Toxicogenomics approaches towards predicting chemical carcinogenicity *in vitro*

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Current carcinogenicity testing strategy

1. **Genotoxicity testing**
   1. *In vitro* (2 or 3 tests on mutagenicity and clastogenicity)
   2. *In vivo* for *in vitro* positives

2. **Carcinogenicity testing**
   1. **For *in vivo* GTX compounds**
   2. **For compounds to which humans will be exposed (drugs, cosmetics, some occupation settings)**

Demands for better tests: examples for genotoxicity and carcinogenicity

- **For pharmaceuticals, the current test battery on genotoxicity (bacterial mutagenesis, *in vitro* mammalian mutagenesis, *in vitro* chromosome aberration analysis and an *in vivo* chromosome stability assay) has been assessed to predict rodent carcinogenicity correctly by not more than 38 % while simultaneously producing high percentages of false positives**

  (*Snyder RD, Green JW. A review of the genotoxicity of marketed pharmaceuticals. Mutat Res. 2001, 488:151-69)*

- **A survey of over 700 chemicals demonstrated that even 75–95% of non-carcinogens gave positive (i.e. false positive) results in at least one test in the *in vitro* test battery**

  (*Kirkland D et al. Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. Mutat Res. 584 (2005) 1–256)*

- **The current rodent cancer bioassays provide inadequate data to estimate human cancer risk at low dose; accuracies of approximately 60 % are achieved**

  (Ames BN et al. Cancer prevention, rodent high-dose cancer tests, and risk assessment. Risk Analysis, 16: 613-617 (1996))

- **50% of all chronically used human pharmaceuticals induce tumors in rodents, but only 20 human pharmaceutical carcinogens have been confirmed by epidemiologic studies**

- **For the important class of non-genotoxic carcinogens, no suitable test model is available**

- **These assays have not been modified substantially since the initiation of their use.**

REACH recommendation with reference to carcinogenicity:

other studies on mechanisms/modes of action, e.g. OMICs studies (toxicogenomics, proteomics, metabonomics and metabolomics): carcinogenesis is associated with multiple changes in gene expression, transcriptional regulation, protein synthesis and other metabolic changes. Specific changes diagnostic of carcinogenic potential have yet to be validated, but these rapidly advancing fields of study may one day permit assessment of a broad array of molecular changes that might be useful in the identification of potential carcinogens.

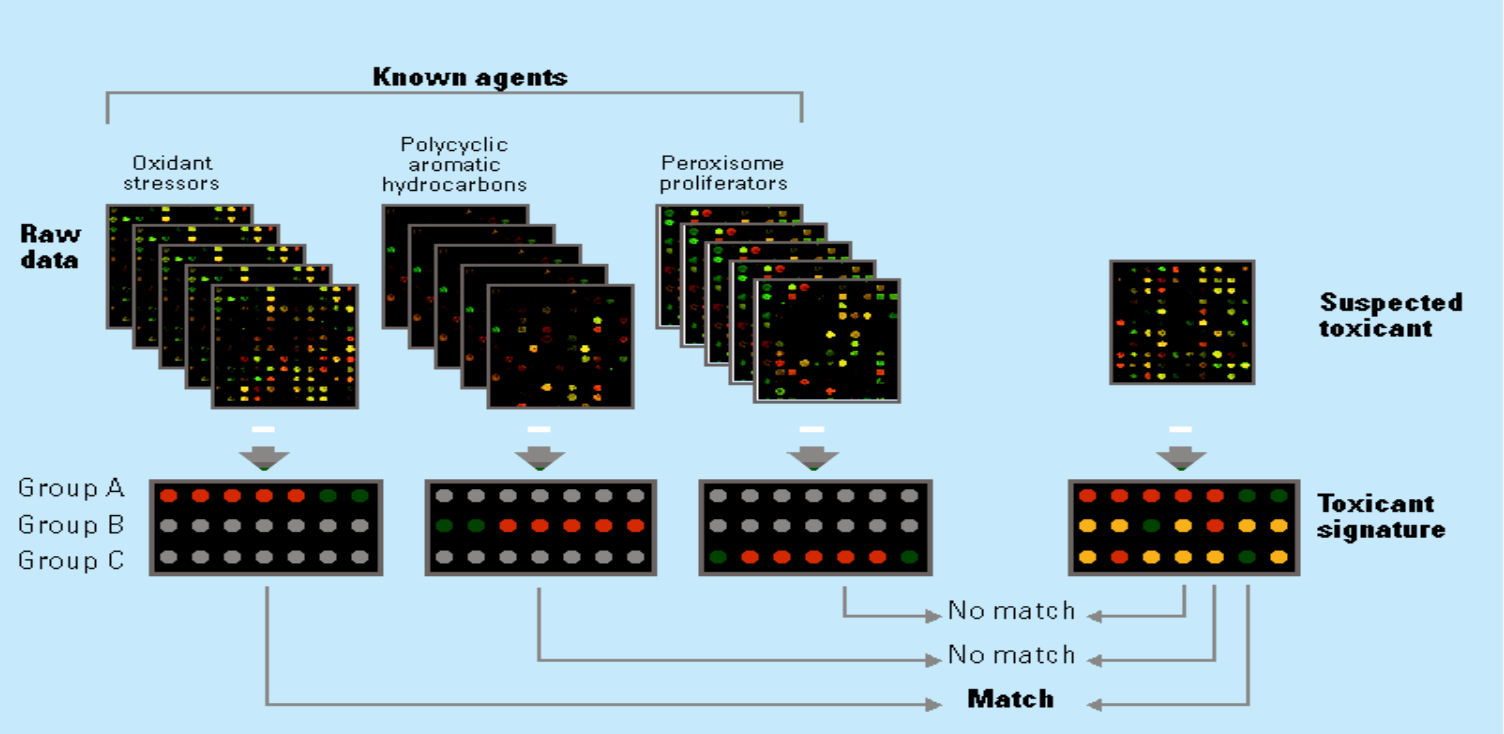
RIP3.3_TGD_FINAL_2007-05-04_Part2.doc Page 384

Major aim of carcinoGENOMICS is to develop *in vitro* methods for assessing the carcinogenic potential of compounds, as an alternative to current rodent bioassays for genotoxicity and carcinogenicity.

KEY TERMS:

- **Metabolome and transcriptome profiling.**
- **Major target organs: the liver, the lung, and the kidney.**
- **Robust *in vitro* systems (rat/human).**
- **Interindividual variability.**
- **Exploring stem cell technology.**
- **Well-defined set of model compounds.**
- **Phenotypic markers for genotoxic and carcinogenic events.**
- **Extensive biostatistics to identify predictive pathways.**
- ***In silico* model of chemical carcinogenesis.**
- **Dedicated high throughput technology**

Potential of toxicogenomics-based screens for toxic class prediction/hazard identification



Using DNA microarrays, gene expression data are derived from exposure of model systems to known toxicants (Group A, B, and C genes). These data are compared to a set of gene expression changes elicited by a suspected toxicant. If the characteristics match, a putative mechanism of action can be assigned to the unknown agent.

Classes of carcinogenic chemicals for which carcinoGENOMICS has developed toxicogenomics-based predictive models *in vitro*

- Genotoxic carcinogens
  - Damage DNA
  - Cause mutations
  - Initiate cancer
- Non-Genotoxic carcinogens
  - No DNA damage
  - Promote cancer
  - Many mechanisms:
    - Cell proliferation stimulation
    - Apoptosis suppression
    - Biotransformation enzyme induction
    - ..

M. Vinken et al. The carcinoGENOMICS project: Critical selection of model compounds for the development of omics-based in vitro carcinogenicity screening assays. Mutat Res. 2008; 659: 202-

