Development of a Human 3D Prostate Microtissue Assay for Anti-androgen Screening

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A Tox21 Cross Partner Project

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Conflict of Interest Disclosures

There is no conflict of interest to declare

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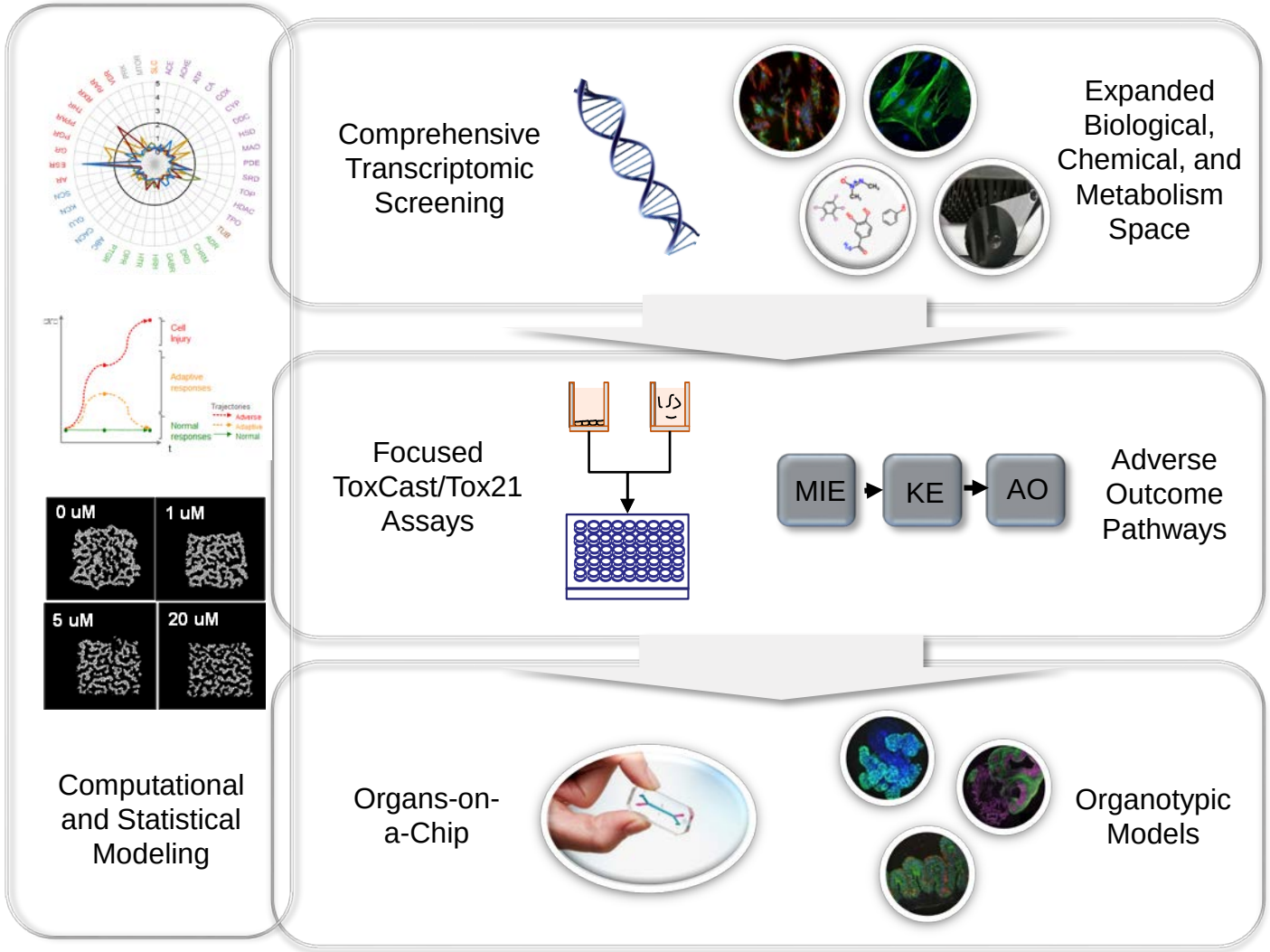
CompTox Roadmap: Tiered Testing Framework for Hazard Characterization

