SESSION 4: DETERMINING THE READINESS OF AN EMERGING BIOMARKER OF EFFECT FOR USE IN ENVIRONMENTAL HEALTH DECISIONS
CONCURRENT BREAKOUT DISCUSSION & DEBATE

ɣH2AX is a histone variant that is phosphorylated in response to double-strand break DNA damage from endogenous or exogenous sources. Upon DNA damage, the phosphorylated protein (ɣH2AX) localizes to chromatin corresponding to individual double-strand breaks. It plays a pivotal role in organizing the response to double strand breaks and other forms of damage by recruiting the DNA repair center complex.

Antibodies have been developed against ɣH2AX, allowing its presence to be observed via a variety of methods, including flow cytometry, enzyme-linked immunoassay, and high-content imaging. The presence of ɣH2AX is sensitive to double strand breaks caused by x-ray radiation as low as 1 mGy[1], suggesting possible utility as a biomarker of genotoxic damage. Because unchecked genotoxic damage can lead to various cancers it is postulated that ɣH2AX may be used to predict the potential for environmental exposures to lead to cancer. Here, we explore two possible uses of ɣH2AX as a biomarker for cancer: (1) assessing pharmaceutical agents for safe human use and (2) monitoring the impact of accidental exposures on populations.

Decision Context 1: Safety Assessment.

U.S. Food and Drug Administration guidance dictates a testing battery approach to predict whether drugs induce genotoxic mutagenicity in humans[2]. The most common implementation of this genetic toxicology battery involves testing the compound in three systems:

1. Bacterial reversion assay (i.e., Ames assay).
2. An in vitro chromosomal damage test (e.g., micronucleus assay) or in vitro mouse lymphoma Tk gene mutation assay.
3. An in vivo micronucleus assay, performed in rats or mice.

Experimental systems using ɣH2AX, because its presence is easily quantified in cells by immunofluorescence, have been suggested as an alternative to this standard battery for assessing genotoxic carcinogenicity. Tomasetig et al.[3] used three human cell lines (Hep3B human hepatoblastoma cells, LS-174T human epithelial colorectal adenocarcinoma cells, and NCI-H358 human bronchioalveolar carcinoma cells) to assess the genotoxic potential of a panel of aryl hydrocarbons. We propose that ɣH2AX, assessed by in-cell Western blot in these three cell systems, is a suitable replacement for the FDA standard battery for genotoxicity.

> *Prompt: ɣH2AX measurement in a panel of human cells should be added to the standard FDA genotoxicity battery.*
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> Breakout Group A. Pro: ɣH2AX **is ready** for use in safety assessment
> Breakout Group B. Con: ɣH2AX measurement **is not ready** for use in safety assessment

[1] https://pubmed.ncbi.nlm.nih.gov/12679524/
[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s2r1-genotoxicity-testing-and-data-interpretation-pharmaceuticals-intended-human-use
[3] https://www.ncbi.nlm.nih.gov/pubmed/32184089

Decision Context 2: Population Monitoring

ɣH2AX is emerging as a potential biomarker of effect in human populations. ɣH2AX is detectable in lymphocytes and can therefore be quantified from peripheral blood. Elevated ɣH2AX has been shown to correlate with occupational exposure to poly arylhydrocarbons and markers of genotoxic damage[4]. Peripheral blood levels of ɣH2AX also correlate with exposures to diagnostic x-ray exposures[5]. Here, circulating concentrations of ɣH2AX are proposed as a biomarker for monitoring cancer risk of exposed populations. The biomarker would be quantified via flow cytometry of peripheral lymphocytes and compared against background population levels.

A facility manufactures a compound that is suspected carcinogen. The weight of evidence indicates that the compound acts by directly interacting with DNA, causing double-strand breaks. Amid concerns for worker safety, the manufacturer is considering adding biomarker monitoring as an added precaution to existing occupational safety considerations already in place.

> *Prompt: The ɣH2AX biomarker is sufficiently developed that it could be used for monitoring potential effects in workers.*
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> Breakout Group C. Pro: ɣH2AX **is ready** for use to screen/monitor an at-risk population
> Breakout Group D. Con: ɣH2AX **is not ready** for use to screen/monitor an at-risk population

Background Reading (Optional)

Bryce S.M., Bernacki D.T., Smith-Roe, S.L., Witt, K.L., Bemis J.C., Dertinger S.D. (2018) Investigating the Generalizability of the MultiFlow DNA Damage Assay and Several Companion Machine Learning Models With a Set of 103 Diverse Test Chemicals. *Toxicol Sci*. 162(1):146-166. https://pubmed.ncbi.nlm.nih.gov/29106658/

Kopp B., Khoury L., Audebert M. Validation of the ɣH2AX biomarker for genotoxicity assessment: a review. Arch Toxicol. 2019;93(8):2103-2114. doi:10.1007/s00204-019-02511-9

Kuo L. J., Yang L. X. (2008) Gamma-H2AX - a novel biomarker for DNA double-strand breaks. In Vivo 22, 305-309, https://www.ncbi.nlm.nih.gov/pubmed/18610740

Rogakou E. P., Boon C., Redon C., Bonner W. M. (1999) Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol 146, 905-916, https://www.ncbi.nlm.nih.gov/pubmed/10477747

Hsieh J.H., Smith-Roe S.L., Huang R., et al. (2019) Identifying Compounds with Genotoxicity Potential Using Tox21 High-Throughput Screening Assays. Chem Res Toxicol. 32(7):1384-1401. doi:10.1021/acs.chemrestox.9b00053

[4] https://ehjournal.biomedcentral.com/articles/10.1186/s12940-016-0182-4
[5] https://pubmed.ncbi.nlm.nih.gov/32154776/