Table 2
Model compounds selected for the first phase of carcinoGENOMICS

Compound	CAS number	ChEBI accession numbers [85,86]	*In vitro* genotoxicity	*In vivo* genotoxicity	Carcinogenicity	IARC	Organ involved
Genotoxic carcinogens							
Aflatoxin B1	1162-65-8	2504	+Ames, +MLA, +UDS, +MNT, +CA [39,41,42,74]	+CA [42]	+Rats, +mice [38,40,41]	1	Li [38,40,41]
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	64091-91-4	32692	+Ames, +UDS, +HPRT [41,42]	+MNT [42]	+Rats, +mice [38,40,41]	2B	Li [38,40,41]
Dimethylnitrosamine	62-75-9	35807	+Ames, +UDS, +HPRT, +MLA, +MNT, +CA [39,41,42,74]	+CA, +MNT [41,42]	+Rats, +mice [38,40,41]	2A	Li, Ki, Lu [38,40,41]
2-Nitrofluorene	607-57-8	1224	+Ames, +MLA, +CA, +MNT, +UDS [37,38]		+Rats [38,41]	2B	Li, Ki [38,40,41]
Benzo[a]pyrene	50-32-8	29865	+Ames, +HPRT, +MLA, +MNT, +CA, +UDS [39,41,42,74]	+CA, +MNT [41,42]	+Rats, +mice [38,40,41]	2A	Li, Ki, Lu [40,41]
Potassium bromate	7758-01-2	38211	+Ames, +MNT, +CA [39,41,74]		+Rats, +mice [38,41]	2B	Ki [38,41]
Streptozotocin	18883-66-4	9288	+Ames, +CA, +HPRT, +MLA [38,41,74]		+Rats, +mice [38,40,41]	2B	Ki [38,40,41]
1,3-Butadiene	106-99-0	39478	+Ames, +HPRT, −MLA [38,41,74]	+CA, +MNT [41]	+Rats, +mice [38,40]	2A	Lu [38,40]
Vinyl chloride	75-01-4	28509	+Ames, +CA [39,41,42,74]	+CA, +MNT [42]	+rats, +mice [38,40]	1	Lu [38,40,41]
Sodium dichromate	10588-01-9	39483	+Ames, +MLA, +CA [39,41,42,74]	+CA [42]	+Rats [38,40]	1	Lu [38,40]
Isobutyl nitrite	542-56-3	46643	+Ames, + MLA, +MNT, +CA [40–42,74]	+CA [40]	+Rats, +mice [38,40]	1	Lu [38,40]
Non-genotoxic carcinogens							
Wy-14,643	50892-23-4	32509	−Ames, −MNT, ±UDS [39–42]		+Rats, +mice [38,40]	–	Li [38,40]
Methapyrilene·HCl	135-23-9	38213	−Ames, ±MLA, +CA [41,42,74]		+Rats [38,40]	–	Li [5,6]
Piperonyl butoxide	51-03-6	32687	−Ames, −UDS, −HPRT +MLA, −CA [41,42,74]		+Rats, +mice [38,40]	3	Li [38,40]
Sodium phenobarbital	57-30-7	8070	−Ames [38,41,42,74]		+Rats, +mice [38]	–	Li [38]
Tetradecanoyl phorbol acetate	16561-29-8	37537	−Ames, −HPRT, −MNT, ±CA [39,41,42,74]		+Mice [41]	–	Li [41]
Ochratoxin A	303-47-9	7719	−Ames, ±UDS, −HPRT, −MLA, ±CA [41,42,74]	−CA [41]	+Rats, +mice [38,40,41]	2B	Ki [38,40,41]
Monuron	150-68-5	38214	−Ames, ±MLA, ±CA [39,41,42]	−CA, +MNT [42]	+Rats, −mice [38,40,41]	3	Ki [38,40,41]
Chlorothalonil	1897-45-6	3639	−Ames, ±MLA, +CA [39–42,74]	−MNT [42]	+Rats, −mice [38,40,41]	2B	Ki [38,40,41]
Bromodichloromethane	75-27-4	34591	−Ames, ±MLA, −UDS, ±CA [39,41,74]	−MNT [38]	+Rats, +mice [38,40,41]	2B	Ki [38,40,41]
S-(1,2-dichlorovinyl)-L-cysteine	627-72-5	46650	±Ames, ±UDS [41]		+Rats* [40]	–	Ki* [40]
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin	1746-01-6	28119	−Ames, ±MLA, ±CA [39,41,42,74]	−CA [42]	+Rats, +mice [38,40]	1	Li, Ki, Lu [38,40]
Cadmium dichloride	10108-64-2	35456	±Ames, ±UDS, +MNT, +MLA, ±HPRT, ±CA [39,41,42,74]	−MNT [42]	+Rats, ±mice [38,40,41]	1	Lu [38,40,41]
Sodium arsenate	7784-46-5	29678	−Ames, ±CA, +MNT, +MLA [41,42]	±MNT [41,42]	−rats, −mice [38]	1	Lu [40]
Asbestos	1332-21-4	46661	−Ames, +CA [39,74]	−MNT, +CA [41]	+Rats [40]	1	Lu [40]
Chloroprene	126-99-8	39481	±Ames [41]	−MNT, −CA [41]	+Rats, +mice [38,40,41]	2B	Lu [38,40,41]
Non-carcinogens							
Nifedipine	21829-25-4	7565	−Ames [41,72]	−NS [72]	−Rats [72]	–	Li, Ki
Tolbutamide	64-77-7	27999	−Ames, −MLA, −CA [41,74]		−Rats, −mice [38,40]	–	Li, Ki
Clonidine	4205-90-7	46631	−Ames, −UDS [41,72]	−NS [72]	−Rats [72]	–	Li, Ki
Sodium diclofenac	15307-79-6	4507	−Ames, −CA, −MLA, −HPRT [41,72]	−NS [72]	−Rats, −mice [72]	–	Li, Ki
D-mannitol	69-65-8	16899	−Ames, −MLA, −CA [41,42,74]	−CA, −MNT [42]	−Rats, −mice [41]	–	Li, Ki
Ethylene	74-85-1	18153	−Ames, −CA [40,42]	−MNT [42]	−Rats, −mice [40]	3	Lu
Beclomethasone dipropionate	5534-09-8	3002	−Ames, −CA, −HPRT [72]		−Rats, −mice [72]	–	Lu
Ipratropium bromide monohydrate	66985-17-9	5957	−Ames, −CA [72]	−NS [72]	−Rats, −mice [72]	–	Lu

Compounds were selected according to the established criteria (see text) and cover a wide range of chemical substances, including industrial chemicals, biocidal products as well as pharmaceuticals. For both kidney and liver carcinogens, ingestion is considered as the route of exposure, whereas inhalation is regarded as the main way of contact for lung carcinogens. Chemicals presented in italics are part of the learning set of compounds ((+) positive outcome; (−) negative outcome; (*) trichloroethylene-associated carcinogenicity; Ames, bacterial reverse mutation assay; CA, chromosome aberration test; CAS, Chemical Abstracts Service; ChEBI, Chemical Entities of Biological Interest; HPRT, hypoxanthine-guanine phosphoribosyltransferase mutation test; IARC, International Agency for Research on Cancer; Ki, kidney; Li, liver; Lu, lung; MLA, mouse lymphoma assay; MNT, micronucleus test; NS, not specified; UDS, unscheduled DNA synthesis test).

Carcinogenomics WP 2 Liver Models

Initial Cell Models:

MODEL	CONCEPT
Rat Hepatocytes +/- TSA	Epigenetic modification of hepatocellular gene expression patterns in order to stabilize liver-specific functionality
HepG2/HepG2up	Re-expression of key liver-enriched transcription factors to re-express important hepatic functions
HepaRG	Undifferentiated cells differentiate into adult hepatocytes under specific culture conditions
DE-Hep	Pluripotent hESC differentiated into the hepatocyte lineage

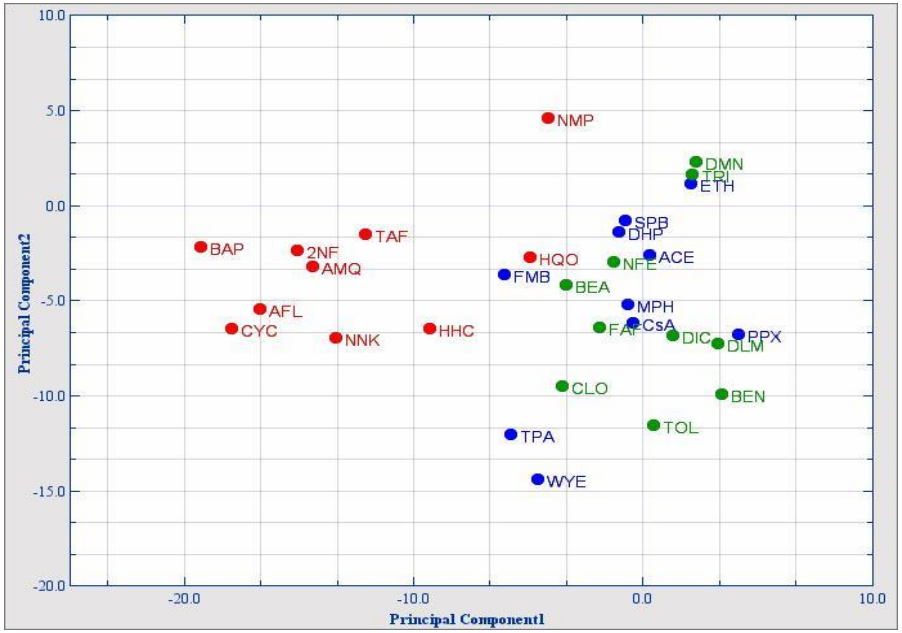
Tatyana Y. Doktorova et al. Carcinogenesis vol.34 no.6 pp.1393–1402, 2013

