MODULE 3

INTRODUCTION TO
HUMAN CELL MODELS OF AGING CORE

• SD-NSC HUMAN COHORT
• CELLULAR MODELS OF AGING

Co-Leaders:

Anthony Molina (UCSD)
Rusty Gage (Salk)
INTRODUCTION TO HUMAN CELL MODELS OF AGING CORE

- INTRODUCTION TO HUMAN CELL MODELS OF AGING CORE
- OVERVIEW OF NOVEL HUMAN LIFESPAN COHORT
- OVERVIEW OF FACILITY RESOURCES
- OVERVIEW OF CELL MODELS
WELL PHENOTYPED COHORT POWERS NOVEL CELL MODELS

- **SD-NSC Cohort will represent the adult human lifespan** and be extensively phenotyped for physical and functional metrics of biological aging
- Subject-specific fibroblast used to generate **iCell models (capture age related phenotypes)** and hiPSCs
- hiPSCs used to generated **niche specific multi-cellular organoid models**
- **iCell and hiPSC models combined** into novel hybrid models of human aging
WELL PHENOTYPED COHORT POWERS NOVEL CELL MODELS

SD-NSC Clinical Cohort

Aim 1

Physical/functional phenotypes of biological age

Aim 2

Generate subject-specific fibroblasts

Induced cell models

Aim 3

Organoid and novel hybrid cell models

Aim 2

hiPSC models

Aim 4

Cellular/molecular phenotypes

Heterogeneity Core

Integrative Models Core

Genotype ↔ Phenotype & Phenotype ↔ Phenotype

Heterogeneity of Aging
What is “representative” or “normal” when it comes to aging?

Major Considerations:
- Healthy vs Normal
- Majority of patients over 65 present with multiple comorbidities

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
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<tbody>
<tr>
<td>- Over 20 yrs of age</td>
<td>- Are pregnant</td>
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<tr>
<td>- Able to consent and participate in the study using English</td>
<td>- Diabetes (fasting glucose &gt;126 mg/dl)</td>
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<tr>
<td>- BMI ≥ 18.5 and ≤30 kg/m2.</td>
<td>- Uncontrolled hypertension (BP &gt; 140/90 mmHg)</td>
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<tr>
<td>- Weight stable for the prior 6 weeks</td>
<td>- Heart or cardiovascular condition, including coronary artery disease, congestive heart failure, diagnosed abnormality of heart rhythm, atrial fibrillation, and/or a history of myocardial infarction</td>
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<tr>
<td>- Normal cognitive function</td>
<td>- Cancer or history of cancer</td>
</tr>
<tr>
<td>- Willing and able to attend two in-person study visits that will include vigorous exercise testing, blood draw, and skin biopsy.</td>
<td>- Dementia or other conditions that may affect cognitive ability</td>
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<tr>
<td>- Willing to wear a wireless accelerometer (Actigraph GT3X) for 14 days</td>
<td>- Sensory or physical impairment that would prevent participation</td>
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<tr>
<td></td>
<td>- Parkinson’s disease, multiple sclerosis, or other neurological condition, including a previous stroke, which may be causing impaired muscle function or mobility</td>
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<tr>
<td></td>
<td>- Medications and supplements that may interfere with measurements or biological outcomes including, but not limited to: metformin, CoQ, glucocorticoids, and medications that may alter cardiac and hemodynamic responses to exercise</td>
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<tr>
<td></td>
<td>- Respiratory disease</td>
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<tr>
<td></td>
<td>- Answers “yes” to one or more questions in the American College of Sports Medicine’s Physical Activity Readiness Questionnaire (PAR-Q) and/or report two or more risk factors for exercise testing</td>
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</table>
Defining Biological Age

- Physical function is among the best predictors of morbidity and all cause mortality

Pooled data from 9 cohort studies
34,485 total participants, 17,528 deaths

- Remaining years of life plotted by age group

10 yr survival Hazard ratio for every 0.1 m/s was 0.88

Studenski JAMA, 2011
Visit 1

Informed Consent During the Covid-19 Pandemic

Blood Sample Collection
Visit 1

Cardiorespiratory Fitness (CPET)

-VO2 max is generally regarded as the best indicator of cardiorespiratory fitness.