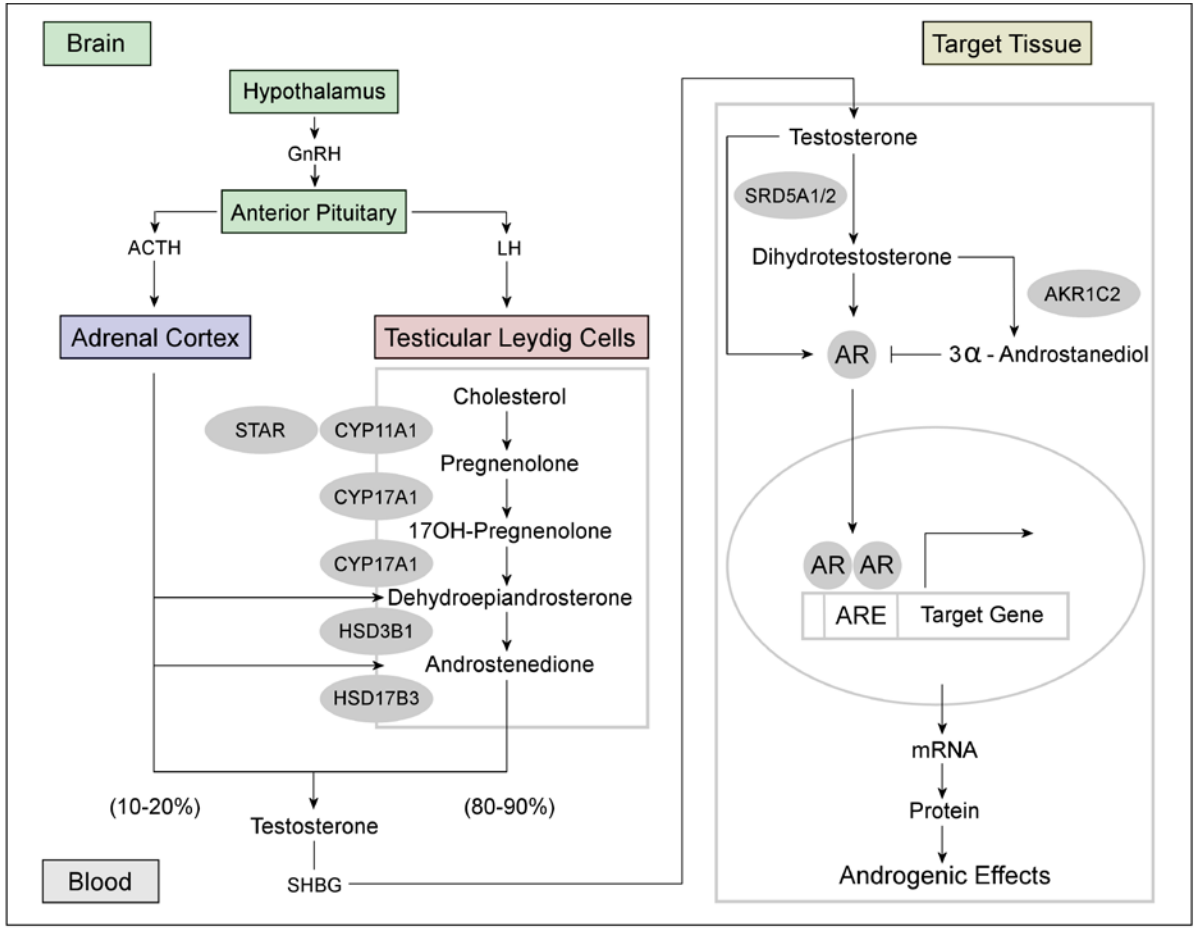


Tier 0
Comprehensive Testing & Characterization

Tier 1
Verification of Affected Processes/ Pathways

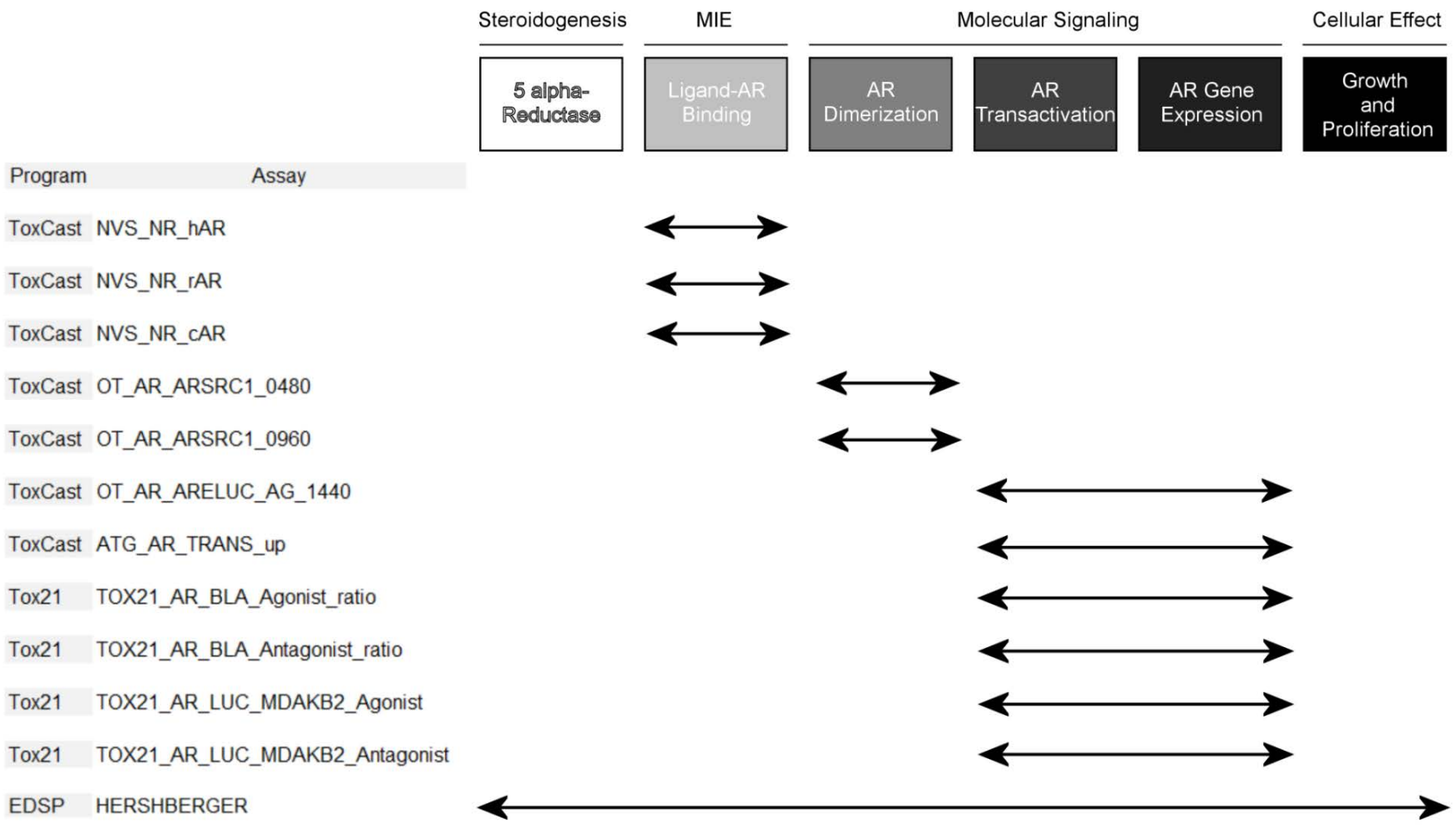
Tier 2
Interpretation of Affected Process/ Pathways and Population Variability

