Center for Drug Evaluation and Research **Food and Drug Administration**

Date: October 24, 2017

ATTN: HESI Committee on Genomics
c/o HESI Executive Director, Syril Pettit
HESI
1156 15th St. NW, 2nd Floor
Washington, DC 20005

Subject: Biomarker Letter of Support

Dear HESI Genomics Committee:

We are issuing this Letter of Support to the HESI Committee on the Application of Genomics to Mechanism-based Risk Assessment (HESI Genomics Committee) to encourage the further study and use of the genomic biomarker panel TGx-DDI intended to facilitate safety genotoxicity hazard assessment in nonclinical studies. For a listing of the components of the TGx-DDI biomarker signature, please see Appendix I.

Although the standard genotoxicity testing battery recommended by the International Conference on Harmonization, ICH S2(R1), has been successful in preventing the introduction of harmful carcinogens to the marketplace, the interpretation of positive genotoxicity findings in the in vitro chromosome damage assays for compounds with otherwise negative results in the Salmonella reverse mutation assay and in vivo micronucleus test is a major challenge to both industry and regulatory agencies. We support the HESI Committee's efforts to develop a rigorous panel of biomarkers to address the need for a reliable follow-up approach for compounds that exhibit these characteristics. We are encouraged by the TGx-DDI biomarker's potential to enhance future testing efficiency and relevance by offering an alternative follow-up test in lieu of an *in vivo* comet assay in some cases.

The currently available data from your presented research and publications support the potential for TGx-DDI biomarker panel to be of value to drug development. Because the positive findings in the in vitro chromosome damage assays could be caused by secondary events such as cytotoxicity that are not relevant to human safety, the TGx-DDI biomarker panel is a promising opportunity to bring additional mechanistic insights and reduce additional follow-up testing. In this capacity, the application of the genomic biomarker TGx-DDI could complement the standard genotoxicity battery ICH S2(R1) option 1. More experience with the use of this biomarker in genetic toxicology testing would be useful to more accurately determine its utility

to complement ICH S2(R1) option 1 battery (e.g., additional case studies of application of TGx-DDI in genotoxicity risk assessment).

The impact of analytical technology on TGx-DDI biomarker performance needs to be evaluated including exploring higher throughput analytical technologies. No specific test system or analytical validation process is endorsed for the TGx-DDI biomarker panel. The analytical performance characteristics should be established in advance of use. The sample stability for the TGx-DDI panel proposed herein should be validated for its intended use conditions. Strong emphasis on applying good scientific, laboratory, and software development practices for quality control and validation of TGx-DDI is imperative.

We encourage further exploration of genomic biomarker TGx-DDI as a potential complement to ICH S2(R1) option 1 battery to address human relevance and risk of compounds that tested positive in the in vitro chromosome assay and negative in both Salmonella and in vivo micronucleus assays. We will consider data collection on this biomarker to be exploratory in nature. The validity of the intended use of these biomarkers has not been fully determined and, therefore, biomarker findings should be interpreted in the context of results for traditional biomarkers and clinical and nonclinical findings. We believe data sharing and integrating data across trials can foster an accelerated path for drug development programs. If sponsors intend to include analyses of this biomarker to support regulatory decision making for a given IND drug development program, they should prospectively discuss the approach to these analyses with the CDER.

Any groups (academia, industry, government) that would like to join in this effort or have information or data that may be useful can contact HESI Executive Director, Syril Pettit, spettit@hesiglobal.org.

Signed:

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Timothy Robison, PhD
Chair, OND Predictive Testing Coordinating Committee for Genotoxicity

Appendix I

The TGx –DDI biomarker panel consists of 64 stress-responsive genes [1] that have been optimized in human TK6 cells, which are commonly employed in standard in vitro toxicology assays. The TGx-DDI biomarker panel was derived as follows:

- **Experimental Cell and mRNA analysis:** TK6 human lymphoblastoid cells were exposed to test agent for 4 hrs alongside concurrent controls, and RNA collected. mRNA expression profiles were derived. Profiles were initially derived by DNA microarrays, but current work is establishing the use of qPCR and high-throughput transcriptional profiling methods.
- **Defining the Biomarker TGx-DDI:** The biomarker is characterized by gene expression changes in a defined set of 64 genes that are primarily regulated by p53. The biomarker was derived from gene expression profiles from an initial training set of 28 prototype chemicals (13 genotoxic, 15 non-genotoxic).
- **Data interpretation:** The analytical approach to application of TGx-DDI consists of three components. (1) nearest shrunken centroids were used for probability analysis (to predict whether the agent is p>0.9 DNA damaging, p>0.9 non-DNA damaging, or not classifiable). (2) 2-dimensional clustering. (3) Principle component analysis with the original 28 reference compounds.

