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A cross-sector call to improve carcinogenicity risk assessment through use of genomic methodologies

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Abstract

Robust genomic approaches are now available to realize improvements in efficiencies and translational relevance of cancer risk assessments for drugs and chemicals. Mechanistic and pathway data generated via genomics provide opportunities to advance beyond historical reliance on apical endpoints of uncertain human relevance. Published research and regulatory evaluations include many examples for which genomic data have been applied to address cancer risk assessment as a health protection endpoint. The alignment of mature, robust, reproducible, and affordable technologies with increasing demands for reduced animal testing sets the stage for this important transition. We present our shared vision for change from leading scientists from academic, government, nonprofit, and industrial sectors and chemical and pharmaceutical safety applications. This call to action builds upon a 2017 workshop on “Advances and Roadblocks for Use of Genomics in Cancer Risk Assessment.” The authors propose a path for implementation of innovative cancer risk assessment including incorporating genomic signatures to assess mechanistic relevance of carcinogenicity and enhanced use of genomics in benchmark dose and point of departure evaluations. Novel opportunities for the chemical and pharmaceutical sectors to combine expertise, resources, and objectives to achieve a common goal of improved human health protection are identified.

Keywords

Cancer risk assessment; Genomics; Toxicogenomics; Cancer; Carcinogenicity; Risk Assessment

1. Introduction

The potential of chemicals (i.e., via environmental exposure in food, water, air, consumer products, and occupational exposures) and pharmaceuticals to cause carcinogenesis is an

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important societal risk and drives significant animal use and financial investment for testing. The most widely accepted approaches to carcinogenicity assessment introduced in the 20th century involve testing rodents for lifetime cancer incidence during or following toxicant exposure. While rodent bioassays are considered broadly protective of human risk for carcinogenicity, they also have many shortcomings (Hoenerhoff et al., 2009; Huff et al., 2008). Novel insights into molecular mechanisms, the role of dose and exposure in activating these mechanisms, and knowledge of human relevant (and nonrelevant) pathways offer timely opportunities to adopt more translational, less animal-intensive, and more exposure-driven approaches to improve cancer assessment (Wolf et al., 2019).

A variety of robust omics approaches, including genomics (sequence analysis), epigenomics, transcriptomics, proteomics, and metabolomics, are available to query the mechanisms underlying toxicological responses and individual susceptibility. Transcriptomics (methods used to profile alterations in RNA expression) is arguably the most widely used toxicogenomic tool. Transcriptomic analyses have provided an increasingly robust body of evidence around factors responsible for perturbing relevant molecular pathways and enabling the development of cancer (Hanahan and Weinberg, 2011). The field's evolution has been fueled by advances in technologies used to measure and analyze global gene expression changes. Methodologies have evolved from limited probe sets on glass slides in the 1990s (Rockett and Dix, 1999), to standardized methodologies for whole transcriptome RNA-sequencing (Auerbach et al., 2015; Marioni et al., 2008), and to high-throughput transcriptomic assays such as TempO-seq or the Nanostring nCounter technology. In parallel, advances in transcriptomic data storage and recommended analytical and reporting best practices [e.g., Microarray Sequencing Quality Control (MAQC) (Shi et al., 2017; Su et al., 2014b), Minimal Information About Microarray Experiment (MIAME)/Minimum Information about a high throughput Sequencing Experiment (MINSEQE) (Brazma, 2009)] have allowed for a measure of standardization. Working groups have been initiated to develop reporting standards (Gant et al., 2017) and good-laboratory practice recommendations (Kauffmann et al., 2017) for regulatory applications. The Organisation for Economic Cooperation and Development (OECD) has initiated projects to develop reporting frameworks for data generation and analysis, to ensure that regulators will have the information required to assess the quality of data generated in a study and evaluate its suitability for use in risk assessment. A variety of robust transcriptomic approaches are now available to identify molecular initiating events and subsequent key events in a mode of action (MOA) framework and to define the associated dose-response characteristics.