Androgen Steroidogenesis and Target Tissue Activity



Altered androgen hormone biosynthesis and metabolism can modulate androgen levels, contributing to endocrine disruption that may result in impaired reproductive and sexual development.

Androgen Screening Battery by AOP Key Event



- Data gaps for *in vitro* testing include 5$^{\alpha}$-Reductase and AR-dependent cellular effects
- Poor coverage across assays for evaluating anti-androgen bioactivity

Tox21 Cross Partner Project: Objective and Goals

- **Objective**
  - Establish a cross-partner collaboration within Tox21 to develop assays for use in evaluating the potential effects of xenobiotics on 5α-reductase function.

- **Goals**
  - Develop and validate a high-throughput assay for screening human 5α-reductase inhibition.

  - Develop a prostate epithelial cell microtissue model for evaluating 5α-reductase inhibition and direct modulation of androgen-dependent signaling.

- **Programmatic Fit**
  - Tox21 Collaboration Roadmap: "…advance the development and deployment of alternative test systems for predicting disruption of human androgen signaling."

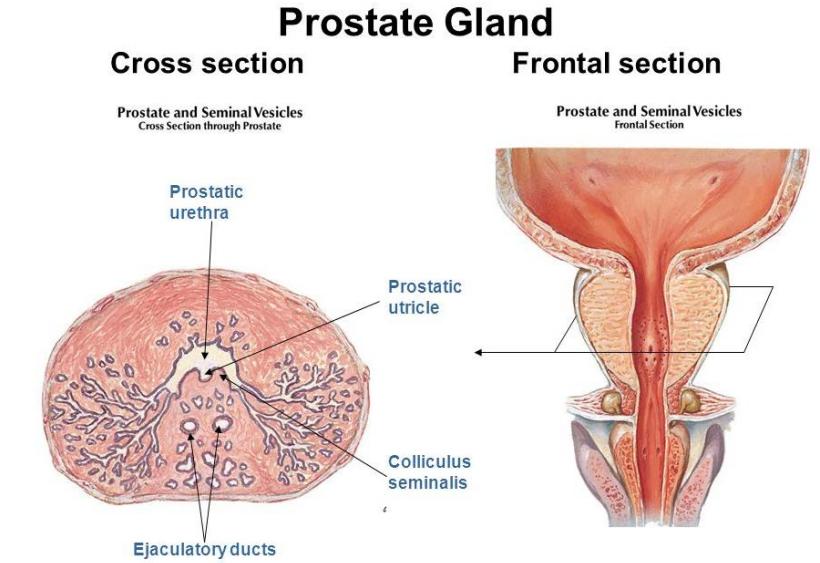
Hershberger Assay

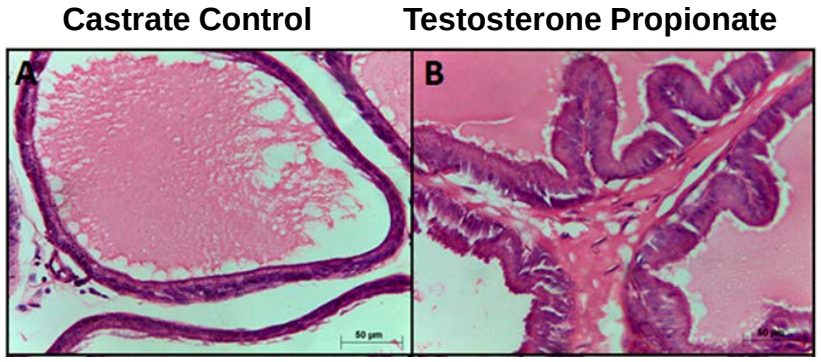
- **Guideline Study:** OECD TG441 (2009), OCSPP Guideline 890.1400 (2011)

