Aminoglycoside-derived Liposomes for Synergistic Drug Delivery

Kaushal Rege
Associate Professor of Chemical Engineering
Arizona State University
Tempe, AZ 85287-6106, USA

Co-PI: Prof. Sandra Gendler, Mayo Clinic Scottsdale, AZ
### Cancer Diseases

#### Estimated New Cases

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>220,800</td>
<td>Breast</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>115,610</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>69,090</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>56,320</td>
<td>Uterine corpus</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>42,670</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>39,850</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>38,270</td>
<td>Melanoma of the skin</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>32,670</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Leukemia</td>
<td>30,900</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>25,510</td>
<td>Kidney &amp; renal pelvis</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>848,200</strong></td>
<td><strong>All Sites</strong></td>
</tr>
</tbody>
</table>

#### Estimated Deaths

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>86,380</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>Prostate</td>
<td>27,540</td>
<td>Breast</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,100</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>Pancreas</td>
<td>20,710</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>17,030</td>
<td>Ovary</td>
</tr>
<tr>
<td>Leukemia</td>
<td>14,210</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,600</td>
<td>Uterine corpus</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>11,510</td>
<td>Non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>11,480</td>
<td>Liver &amp; intrahepatic bile duct</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>9,070</td>
<td>Brain &amp; other nervous system</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>312,150</strong></td>
<td><strong>All Sites</strong></td>
</tr>
</tbody>
</table>

> 2 women die of breast cancer **every day** in AZ

---

**Cancer statistics, 2015.**

Triple Negative Breast Cancer (TNBC)

- “Triple-negative”: Lack of estrogen, progesterone, and HER2 receptors → no targeted therapies.
  
  Toh TB et al. *Mol Pharm.* 2014, 11(8), 2683-91

- Diagnosed in 15-30% of all breast cancer cases. Aggressive and high mortality

- Standard treatment: surgery with adjuvant chemotherapy and radiation therapy.
  

No targeted strategies for TNBC in the clinic

Urgent need for effective and targeted therapeutics for TNBC
Nanoparticle-mediated Drug Delivery

1. Can minimize cardiotoxicity (e.g. FDA-approved Doxil®)

2. Can Facilitate
   i. Longer circulation time in the body
   ii. Targeted delivery to tumors
   iii. Delivery of multiple drugs

Our Proposed Approach

Agent for targeting TNBC cells
e.g. folic acid and antifolates

Encapsulated single or synergistic drug combinations
e.g. Mitoxantrone (*) (FDA-approved)

100 – 200 nm diameter

Background Studies in the Rege Lab - I

TRAIL selectively kills cancer cells but

Cells are / can develop a resistance to TRAIL

We identified the combination of mitoxantrone + TRAIL as a novel synergistic treatment in multiple cancer cell lines(*)

Micelles (~15 nm; untargeted) were used to deliver mitoxantrone to cancer cells(#)

Poor stability

Difficult to load multiple drugs

Aminoglycosides are amine-containing sugars used as antibiotics (e.g. neomycin in Neosporin®)

*Background Studies in the Rege Lab - II*

R₁ = Aminoglycoside Monomer
R₂ = Glycerol diglycidyl linker
R₃ = Alkyl group (C₆, C₁₄, C₁₈)


**J. Control. Release** 2014; 176: 35–43.

**Int. J. Pharm.** 2015; 489: 18-29.

Aminoglycoside Lipopolymers ➔ Mitoxantrone-loaded Liposomes

- Aminoglycoside Lipopolymers + Co-lipids and Drug (Mitoxantrone) dissolved in chloroform/methanol and dried.
- Swollen overnight in water.
- Vortexed and sonicated for 1-2 minutes
Liposomes

- Five different types of mitoxantrone-loaded aminoglycoside liposomes
- Hydrodynamic Diameters: 110 – 160 nm
- Zeta Potential Values: +32-36 mV (positively charged)

<table>
<thead>
<tr>
<th>Liposome Formulation</th>
<th>HYDRODYNAMIC DIAMETER (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One month</td>
<td>Two months</td>
</tr>
<tr>
<td>1</td>
<td>132 ± 1</td>
<td>128 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>127 ± 1.5</td>
<td>132 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>138 ± 1.9</td>
<td>136 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>158 ± 2</td>
<td>140 ± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>143 ± 1.3</td>
<td>135 ± 1.7</td>
</tr>
</tbody>
</table>

Stable Drug-loaded Nanoparticles
I.
Mitoxantrone-loaded liposomes (Single-agent)
TNBC Cells: Mitoxantrone Liposomes

CTRL: liposomes without drug (empty liposomes)
Mitox: free (unencapsulated) mitoxantrone

Near-complete ablation of TNBC cells in culture
Mitoxantone-Liposomes are also Effective in Prostate and Bladder Cancer Cells

Prostate and Bladder Cancer Cells

Prostate Cancer Cells

Bladder Cancer Cells

* p ≤ 0.05, Compared Mitoxantrone containing liposomes Vs Free Mitox
II.

Efficacy of Mitoxantrone-loaded liposomes + TRAIL protein (delivered separately)
TNBC Cells: Mitoxantrone Liposomes + TRAIL

TRAIL + Mitoxantrone-Liposomes are effective for ablation of TNBC Cells

TRAIL alone (indicates cancer cell resistance)

** p ≤ 0.05, Compared combination of Mitoxantrone containing liposomes + TRAIL vs Free TRAIL
Prostate and Bladder Cancer Cells

Prostate Cancer Cells

UM-UC-3 48h

Bladder Cancer Cells

Similar results in other cancer cells
II.