Despite methodological advances and regulatory efforts to provide guidance, routine use of toxicogenomic data to support carcinogenicity assessment has been limited (e.g., Yauk et al., 2019). To understand and remove some of the potential hurdles, the nonprofit Health and Environmental Sciences Institute (HESI) Emerging Systems Toxicology in the Assessment of Risk (eSTAR) committee convened a forum, in cooperation with McGill University and Health Canada, on "Advances and Roadblocks for Use of Genomics in Cancer Risk Assessment." By convening scientists from the pharmaceutical and chemical sectors along with experts from academia, government, and industry, the workshop enabled the identification of intersecting opportunities that could be used to drive implementation of toxicogenomics to advance cancer risk assessment. During the workshop, participants

observed that a major hurdle to implementation is a lack of consensus on defined applications for, and interpretations of, toxicogenomic data in cancer risk assessment. Such clarity will be required to move beyond the currently limited use of toxicogenomic data in generating testable hypotheses and derisking critical observations in lifetime rodent studies. This report discusses the workshop recommendations with the addition of contemporary applications in the pharmaceutical and chemical sectors. We offer a call to action to enhance implementation of genomics in carcinogenicity risk assessment and to reduce future reliance on conventional 2-year rodent carcinogenicity testing.

2. Contemporary regulatory applications

The nature and extent of toxicogenomics data use in the pharmaceutical industry has been inconsistent over the years since initial adoption in the late 1990s. However, there is a current resurgence in interest given the availability of improved technologies, analytical approaches, and evidence of robust translational value in specific contexts of use in cancer risk assessment. This resurgence is paralleled by increasingly routine application of toxicogenomics toward safety evaluation of industrial and consumer chemical products. This momentum is driven, in part, by the need to embrace emerging technologies and tools in the consumer product and chemicals safety arena to address legislative requirements and public pressure to reduce testing in whole animals. The effort is aided by a broadly recognized desire to introduce efficiencies in assessing chemical risks with regard to shortening time of testing and reducing cost.

Although there are commonalities, the application domains of toxicogenomic data in pharmaceuticals versus chemical assessments can also be unique. The increased use of toxicogenomic approaches in the chemical and pharmaceutical sectors provides an opportunity to leverage and cross-purpose these learnings. The more extensive regulatory experience for incorporating toxicogenomic data in the chemical sector may provide opportunities to advance novel applications in the pharmaceutical regulatory sector. In contrast, the routine use of toxicogenomics to inform mechanistic studies and biomarker development in pharmaceutical research and development may be translated into cutting-edge predictive toxicology tools for chemical assessment. Further discussion of opportunities to advance the field of toxicogenomics and implement near-term tools/solutions for contemporary challenges in cancer risk assessment follows below.

2.1. Applications and opportunities in common to chemicals and pharmaceuticals testing

Within the agriculture, chemicals, and pharmaceuticals sector, the primary use of toxicogenomic information to date has been in supporting MOA analyses. This applies to understanding both carcinogenic mechanisms and human relevance, but also to the full spectrum of potential toxicological outcomes. In these studies, investigators observe coordinated changes in transcripts ascribed to specific molecular pathways or biological functions to make inferences around the biological response of the exposed tissue following chemical exposure. Integration of pathway-level perturbation data with observed apical effects contributes to the overall weight of evidence for a critical effect. What is often not straightforward is deriving a biological interpretation from the gene expression changes.

However, molecular pathway analysis software tools, such as Ingenuity Pathway Analysis and GeneGO MetaCore, are useful for data reduction and interpretation. Such tools provide curated gene sets and utilize computational approaches to assess pathway representation of differentially expressed gene transcripts in the dataset. Indeed, the primary use of toxicogenomic data at the US Environmental Protection Agency (EPA) (e.g., the EPA Integrated Risk Information System or IRIS) and at Health Canada has been in supporting MOA (Bourdon-Lacombe et al., 2015; Yauk et al., 2019) and there is clear overlap in these types of analyses across the pharmaceutical and agricultural-chemical sectors. Moving forward, pathway analysis to define MOA will continue to be a key application for genomic data across sectors.