-Safety
  - Persons with counterindications will be excluded from the study
  - Monitoring (Physician, Exercise Physiologists)
    - EKG, Blood Pressure

-Age/Ability appropriate
  - Exercise Modalities
Short Physical Performance Battery

1. Balance Tests
   - Side-by-Side Stand
     Feet together side-by-side for 10 sec ≤ 10 sec (0 pt)
     10 sec (1 pt)
   - Semi-Tandem Stand
     Heel of one foot against side of big toe of the other for 10 sec ≤ 10 sec (0 pt)
     10 sec (1 pt)
   - Tandem Stand
     Feet aligned heel to toe for 10 sec 10 sec (+2 pt)
     3-8.99 sec (+1 pt)
     >8.7 sec (0 pt)

2. Gait Speed Test
   Measures the time required to walk 4 meters at a normal pace (use best of 2 times)
   1m 2m 3m 4m
   < 6.21-6.70 sec 2 pt
   >6.7 sec Unable 0 pt

3. Chair Stand Test
   Pre-test
   Participants fold their arms across their chest and try to stand up once from a chair
   Able ≤ 11.19 sec 4 pt
   11.20-13.69 sec 3 pt
   >13.70-16.69 sec 2 pt
   >16.7 sec 1 pt
   >60 sec or Unable 0 pt

Limitations
- Ceiling Effects
Visit 2
Dual-energy X-ray absorptiometry (DXA)

Body Composition
Bone Mineral Density
Visit 2

Physical Performance

Gait Speed
- 6mwd (fast)
- 2.5 mwd (usual)

Leg Strength (Biodex)

Grip Strength
Visit 2-3

Actigraphy
monitoring rest/activity cycles
2 weeks
Visit 3

Muscle Composition

Size and Adiposity
Visit 3

Sensory Abilities
Visit 3

Cognition

NIH TOOLBOX

DOG  HORSE

Emotion Sensation Cognition

Reading

7 5

D 3
## Biological Samples

SD-NSC Biorepository  
Rolling Enrollment (2021-2023)

<table>
<thead>
<tr>
<th>Blood</th>
<th></th>
<th>Limitations of SD-NSC Cohort</th>
</tr>
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<tbody>
<tr>
<td>- Platelets</td>
<td></td>
<td>- Small Size (N=40)</td>
</tr>
<tr>
<td>- WBCs</td>
<td></td>
<td>- Inclusion/Exclusion Criteria</td>
</tr>
<tr>
<td>- Plasma</td>
<td></td>
<td>- Diversity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Sex</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Race/Ethnicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Suitability of measures for broad age range</td>
</tr>
<tr>
<td>Cells</td>
<td>- Primary dermal fibroblast</td>
<td><em>Opportunities to expand</em></td>
</tr>
<tr>
<td></td>
<td>- reprogrammed iPSCs (stem cells)</td>
<td>- # participants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Representation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Measurements</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Biospecimens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Longitudinal Follow Up</td>
</tr>
</tbody>
</table>

*Based on future grant funding*
Mitochondrial Bioenergetics – Blood Cells

**Agilent Seahorse XF Analyzer**
- High Throughput
- Intact cells
- 4 injection ports

**Oroboros Oxygraph-2K**
- High resolution
- Intact and permeabilized cells/tissues
- Unlimited injections

www.predictivebiomarkers.org
www.molinalab.com
Skin Punch Biopsy

SD-NSC Cell Models Core @Salk
WELL VERSED IN BEST PRACTICES FOR HUMAN CELL MODELS

Derive → Bank → Characterize → Model

Punch biopsy

Human Somatic Cells

2-4 weeks

Pluripotency Marker Expression
Germ Layer Differentiation
Viral Silencing
Stable Karyotype

Validate Pluripotency

Isolate Individual Clones

10 Days - 4 Weeks

Colonies Emerging

2-3 Weeks

Expand and Freeze Clonal Lines

1-2 Months

Validated iPSC Line

Reprogramming Technology

Retro Virus
Lenti Virus
Sendai Virus
Episomal
microRNA/mRNA
Self-replicating RNA

Integrating Virus
Integrating Virus
Non-integrating RNA Virus
Non-integrating/Non-viral DNA
Non-integrating/Non-viral RNA
Non-integrating/Non-viral RNA

3-4 Weeks
3-4 Weeks
3-4 Weeks
3-4 Weeks
10-15 Days
3-4 Weeks

Targeted Differentiation
Disease Modeling
Developmental Biology
Tissue Engineering
Tiered Banking Ensures Prolonged Access to Cell Resources