Misclassifications of carcinogens in HepaRG

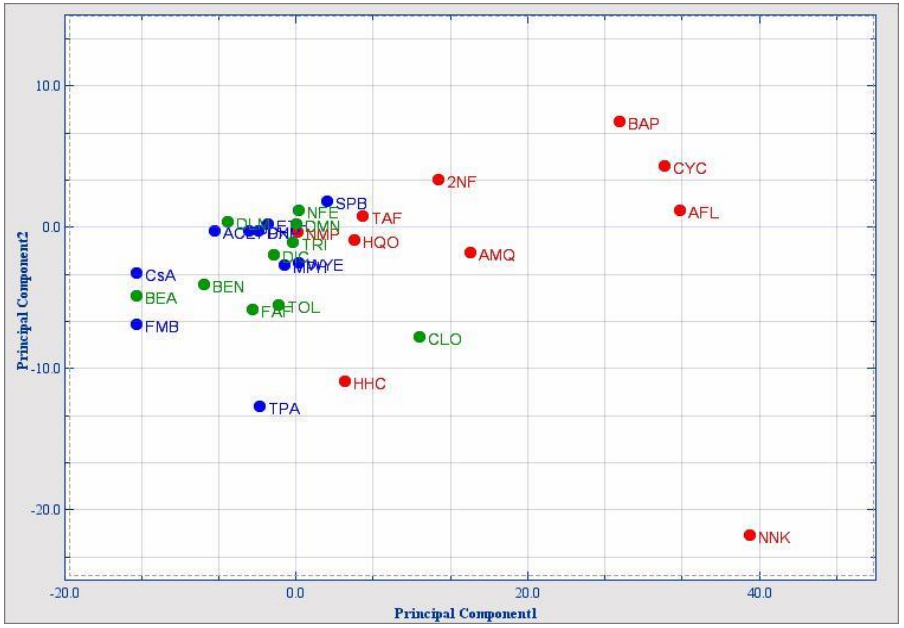
% of correctly classified experiments / groups											
		# of exp			Exp wise CV			Group wise CV			
Normalization	DMSO	Phase I	Phase II	Phase I & II	Phase I	Phase II	Phase I & II	Phase I	Phase II	Phase I & II
none	-	156	90	246	95	77	87	92	69	81
none	+	174	128	302	93	71	83	92	62	77
half-z	-	156	90	246	97	81	91	93	73	82
half-z	+	174	128	302	96	75	84	89	57	78
relative	-	156	90	246	99	77	88	95	66	78
relative	+	174	128	302	98	73	86	93	59	73

- Cross validation results obtained with the compounds from phase I (n=15), phase II (n=15), and phase I & II
- Best results after a 24 h incubation period, rather than after 72 h
- The numbers of misclassified experiments are slightly higher upon including the samples from both phases
- This can be attributed to the higher misclassification rates using the experiments from phase II

Classifier construction from Hepa RG phase I + II data



PCA based on pathways by applyng Consensus dB
N=233
ANOVA p-value<0.05



PCA based on genes
N=3,540
ANOVA p-value<0.05

Carcinogenomics WP 3 Kidney Models :

Initial Cell Models:

Human proximal tubular epithelial cells:

- Human Primary Cells
- HK-2 human cell line
  - Human pamplona virus transformed
- RPTEC/TERT1 human cell line
  - transfected with human telomerase (hTERT) (~ telomerase positive)

Rat proximal tubular epithelial cells:

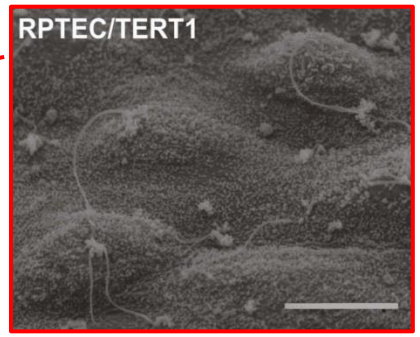
- NRK-52E cell line

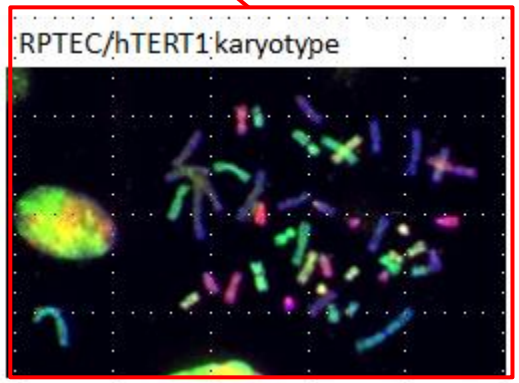
The Human - RPTEC/TERT1 Kidney model

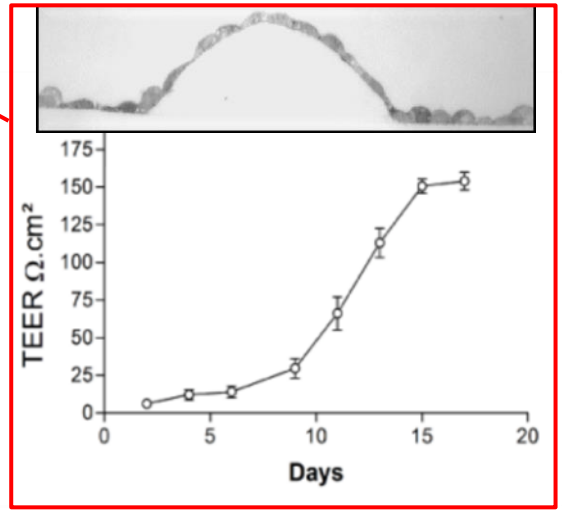
M. Wieser et al. Am. J. Physiol. Am J Physiol Renal Physiol. 2008, 295:F1365-75.

robust human proximal tubular epithelial cell model selected and optimized based on:

- Morphology and characteristics
- Barrier function
- Genetic stability
- Metabolic characterization
- Transcriptomic profiling







Misclassification rates of the RPTEC/TERT1 Kidney model

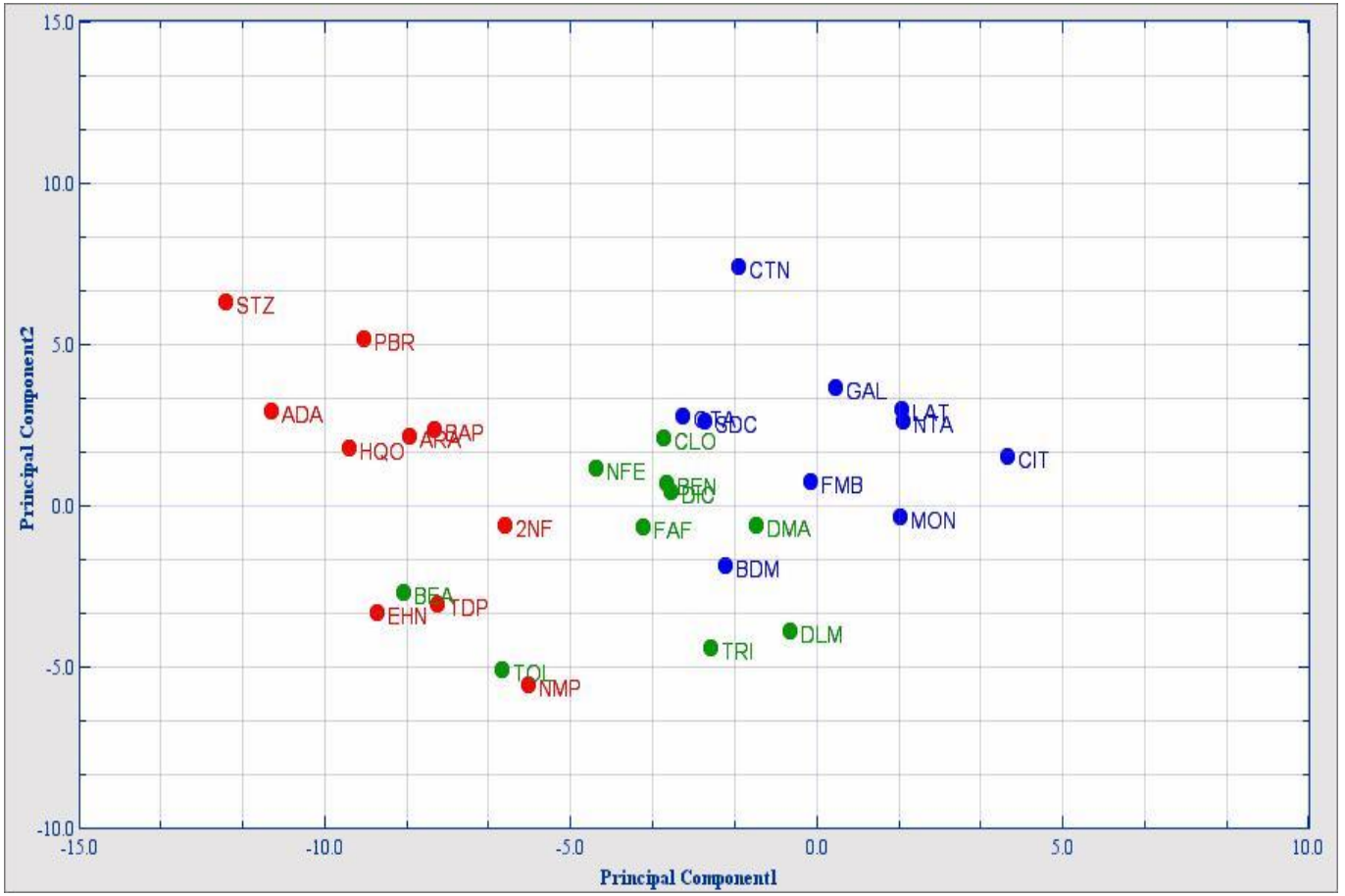
Normalization	DMSO	# of exp	Exp wise CV	Group wise CV
6h experiments				
none	-	96	71	
none	+	117	66	
relative	-	96	72	
relative	+	117	74	
24h experiments				
none	-	96	77	
none	+	117	71	
relative	-	96	79	
relative	+	117	77	
72h experiments				
none	-	96	84	35
none	+	117	77	
relative	-	96	84	31
relative	+	117	82	