More rapid extraction of MOA information from complex transcriptomic datasets is achieved through the use of transcriptomic biomarker signatures. Transcriptomic biomarkers provide an efficient and nonsubjective way to extract MOA information from complex biosets. For example, the TGx-DDI biomarker is a 64-gene transcriptomic biomarker that is used to differentiate DNA damage-inducing from non–DNA damage-inducing agents in human cells in culture (Buick et al., 2015; Li et al., 2015; Yauk et al., 2016). The biomarker has been extensively validated and demonstrated to perform accurately on modern high-throughput transcript profiling platforms (Li et al., 2017). In addition, it has been used in case studies of both pharmaceuticals and chemicals to support whether a toxicant operates through a genotoxic MOA that is relevant to humans (Moffat et al., 2015). Another powerful example of the practical application of such a biomarker-based approach was recently published by Rooney et al. (2018). The authors developed and applied rodent in vivo transcriptomic biomarkers of genotoxicity, cytotoxicity, aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), estrogen receptor, and peroxisome proliferator-activated receptor α (PPARα) and associated downstream effects (oxidative stress, cell proliferation, liver to body weights). The accuracy of the biomarkers in predicting these key events ranged from 91% to 98% and the authors found that tumorigenic doses of the chemicals gave the highest-ranking scores. These studies demonstrate that transcriptomic signatures provide a useful quantitative approach to predict carcinogenicity and have potential to inform associated human relevance from short-term studies that is relevant to both pharmaceutical and chemical evaluation. Additional foundational work has demonstrated that these types of signatures/biomarkers are highly consistent across technologies and can, in some cases, even be cell-type agnostic (e.g., Buick et al., 2015; Li et al., 2017; Su et al., 2014a). Thus, transcriptional biomarkers provide a clear example of realizable/viable opportunities for immediate use. The TGx-DDI biomarker is already undergoing formal validation through the US Food and Drug Administration’s Biomarker Qualification Program. The workshop participants noted that other relevant transcriptomic signatures are also available for validation. Immediate efforts should focus on identifying these biomarkers, defining how validation should proceed, and undertaking qualification exercises to enable formal application in regulatory decision-making in the near term.

A tool that was envisioned to facilitate the use of toxicogenomic data in risk assessment, in particular in the area of MOA development and predictive toxicology, is the adverse outcome pathway (AOP) framework. AOPs describe a sequential series of events that begin at the molecular level and proceed through to organ, individual and population levels, to

describe how toxicants exert their adverse effects (OECD, 2018). The framework provides a construct to organize information and knowledge and assess the weight of evidence supporting a pathway. Specific pathway perturbations can be used to measure key events in an AOP enabling use in risk assessment (e.g., in integrated approaches to testing and assessment; OECD, 2019). A variety of groups have explored the use of transcriptional data in assessing AOPs. Databases, such as the Comparative Toxicogenomics Database (https://www.ctdbase.org), seek to curate these data, describing the relationships between xenobiotic agents, gene transcripts or proteins, disease states, phenotypes, gene ontology annotations, pathways, and interaction modules. Case studies have demonstrated how such databases can be used to construct AOPs (Davis et al., 2018). Quantitative AOPs (i.e., those that define the thresholds and response relationships across key events) provide the empirical data and mathematical models for predictive toxicology (Foran et al., 2019; Perkins et al., 2019; Wittwehr et al., 2017). To date, AOPs have been a focus primarily in the chemicals sector, although it is clear that many of the pathways being developed encompass MOAs that would also be relevant to pharmaceuticals. AOPs provide a clear opportunity and tool for cross-sector collaboration to advance the use of predictive toxicogenomic information in risk assessment. Resources should be invested to develop relevant AOPs for predominant MOAs in carcinogenesis.

2.2. Applications and opportunities in pharmaceutical safety applications

While there appear to be very few examples of genomic data being submitted in support of pharmaceutical regulatory submissions, the integration of transcriptomic, epigenomic, and genetic indicators of neoplastic risk offers great potential to strengthen MOA-based cancer risk assessment of therapeutics (Fielden et al., 2018; Moggs et al., 2016; Peffer et al., 2018; Rooney et al., 2018; Terranova et al., 2017). Applications in drug development include the following: 1) elucidation of on- and/or off-target mechanisms to support potential mitigation of positive rodent carcinogenicity findings (e.g., through demonstration of species-specific molecular pathways); 2) preclinical biomarker discovery to support decision-making; and 3) enhanced cancer risk assessment of drug targets through genotype-phenotype association data.

