carcinoGENOMICS
a Project of the European Union

Necessity of regulators and authorities' input into FP-projects such as carcinoGENOMICS

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Increasing demands on chemical risk assessment:

- **The risk of false negatives:**
  high failure rate of new drug candidates due to unmanageable toxicity, accounting for approximately 30% of this attrition

- **The risk of false positives:**
  the EU REACH policy program on industrial chemicals
  - Existing and new substances should in the future be subject to the same procedure under a ***single system***.
  - Large amounts of additional tests required before 2018
    - 30,000 existing chemicals already placed on the market since before 1981 and sold at > 1 tonne per year
    - Estimated costs: 2,5 – 6,5 billion Euros
    - Estimated use of animals: 8 – 20 million

- **EU-wide ban on animal use in cosmetics development**
  - Council Directive 92/32/EEC (7th Amendment): testing ban prohibiting animal testing of cosmetic products in the EU.

The potential of Toxicogenomics:

- **“Ultimately, exploitation of microarray-based biomarkers will help bring about the transition from population-based medical treatment to true personalized medicine”.**
  - *Daniel A. Casciano, former director of the US FDA’s National Center for Toxicological Research, in: Nature Biotechnology (2006)*

- **“The advent of genomics and the burgeoning amount of genomics-related data presents opportunities and challenges to the EPA in fulfilling its regulatory and risk-assessment responsibilities”.**
  - *David J Dix, US EPA, National Center for Computational Toxicology, in: Nature Biotechnology (2006)*

Impact of microarray data quality on genomic data submissions to the FDA

Felix W Frueh

How can microarray data best be exploited and integrated into the regulatory decision-making process?

Five years ago, the completion of the sequencing of the human genome was announced[1,2], triggering many comments about the value of this knowledge for new approaches and insights into drug development. However, although genomics is used in an increasing number of drug development programs, the genomics-led 'revolution' in drug development has not happened yet. This can be attributed to a variety of reasons; one reason is the lack of a thorough evaluation of the quality of novel technologies such as DNA microarrays as well as the manner in which the results of such experiments are analyzed and interpreted.

To investigate the challenges presented to regulators by microarray data, the US Food and Drug Administration (FDA) spearheaded the formation of the MicroArray Quality Consortium (MAQC), which brings together researchers from the government, industry and academia to assess the key factors contributing to variability and reproducibility of microarray data. Ultimately, the data from this initiative will help determine a new set of standards and guidelines for the use of DNA microarray data.

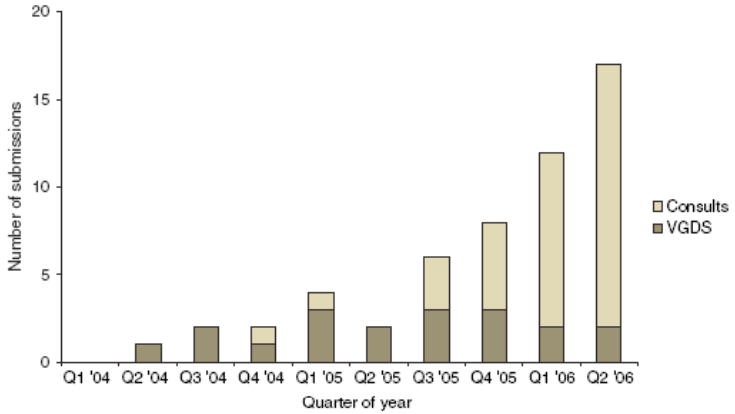

Figure 1 Increase in formal requests (consults) for genomic data review (data submitted as part of regular INDs, NDAs or BLAs) to the Office of Clinical Pharmacology, and voluntary genomic data submissions (VGDS) to the FDA, since 2004. IND, investigational new drug; NDA, new drug application; BLA, biologic license application.

Genomic data matures

Several factors have encouraged the adoption and integration of genomic data in drug development and regulatory assessment, including a better understanding of disease pathophysiology and targeted drug molecules to sites of action. However, there are challenges to further expansion of genomics use; one key issue frequently discussed is that genomic science has evolved more quickly than technologies suitable for generating consistent, high-quality genomic data. Before 2004, genomic information was largely absent from the investigational new drug submissions or new drug applications received by the FDA; today, that situation is changing (**Fig. 1**). This more than likely reflects the timelines associated with the drug development process overall and the integration of genomics within that process. It is therefore logical that by this time, we should be starting to see an increase in submissions to the FDA containing genomic information; indeed, the number of data submissions containing genomic information is increasing significantly (**Fig. 1**).