- **Background:**
  - Androgens are essential for sex determination via development of the male reproductive system and maturation of accessory sex organs (ASO).
  - The Hershberger rat bioassay was published in 1953 to evaluate disruption of this process.
  - Adopted as a component of EDSP in 1998, first test orders began in 2009. To date, ~136 unique chemicals have been screened.

- **Purpose:** Short-term *in vivo* bioassay to identify chemical substances with **androgenic**, **anti-androgenic**, and **5α-reductase inhibition** activity

Targeting Hershberger Accessory Sex Organs *In Vitro*: Prostate Epithelia 3D Microtissue

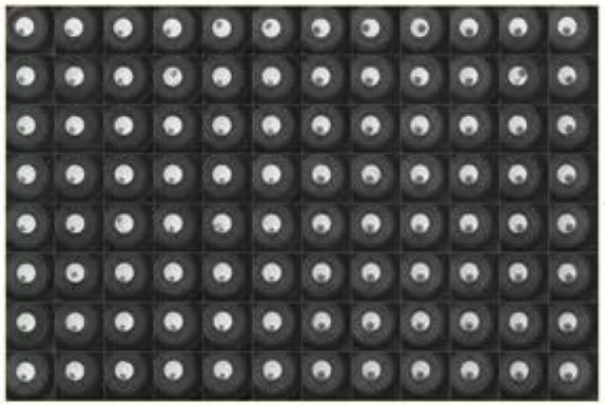




Sci Rep. 2015 Oct 23;5:15639

- **Reproductive Function**: tubulalveolar exocrine gland that secretes alkaline fluid to support semen volume and stability
- **Primary Cell Types**: epithelial, stromal myoblasts and fibroblast, immune, and endothelial cells
- **Androgen Dependence**: Prostate epithelial cells (PEC) contain a physiological androgen receptor signaling pathway
- **Steroidogenesis**: PEC maintain a high level of 5α-reductase expression.
- **Cellular Functions**: Secretion of AR-dependent Prostate Specific Antigen (PSA) and proliferation of PECs cells are modulated by AR activation.

Assay Concept: A Multiplexed Phenotypic Assay for Capturing AOP KEs in Androgen Signaling



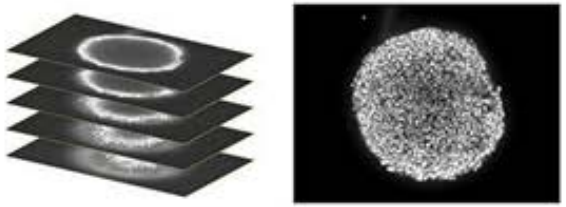
Lactate Dehydrogenase

Cell Titer-Glo (ATP)

