significant delayed effects in lung, kidney, and heart in survivors of H-ARS. Assessing the role of MCMs against the H-ARS evolved to assessing long-term recovery of HSC biology, the modified dose- and time-dependent thresholds for DEARE, and long-term survival.

Hence, the well-characterized animal model research platforms have the capability to assess the efficacy of organ-specific MCMs on multiorgan damage as well as combined MCM efficacy throughout the time course of the MOI characteristic of ARS and DEARE.

Model validation: mouse, rat, NHP—MCART teams and colleagues developed small- and large-animal models of ARS and DEARE that are product independent and may be proposed to the FDA as drug development tools (US FDA 2014). These models adhered to key components of the validation process relative to phenomenological, predictive, and construct validity (Willner 1991; Van Dam and De Deyn 2006; van der Staay 2006; Varga et al. 2010; MacVittie 2014). The models have been validated through established species-specific, organ-specific, and dose-dependent dose-response relationships characterized by

LD_{50} values and respective slopes, and repeated use and development at multiple research sites as well as to establish the efficacy of MCMs under a specific context of use, i.e., assessing the efficacy of MCMs to mitigate lethal H-ARS, RILI, and combined organ injury consequent to acute radiation exposure (Travis et al. 1980; Farese et al. 2012a and b; Plett et al. 2012, 2014; Farese et al. 2013; Chua et al. 2014; Fish et al. 2016).

Animal models and the human response to radiation and treatment: predictive validity—Dabjan and colleagues, in a recent review article on lung injury in mouse models, asked an insightful question: "So what really happens in humans that should be addressed in an animal model?" (Dabjan et al. 2016). The question is relevant to the major subsyndromes and associated MOI consequent to acute, potentially lethal radiation exposure. The exposure conditions relative to the context of use are complicit in the difficulty of developing valid animal models of the human response to acute radiation exposure and treatment. The human database is anecdotal and extremely variable due to required retrospective dose analysis and delay in clinical triage, diagnosis, and treatment. The ability to establish a human model of acute radiation exposure for ARS and DEARE relied on published and unpublished reports from human exposures in relevant clinical protocols for accidental acute exposures to workers and emergency personnel and nuclear weapons (Wiernik 1966a–c; Ricks and Fry 1988; Anno et al. 1989, 2003; Baranov and Guskova 1990; Baranov et al. 1994; Meineke and Fliedner 2005; Igaki et al. 2008; Dainiak et al. 2011a and b; Doerr et al. 2014; Graessle et al. 2015).

Additionally, the predictive ability of the NHP models of H-ARS to mimic the human response to acute radiation and treatment has been supported through analysis of radiation accident victims contained in the System for Evaluation and Archiving of Radiation Accidents Based on Case Histories (SEARCH) database in addition to the Moscow-Ulm Radiation Accident Database (MURAD) (Fliedner et al. 2002; Doerr et al. 2014; Graessle et al. 2015). Validation and predictive ability of the NHP animal models for GI-ARS and H-ARS was accomplished through substantive use and phenomenologic equivalence relative to clinical time course, morbidity, cellular effects, and histopathology. Equivalent NHP models of TBI-induced H-ARS, to include medical management, have been established by Yu et al. (2015) and Thrall et al. (2015).

The most relevant clinical reports that described acute radiation-induced lung injury are from the Princess Margaret Hospital. The reports included cancer patients that received upper hemibody irradiation (UHBI) in the dose range of 8.0–12.0 Gy at comparative dose rates of ^{60}Co gamma radiation or linac-derived photons. The clinical database underscored the severity of radiation-induced pneumonitis that was associated with mortality (Prato et al. 1977; Fryer et al. 1978; Van Dyk et al. 1981). We note that pneumonitis was defined as presence of cough, dyspnea, and characteristic radiographic x-ray analysis. The absence of histopathological evidence of pneumonitis and fibrosis limited the correlation with the nonhuman primate database. Other investigators provided studies that underscored the critical control of variables such as species and strain differences in mouse and rat models of radiation-induced lung injury (Prato et al. 1977; Fryer et al. 1978; Van Dyk et al. 1981; Down 1986; Jackson et al. 2012, 2014; Medhora et al. 2015; Dabjan et al. 2016).

Rodent models have been the informative base for assessing the effects of acute radiation exposure on the lung and heart. Medhora and colleagues established WTLI and PBI/BM-sparing models in the rat to investigate both acute and delayed radiation effects in the context of assessing MCM efficacy, combined organ injury, and biomarker development (Medhora et al. 2015, 2016; Fish et al. 2016; Jacobs et al. 2019). The WTLI model provided a platform for determining the combined injury of lung and heart via assessment of lung function and structure relative to that of the heart using an echocardiographic methodology (Medhora et al. 2015). Ghobadi et al. had used a similar model of targeted proton irradiation to the lung and heart to establish a data set that suggested that heart irradiation reduced the threshold for the delayed RILI (Ghobadi et al. 2011, 2012). Additionally, the PBI/BM-sparing model in the rat underscored its tactical value in permitting determination of MCM efficacy to mitigate injury to the lung and kidneys, in addition to linking ARS and DEARE via use of granulocyte colony-stimulating factor (G-CSF) and medical management to mitigate against H-ARS in the context of combined MCM administration against delayed MOI (Fish et al. 2016). Additionally, lung and cardiac injury analysis was initiated in NHP models of WTLI and PBI with 5% bone marrow sparing (PBI/BM5) exposure protocols (de Faria et al. 2015).