- **Human RPTEC/TERT1 cells were treated for 6h, 24h, and 72h with 1 concentration**
- **Each treatment was performed in at least 3 replicates**
- **Each tox class (GTX, Non-GTX, Non Carcinogen) is represented by 10 compounds**
- **Lowest misclassification rates obtained using:**
  - All experiments
  - 72 h experiments

Classifier construction in the RPTEC/TERT1 human *in vitro* model using the Consensus dB pathway finding tool

-> Classifier was based on 149 pre-defined human pathways (ANOVA p-value<0.05) and 30 chemicals

-> Additional blinded compounds were correctly classified with respect to all three toxicity classes



SIXTH FRAMEWORK PROGRAMME

IP PL
037712

carcinoGENOMICS
a Project of the European Union

Reproducibility assessment of ‘omic-based test methods

- of the HepaRG and RPTEC/TERT1 test models
- three encoded chemicals per model
- by three independent labs
- transcriptome analysis by single lab
- multiple bioinformatics methods
  - evaluation of response gene lists
  - correlation analyses
  - multivariate statistical methods (SVM classification)

EURL ECVAM

European Union Reference Laboratory for Alternative Methods to Animal Testing

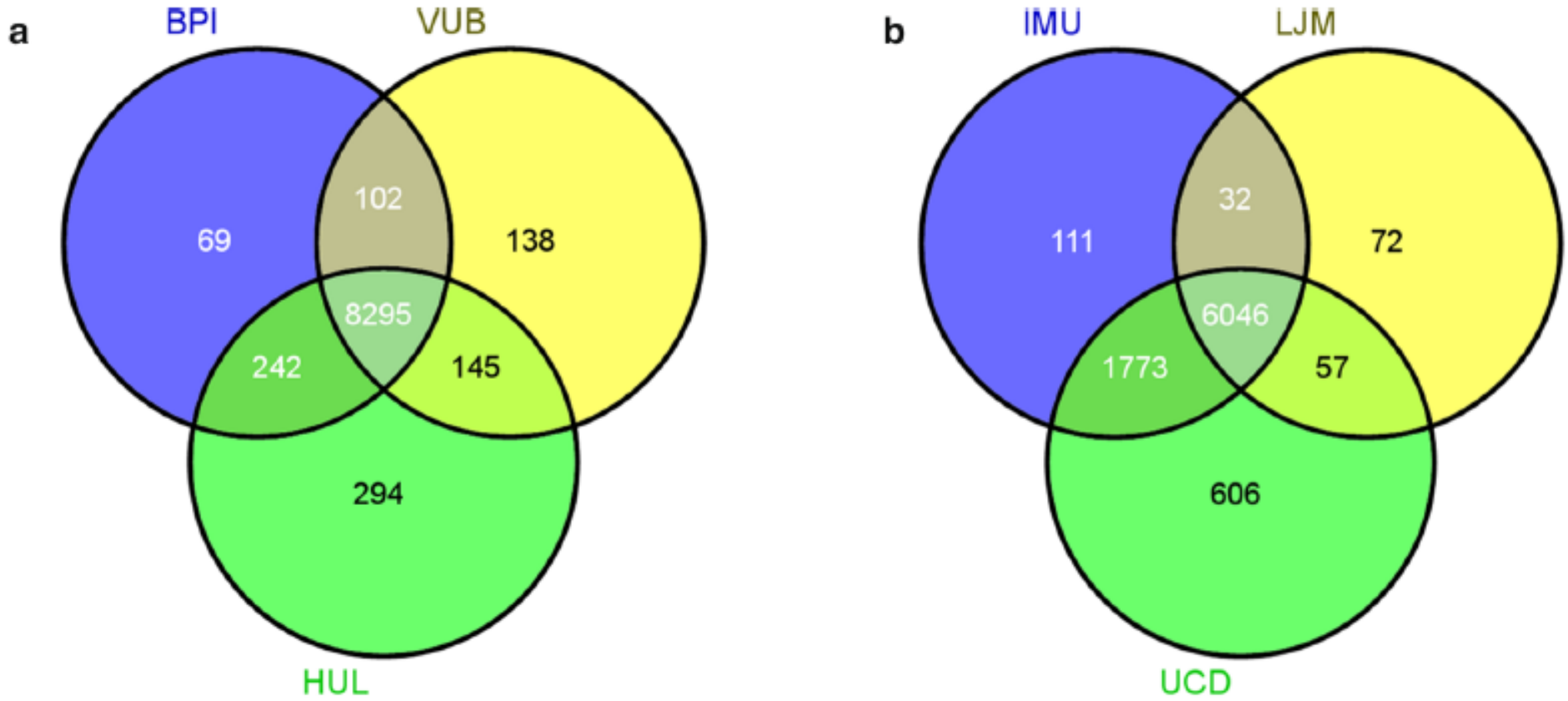
Institute for Health and Consumer Protection (IHCP)

European Commission

Joint Research Centre, Ispra (Italy)

R. Herwig et al. Arch Toxicol. 2015 Nov 2. [Epub ahead of print]

Inter-laboratory study of human in vitro toxicogenomics-based tests as alternative methods for evaluating chemical carcinogenicity: a bioinformatics perspective.



a VENN diagram of genes expressed in the HepaRG assay measured in the three different laboratories.

b VENN diagram of genes expressed in the RPTEC/ TERT1 assay measured in the three laboratories.

Carcinogenesis vol.33 no.7 pp.1421–1429, 2012
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A transcriptomics-based *in vitro* assay for predicting chemical genotoxicity *in vivo*

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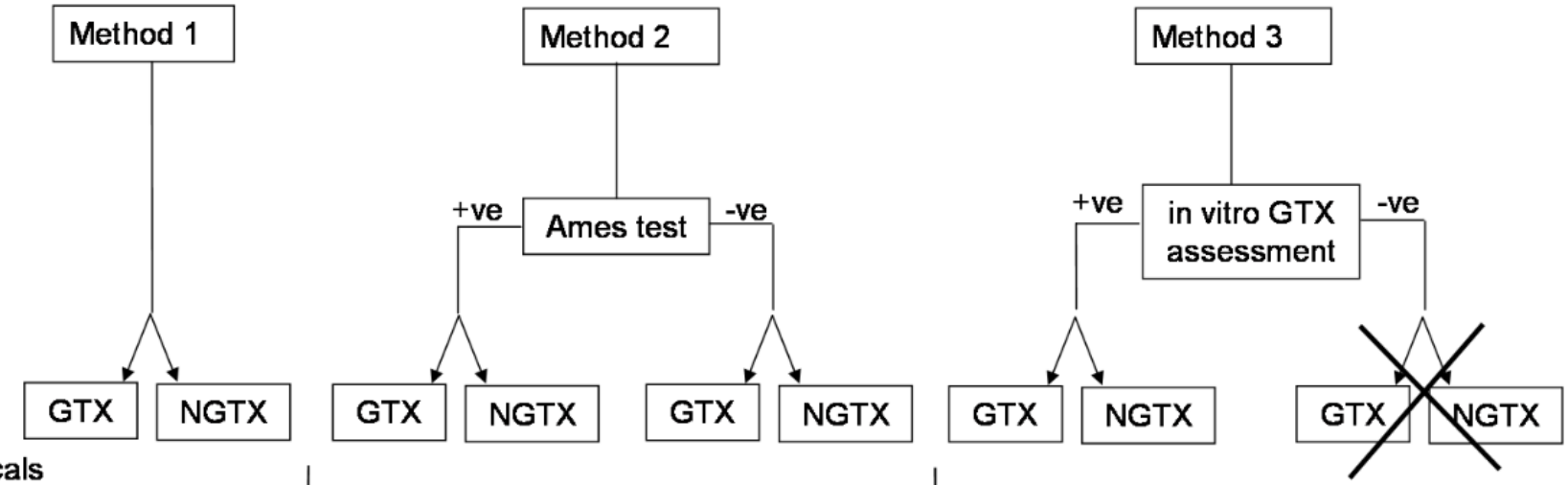
The lack of accurate *in vitro* assays for predicting *in vivo* toxicity of chemicals together with new legislations demanding replacement and reduction of animal testing has triggered the development of alternative methods. This study aimed at developing a transcriptomics-based *in vitro* prediction assay for *in vivo* genotoxicity. Transcriptomics changes induced in the human liver cell line HepG2 by 34 compounds after treatment for 12, 24, and 48 h were used for the selection of gene-sets that are capable of discriminating between *in vivo* genotoxins (GTX) and *in vivo* nongenotoxins (NGTX). By combining transcriptomics with publicly available results for these chemicals from standard *in vitro* genotoxicity studies, we developed several prediction models. These models were validated by using an additional set of 28 chemicals. The best prediction was achieved after stratification of chemicals according to results from the Ames bacterial gene mutation assay prior to transcriptomics evaluation after 24 h of treatment. A total of 33 genes were selected