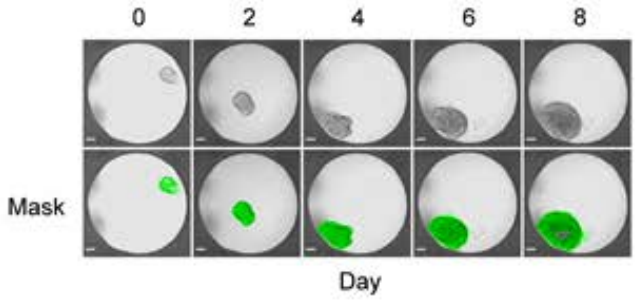
Cytotoxicity

Prostate Specific Antigen ELISA

AR Pathway Bioactivity

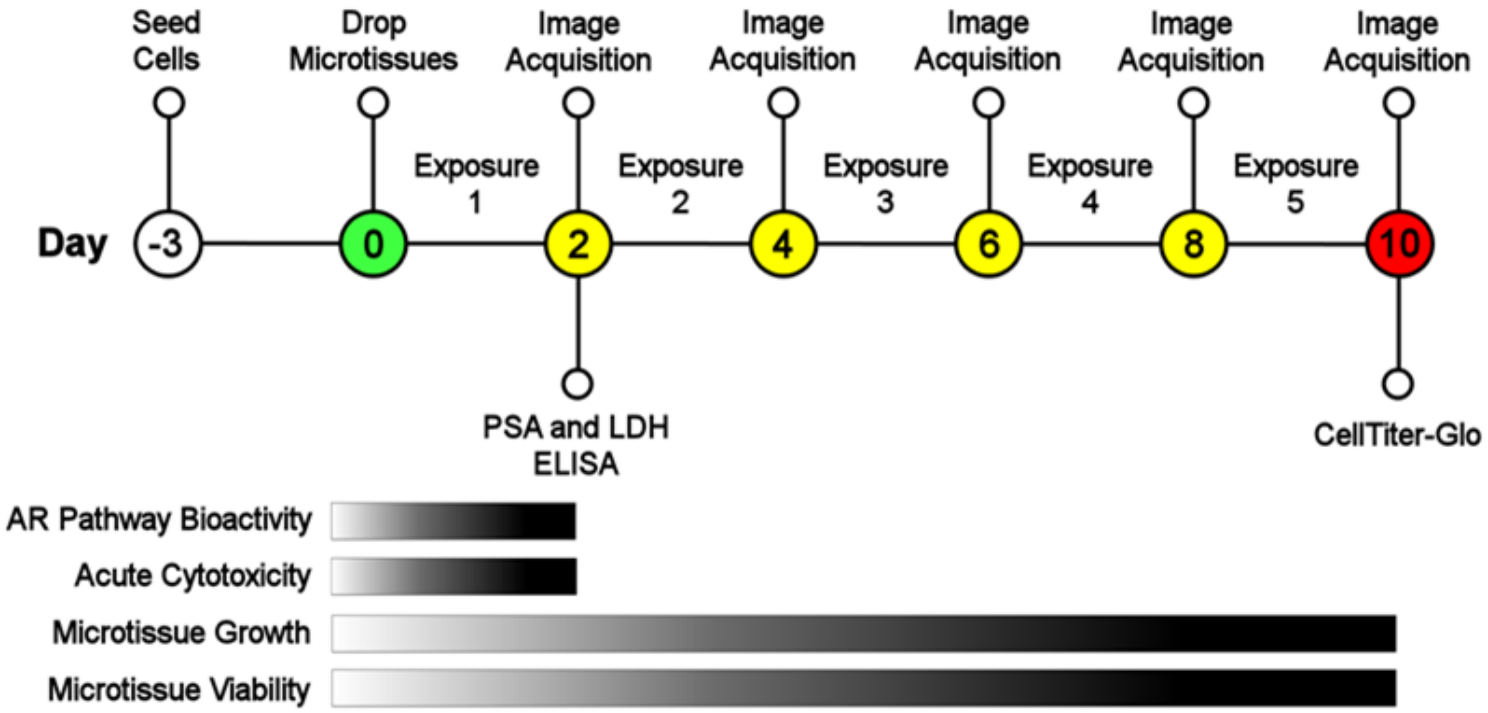


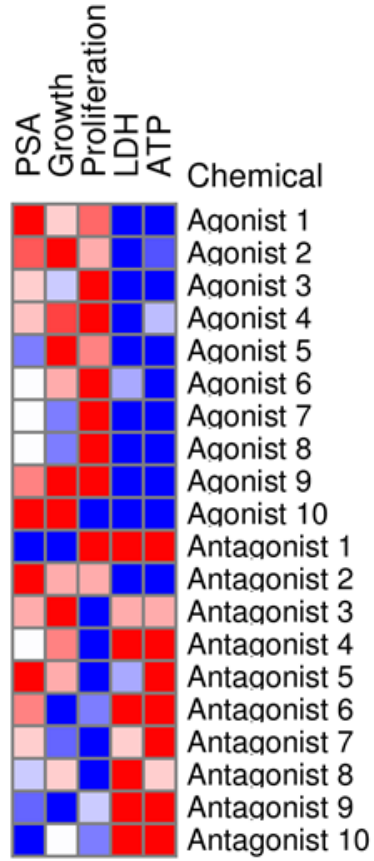
Cell Proliferation



Microtissue Hypertrophy

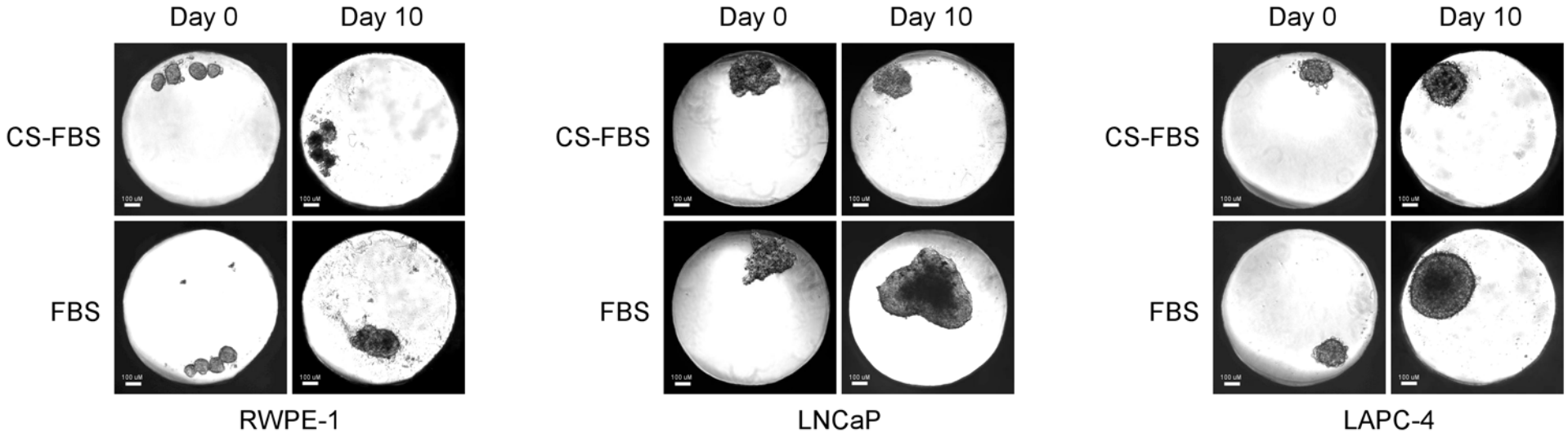