As described above, a major area of focus has been in the quest for predictive transcriptomic biomarkers that reflect early molecular changes associated with carcinogenesis. Indeed, there are many examples of how such biomarkers can be used to understand MOA and human relevance for cancer risk assessment. However, transcriptomic biomarker development has been limited by several factors, including the diverse range of tissue-, strain-, and species-specific tumors that are observed in rodent carcinogenicity studies. Furthermore, the potential for contributions to rodent carcinogenicity from both on- or off-target properties of therapeutics makes the determination of MOA and assessment of human relevance very challenging. Nevertheless, mechanistic studies that integrate genomic and phenotypic endpoints have been successfully used to support the interpretation of drug-induced tumors and can provide particularly valuable perspectives on potential relevance for humans in pharmaceutical testing (Table 1). For example, in the case of rodent liver tumors associated with defined nuclear receptor-mediated MOAs (e.g., activation of CAR, PPARα, and/or AhR), there is a clear opportunity to leverage established preclinical toxicogenomic

biomarkers to support decision-making (Gusenleitner et al., 2014; Peffer et al., 2018; Rooney et al., 2018). Two primary uses for these transcriptomic biomarkers can be envisioned: 1) to derisk positive carcinogenicity findings through the demonstration that the MOA is not relevant to humans; and ultimately 2) to provide a rationale for a potential waiver of rat carcinogenicity studies (ICH, 2016). The latter is supported by evidence that both histologic signals that emerge from chronic rat studies (Sistare et al., 2011), as well as all available knowledge of the intended pharmacologic target (van der Laan et al., 2016a, 2016b), can be good predictors of risk for carcinogenesis and will provide early direction for such molecular investigations.

During the 2017 workshop, particular attention was paid to a prospective regulatory study that was initiated 6 years ago within the framework of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The goal of the study is to evaluate the predictive value of pharmacological and toxicological data available at the end of Phase II to estimate the outcome of a rat 2-year carcinogenesis study in order to obtain a (only virtual) waiver. These datasets generally did not include toxicogenomic data and utilized a NegCarc (Negative for Endocrine, Genotoxicity, and Chronic Study Associated Histopathologic Risk Factors for Carcinogenicity) approach focusing on the absence of toxicity—that is, absence of histologic risk factors for carcinogenicity in 6-month rat toxicity studies (Bourcier et al., 2015; Sistare et al., 2011). This was extended by analysis of the pharmacological properties of the compounds in this database and its relationship with the observation of tumors in specific organs. Participants noted that data on the pharmacological profile of the compound are highly important for both negative and positive prediction (van der Laan et al., 2016a, 2016b). A positive prediction should be given when the stimulation of the pharmacological target would lead to a proliferative or transformative action. A negative prediction based on pharmacology might be more difficult to prove, as the absence of an effect might require more effort.

The basis for an evidentiary standard in a presumptive negative toxicogenomic finding may be challenging and should be supported by additional data. The relation between pharmacological profile and tumor response should be understood in the framework of the key factors that are descriptive of cancer, as spelled out in more detail and acknowledged in section 1 (Hanahan and Weinberg, 2011). Pathway analysis is important to support the relation between the pharmacological effector and the intracellular response following that effector stimulation. In addition, the following question should be answered: Is there a direct or indirect stimulation of cell growth and proliferation as a consequence of the pharmacological effector function? For first-in-class compounds, a higher evidentiary standard may be needed to confirm that these compounds would not induce some type of proliferation or transformation resulting in a mutationally conveyed clonal growth advantage leading to tumorigenesis. Key questions that may be answered are the stimulation of specific AOPs that lead to tumor promotion (i.e., cell proliferation, inflammation, or immunosuppression). As also outlined in section 2.1, modulation of such AOPs by pharmaceuticals or other chemicals may be an alert triggering further investigation on the dose-response relationship and translatability of the mechanism to humans. In this setting, “omics-methodology” might be of significant utility to not only evaluate whether a critical effect is present in rodents but to also assess its relevance for humans.

In addition to deploying genomic approaches for evaluating the carcinogenic potential of compounds during drug development, there is also a trend toward leveraging genetic models and genome resources to investigate the potential association of intended drug pharmacologic and/or pathway target modulation with tumorigenic phenotypes (Fielden et al., 2018; Moggs et al., 2016). The power of integrating drug target/pathway (epi)genotype-phenotype associations is exemplified by an analysis of the potential of therapeutic fumarates to lead to oncogenic outcomes (Fuhler et al., 2017). Human mutations in fumarate hydratase (Fuhler et al., 2017) have been associated with renal cell cancer, and a putative mechanism has been proposed based on mechanistic studies in cellular models involving fumarate accumulation followed by inhibition of alpha-ketoglutarate–dependent TET dioxygenases and resultant alteration in the DNA methylation status of genes that regulate epithelial-to-mesenchymal transition. In addition, it is noteworthy that several years prior to these molecular pathway-related insights, oral administration of dimethyl fumarate to mice and rats in 2-year carcinogenicity studies was reported to result in kidney adenomas and carcinomas (https://www.ema.europa.eu/en/medicines/human/EPAR/tecfidera).