The most commonly used assays for detecting GTX compounds *in vitro* are the bacterial gene mutation assay (Ames test) (9), the mammalian micronuclei (MN), the chromosomal aberration (CA), and the mouse lymphoma assays (MLA). For chemicals that are genotoxic *in vitro,* regulatory authorities may require the *in vivo* evaluation of genotoxic properties in rodents (EC 1907/2006) (10). However, often the conventional *in vitro* test battery does not correspond with *in vivo* findings and thus fails to correctly predict the *in vivo* genotoxic and carcinogenic potential of compounds (10,11). Consequently, the high false positive rate (50% in some cases) (11) of *in vitro* genotoxicity assays results in a relatively high number of unnecessary animal experiments, which inflict considerable costs and raise ethical issues. Thus, a more reliable *in vitro* assay for predicting *in vivo* genotoxicity is urgently required. The socioeconomic necessity to reduce animal experiments (12) inspired the search for alternative *in vitro* methods thereby exploring novel technological approaches, such as toxicogenomics (13). Toxicogenomics-based approaches have been explored for the development of genotoxicity classification tools showing that they are indeed capable of discriminating GTX from NGTX compounds (14–17).

Therefore, the aim of this study was to improve prediction of *in vivo* genotoxicity using an *in vitro* transcriptomics-based method. The human hepatic cell line HepG2 was selected for this purpose, as it expresses many drug metabolizing enzymes after chemical exposures (18–22); lacks mutations in p53 (23) and has been successfully applied in the phenotypic detection of genotoxic effects of chemicals

Genotoxicity prediction approaches: stratification of compounds based on *in vitro* GTX tests



Number of chemicals												
Training	12	22	7	6	5	16	11	13	1	9
Validation	11	17	11	2	-	15	11	6	-	11
Classifiers										
12h	12		17		8		14		-	
24h	44		33		22		42		-	
48h	19		25		23		10		-	

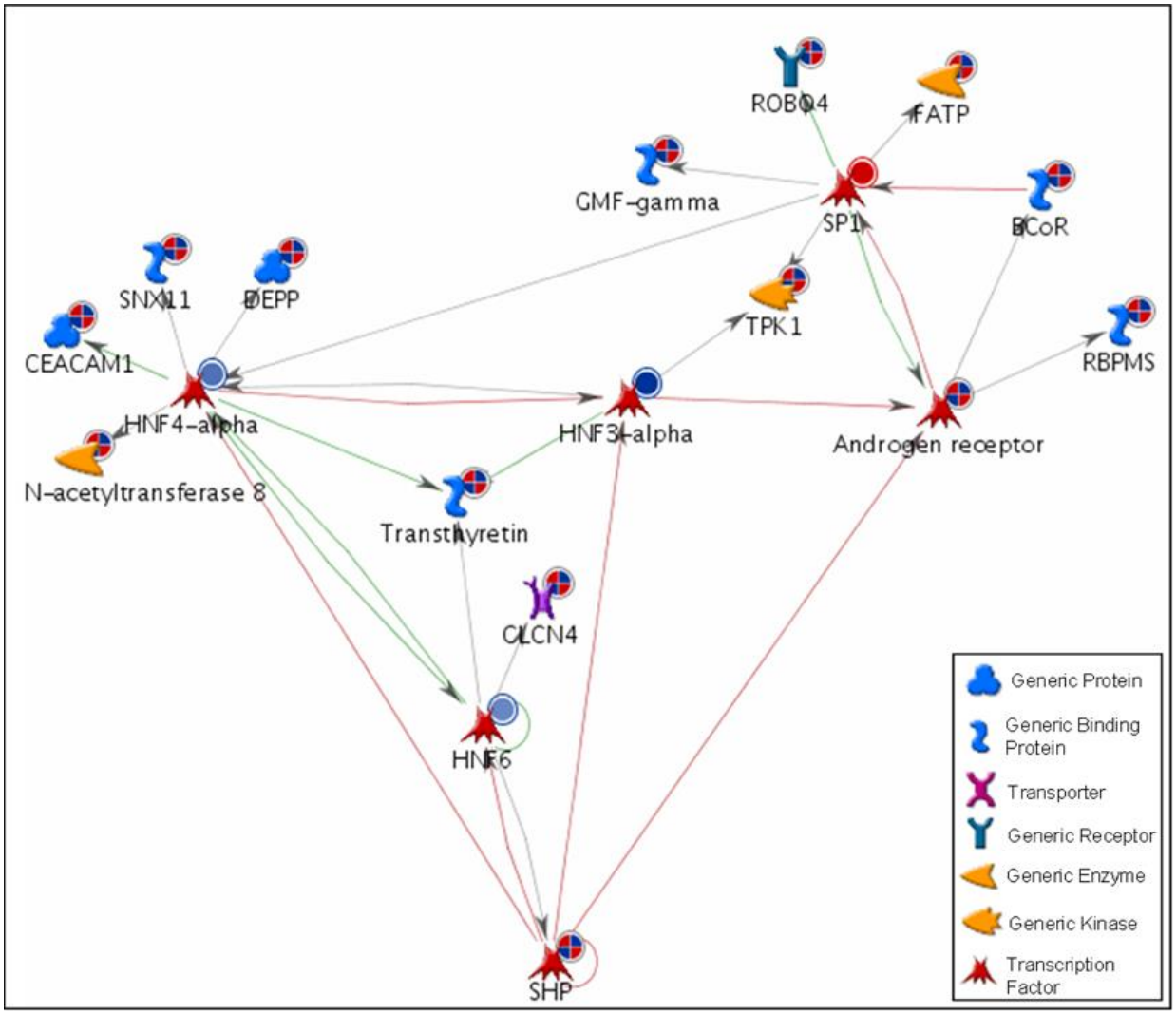
Table 3: Comparison of the performance for predicting *in vivo* genotoxicity of the transcriptomics-based assay upon 24h of exposure and Ames stratification of chemicals with conventional *in vitro* genotoxicity assays and combinations thereof

	Ames [a]	Ames + GE[a]	MLA [b]	Ames + MLA[b]	Ames + GE[b]	MN/CA[c]	Ames + MN/CA[c]	Ames + GE[c]	Ames + MLA/MN/CA [d]	Ames + GE[a]
Accuracy	**77%**	**89%**	**60%**	**60%**	**91%**	**63%**	**62%**	**88%**	**68%**	**89%**
Sensitivity	**78%**	**91%**	**94%**	**94%**	**100%**	**96%**	**96%**	**91%**	**96%**	**91%**
False negative rate	**22%**	**9%**	**6%**	**6%**	**0%**	**4%**	**4%**	**9%**	**4%**	**9%**
Specificity	**77%**	**87%**	**42%**	**42%**	**97%**	**46%**	**40%**	**86%**	**51%**	**87%**
False Positive rate	**23%**	**13%**	**58%**	**58%**	**3%**	**54%**	**60%**	**14%**	**49%**	**13%**

MLA: Mouse Lympoma Assay, GE: Gene expression, MN: Micronuclei Assay, CA: Chromosomal Aberrations

[a]: based on 62 compounds with available Ames results; [b]: based on 47 compounds with available MLA results; [c]: based on 60 compounds with available MN or CA (or both results); [d]: based on 62 compounds with data in at least one of the four in vitro assays.

Direct Interactions Network for Transcription Factors and their targets among the 33 classifiers of Method 2 for Ames-positive compounds.



