



The NTP HTS Initiative

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Implementation of the Vision: The NTP Roadmap

The NTP Roadmap places an increased emphasis on the use of alternative assays for targeting the key pathways, molecular events, and/or processes linked to disease or injury and attempts to incorporate them into a research and testing framework.

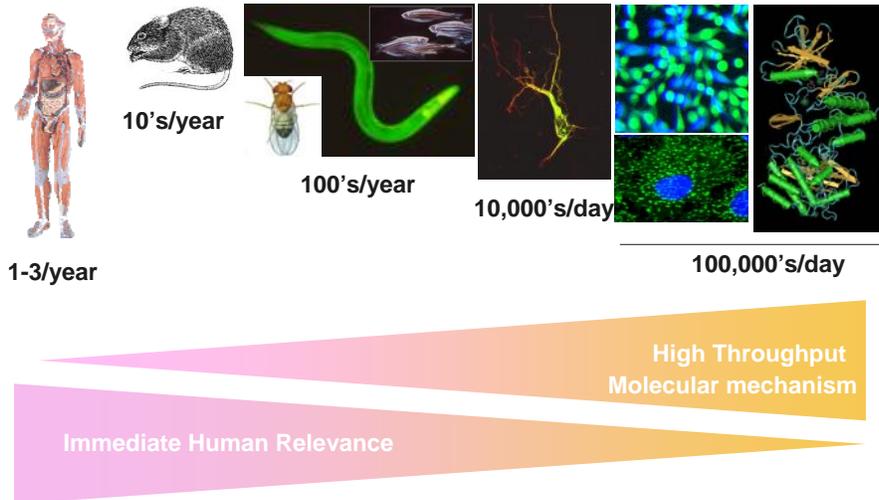
Roadmap Goals

- The NTP plans to convene the first HTS working group within the first 8 months of 2005 and have an HTS facility active within 20 months. NTP staff will identify chemicals for HTS that will include appropriate chemicals from the 500 plus agents evaluated in NTP carcinogenicity and reproductive and developmental toxicity studies.
- Activities and assays developed under the NTP Roadmap will be done in cooperation and consultation with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to maximize their value to regulatory agencies.

To meet the challenge of 21st century toxicology, the NTP Roadmap includes a major new initiative to develop a high throughput screening (HTS) program with 3 main goals:

- Identify mechanisms of action for further investigation
- Develop predictive models for in vivo biological response (esp. human)
- Prioritize substances for further in-depth toxicological evaluation

What can be screened?



NTP High Throughput Screening Assays Workshop

December 14 - 15, 2005, Arlington, VA

(<http://ntp.niehs.nih.gov/index.cfm?objectid=E13C3474-F1F6-975E-7CA9B6918AE38EF4>)

- **Selection of Targets and Assays for High Throughput Screening (HTS)**
 - **Co-chairs:** Dr. Kate Johnston (Cellumen Inc.), Dr. Tim Zacharewski (Michigan State Univ.)
- **Chemical Selection, Study Design and Analytical Methods**
 - **Co-chairs:** Dr. Christopher Lipinski (Pfizer Global R&D), Dr. William Janzen (Amphora Discovery Corp.)
- **Data Storage, Analysis, and Interpretation**
 - **Co-Chairs:** Dr. Pauline Gee (CeMines Inc.), Dr. Alexander Tropsha (Univ. North Carolina at Chapel Hill)
- **Application of Data from HTS Assays in Regulatory Decision-making**
 - **Co-Chairs:** Dr. Jonathan Freedman (NIEHS), Dr. Hillary Carpenter (Minnesota Department of Health)

Use of HTS data in Regulatory Decision-Making

Presently

- Cannot be used for making regulatory decisions
- Could be used in priority setting for further evaluation

Requirements for use

- Validation
- Uncertainty analysis
- Predictive ADME
- Chemicals must be tested in a large number of assays

Criteria for Regulatory Acceptance of HTS Assays

- Relevant (fits into exposure-disease continuum)
- Reliable (a workable assay)
- Repeatable (consistent results within/among labs)
- Recognized (acceptance by a large diverse audience)
- Realistic (outcome used for decision-making).
- Predictability - must provide information on sensitivity and specificity for a well-characterized reference set of agents