The drug regulatory authorities in this ICH process have written status reports (ICH, 2017, 2019) to reflect intermittently on the s that have been evaluated. In the 2017 report, it was indicated that there is lack of “omic-methodology” in the submitted carcinogenicity assessment reports. With further emphasis on early MOA analysis and incentive from a real waiver of a 2-year bioassay, it can be expected that the inclusion of toxicogenomic information as supporting data will increase.

A reasonable vision for the future of carcinogenicity testing must consider strategies defined both by known on-target pharmacologic pathways, as well as all evidence for those off-target drug liabilities that have been identified to raise reasonable concerns of carcinogenic potential. Molecular transcriptomic biomarkers of known mechanisms of tumorigenesis that have been cataloged over decades of pharmaceutical testing will provide a strong foothold to this vision. There is now a timely opportunity to generate, centralize, and publicly disseminate rigorous and reproducible data on these transcriptomic biomarkers. This effort will be required to support their reliable and consistent use in pharmaceutical or other safety assessment decision-making and carcinogenicity testing strategies. There is also a need to develop foundational datasets that establish the strengths and limitations for new technologies (e.g., error-corrected sequencing) to complement transcriptomic mechanistic insights, by identifying when sustained pharmacologic or toxicologic action will drive genesis of growth advantaged clonal cellular populations in tissues by generating critical mutations in key driver genes (Schmitt et al., 2012). Tissue samples from standard subchronic and chronic rat studies can be leveraged for these assessments and inform larger discussions about the routine need for 2-year rodent studies.

2.3. Applications and opportunities in chemical safety applications

The tools being developed and deployed above will be critical to advancing chemical risk assessment. However, a particular area of challenge that is unique to the chemicals sector is the prevalence of thousands of chemicals in commerce that have not been subjected to conventional toxicological tests (i.e., legacy chemicals). Such data-poor chemicals are in

urgent need of data to support toxicological assessment. Advances in high-throughput sequencing technologies have made screening and prioritization possible with transcriptomic data to address these data-poor substances (e.g., through use of NanoString and TempO-seq technologies). Such an approach requires not only extraction of hazard and MOA information but also the ability to derive a point of departure (POD), or the dose at which an effect is observed. In this context, benchmark dose (BMD) modeling has been adopted to evaluate both in vivo and in vitro dose response to identify transcriptional PODs for screening chemicals (NTP, 2018). The underlying principle is that environmental encounters with chemicals ideally should not result in gene expression changes in human tissues. In these studies, a transcriptional POD is calculated based on gene expression data, either singly or grouped into transcriptional pathways, using a free software program called BMDExpress (https://github.com/auerbachs/BMDExpress-2/wiki; Phillips et al., 2019; Yang et al., 2007). Assessment of a transcriptional POD can be used to compare potencies to prototype toxicants or coupled with estimates of human in vivo internal exposure to the chemical (for in vitro studies through in vivo to in vitro extrapolation) to determine which chemicals pose a greater risk to public health and thus should be prioritized for further testing. The pipeline for transcriptional POD determination is still emerging across agencies, but a recent report outlines the approach that the US National Toxicology Program (NTP) is following based on feedback from an external expert panel review and thus makes strides toward best practices (NTP, 2018).