On the basis of 20 voluntary genomic data submissions that have been submitted to the FDA so far, it appears that the technologies for generating genomic data have only recently become a commodity of broader application. Recently, the integration of large-scale screening approaches (e.g., gene expression profiling or whole genome single-nucleotide polymorphism (SNP) scans has been observed in different stages of drug discovery and now also in drug development. Consequently, at this point, the generation and exploitation of genomic data generated from such large-scale efforts in modern drug development requires a regulatory environment adequately equipped to review such data.

The agency responds

Shortly after the human genome sequence was announced, a seminal paper by the FDA's Lesko and Woodcock[3] was published highlighting

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TOXICOLOGICAL SCIENCES 95(1), 5–12 (2007)
doi:10.1093/toxsci/kfl103
Advance Access publication September 8, 2006

FORUM

The ToxCast Program for Prioritizing Toxicity Testing of Environmental Chemicals

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Received May 24, 2006; accepted August 30, 2006

The U.S. Environmental Protection Agency (EPA) is developing methods for utilizing computational chemistry, high-throughput screening (HTS), and various toxicogenomic technologies to predict potential for toxicity and prioritize limited testing resources toward chemicals that likely represent the greatest hazard to human health and the environment. This chemical prioritization research program, entitled "ToxCast," is being initiated with the purpose of developing the ability to forecast toxicity based on bioactivity profiling. The proof-of-concept phase of ToxCast will focus upon chemicals with an existing, rich toxicological database in order to provide an interpretive context for the ToxCast data. This set of several hundred reference chemicals will represent numerous structural classes and phenotypic outcomes, including tumorigens, developmental and reproductive toxicants, neurotoxicants, and immunotoxicants. The ToxCast program will evaluate chemical properties and bioactivity profiles across a broad spectrum of data domains: physical-chemical, predicted biological activities based on existing structure-activity models, biochemical properties based on HTS assays, cell-based phenotypic assays, and genomic and metabolomic analyses of cells. These data will be generated through a series of external contracts, along with collaborations across EPA, with the National Toxicology Program, and with the National Institutes of Health Chemical Genomics Center. The resulting multidimensional data set provides an informatics challenge requiring appropriate computational methods for integrating various chemical, biological, and toxicological data into profiles and models predicting toxicity.

Key Words: high-throughput screening; toxicogenomics; chemoinformatics; bioinformatics.

prioritize the use of testing resources toward those chemicals and endpoints that present the greatest likelihood of risk to human health and the environment. This need can be addressed through the experience of the pharmaceutical industry in the use of state of the art, high-throughput screening (HTS), toxicogenomics, and computational chemistry tools for the discovery of new drugs (Table 1), with appropriate adjustments to the needs of environmental toxicology. Thus, a research program entitled "ToxCast" has been initiated within EPA to develop an ability to forecast toxicity based on bioactivity profiling. Ultimately, ToxCast's purpose is to develop methods of prioritizing chemicals for further screening and testing to assist EPA programs in the management and regulation of environmental contaminants.

Over the past decade, HTS has developed into a primary tool for drug discovery based upon bioactivity screening of the drugable proteome (Fliri *et al.*, 2005b; Janzen and Hodge, 2006). On a more limited scale, HTS has also been adapted to agrochemical discovery for the analysis of target species and model organisms (Smith *et al.*, 2005; Tietjen *et al.*, 2005). Recently, HTS applications to toxicology have been expanding as a useful complement to traditional toxicology (Bhogal *et al.*, 2005; Fliri *et al.*, 2005a; Kikkawa *et al.*, 2006). In the federal sector, the National Institutes of Health Chemical Genomics Center (NCGC) has been established (http://www.ncgc.nih.gov/). The NCGC is using industrial-scale HTS technologies to collect data that is useful for developing small-molecule chemical probes for basic biological research (Austin *et al.*

Amendment to the latest consolidated version of the REACH legislation:

- The Commission aims to reduce the number of animals used for testing, by half
- (39) The Commission, Member States, industry and other stakeholders should continue to contribute to the promotion of alternative test methods on an international and national level including computer supported methodologies, in vitro methodologies, such as appropriate, those based on toxicogenomics, and other relevant methodologies. The Community's strategy to promote alternative test methods is a priority and the Commission should ensure that within its future Research Framework Programmes and initiatives such as the Community Action Plan on the Protection and Welfare of Animals 2006-2010 this remains a priority topic. Participation of stakeholders and initiatives involving all interested parties should be sought.