NIH Molecular Libraries Initiative

<http://nihroadmap.nih.gov/molecularlibraries/>

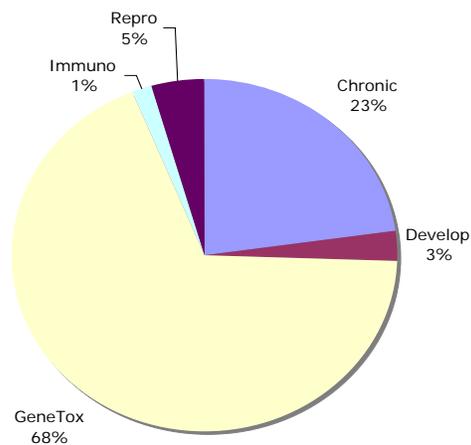
- The MLI is using HTS methods to identify small molecules that can be optimized as chemical probes to study the functions of genes, cells, and biochemical pathways.
- In mid-2005, NTP became a formal participant in the MLI by establishing a collaboration with Drs. Chris Austin and Jim Inglese of the NIH Chemical Genomics Center (NCGC) (<http://www.ncgc.nih.gov/>)
- Thus, the NTP has the opportunity to link data generated from HTS assays for biological activity to data produced by the NTP's toxicology testing program.



The NTP “1408”

- Provided as 10 mM solutions dissolved in DMSO
- 55 Duplicates
- Includes nearly every chemical class
- Molecular weights range from ~100 to ~400
- All have pre-existing toxicity data
 - 1206 with NTP test data
 - 147 are reference substances identified by NICEATM for the validation of alternative *in vitro* test methods (e.g., dermal corrosion, acute toxicity, endocrine activity).
- Includes solvents, fire retardants, preservatives, flavoring agents, plasticizers, therapeutic agents, inorganic and organic pollutants, drinking water disinfection byproducts, pesticides, and natural products
- In negotiation to provide compounds as a speciality set to MLI for use by all Centers

NTP Distribution



**The 1206 -
distribution
among Individual
NTP assays**



**The 1206 -
distribution
among NTP
assays, by
number of
assays tested**

Studies	Count
none	12
Chronic	12
Chronic & Devel	1
Chronic & GeneTox	305
Chronic & Devel & GeneTox	11
Chronic & Immuno & GeneTox	6
Chronic & GeneTox & Repro	34
Chronic & Devel & GeneTox & Immuno	1
Chronic & Devel & GeneTox & Repro	12
Chronic & GeneTox & Immuno & Repro	5
Chronic & Devel & GeneTox & Immuno & Repro	2
Devel	2
Devel & GeneTox	10
Devel & Repro	1
Devel & GeneTox & Repro	4
GeneTox	760
GeneTox & Immuno	7
GeneTox & Repro	9
GeneTox & Immuno & Repro	1
Immuno	2
Immuno & Repro	1
Repro	8
Total	1206



The Next 1408

- IRIS, Carcinogenic Potency, and HPV databases merged and duplicates subtracted
- Subtracted first 1408
- Subtracted MW<80; MW>700
- Added on-plate duplicates from first 1408
- Added compounds that didn't make the first set (arrived late, etc)
- Solicited suggestions from NIEHS community
- Desire to include structurally-related chemicals that cover the complete activity range
- Being whittled down to 2000 or so to be ordered

HTS assays supplied to the NCGC

- **Apoptosis Assays**
 - Caspase-Glo® 3/7 Assay
 - Caspase-Glo® 9 Assay
 - Caspase-Glo® 8 Assay
- **Cytotoxicity Assays**
 - CellTiter-Glo® Luminescent Cell Viability Assay (measures ATP levels)
 - Cytotox-ONE™ Homogeneous Membrane Integrity Assay (measures release of lactate dehydrogenase from membrane-damaged cells)
- **P-glycoprotein (Pgp) ATPase Assay (aka MDR1 or ABCB1)**
 - Pgp-Glo™ Assay

Cell lines being screened at the NCGC against NTP assays

Human cell lines

HEK 293	Transformed kidney
HepG2	Hepatoma
SH-SY5Y	Neuroblastoma
Jurkat	Acute T-cell leukemia
BJ	Foreskin fibroblasts
HUV-EC-C	Umbilical vein vascular endothelium
MRC-5	Lung fibroblasts
SK-N-SH**	Neuroblastoma (will not be included in future screens)