Gene interactions are indicated by green = activation, red = inhibition, grey = unspecified. Red circles indicate up-regulation and blue circles down-regulation after both GTX and NGTX treatments; 'checkerboard' colors indicate mixed expression between GTX and NGTX compounds.

Comments from ECVAM to the Genomics-genotox assay

- Strengths of the test method include:
  - An apparently good sensitivity regarding the prediction of *in vivo* genotoxins.
  - A better specificity than the current *in vitro* battery with similar values of sensitivity.
  - The use of a human p53 competent cell line.

- Limitations of the test method include:
  - The cell line employed lacks significant metabolic biotransformation capacity which could result in some pro-genotoxins being classified as NGTX.
  - The functional role of the genes employed in the classifiers is not established.
  - A significant limitation of the performance evaluation of the test method is the fact that the set of reference chemicals used has not a sufficient representation of NGTX chemicals for the Ames positive and GTX chemicals for the Ames-negative classes.

Moving forward in human cancer risk assessment.

Paules RS, Aubrecht J, Corvi R, Garthoff B, Kleinjans JC.
Environ Health Perspect. 2011 Jun;119(6):739-43

- Although exciting progress is being made using genomics approaches, a new paradigm that uses these methods and human material when possible would provide mechanistic insights that may inform new predictive approaches (e.g., in vitro assays) and facilitate the development of genomics-derived biomarkers.
- Regulators appear to be willing to accept such approaches where use is clearly defined, evidence is strong, and approaches are qualified for regulatory use.

Hepatic and Cardiac Toxicity Systems

http://www.hecatos.eu/

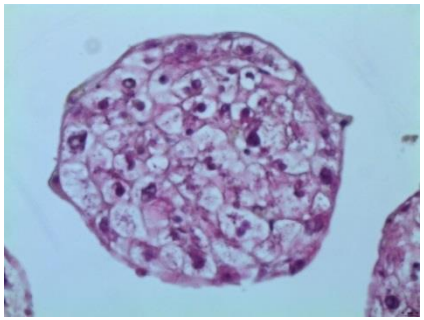
Building on success:

- **Main goal is to create multi-scaled *in silico* models for predicting preclinical toxicity, by:**
  - **Improving the biology**
  - By using complex 3D human cell models including organotypic primary cells instead of cell lines
  - By generating data at physiologically relevant doses, from multiple, more advanced 'omics platforms, and in combination with dedicated functional assays, thus enabling capturing a much wider range of intracellular mechanisms of toxicity
  - **Improving the relevance of *in vitro* responses for humans *in vivo***
  - By validating *in vitro* readouts on toxic mechanisms by analyzing organ biopsies from drug-treated patients
  - **Improving the prediction for human safety**
  - By developing multi-scaled *in silico* models using this wealth of data, thus capturing responses from the (sub-)cellular to the organ and organism level
  - By populating these models with "big data" from other publicly accessible data bases on toxicogenomics (EU FP7 diXa) and chemoinformatics (ChEMBL)

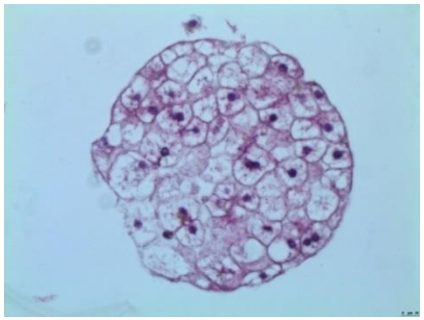
Human liver microtissues: cryopreserved hepatocytes and macrophages

HE

Day 7

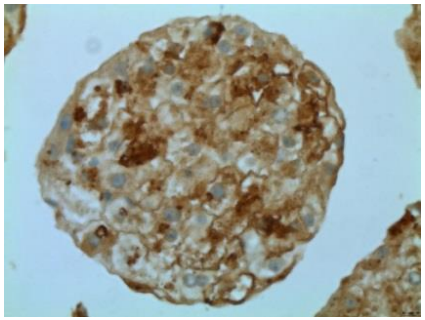


Day 28



CYP 3A4

Day 7



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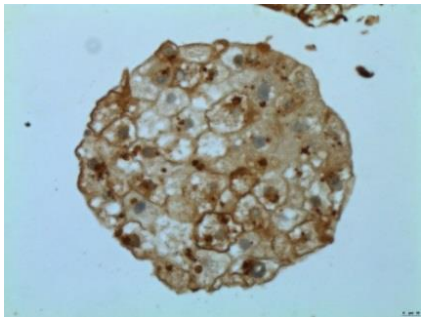
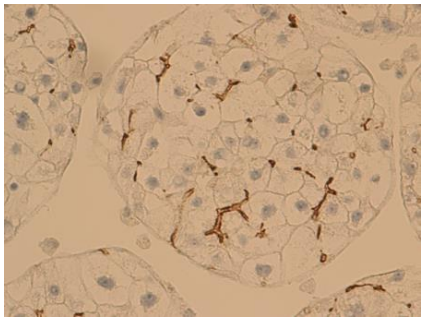
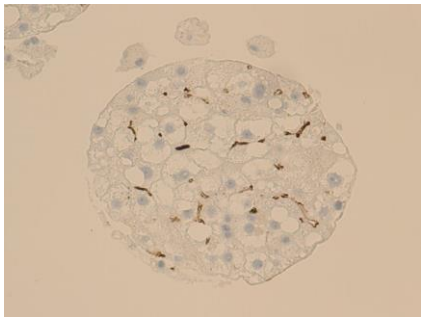
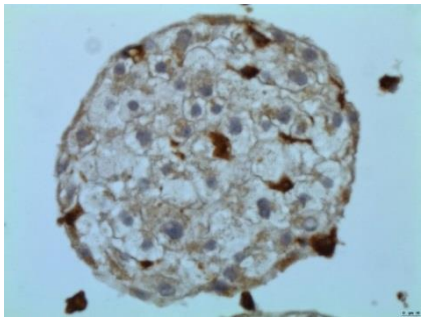