Prostate Microtissue Assay Workflow





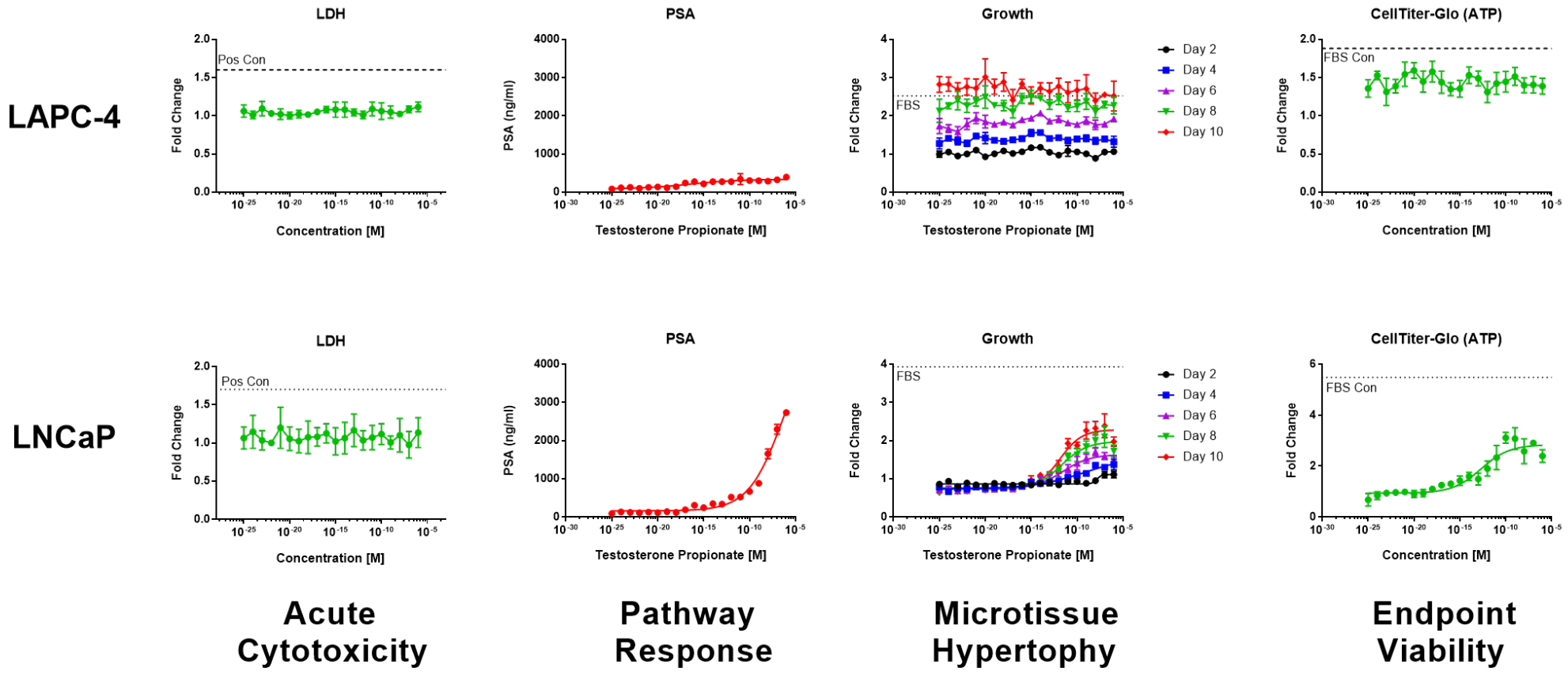
- Workflow simulation of the Hershberger assay
- Multiplexed approach to integrate pathway-level bioactivity with a tissue-like endpoint
- Weight-of-evidence across multiple endpoints to determine AR-dependent toxicodynamics