Rodent cell lines

Primary Renal Proximal tubule cells – rat	
H-4-II-E	Liver carcinoma – rat
N2a	Neuroblastoma – mouse
Buffy coat	Lymphocytes – rat
NIH 3T3	Embryonic fibroblasts -- mouse

Summary of NCGC Testing Conducted to Date and PubChem Status

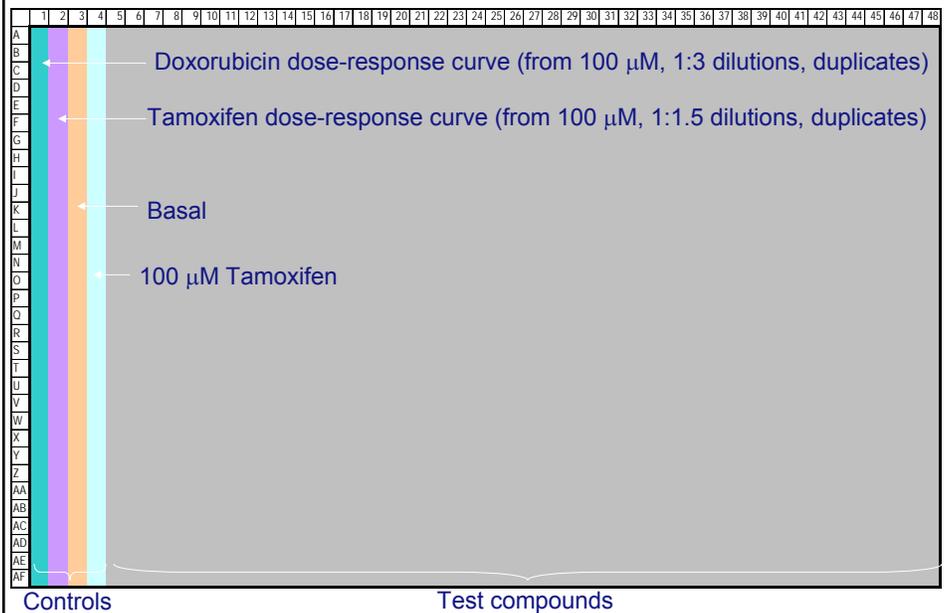
Human cell lines	CellTiter-Glo	PubChem	Caspase-3	LDH
Hek 293	Mar-06	✓	Jun-06	
HepG2	Jan-06	✓	Jun-06	Assay development
SH-SY5Y	Apr-06		Aug-06	
Jurkat	Jan-06	✓	Jun-06	
BJ (skin fibroblasts)	Mar-06	✓		
HUV-EC-C	Jul-06		Jul-06	
MRC-5 (lung fibroblasts)	Mar-06	✓		
SK-N-SH*	Mar-06	✓		
Rodent cell lines	CellTiter-Glo	PubChem	Caspase-3	LDH
Renal proximal tubule cells (rat)	Sep-06 **		Sep-06 **	Assay developed
H-4-II-E (rat)	Jun-06		Jun-06	
N2a (mouse)	Jun-06		Aug-06	
Buffy coat (rat)				
NIH 3T3 (mouse)	Jun-06		Jul-06	