Several promising case studies provide enthusiastic support for the approach described above. A key finding of these studies is that the dose at which transcriptional activity is initiated in short-term rodent tests is highly concordant with the dose (i.e., BMD) at which the lowest adverse apical effects, including cancer, occur in chronic animal tests (e.g., Thomas et al., 2013). This has led to an emerging idea to use short-term in vivo transcriptional studies in emergency situations where a provisional POD is quickly needed. In these studies, rodents are exposed to a variety of dose levels of a chemical, (usually) livers are extracted as a sentinel organ, and transcriptional POD data are assessed. The lowest transcriptional pathway BMD is considered to be the dose at which the chemical is expected to exert an effect. The NTP has established a reporting framework for short-term rat toxicogenomic studies, which is exemplified by the recent transcriptional potency study of the aromatic phosphate flame retardant triphenyl phosphate (Gusenleitner et al., 2014). Such studies have the potential to impact both chemical testing prioritization and dose selection for longer-term toxicity tests. Thus, there is great opportunity to use transcriptional BMD analysis to screen chemicals to establish acceptable exposure levels based on identifying the dose below which there is no biological activity, even in the absence of MOA information.

A notable implementation of this approach followed the release of approximately 10,000 gallons of liquid mixture containing crude methylcyclohexanemethanol (MCHM) into the Elk River, contaminating the drinking water supply for residents of Charleston, West Virginia. The Centers for Disease Control (CDC) requested that the NTP perform studies in a 1-year time frame to address lingering toxicity-related questions around chemicals involved in the spill, owing to reports of residents manifesting symptoms of chemical exposure including skin rash or irritation, diarrhea, nausea, and respiratory illness. An initial CDC recommended drinking water screening level was set at 1 ppm (0.1 mg/kg/day), owing

to a lack of available toxicological data on MCHM. In short-term (5-day) rat toxicogenomic studies in which a range of doses were tested (six doses, ranging from 1 to 2000 mg/kg/day), the transcriptome was measured in the liver and BMD modeling was applied to identify transcriptional PODs for individual transcripts and aggregate pathways. Concordant with a battery of related tests that included rat developmental toxicity studies, short-term toxicogenomic studies in adult rats suggested a POD of 100 mg/kg/day, corresponding to 1000 ppm in drinking water for an infant (CDC, 2014). The NTP thus concluded that sensitive molecular changes found in rats would be unlikely to occur in humans at the screening level (NTP, 2016) recommended by the CDC at the time of the spill.

Other applications unique to the data-poor chemicals area is use of toxicogenomic data in justification of chemical groupings and read-across (e.g., Grimm et al., 2016). With a growing database of transcriptional profiles against which to identify perturbed signatures or correlations in expression profiles (e.g., through connectivity mapping; Caiment et al., 2013), it is easy to foresee how these approaches could prove to be highly informative during initial stages of chemical assessment.

Overall, it is realistic to envision that transcriptomic data will be available in the upcoming years, through initiatives undertaken within the US EPA and elsewhere, for hundreds of substances requiring evaluation. The resulting public database could serve as an opportunity for which transcriptional biomarkers for key events in AOPs for carcinogenicity assessment may be developed, as many of the chemicals initially tested in high-throughput transcriptomic screens will have concordant ToxCast and Tox21 data, and in some cases, in vivo and exposure data. At the same time, parallel efforts within the NTP Carcinogenicity Health Effect Innovation Center, industry, HESI eSTAR Committee, and related agencies and consortia toward modernizing carcinogenicity testing should include transcriptomics as an endpoint toward the overall weight-of-evidence approach.

3. Proposed collaborative path forward to enhance use of toxicogenomics in risk assessment across sectors

Our broad overview of recent advances in toxicogenomics reveals some clear areas where collaborative focus from both the pharmaceutical and chemicals sector could mutually benefit and improve cancer risk assessment approaches. In particular, three synergistic areas emerged from our discussions for immediate attention: AOP development, genomic dose-response modeling, and toxicogenomic biomarkers. AOP development and genomic dose-response modeling have been a predominant focus for chemicals risk assessment; in contrast, the development and application of predictive toxicogenomic biomarkers has been primarily driven by pharmaceutical applications. The working group envisions a future where these sectors work together to define the events occurring in expert-endorsed AOPs that describe links between molecular initiating events and the associated transcriptomic response thresholds (along with other related pathologic or toxicological evidence) that must be surpassed to lead to downstream adverse events and cancer. Threshold doses would be derived through computational dose-response modeling to enable the robust and quantitative application of toxicogenomics to predict cancer outcomes. In parallel, experience gained in

biomarker development and validation primarily in pharmaceutical contexts could be harnessed to create panels of *in vitro* and *in vivo* toxicogenomic biomarkers aligned against critical/predictive molecular initiating events. Such biomarkers could enable rapid hazard identification and, through integration with quantitative AOPs and possibly other toxicological or pathological evidence, could be used for predictive toxicology to refine or replace animal testing. Below, we broadly describe how the respective communities should collaborate in these areas to advance this vision.