Cell Line Evaluation: Microtissue Formation and Growth



Designation	Organ	Type	Disease	AR	SRD5A1	SRD5A2	PSA	Source
RWPE-1	Prostate	Epithelial	Normal	WT	NA	NA	Yes	ATCC (CRL-11609)
LNCaP FGC	Prostate	Epithelial	Adenocarcinoma	Mutant	High	Low	Yes	ATCC (CRL-1740)
LAPC-4	Prostate	Epithelial	Primary transitional cell carcinoma	WT	High	Low	Yes	Charles Sawyer (UCLA)

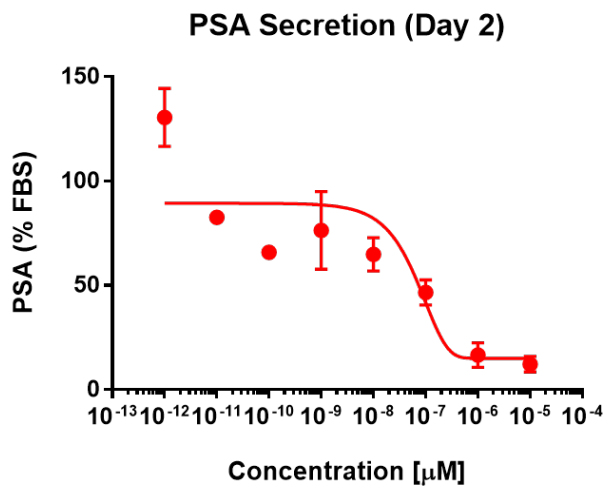
Agonist Mode: Prostate Cell Line Responses to Hershberger Reference Androgen



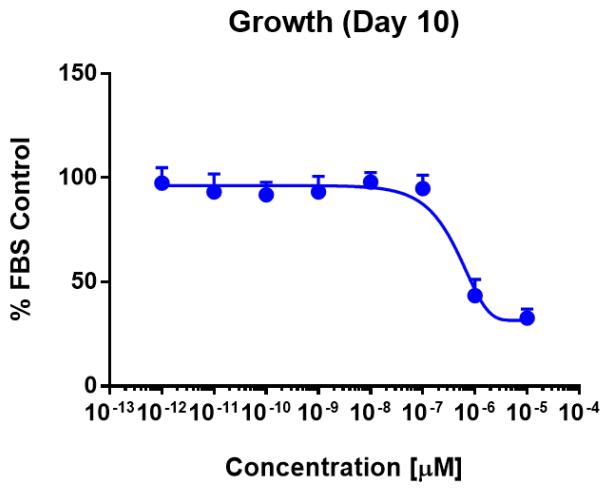
- Linking pathway level potency (MIE) to a tissue-like endpoint response in a single test system
- Measurement of acute and chronic cytotoxicity responses across a 10-day assay
- Data highlights significant differences in testosterone dependence across human cell lines

Antagonist Mode: 5α-reductase Inhibitor Reduces AR Signaling and Microtissue Growth

