



Selection of Targets and Assays for High Throughput Screening (HTS)

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Critical Constituents and Targets

What are the critical cellular constituents or pathways that are likely to be linked to an *in vivo* toxic response?

Guiding principals:

- ◆ **Initial holistic approach:**
 - Apical function > pathway > isolated target
 - Function (phenotypic) > cellular pathway > target
- ◆ **Integrate transcript profiling to guide selection of relevant pathways & targets**
- ◆ **Multi-dose, multi-time point assays required**
- ◆ **Whenever possible, test metabolites & explore cell backgrounds with metabolizing capabilities e.g., MCL5**

Critical Constituents and Targets

Guiding Principals

- ◆ **Select most physiologically-relevant model:**
 - **Organisms, (worms and fish): feasible for HTS but relevance to mammalian systems is problematic**
 - **Tissues: not feasible for HTS**
 - **Co-cultures: intriguing, EXPLORE**
 - **Primary cultures: technically challenging, but preferred; use both rodent and human when possible, possibly stem or progenitor cells**
 - **Cell lines: non-transformed, screen both human and rodent**

Critical Constituents and Targets

All including:

- ◆ - Apoptosis
- ◆ - Proliferation (PCNA, Ki67, centriole number)
- ◆ - Oxidative stress
- ◆ - Mitochondrial activity
- ◆ - Receptor interactions (nuclear and membrane)
- ◆ - Metabolism
- ◆ - Signal transduction
- ◆ - DNA damage
- ◆ - Cytoskeletal/differentiation
- ◆ - Lipid metabolism and transport channels
- ◆ -Transporters
- ◆ - Chromatin remodeling
- ◆ - Necrosis/membrane integrity
- ◆ - Cytokines
- ◆ - Cell-cell communication
- ◆ - Adhesion

Endpoints for Carcinogenesis

Which pathways are the most important to study to understand the pathways leading to cancer?

- ◆ **Function first: phenotypic assays for cellular proliferation, death and environmental response**
- ◆ **Critical pathways for these functions:**
 - **Apoptosis**
 - **Proliferation, cell-cycle control**
 - **DNA damage and repair**
 - **Chromatin remodeling**
 - **Signal transduction modulation**
 - **SAPKs, MAPKs, Inflammatory response**

Endpoints for Carcinogenesis

Apoptosis

- ◆ **Models**
 - Rodent: Rat1, NIH 3T3, primary hepatocytes
 - Human: HepG2, TK6 (p53 WT& its p53⁻ pair), MCL5
 - +/- p53 pair
- ◆ **Measure both pro-apoptotic & inhibitory activities**
- ◆ **Assays:**
 - Early & late markers of apoptosis
 - Multi-dose & multi time points

Endpoints for Carcinogenesis

Apoptosis assays:

- **Cytochrome C release**
- **AIF release**
- **annexinV binding**
- **Caspase activity: 3,7,8,9**
- **Mitochondrial membrane potential**
- **Nuclear fragmentation**

Appropriate Endpoints for Carcinogenesis

Proliferation, Cell-cycle control

◆ **Models**

- **Rodent: Primary hepatocytes, MEFs,**
- **Human: HepG2, human foreskin fibroblasts, MCL5**

◆ **Assays**

- **Cell number: cell count, nuclei count**
- **DNA content: Hoechst intensity, thymidine or uridine incorporation**
- **Cell cycle markers: histone phosphorylation, DNA content (2n, 4n), microtubule stability**

Endpoints for Carcinogenesis

DNA Damage

- ◆ **Models**
 - Rodent: L5178Y, CHO
 - Human: HepG2, TK6
- ◆ **Assays**
 - H2AX phosphorylation
 - P53 activation: phosphorylation, translocation
 - Nuclear morphology
 - Micronucleus
 - DNA fragmentation COMET

Endpoints for Carcinogenesis

Chromatin remodeling

◆ Models

- Rodent
- Human

◆ Assays

- De-repression of CMV-driven GFP expression
- Excision repair enzyme activity

Endpoints for Carcinogenesis

Signal Transduction Pathway Modulation

◆ Models

- Rodent CHO
- Human HepG2, U2OS

◆ Assays

- Kinase activity SAPKs, MAPKs : phosphorylation (Abs - imaging, cell ELISAs)
- Transcription factor activity (reporters), phosphorylation (Abs - imaging, cell ELISAs), translocation and stability (Abs or chimeras - imaging)

Toxicology - Wish List

- ◆ **DNA repair assays**
- ◆ **HTS-compatible lipid metabolism assays**
- ◆ **Differentiation methods for stem cells and a mechanism to identify a population**
- ◆ **Fixable probes for mitochondrial membrane potential, oxygen tension, lipid content**
- ◆ **Assays of more than 48 hrs duration**
- ◆ **HTS-compatible hERG assay (ion channel blocking)**

Toxicology - Wish List, continued

Novel assays to measure Toxicologically-significant endpoints in HTS

- ◆ Nuclear Receptors
- ◆ G-protein Coupled Receptors
- ◆ Toll Rc modulation
- ◆ TRKs (growth factor rc)
- ◆ Cell backgrounds with enhanced metabolizing capability
- ◆ Notch signaling assays
- ◆ Card-carrying toxicologists responding to the RFA

Assay Development

- ◆ Assay Development for High Throughput Molecular Screening (R03/R21)
Reissue for FY2006 of RFA-RM-05-011
- ◆ Mark Scheideler, Program Director for Molecular Libraries Initiative
 - Mark Scheideler
301 496-1779
- ◆ Opportunity to submit mechanism-focused Toxicology assays to MLSCN

Assays in the Queue

- ◆ Caspase 3/7, 8 & 9 activation
- ◆ pGP
- ◆ Cell viability
- ◆ Heat Shock Protein 90
- ◆ GPCRs: Formyl peptide Rc, 5HT1E
- ◆ NfκB pathway
- ◆ TNF alpha signaling
- ◆ Channels: GIRK, voltage gated K⁺
- ◆ Htt protein aggregation and degradation