AOPs:

AOP development will require describing mutually agreed-upon molecular initiating and key events involved in different pathways to cancer, the methods used to measure each event, the weight of evidence supporting the relationship between key events and the overall outcomes, and expert-informed consensus on the human relevance of the pathways. Such efforts would clearly lead to more harmonized and focused methodology development and application. AOP development requires collaborative multi-disciplinary efforts to transparently document the linkages between molecular, cellular, organ, tissue and individual level responses to toxicants. Publication in the AOP-wiki (https://aopwiki.org) enables real-time updating of pathways with the most recent empirical data and methodologies for key event analysis, and facilitates collaboration and crowd-sourcing for further development and application. Given the modular nature of AOP development (built in individual key event and key event relationship modules), the creation of a few foundational AOPs could greatly reduce the workload for creating other AOPs, through re-use/sharing of central modules. Initial AOPs should cover targets that are relevant to both chemicals and pharmaceutical sectors. We thus advocate that the pharmaceuticals sector become involved in *de novo* development, refinement, review, and endorsing of the AOPs of mutual interest to the chemicals sector.

Genomic dose-response evaluation:

User-friendly computational tools to establish dose-response relationships for every gene/pathway in the transcriptome have advanced tremendously over the past decade in the chemicals arena, with little uptake in pharmaceuticals. The methodologies for BMD modeling of both genomic and apical endpoints is mature. It is clear that mathematical modeling of transcriptomic data can now be applied to readily identify the doses at which effects at other levels of biological organization manifest (described above). Quantitatively establishing the extent to which transcriptional alterations within a pathway are required before downstream changes at the organ/tissue level occur will be critical for the use of transcriptional biomarkers in risk assessment and for future replacement of longer-term animal tests (i.e., predictive toxicogenomics in shorter-term assays or serially collected during interim timepoints in longer-term *in vivo* studies). To fully harness the power of genomic dose-response modeling for this purpose, the expert communities must establish consensus on best practices and apply these tools together toward this objective.

Toxicogenomic biomarkers:

The sectors should clearly come together to identify robust toxicogenomic biomarkers (e.g., gene expression signatures) that predict the relevant molecular initiating and key events described in AOPs that lead to cancer outcomes. Transcriptomic signatures have been

demonstrated to be powerful predictors of toxicological changes that can be integrated in risk assessment. Priority signatures should be subject to validation or performance assessment to characterize their specificity/sensitivity and to define their domains of application. The data are currently available to mine and validate transcriptomic biomarkers. As described above, validation of several such signatures effectively establishes the framework by which new toxicogenomic signatures can be more readily qualified for use in different risk assessment contexts. For example, *in vitro* transcriptomic biomarkers can be used for lead prioritization and in read-across, using gene expression databases and datasets to identify potential hazards. In contrast, the integration of highly predictive biomarkers with short-term rodent tests could reduce the need for rodent cancer bioassays in certain regulatory decision-making contexts in chemicals and pharmaceutical assessment.

The availability of quantitative, predictive toxicogenomic biomarkers aligned against cancer-outcome AOPs will have many applications in risk assessment in both the pharmaceutical and chemicals sectors. Once constructed, validated and endorsed, the relevant industry/regulatory authorities will need to determine and agree upon the suitable contexts of use across decision-making processes. We call upon the communities to work together to achieve this vision. Below we describe efforts of the HESI eSTAR committee to advance this objective.

4. Conclusions

The contemporary state of the science and an international workshop demonstrated that it is clearly time to harvest the wealth of genomic data and associated tools for risk assessment and develop consensus on application and interpretation. Now is the time to merge core scientific priorities across chemical and pharmaceutical disciplines with academic experts and regulatory stakeholders. It is time to collaborate to develop AOPs with aligned toxicogenomic biomarkers for essential key events, and define associated thresholds that can reliably inform dose-responsive tumor biology. And there is ample opportunity because regulatory authorities now have the required infrastructure to accept the data, have appealed for submissions to include these data, and have explained what is needed to qualify such tools and tissue biomarkers (FDA, 2018; Health Canada, 2019).