Meeting Report: Validation of Toxicogenomics-Based Test Systems: ECVAM–ICCVAM/NICEATM Considerations for Regulatory Use

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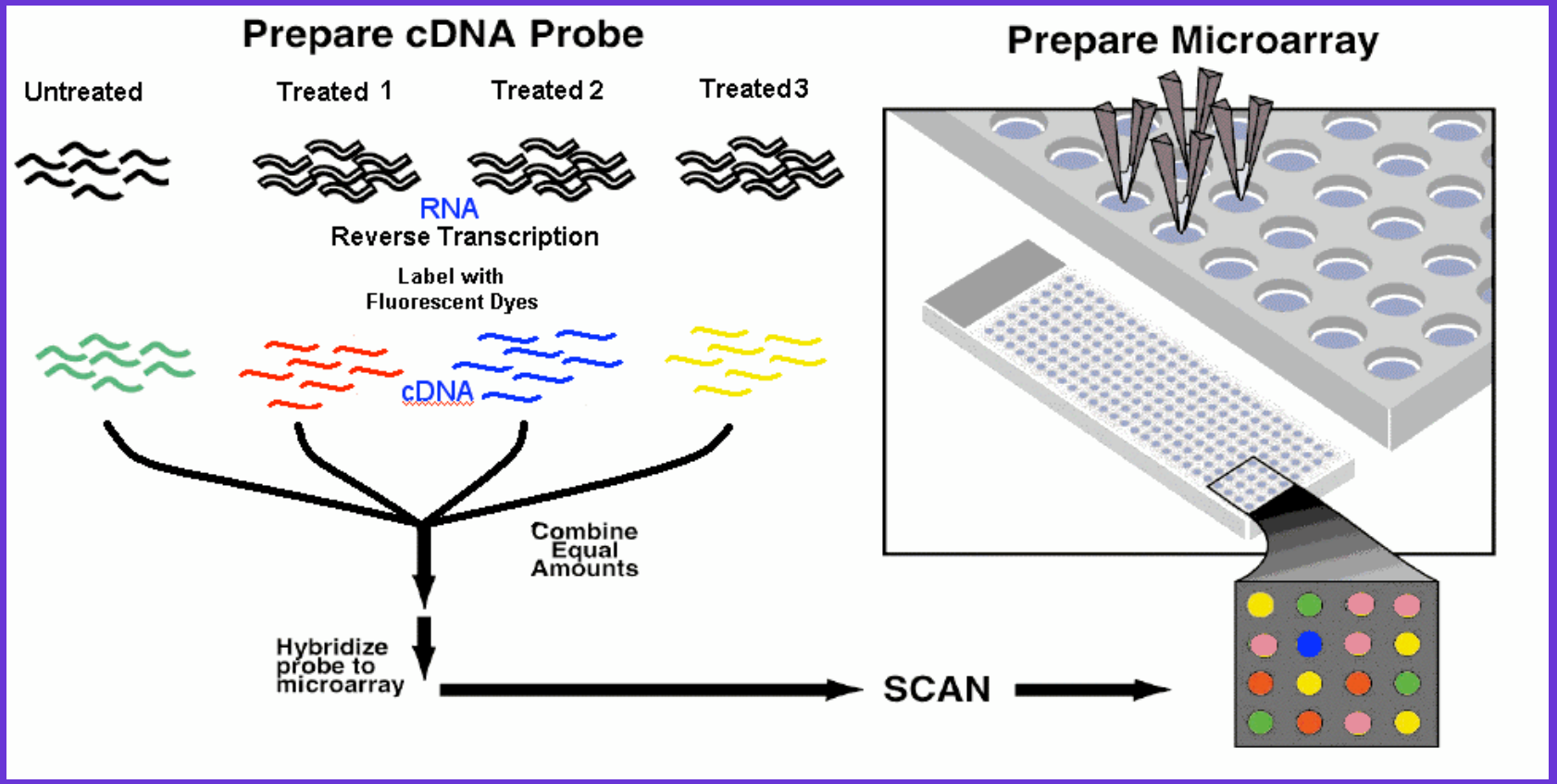
This is the report of the first workshop "Validation of Toxicogenomics-Based Test Systems" held 11–12 December 2003 in Ispra, Italy. The workshop was hosted by the European Centre for the Validation of Alternative Methods (ECVAM) and organized jointly by ECVAM, the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The primary aim of the workshop was for participants to discuss and define principles applicable to the validation of toxicogenomics platforms as well as validation of specific toxicologic test methods that incorporate toxicogenomics technologies. The workshop was viewed as an opportunity for initiating a dialogue between technologic experts, regulators, and the principal validation bodies and for identifying those factors to which the validation process would be applicable. It was felt that to do so now, as the technology is evolving and associated challenges are identified, would be a basis for the future validation of the technology when it reaches the appropriate stage. Because of the complexity of the issue, different aspects of the validation of toxicogenomics-based test methods were covered. The three focus areas include *a*) biologic validation of toxicogenomics-based test methods for regulatory decision making, *b*) technical and bioinformatics aspects related to validation, and *c*) validation issues as they relate to regulatory acceptance and use of toxicogenomics-based test methods. In this report we summarize the discussions and describe in detail the recommendations for future direction and priorities. *Key words:* acceptance, alternatives, biomarker, predictive test, regulatory use, standardization, toxicogenomics, toxicology, validation. *Environ Health Perspect* 114:420–429 (2006). doi:10.1289/ehp.8247 available via *http://dx.doi.org/* [Online 17 August 2005]