Other NCGC HTS Assays

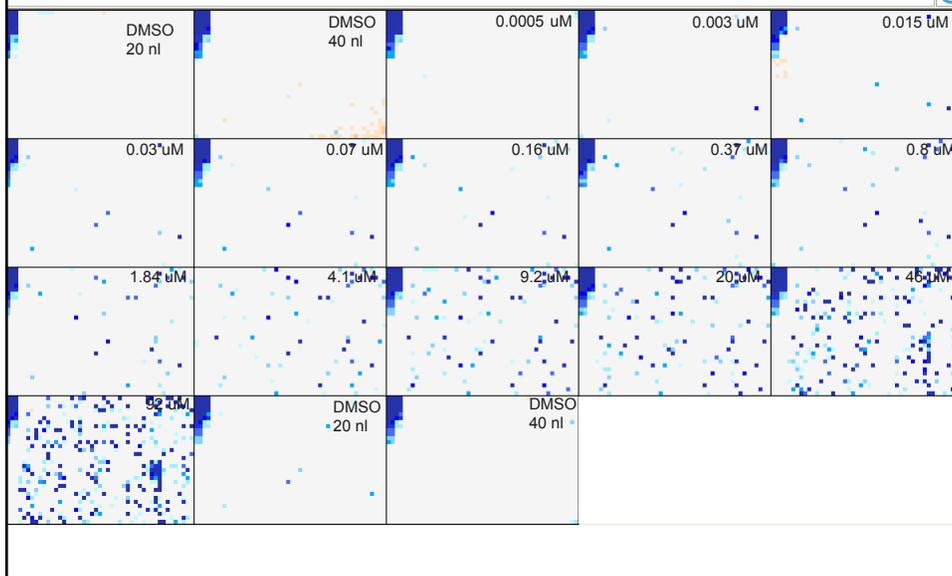
Project Name	Disease application (if any)
Acrosome reaction GFP	
Anthrax LF BLA	Anthrax
ATR Activation	Ataxia telangiectasia (OMIM 607585)
β-AdrR PCA bifurcated GFP	
β-lactamase (AmpC)	
β-glucocerebrosidase FI	Gaucher disease (OMIM 230800)
β-Thal mRNA splicing GFP	Beta-thalassemia
Caspase 3	
Cell signaling AP-1-BLA	
Cell signaling CRE-BLA	
Cell signaling HRE-BLA	
Cell signaling M1 NTR	
Cell signaling NFAT-BLA	
Cell signaling SIE-BLA	
Cell Translocation GR-EFC	
Cell Translocation GR-GFP	
Cell Translocation p65 HaloTag	
Cellular Toxicity (ATP level)	
Cellular Toxicity (LDH level)	
cLANA	HSV
Cpd aggregation FRET-1 (AggFRET)	
DNA damage GFP-x gene	
Drosophila Fat cell GFP	
ER/GR Translocation	
Fluor-DNA displacement-1	
Fluorescent Profiling-1	
GPVI Luciferase	
HIV Nucleocapsid FP	HIV
Hsp90 co-chaperone interaction	
Huntingtin PC12 cell toxicity	Huntington's Disease (OMIM 143100)
IkBα Cell sensor Dual Luc	Rare lymphomas
JNK ALPHAScreen	

**Other
NCGC HTS
Assays**

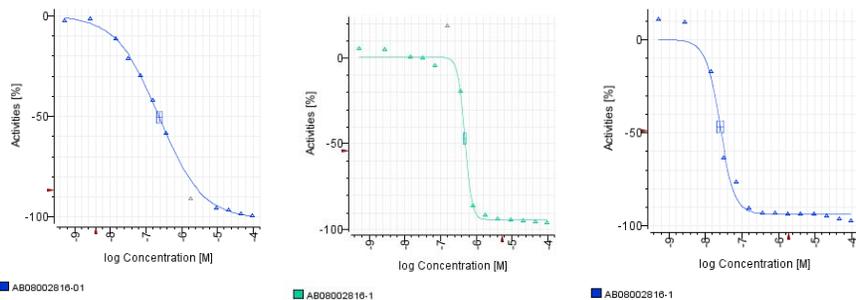
Project Name	Disease application (if any)
Locus Derepression Assay-1 GFP	
Luciferase profiling	
Malarial PSAC	Malaria
Multi-protein DNA Replication System	
O-Glc NAc Transferase	
Opsin trafficking ALPHA	Retinitis pigmentosa (OMIM 180380)
orphan GPCR -ADHD	ADHD
Oxidoreductase HADH2	
Oxidoreductase DCXR	
Oxidoreductase HSD17b4	
Oxidoreductase retSDR3	
Oxidoreductase SPR	
P450 CYP1A2, Luc	
P450 CYP2C9, Luc	
P450 CYP2C19, Luc	
P450 CYP2D6, Luc	
P450 CYP3A4, Luc	
Pantothenate Kinase	Tuberculosis
Peroxioredoxins (Tgr-Trx-Prx)	Schistosomiasis
PI5K4Pbeta	Diabetes
Progeria mRNA splicing GFP/RFP	Progeria (OMIM 176670)
Proteosome ubiquitin-GFP	Various
PyruvateKinase Luc	Hemolytic anemia (OMIM 266200)
RAS-RAF PCA bifurcated GFP	
Sialic aciduria	Sialuria (OMIM 269921)
SMA Cellular promoter act BLA	Spinal Muscular Atrophy (OMIM 253300)
Tau polymerization	Alzheimer, Frontotemporal dementia (OMIM 600274)
TF assay-cancer	Cancer
TPO Luciferase	Thrombocytopenia
Ubiquitin Pathway	Various
YieE FP	