List of the 64 stress-responsive genes that compose the TGx-DDI biomarker panel (Genbank information found at https://www.ncbi.nlm.nih.gov/gene)

Gene Symbol	Description	Genbank Accession/ Gene ID
ACTA2	actin, alpha 2, smooth muscle, aorta	NM_001613
AEN	apoptosis enhancing nuclease	NM_022767
ARRDC4	arrestin domain containing 4	NM_183376
B3GNT2	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	NM_006577
BLOC1S2	biogenesis of lysosomal organelles complex-1, subunit 2	NM_001001342
BRMS1L	breast cancer metastasis-suppressor 1-like	NM_032352
BTG2	BTG family, member 2	NM_006763
C12orf5	chromosome 12 open reading frame 5	NM_020375
CBLB	Cas-Br-M (murine) ecotropic retroviral transforming sequence b	NM_170662
CCP110	centriolar coiled coil protein 110kDa	NM_014711
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	NM_078467
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	NM_005195
CENPE	centromere protein E, 312kDa	NM_001813
COIL	coilin	NM_004645
DAAM1	dishevelled associated activator of morphogenesis 1	NM_014992
DCP1B	DCP1 decapping enzyme homolog B (S. cerevisiae)	NM_152640
DDB2	damage-specific DNA binding protein 2, 48kDa	NM_000107
DUSP14	dual specificity phosphatase 14	NM_007026
E2F7	E2F transcription factor 7	NM_203394
E2F8	E2F transcription factor 8	NM_024680

EI24	etoposide induced 2.4 mRNA	NM_004879
FAM123B	family with sequence similarity 123B	NM_152424
FBXO22	F-box protein 22	NM_147188
GADD45A	growth arrest and DNA-damage-inducible, alpha	NM_001924
GXYLT1	glucoside xylosyltransferase 1	NM_173601
HIST1H1E	histone cluster 1, H1e	NM_005321
HIST1H2BB	histone cluster 1, H2bb	NM_021062
HIST1H2BC	histone cluster 1, H2bc	NM_003526
HIST1H2BG	histone cluster 1, H2bg	NM_003518
HIST1H2BI	histone cluster 1, H2bi	NM_003525
HIST1H2M	histone cluster 1, H2bm	NM_003521
HIST1H2BN	histone cluster 1, H2bn	NM_003520
HIST1H3D	histone cluster 1, H3d	NM_003530
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	NM_002166
IKBIP	IKBKB interacting protein	NM_153687
ITPKC	inositol-trisphosphate 3-kinase C	NM_025194
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	NM_002222
LCE1E	late cornified envelope 1E	NM_178353
LRRFIP2	leucine rich repeat (in FLII) interacting protein 2	NM_006309
MDM2	Mdm2 p53 binding protein homolog (mouse)	NM_002392
MEX3B	mex-3 homolog B (C. elegans)	NM_032246
NLRX1	NLR family member X1	NM_170722
PCDH8	protocadherin 8	NM_002590
PHLDA3	pleckstrin homology-like domain, family A, member 3	NM_012396
PLK3	polo-like kinase 3	NM_004073
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	NM_003620
PRKAB1	protein kinase, AMP-activated, beta 1 non-catalytic subunit	NM_006253
PRKAB2	protein kinase, AMP-activated, beta 2 non-catalytic subunit	NM_005399
PTGER4	prostaglandin E receptor 4 (subtype EP4)	NM_000958
RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	NM_014247
RBM12B	RNA binding motif protein 12B	NM_203390
RPS27L	ribosomal protein S27-like	NM_015920
RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	NM_015713
SEL1L	sel-1 suppressor of lin-12-like (C. elegans)	NM_005065
SEMG2	semenogelin II	NM_003008
SERTAD1	SERTA domain containing 1	NM_013376
SMAD5	SMAD family member 5	NM_001001419
TM7SF3	transmembrane 7 superfamily member 3	NM_016551
TNFRSF17	tumor necrosis factor receptor superfamily, member 17	NM_001192

TOPORS	topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase	NM_005802
TP53I3	tumor protein p53 inducible protein 3	NM_004881
TRIAP1	TP53 regulated inhibitor of apoptosis 1	NM_016399
TRIM22	tripartite motif containing 22	NM_006074
USP41	Ubiquitin Specific Peptidase 41	NM_937988