The basis of well-characterized animal models has been addressed above relative to establishing a DRR and all critical components thereof. Adherence to the continued definition of these components, regardless of the numerous animal- and species-dependent variables, will support a more valid understanding of the animal model and its limitations in predicting the human response to acute, potentially lethal radiation and treatment of acute and delayed MOI.

Continued refinement of the animal model research platform is focused on the concomitant evolution of multiorgan damage in ARS and DEARE. The strategic approach is centered on (1) definition of the time to onset of organ sequelae (the latent period); (2) the interactive biology during the latent period for organ-specific acute and delayed effects; (3) the natural history of organ-specific and multiorgan damage; (4) identification of definitive biomarkers for organ damage; (5) development and refinement of organ imaging methods for noninvasive, longitudinal assessment of organ injury; (6) clarification of the mechanism of action for single-organ injury or MOI; and (7) the latency, incidence, and severity of delayed multiorgan pathology, such as fibrosis. This approach provides an overall plan for assessing long-term efficacy or toxicity of MCMs and resolution of radiation-induced MOI. The MCM can be defined in terms of efficacy against specific organ damage, as well as its ability to mitigate similar damage in multiple organs against a backdrop of comorbidities, prolonged enteropathy, and immune suppression. In summary, the models provide a more comprehensive view relative to the concomitant nature of acute and delayed effects of acute radiation exposure on MOI.

A comprehensive understanding of radiation effects, both acute and delayed, permits a study design for MCM efficacy to include consideration of current knowledge gaps such as determining the effect of (1) an organ-specific MCM on other organ injury, (2) combined MCM administration on MOI, (3) MCM administration during the concomitant evolution of MOI within ARS and DEARE, (4) the effect of a consensus MCM with a mechanism

of action for similar/equivalent sequelae (fibrosis) in multiple organs, and (5) extending the study duration to permit adequate determination of the progression and resolution of the organ-specific injury in control and MCM-treated cohorts. Considerations relative to time of onset (latency), incidence, severity, progression, duration, and resolution of the sequelae are important to determine if the primary and secondary end points, such as key signs of morbidity and/or mortality, are stabilized/resolved over the study duration (US FDA 2015). These are important parameters that will aid in defining prospective studies to assess the true treatment effect for MCM efficacy.

BIOMARKERS: A MULTIDISCIPLINARY APPROACH TO PREDICT CLINICAL OUTCOME IN NHP MODELS OF MOI

A key aspect of the strategic approach employed by the MCART team and colleagues has been to evaluate biomarkers in the context of the clinical signs and symptomatology to more effectively manage the triage and treatment of exposed individuals. The identification of biomarkers not only advances the characterization of animal models as drug development tools but also aligns with the guidance provided by the US FDA that would enable biomarkers to be used in support of MCM development under the Animal Rule (US FDA 2015). Here, key biomarker activities include (1) defining biomarkers according to the natural history of the radiation syndrome by identifying, characterizing, and validating syndrome-specific biomarkers; (2) evaluating candidate biomarkers for utility as end points in assessment of the disease condition or the efficacy of the MCM; (3) identifying biomarkers that would be used as a trigger-to-treat because they correlate with pathophysiology; (4) evaluating syndrome and MCM mechanism of action according to biomarkers; and (5) using biomarkers to translate and select an effective MCM dose in humans. A multidisciplinary approach is needed to both identify and characterize biomarkers that correlate with clinical outcome and to understand how biomarkers may be used to define the natural history and interactions of biological systems in both organ-specific and MOI contexts. Mass spectrometry-based approaches have been developed and applied due to their analytical versatility and rigor. The MCART team and colleagues have employed a systems biology-based approach though combining “omics-based” approaches (metabolomics, proteomics, lipidomics), targeted profiling to quantify specific biomarkers, and matrix-assisted laser desorption ionization-mass spectrometry ionization (MALDI-MSI) for spatial localization of molecules to define biomarkers and/or mechanisms (Carter et al. 2019; Huang et al. 2019a and b; Jones et al. 2019a and b).