Motivated by the workshop and call to action, the HESI eSTAR Committee recently launched a subcommittee to implement genomic strategies within the ICH framework for cancer risk assessment. This newly formed eSTAR Carcinogenomics Working Group will collaborate across >20 institutions to extract public and shared private toxicogenomic data, identify or develop toxicogenomic biomarkers to address critical testing and data gaps, and critically assess performance attributes and limitations. After successfully establishing broad stakeholder alignment, regulatory qualification will be sought for targeted sets of transcriptomic signatures that inform established mechanisms of rodent carcinogenicity. This working group will work collaboratively with the HESI Genetic Toxicology Testing Committee to generate complementary data and methods to assess cancer driver gene mutated subclonal populations using ultrasensitive DNA sequencing technology. The formation of consortia of highly engaged stakeholders (including formal regulatory biomarker qualification programs) is an essential step toward realizing toxicogenomics'

contribution to the shared goal of implementing more accurate and efficient cancer risk assessments. Armed with a robust understanding of the biology underlying transcriptional alterations and spurred by the incentive of delivering protective and reliable assessments, the hard work of aligning toxicogenomics data and defined contexts for use and cancer risk assessment decision-making must begin.

While seemingly disparate, chemical and pharmaceutical safety sectors will both benefit by mutual collaboration on cultivating genomic endpoints for carcinogenicity studies. These data have the potential to both shorten the time scales needed to evaluate the carcinogenicity potential of compounds and to provide human risk contextualization of rodent bioassay results. By working together toward this common goal, the future of carcinogenicity testing for the 21st century and beyond will be fully realized.

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Abbreviations:

AhR	aryl hydrocarbon receptor
AOP	adverse outcome pathway
BMD	benchmark dose
CAR	constitutive androstane receptor
CDC	Centers for Disease Control and Prevention
EPA	U.S. Environmental Protection Agency
eSTAR	Emerging Systems Toxicology in the Assessment of Risk
HESI	Health and Environmental Sciences Institute
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IRIS	Integrated Risk Information System
MCHM	methylcyclohexanemethanol
MOA	mode of action
NegCarc	Negative for Endocrine, Genotoxicity, and Chronic Study Associated Histopathologic Risk Factors for Carcinogenicity

NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
POD	point of departure
PPARα	peroxisome proliferator-activated receptor α

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Table 1:

Utility of genomics for de-risking positive findings in rodent 2-year carcinogenicity studies - pharmaceutical development case studies

Rodent 2-year carcinogenicity study outcomes	Proposed mode of action	Genomic data	Human relevance?	Impact
Histiocytic sarcoma in multiple tissues (mouse; not rat)	Unknown	1) Deep sequencing of mouse histiocytic sarcomas excluded viral-based tumor etiology. 2) Early onset and persistent activation of IFN γ-associated gene expression signature followed by late onset of histiocytic sarcoma-associated gene expression signature in affected mouse tissues (not observed in rats or in PBMCs from patients in a clinical phase IIb study)	Likely human irrelevant (based on species differences in molecular pathways associated with mouse histiocytic sarcomas)	Reassured health authority and supported further clinical development.
Liver/thyroid tumors (mouse and rat)	CAR activator	1) CAR activator-induced gene expression signatures observed in liver (Peffer et al., 2018; Rooney et al., 2018) from rat 4 week repeat dose toxicity study 2) Absence of AhR-, PPARα, activator-induced gene expression signatures in liver from rat 4 week repeat dose toxicity study (Rooney et al., 2018)	Human-irrelevant mode of action based on established species differences in CAR-mediated proliferative responses and human epidemiological data on phenobarbital	Reassured health authority and supported further clinical development; Integration of genomics and histopathology data from subchronic toxicology studies would also have supported a Category 3a carcinogenicity study waiver under the proposed ICH S1 revision.
Liver/ lung tumors (mouse and rat)	Sustained AhR activation	Careful attention to dose-response with regard to thresholds and kinetics of CYP1A1 and CYP1A2 activation as liver tissue transcriptomic biomarkers of AhR activation tone (Qin et al., 2019; Taylor et al., 2015)	Likely human relevant but only when a therapeutic dose level may be associated with sustained activation above a defined threshold	Reassures both sponsor and health authorities when hypertrophy/ hyperplasia seen in a chronic study is not associated with sustained suprathreshold AhR activity

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