methods based on toxicogenomics and to evaluate the scientific validity and regulatory applicability of such test methods. It is envisioned that the entire validation process will be more complex and challenging than that typically encountered thus far for other alternative test methods. This is because not only will the technology itself need to be standardized and validated, but the methods that are based upon the technology and their predictive aspects will also need to undergo validation if they are to be employed in regulatory decision-making processes. In addition the validation process must be able to accommodate the anticipated rapid changes in technology that could affect the performance of the test method and its reliability for a specific purpose.

Toxicogenomics-based methods are being widely applied in toxicology and biomedical research. Because data are already being generated using these technologies, it is both timely

MICROARRAY technology for gene expression analysis



•Toxicogenomics Research Consortium. *In: Nature Methods (2005)*
"Reproducibility for most platforms within any laboratory is typically good. Microarray results can be comparable across multiple laboratories, especially when a common platform and a set of procedures are used."
•MicroArray Quality Control (MAQC) project. *In: Nature Biotechnology (2006)*
"We show intraplatform consistency across test sites as well as a high level of interplatform concordance in terms of genes identified as differentially expressed".

Applications of toxicogenomics to classifying genotoxic and non-genotoxic carcinogens

***In vivo* studies**

- Nie AY et al. Predictive toxicogenomics approaches reveal underlying molecular mechanisms of nongenotoxic carcinogenicity. *Mol. Carcinog. 2006*
- Ellinger-Ziegelbauer H. et al. Comparison of the expression profiles induced by genotoxic and nongenotoxic carcinogens in rat liver. *Mutation Research 575 (2005) 61–84*
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***In vitro* studies**