Accordingly, the MCART team has worked to advance the clinical and regulatory utility of candidate biomarkers by validating them for sensitivity and selectivity, robustness of response, and stability of response for radiation-induced injury (Jones et al. 2014, 2015a and b). The potential for biomarkers to be diagnostic (absence/presence of radiation-induced disease state), prognostic (indicative of progression of natural history of radiation-induced disease state), or predictive (prediction of clinical end points at earlier time points), as well as the potential to serve as a pharmacodynamic biomarker (indicative of biological response to an intervention), have been explored (Carter et al. 2019; Huang et al. 2019a and b; Jones et al. 2019a and b). The mechanistic significance of biomarkers continues to be determined

through literature, statistical modeling, pathway analysis, and interpretation of the biomarker response in the context of clinical and histopathological outcomes. The forward objectives for MCART biomarker studies are that biomarkers provide additional mechanistic insight into radiation models of organ-specific injury and MOI and that advancement efforts yield an FDA-qualified biomarker whose conditions of qualified use and mode of measurement are determined and defined (US FDA 2014). Currently, a gap in knowledge is that there are no FDA-qualified biomarkers for radiation-induced injury available for use in making regulatory or patient treatment decisions. The multifaceted database of biomarker data is a necessity to close these gaps and is an essential complement to the well-defined animal models established by the MCART team and colleagues.

ISSUE SUMMARY: AN EXPANDED DATABASE RELATIVE TO MCM AND BIOMARKER DEVELOPMENT; GAPS IN KNOWLEDGE

The animal model research platform described herein has provided an extended database that will link ARS and DEARE relative to the natural history of organ-specific injury and MOI, systems biology, and MCM efficacy. This team of researchers has provided the essential basics of animal model development in their relevant species, the mouse, rat, and NHP. The studies have established species- and organ-specific dose-response relationships for ARS and DEARE that will permit continued effort to close several key gaps in knowledge relative to MCM development. These are to establish (1) the natural history of the multiorgan injury characteristic of DEARE, e.g., prolonged GI, lung, kidney, and heart injury; (2) the interactive, causative, and/or associated link of MOI relative to morbidity and mortality; (3) the true effect of radiation-induced MOI by extending the study duration to assess stable MOI end points; (4) if there is a link between the MOI of ARS and DEARE; (5) the dose- and time-dependent threshold trigger-points for initiation of overt MOI; and (6) the effect of organ-specific or systems-dependent MCMs on MOI within ARS and/or DEARE.

CONTRIBUTORS TO THE MARCH AND APRIL 2019 ISSUES OF *HEALTH PHYSICS*—ARS, DEARE, AND MULTIPLE-ORGAN INJURY: A STRATEGIC AND TACTICAL APPROACH TO LINK RADIATION EFFECTS, ANIMAL MODELS, MEDICAL COUNTERMEASURES, AND BIOMARKER DEVELOPMENT TO PREDICT CLINICAL OUTCOME

MacVittie et al. have contributed a systematic review of acute GI-ARS in rhesus macaques and two papers focused on (1) the concomitant GI-ARS and H-ARS in an NHP model of PBI/BM sparing reduced to approximately 2.5% administered either Neupogen® (Amgen, Inc., Thousand Oaks, California, US) or Neulasta® (Amgen) in delayed schedules. The approximate 2.5% BM sparing may represent the threshold amount required for enhanced recovery post high-dose lethal exposure to the NHP; and (2) the time course of radiation-induced lung injury in NHPs that received early administration of Neupogen. The latency, incidence, or progression of RILI were not observed to be influenced by Neupogen

administration. These results were supported by the histopathological analysis provided by Dr. George Parker.

Karla Thrall and colleagues established a DRR for acute radiation-induced lung injury in female rhesus macaques. This model validated the University of Maryland, Baltimore, WTLI rhesus macaque model conducted in male macaques and importantly confirmed that a sex-based effect is not evident in the NHP model.

Eric Cohen followed up on his recent publication defining both AKI and CKI in the irradiated NHP, determining that there is no effect of Neupogen administration on either AKI or CKI. Eric, George Parker, and colleagues continued to define the incidence and evolution of clinical, cellular, and histopathology of acute radiation-induced fibrosis within the kidney.

George Parker and respective colleagues provided a comprehensive histopathological perspective on clinical and cellular damage in the lung, kidney, and GI systems. Parker's analysis added support to the longitudinal, clinical, and radiographic evidence of pneumonitis and fibrosis described in the part 1 papers by Tom MacVittie, Eric Cohen, and colleagues.

Christie Orschell and her team continued to provide valuable insights into definitive residual, functional damage to hematopoietic stem cells in their models of long-term survivors after high-dose H-ARS.

Meetha Medhora and her team used the leg-out PBI model in the rat to provide insights on two key questions relative to radiation effects and MCM efficacy. Two papers are presented that compare the effect of the MCM lisinopril on age subsets, geriatric and juvenile. A second paper provided additional information on the combined lung and heart injury after high-dose exposure.

Maureen Kane and her team at the University of Maryland School of Pharmacy Mass Spectrometry Center describe their approach to biomarker analysis as a multidisciplinary systems biology platform driven by mass spectrometry-based techniques. They explore the interactive biology during the latent period of DEARE for lung using proteomics to identify initiating events. A similar proteomic approach provides perspective to the natural history of GI-ARS. Metabolomic approaches extend previous work toward identifying definitive biomarkers of organ damage through establishing tissue-plasma correlations and investigating the confounding effect of sex. Lastly, spatial differences in target molecules localized to specific tissue features across the natural history of GI-ARS in NHPs were detected using MALDI-MSI.

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