qHTS results map for Jurkat cell screen



Cell viability dose response



NGGC provides normalized and corrected values and computes AC50

Each dilution is assayed in a separate plate, with in-plate controls
 Doxorubicin and Tomaxifen dose response in duplicate
 Column of Basal, column of 100 uM Tomaxifen is used to define 0% and 100% "death"

NTP National Toxicology Program PubChem

© AID 427 - PubChem BioAssay Summary

Address: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=427>

NCBI PubChem National Library of Medicine

BioAssay Summary

AID: 427
 Name: Cell Viability - Hek293
 Data Source: NCGC

Test Results: [Show](#) [Select](#) [Plot](#)

Links: [Description](#) [Protocol](#) [Comment](#) [Definitions](#)

Links:

- Compounds: All: 1335 Active: 74 Inactive: 1180 Inconclusive: 86
- Substances: All: 1408 Active: 80 Inactive: 1241 Inconclusive: 87
- Taxonomy: 1 Link

Description:

NIH Chemical Genomics Center (NCGC)
 NIH Molecular Libraries Screening Centers Network (MLSCN)
 National Institutes of Environmental Health Sciences (NIEHS)
 National Toxicology Program (NTP)

NTP National Toxicology Program PubChem

© AID 427 - PubChem BioAssay Summary

Address: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=427#Description>

Descriptions:

NIH Chemical Genomics Center (NCGC)
 NIH Molecular Libraries Screening Centers Network (MLSCN)
 National Institutes of Environmental Health Sciences (NIEHS)
 National Toxicology Program (NTP)

NCGC Assay Overview:

We have developed a 1536-well cell-based assay for quantitative high throughput screening (qHTS) against a number of cell lines to determine in vitro cytotoxicity of small molecules. This particular assay uses the Hek 293 cell line which is derived from human embryonic kidney cells (transformed with adenovirus).

Protocol:

NCGC Assay Protocol Summary:

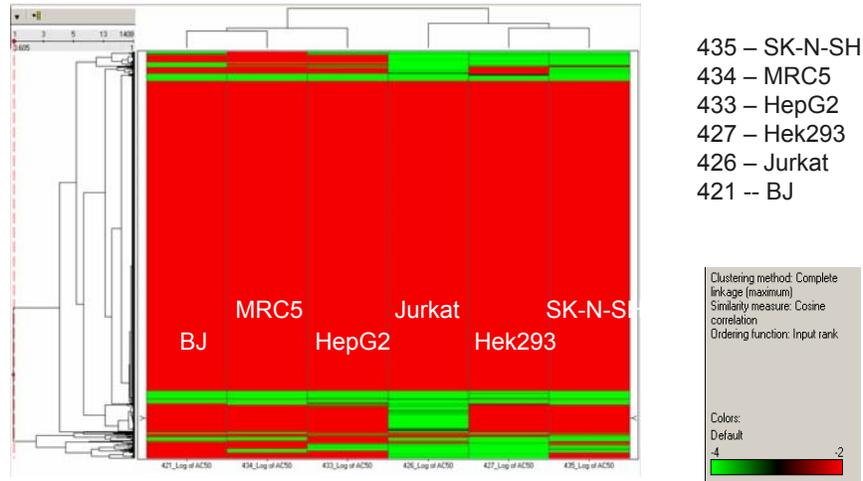
The CellTiter-Glo luminescent cell viability assay (Promega) is a homogeneous method to measure the number of viable cells in culture. The end point readout of this assay is based on quantitation of intracellular ATP, an indicator of metabolic activity, using the luciferase reaction. Luciferase catalyzes the oxidation of beetle Luciferin to oxyluciferin and light in the presence of ATP. The luminescent signal is proportional to amount of ATP present.