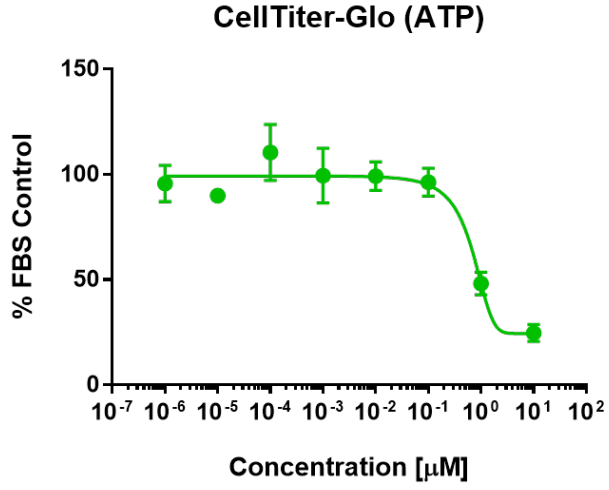
LNCaP



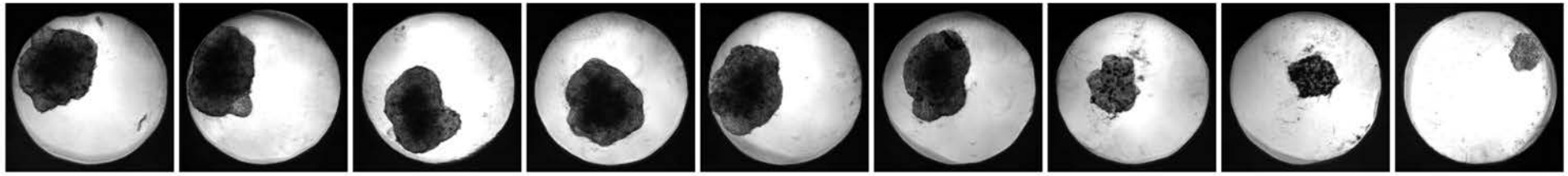
Pathway Response



Microtissue Hypertrophy

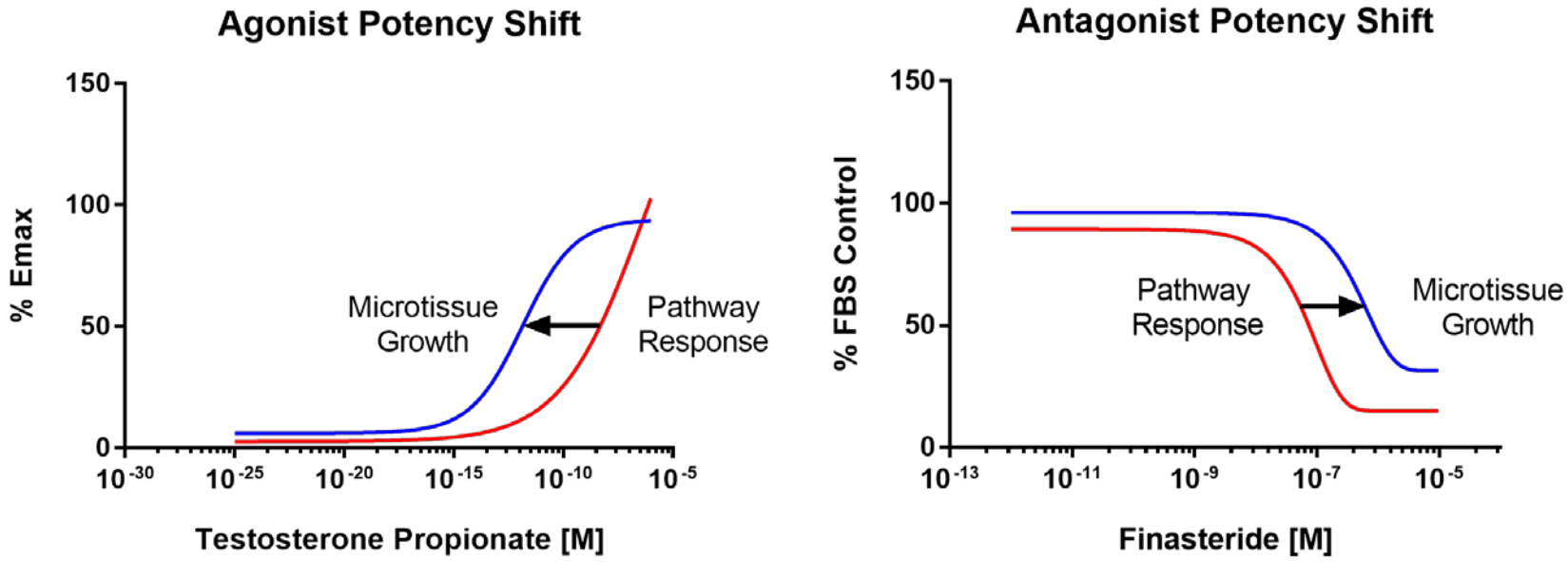


Endpoint Viability



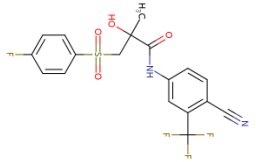
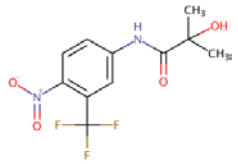
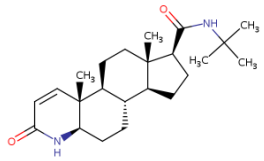
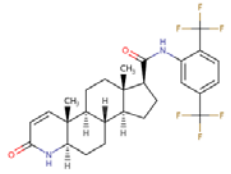
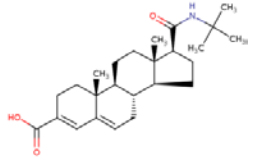
Potency-Bioactivity Relationship: Contrasting Short Term Bioactivity with Repeat Exposure Outcomes

LNCaP



- Potency assessments reveal shifts between early AR pathway level responses (Day 2) and terminal microtissue growth (Day 10)
  - **Agonist mode**: Microtissue growth is ***more*** sensitive to repeated androgen exposure over time
  - **Antagonist mode**: Microtissue growth is ***less*** sensitive to repeated 5$^{\alpha}$-reductase inhibitor exposure over time

Reference Chemical Evaluation for Anti-androgen and 5α-reductase Inhibitors in LNCaP Cells

Test Compound	Structure	MOA	LDH	Pathway IC50	Growth IC50	ATP IC50
Bicalutamide		AR Inhibitor	No Effect	0.4	2.3	3.3
Hydroxyflutamide		AR Inhibitor	No Effect	ND	42.1	51.9
Finasteride		5α-Reductase	No Effect	0.3	1.6	3.3
Dutasteride		5α-Reductase	No Effect	8.2	79.5	44.5
Epristeride		5α-Reductase	No Effect	ND	ND	6.1

Conclusions

- The hanging drop technology is a sound approach for screening chemical effects on prostate spheroid androgen activation, growth, and cytotoxicity in 3D cell culture.

- A multiplexed approach to assay endpoint evaluation integrates AOP key event from pathway-level bioactivity to a tissue-like endpoint

- Potency shifts, assessed in the context of cytotoxic effects, may refine the interpretation of AR antagonism