Tier 1 - Process punch biopsy and establish primary cell bank
- Small blood collection to match STR
- Dermal punch is processed, expanded, and banked
- Primary fibroblast bank (6 vials)
- STR fingerprinting and myco testing

Tier 2 - Establish secondary fibroblast bank and reprogram to
- Expanded secondary fibroblast bank for distribution (20-30 vials)
- STR fingerprinting and myco testing
- 3 clonal iPSC lines per subject
- 1 clonal line is expanded for secondary bank and characterization
- Primary and secondary iPSC banks
- STR fingerprinting and myco testing
- iPSC characterization (pluri, germ layer, normal KT)
Fibroblasts and hiPSCs available in late 2021

*Early sharing through HCMA Core (SD-NSC Website)*

*Plan to deposit with national repository for broader distribution (NIA Repository at Coriell)*

Protocols for basic fibroblast/hiPSC maintenance available mid 2021

*Formal written and image-enhance protocols (SD-NSC Website)*
SAMPLE OF IMAGE-ENHANCED PROTOCOLS

Coming soon to the SD-NSC Website

SAN DIEGO NATHAN SHOCK CENTER

PASSAGING hPSCs USING VERSENE (EDTA)

Healthy hPSCs have 4 key characteristics

1. They grow in colonies
2. Colonies have smooth, defined boarders
3. Cells are small and compact
4. The nuclei are large, taking up most of the cell

SD-NSC Human Cell Models of Aging

Published: March 2021

Research reported in this publication was supported by the National Institute on Aging of the National Institutes of Health under Award Number P50AG066839.
iCell Models Maintain Age Related Phenotypes

Fibroblasts

Age-dependent AD genes

Age-independent AD genes

Normal aging genes

(Mertens et al. CSC 2015 and data bases)

Fib-Induced Neurons

iCell Models

IPSC

iPSC Derived Cells are Rejuvenated

Age Related Phenotypes Maintained
hiPSCs Recreate Niche Specific Dynamics Through Organoid Models

2D hiPSCs → 3D, Matrix, Morphogens → Time → 3D Organoids w/Multi-Cellular Structural Complexity
HYBRID MODELS COMBINE THE BEST OF BOTH WORLDS

Age Related Phenotypes

Fibroblasts

Age-dependent AD genes

Age-independent AD genes

IPSC

Normal aging genes

_Reprogramming_

Fib-Induced Neurons

Age Related Phenotypes

Maintained

iPSC Derived Cells are Rejuvenated

Niche Specific Multi-Cellular Dynamics

Hybrid Models of Human Aging

Aged Niche in a Dish
Induced cell models available in 2022
*Neurons (iN) and Vascular Endothelial Cells (iVECs)*

*Detailed Protocols (SD-NSC Website)*
*Updates made available (SD-NSC Website)*

Organoid and Hybrid Cell Models in 2023-2024

*In development – HCMA Core partnering with key SD-NSC researchers (Rusty Gage)*
*Keep fingers crossed and stay tuned!*
ESTIMATED TIMELINE FOR CELL RESOURCES AND MODELS

- **Aim 1**: SD-NSC Cohort
- **Aim 2**: Derive Fibroblasts and hiPSCs
- **Aim 3**: Induced Cell Models
- **Aim 4**: Org & Hybrid Models

Timeline:
- 2021
- 2022
- 2023
- 2024
SD-NSC COHORT POWERS IT ALL...

SD-NSC Clinical Cohort

Aim 1

Physical/functional phenotypes of biological age

Generate subject-specific fibroblasts

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Induced cell models

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Organoid and novel hybrid cell models

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Cellular/molecular phenotypes

Heterogeneity Core

Integrative Models Core

Genotype ↔ Phenotype & Phenotype ↔ Phenotype

Heterogeneity of Aging
Q&A

MODULE 3 – HUMAN CELL MODELS OF AGING CORE

Please type your questions in the chat
Overview of Facility, Equipment, and Resources

- 2500 sqft High Volume TC facility
- 13 hoods, 24 incubators
- Vapor-phase cryostorage
- Established 2007
- Newly renovated in 2012
Overview of Facility, Equipment, and Resources

The Mission: Lower the bar to access advance state of the art human cell based models

- Equipment (Live Imaging, Metabolism)
- Validated Media and Reagents
- Training and Project Support
Overview of Facility, Equipment, and Resources

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