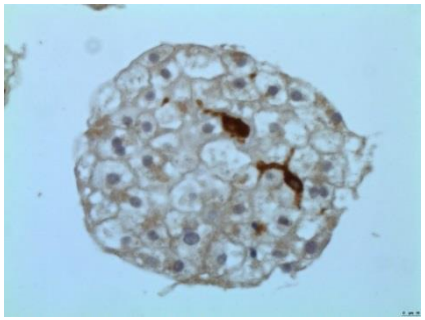
BSEP



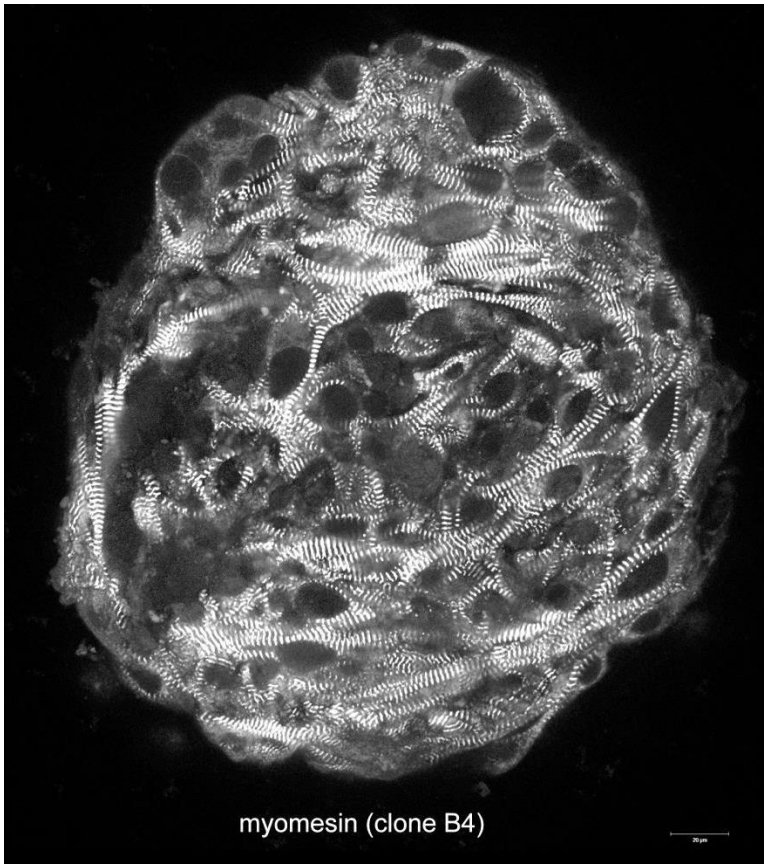


CD 68

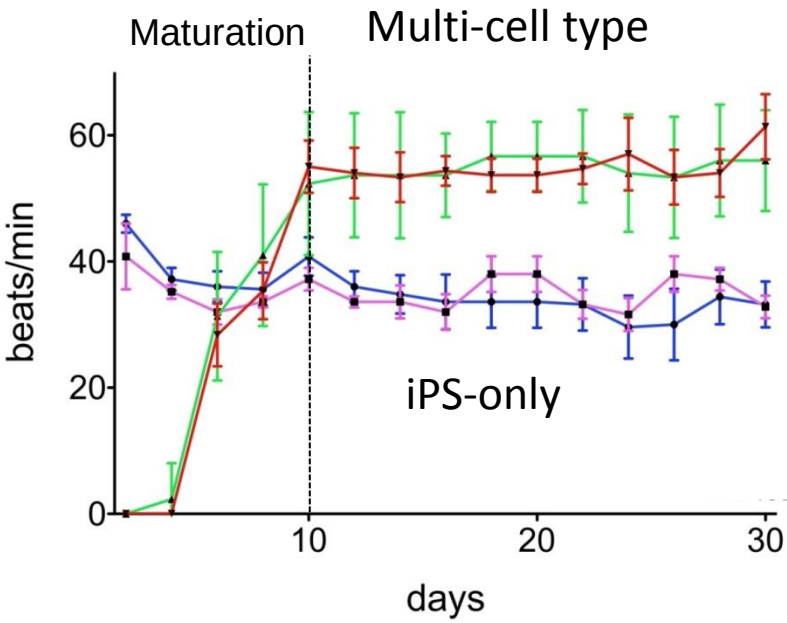




iPS-derived multi-cell type cardiac microtissues plus myofibroblasts

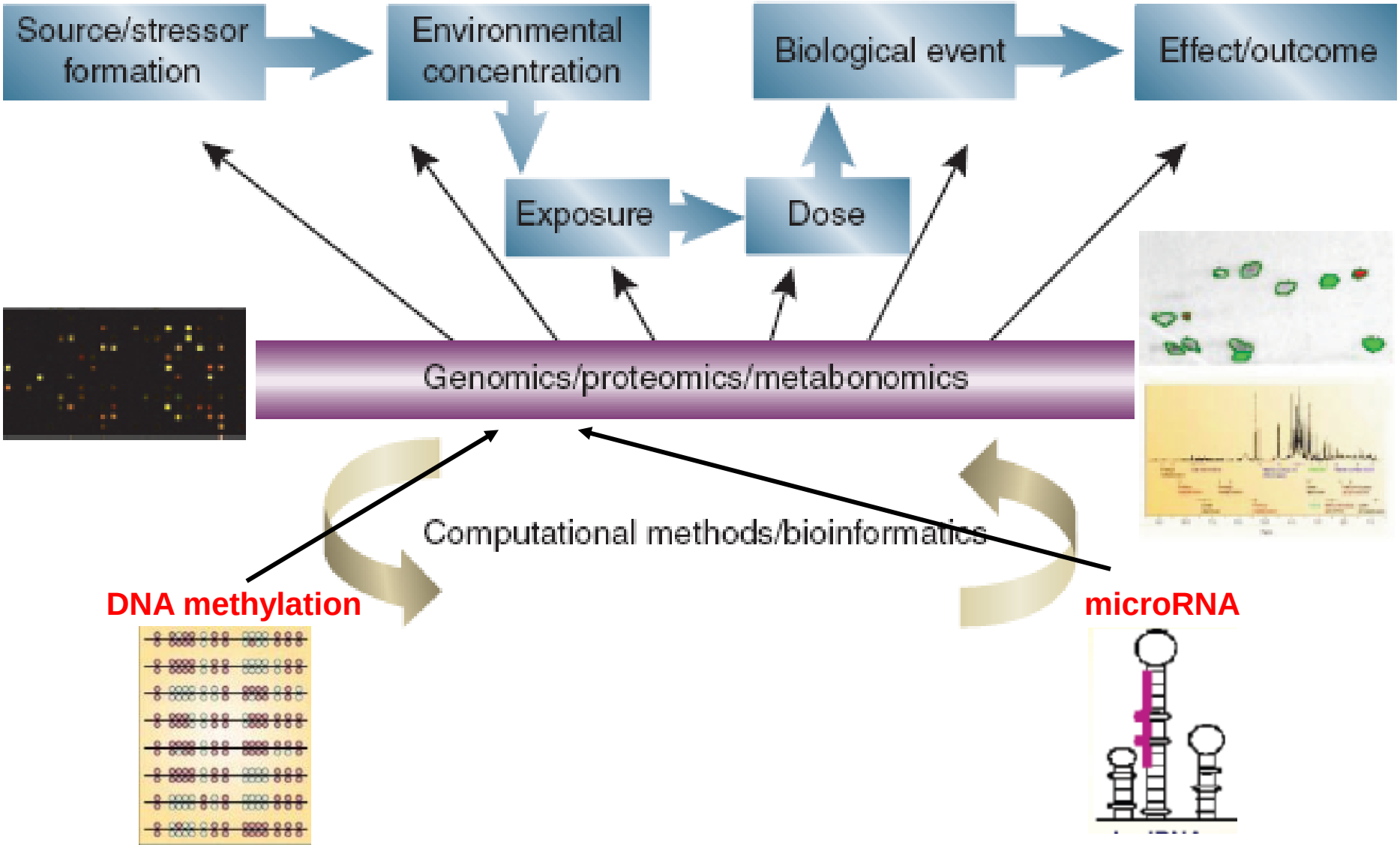


Myomesin



Data generated by Christian Zuppinger, University Hospital Bern

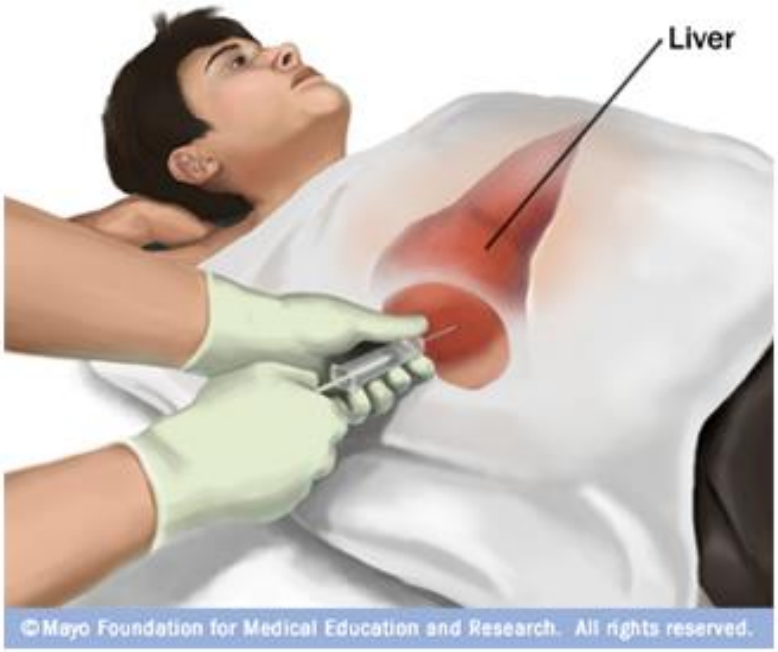
Integration of transcriptomics, proteomics, metabonomics with epigenetics and μRNA and bioinformatics in predictive toxicology



Clinical samples (liver tissue)

1

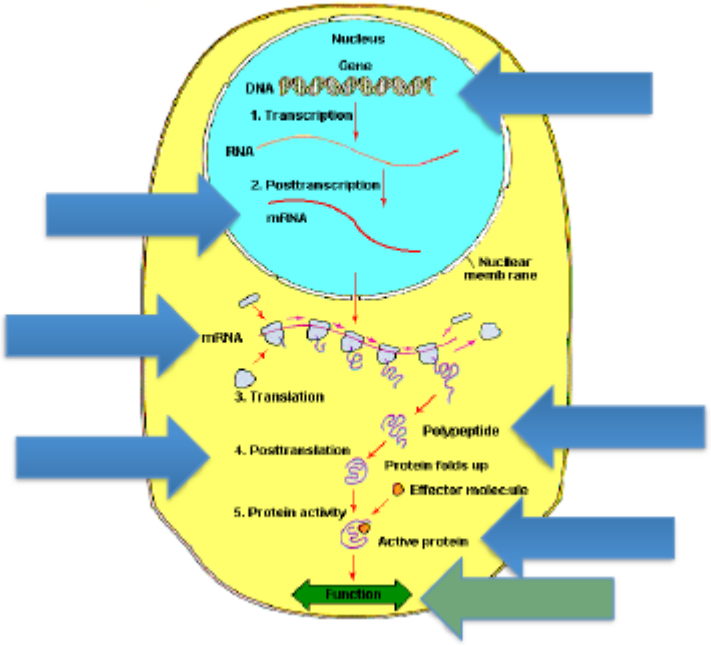
2



3

‘Omics analyses: Generation of ‘Omics data at all molecular levels with subcellular (mitochondrial) resolution