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- Harris AJ et al. Comparison of basal gene expression profiles and effects of hepatocarcinogens on gene expression in cultured primary human hepatocytes and HepG2 cells. *Mutation Research 549 (2004) 79–99*
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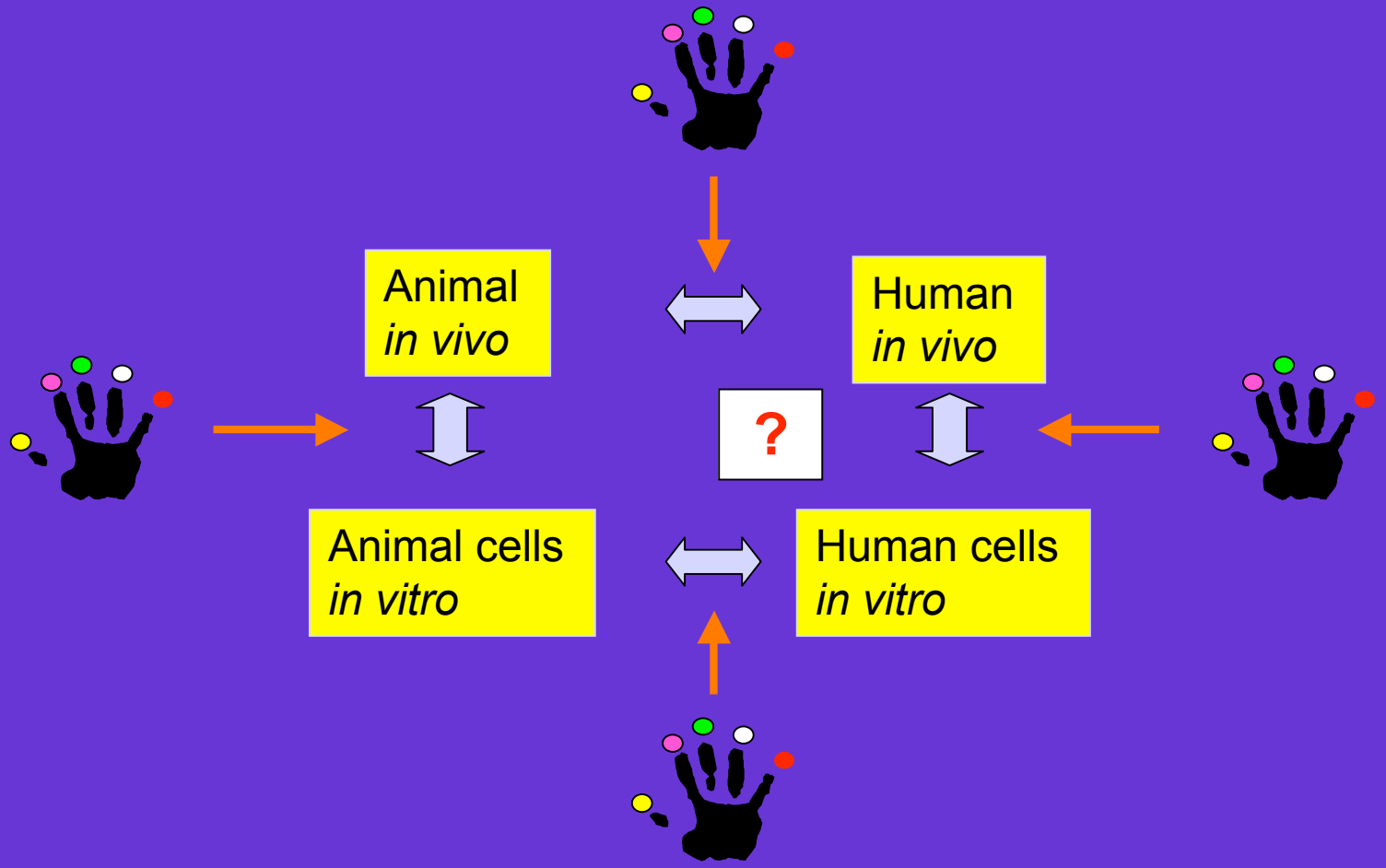
Development of alternatives to animal toxicity models: *in vitro-in vivo* and inter-species extrapolation in validating toxicogenomics-based predictive screens



How to integrate ‘omics information in a REACH testing strategy?

- In conjunction with other alternative methods in a weight of evidence evaluation
- Elucidate mode/mechanism of action

Statements

- The success of the incorporation of the new methods into risk assessment strategies will be intensified by an active cooperation with risk assessors and regulatory bodies
- For that, it is necessary that risk assessors should become accustomed to the new genomics and bioinformatics technologies
- Data requirements by the international risk assessment community should be identified
- Such requirements will additionally guide the development of research
- Consequently, an iterative collaboration between toxicogenomicists and regulators should be established