NCATS Intramural Research Program in the HEAL Initiative:
Developing Human-based Testing Platforms and Novel Drugs for
Pain, Addiction, and Overdose

**NOT-TR-19-018** NCATS is accepting pre-proposal applications!

**Mission:** Speed and facilitate the development of new treatments for pain, opioid misuse and opioid overdose

**Productive Partnership(s)**
- Milestone driven
- Agile and iterative
- Synergistic
- Mission-specific

**NCATS brings:**
- Access to resources and technology not easily accessible
- Automation
- Industry and other expertise
- Agile process
- Program management

**Collaborator brings:**
- Target, probe, or compound
- Subject matter expertise
- Necessary resources
- Existing data
Human iPSC-Derived Neurons for Pain and Reward Pathways

Collaborators can work with NCATS Stem Cell Translation Lab to develop iPSC-derived cellular platforms for improved prediction of *in vivo* human effects of lead compounds.

### Capabilities:

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<tr>
<th>Access to relevant human cell types</th>
<th>Advanced imaging technologies for functional cell characterization</th>
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<td>Sensory neurons (nociceptors) and other neuronal subtypes</td>
<td>High-content confocal, calcium imaging, optogenetics</td>
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<td>High-throughput electrophysiology methods</td>
<td>Measurement of signaling pathways, metabolism &amp; specific targets</td>
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<td>Cyclic AMP, PKA activity, CREB phosphorylation, energy metabolism</td>
<td>Longitudinal tracking of cell behavior</td>
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<td>Multiple measurements over days, weeks or months</td>
<td>Combined single-cell transcriptomic &amp; proteomic analyses</td>
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<td>Drug response in individual nociceptors and other neuronal phenotypes</td>
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Induced Pluripotent Stem Cells (iPSCs)

- Reprogram and expand
- Isolate PBMCs (blood draw) or Fibroblasts
- Can be differentiated into a variety of lineages
  - Cardiomyocytes
  - Muscles
  - Neurons
  - Blood Cells
  - Hepatocytes

*2012 Nobel Prize*

~30 Days
Generation of Human iPSCs for the NIH HEAL Initiative

- 4-5ml blood draw
- Isolate PBMCs
- Reprogram and expand
- Establish stable patient derived iPSCs
- 2-D Neuron Cultures
- 3-D Neural spheroids
- 3-D Minibrain Organoids
Highly Efficient Human Nociceptor Differentiation Protocol

NF200/BRN3A/DNA

Nociceptor Neurons

Highly Efficient Human Nociceptor Differentiation Protocol
3-D Bioprinted Tissue Models

Collaborators can work with NCATS 3-D Biofabrication Laboratory to biofabricate multicellular functional tissues using human primary or iPSC-derived cells that are better models of human disease state and response to new drugs.

**Capabilities**

- **Tissue engineering technologies**
  - Development of tissues-in-a-well

- **Automated production of iPS cell-derived cells**
  - To reproducibly scale up production of human tissue relevant cells

- **3D biprinters**
  - To create spatial cellular patterns in tissues, e.g., neuronal circuits, neurovascular unit, innervated tissues

- **Spatially defined and physiologically relevant tissue models**

- **Validation of 3D organoid cultures**
  - Neural spheroids for compound screening

- **Assays using 3D tissue models**
  - High-content confocal, calcium imaging, optogenetics, multielectrode arrays, neurotransmitters biosensors
Development of high-throughput tissue clearing protocols for high-content, image and functional activity analyses in human iPSC-derived neural spheroids

Clearing can be used to improve 3D tissue visualization and analysis

Application of clearing to iPSC-derived neural spheroids

Functional imaging of spontaneous and synchronized calcium oscillations in iPSC-derived neural spheroids:

- Untreated
- Compound 1
- Compound 2

Boutin, et al, Sci Rep, 2018
Human iPSC-Derived Advanced Brain Organoid Models

Differentiation, embryoid body formation, further maturation under agitation/stirring

iPSCs → PKCλ, CTIP2, DAPI

Day 52
Forebrain Organoid

TH, FOXA2, DAPI

Day 40
Midbrain Organoid

3-Dimensional

*In vivo* architecture

Cell-identity

Protocol adapted from Qian et al., Nature Prot. 2018; Cell 2016
Development of Pharmacological Probes for Novel Targets

Access NCATS resources and expertise in assay development and quantitative high-throughput screening to identify promising compounds to modulate novel targets; optimize compound properties to probe novel targets.

Capabilities

HTS assay adaptation, development

- GPCR and ion channel assays and high-content image-based assays

Drug repurposing libraries

- All FDA approved compounds (>2,400), as well as >150,000 in annotated/diversity collections, HEAL-focused library

Counterscreen & confirmatory assays

- Molecular modeling and docking, Machine learning, High content image analysis

Cheminformatics platforms

- Largest medicinal chemistry program at NIH, > 30 fume hoods, > 20,000 molecules made

Medicinal chemistry

- Aqueous kinetic solubility, rodent & human liver microsomal stability & PAMPA permeability

ADMET Assays
Development of Investigational Drugs Ready for Clinical Testing

Joint project teams develop prototype therapeutics into IND-enabled small molecules, biologics, and gene and cell therapies ready for clinical testing.

Capabilities

- **Target validation and lead optimization**: To finalize declaration of clinical candidates
- **Pharmacokinetics/pharmacodynamics**
- **GLP safety evaluation and toxicology**
- **Therapeutic modality expertise**: Including small molecules, biologics and gene and cell therapies
- **GMP manufacturing and formulation**: To scale up the production of the compound for clinical testing
- **Repurposing of approved therapies**
Tissue Chip
RFA TR-19-003

The goal of this FOA is to promote the development of in vitro microphysiological systems to model human nervous and non-nervous tissues that recapitulate the mechanisms or effects of nociception/pain-relevant signaling, addiction, or opioid use disorders (OUDs), and/or their respective therapies and treatments.