Using the CellTiter-Glo luminescent cell viability assay, the amount of cellular ATP was measured in the Hek293 cell line with complete culture medium following compound treatment for 40 hours. The assay was performed in opaque white Kalypsys 1536-well plates. In the screen, temozolomide and doxorubicin were used as positive controls. Library compounds were measured for their ability to cause acute toxicity in the cell line, as reflected by a decrease in intracellular ATP levels, in a concentration-dependent manner. Data were normalized to the controls for basal activity (DMSO only) and 100% inhibition (100 nM temozolomide). ACS0 values were determined from concentration-response data modeled with the standard Hill equation.

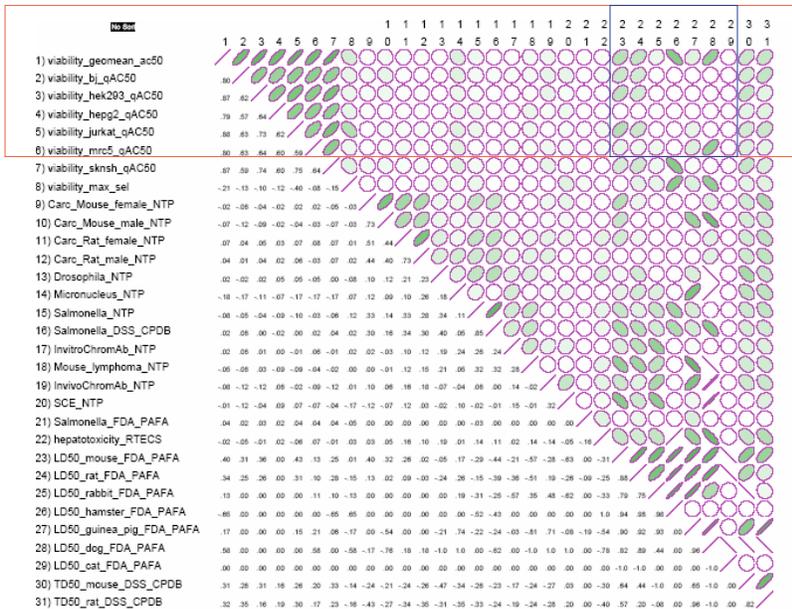
qHTS protocol for CellTiter-Glo-Hek 293 cellular assay

[Step]	[Parameter]	[Value]	[Description]
1.	Reagent:	5 uL; 1:500	Hek293 cells/well
2.	Time:	5 hr;	37°C incubation
3.	Compounds:	23 nL; 0.59 nM to 92 uM	
4.	Controls:	23 nL; Temozolomide 0.34 uM to 130 uM & Doxorubicin 0.02 nM to 100 uM	
5.	Time:	40 hr;	37°C incubation
6.	Reagent:	5 uL; CellTiter-Glo reagent	
7.	Time:	20 min;	Room Temperature
8.	Detection:	Luminescence;	Viewlux plate reader

Heatmap: log(AC50) organized by correlation



remove the ~ 1000 compounds that were not active (AC50 = -2)



NTP-EPA Collaboration on HTS Assays

- In Dec. 2005, a collaboration was established between the NTP HTS Faculty and the EPA Chemical Prioritization Community of Practice (CPCP) to jointly evaluate HTS assays and other model systems for their use in toxicological investigations and in chemical prioritization.
- In Jan, 2006, joint NTP/EPA subcommittees were established to address specific topics related to HTS
 - Toxicity targets and bioactivity assays
 - Co-Chairs: Kristine Witt (NIEHS) and Keith Houck (EPA)
 - Chemical selection
 - Co-Chairs: Cynthia Smith (NIEHS) and David Dix (EPA)
 - Informatics
 - Co-Chairs: Jennifer Fostel (NIEHS) and Ann Richard (EPA)

Future Directions

- **Expand the number of compounds**
 - Structurally-related compounds with a range of toxicities
 - Parent and metabolites
 - Mixtures
- **Expand the number of HTS assays**
 - P450s (CYP1A2, CYP3A4, CYP2D6)
 - Critical Pathways: AP1, STAT, NFAT, HRE, NFkB, nuclear hormone signaling
 - Other NCGC assays
 - Other MLI assays
 - Other NTP selected assays
- **Expand the number of cell types**
 - Primary cells
 - Different species
- **Expand chem-bio-informatic capabilities**

Determining the Mechanistic Basis for Toxicity through HTS