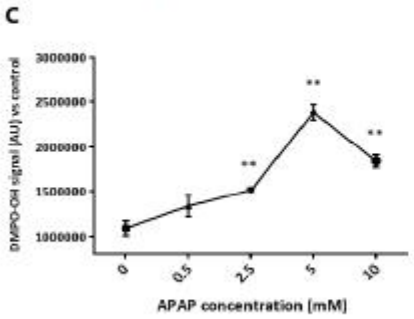
- Transcriptomics and Epigenomics through DNA/RNA sequencing
- Quantitative Proteomics and Phospho-proteomics (LC-MS/MS)
- Metabolomics and Flux analysis (NMR, LC/GC-MS)
- Functional Validation through RNAi technology



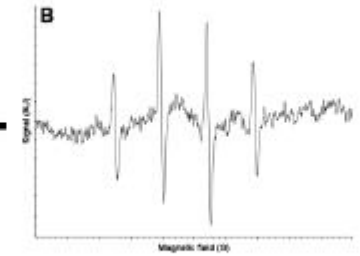
Analyses: functional analyses

- Functional Analysis
  - Mitochondrial Dysfunction
    - O2 Consumption
    - Extracellualr Acidification
    - Intracellular Oxygen
    - MMP
  - Apoptosis
    - Caspase 3/9
  - Cytokine Release
    - IL6 Release
  - ROS
    - Fluorometric
    - ESR
  - Electrophyiology
    - xCELLigence

Mitochondrial $O_2^{\cdot-}$/$OH^{\cdot}$ formation

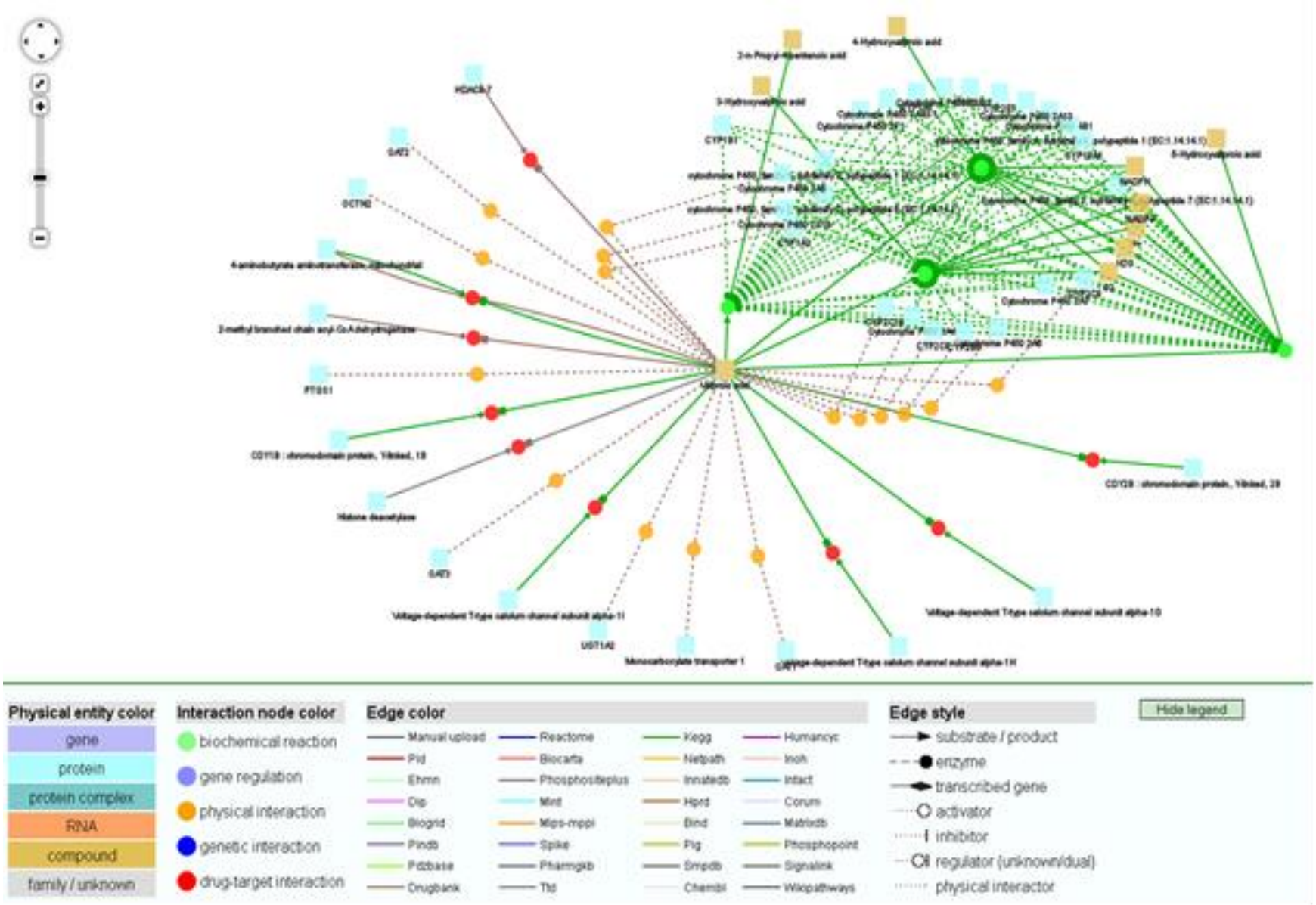


Radical identification/quantification



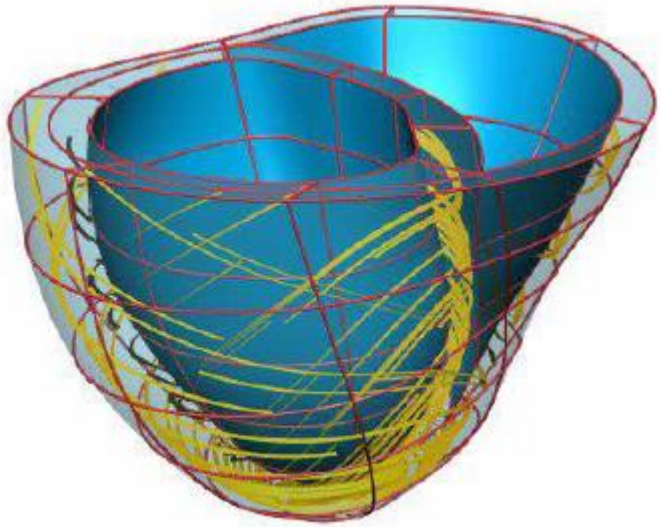
ESR: Direct detection of radicals

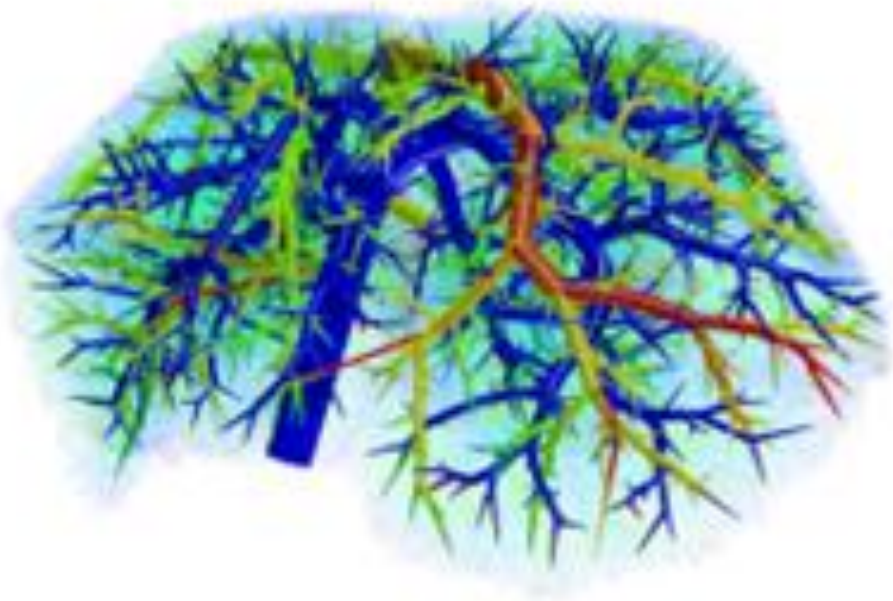
Creating multi-scaled *in silico* models. Step 1: molecular networks



HECATOS

Creating multi-scaled *in silico* models.
Step 2: physiological networks





Many thanks to

- The people of the Dept of Toxicogenomics at Maastricht University
- The carcinoGENOMICS consortium
- The HeCaToS consortium

AND TO YOU ALL !!