Mitoxantrone-loaded liposomes

+ PARP inhibitors

(delivered separately)
Parp inhibitors

Mitoxantrone induces DNA double-strand breaks

PARP is an enzyme that repairs double-strand breaks

Inhibition of PARP + mitoxantrone ➞ enemy’s enemy is my friend
PARP Inhibitors (PARPi)

Targeted activity in BRCA-mutated cancers including TNBC

Olaparib
- FDA approved PARP inhibitor for the treatment of Ovarian cancer

Veliparib
- Anti-cancer drug for treating metastatic melanoma and breast cancer

DNA damaging drug: Mitoxantrone

Drug that prevents DNA repair: PARP inhibitors
**Similar results in bladder and prostate cancer cells**

**p ≤ 0.05, Compared combination of Mitoxantrone containing liposomes + Olaparib Vs Free Olaparib**
Near-complete ablation of TNBC cells in culture
Summary & Ongoing Research

- Synthesis and characterization of mitoxantrone-loaded, aminoglycoside-derived liposomes
  - Mechanistic studies of mitoxantrone-induced cancer cell death are underway

- Demonstration of efficacy of mitoxantrone-liposomes and their combinations with TRAIL and PARP inhibitors.
  - Additional dose studies underway

- Preliminary / Ongoing Studies:
  - Folate-conjugated polymers for targeting TNBC cells: synthesis, characterization, and targeted uptake
  - Generation of liposomes encapsulating PARP inhibitors
  - Establishment of the orthotopic TNBC tumor model in mice (Prof. Gendler) for evaluating effective treatments
Acknowledgments

Dr. Sudhakar Godeshala, Postdoctoral Research Fellow (ASU)

Dr. Taraka Sai Pavan Grandhi and Dr. Bhavani Miryala (ASU) for discussions

Prof. Deirdre Meldrum, Director, Center for Bisignatures Discovery Automation, Biodesign Institute, ASU for access to equipment

Arizona Biomedical Research Commission (ABRC)
Biomedical Investigator Grant (BIG) in collaboration with Prof. Sandra Gendler, Mayo Clinic, Scottsdale, AZ.
Identification and Functional Characterization of Novel Neuromuscular Disease-Causing Variants in Arizona Infants and Children

Lisa Baumbach-Reardon, Ph.D.

TGen

Arizona Biomedical Research Commission Award 2014-2017
Dr. Lisa Baumbach-Reardon

Education, Training and Experience

• Ph.D., Biochemistry and Molecular Biology, Univ. of Florida, Gainesville

• Postdoctoral Training, Baylor College Of Medicine Thomas Caskey—DNA Diagnostics and genotypic studies of DMD/BMD patients

• Fellowship in Human Genetics- Univ. of Colorado, Denver Resulted in ABMG Board dual certification in Clinical Molecular Genetics and Biochemical Genetics

Faculty member at University of Miami/Miller School of Medicine – 20 yrs. Primary Research interests- Neurogenetics, Human Genetics, Rare diseases—which led us to the X-Linked SMA (XL-SMA) Story

2011—Moved to Tgen – start the new Dorrance CLIA Lab and continue our exciting research in XL-SMA and other rare neurogenetic disorders.
ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization

The underlying premise of these studies is that investigating rare diseases will lead to greater understanding of common disease mechanisms
Why Study Neuromuscular Disease?

- There are numerous forms of neuromuscular disease that affect both adults and children
- They are often fatal or debilitating
- There is great phenotypic and genetic variability thus diagnosis is not easy

What is Neuromuscular Disease (NMD)?

- A neuromuscular disease is a disorder that affects the peripheral nervous system
- Peripheral nervous system includes muscles, the neuromuscular junction, peripheral nerves in the limbs, and the motor-nerve cells in the spinal cord
- Patients with neuromuscular diseases may present with:
  - weakness
  - loss of muscle bulk
  - muscle twitching, cramping
  - numbness, tingling, and other symptoms
Anterior Horn Cell Disease—2 types

Spinal Muscular Atrophy (SMA)

Features of Disease:
- SMA is an autosomal recessive disorder affecting ~1 in 10,000 live births
- Most common genetic cause of infant mortality
- Mutation in Survival Motor Neuron (SMN) gene results in decreased levels of SMN protein
- Carrier frequency is ~1 in 40 to 1 in 60
- Attacks motor neurons which control voluntary muscles
- Anterior horn cells (lower motor neurons) in base of brain & spinal cord gradually degenerate
- Results in muscle weakness and atrophy
- Respiratory failure from diaphragmatic muscle involvement late in the disease

X-linked Spinal Muscular Atrophy (XL-SMA)

Features of Disease:
- X-linked
  - No reported phenotype in female carriers
  - Usually infantile lethal in males
- Severe congenital hypotonia (muscle weakness)
- Contractures / Arthrogryposis (flexed joints)
- Bone fractures at birth (sometimes)

In 2008 we identified UBA1 mutations as the genetic cause of XL-SMA

Ramser et al 2008 Am J Hum Genet 82:188-93
ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization
Whole exome sequencing

- Sequence all ~25,000 genes at once
- Use supercomputer to identify changes in the sequence that cause SMA and related neuromuscular disease.
Summary of ABRC exome studies 2014-2016

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Families enrolled in exome studies</td>
<td>18</td>
</tr>
<tr>
<td>Families with exomes completed</td>
<td>12</td>
</tr>
<tr>
<td>Individuals in exome studies</td>
<td>70</td>
</tr>
<tr>
<td>Individual exomes completed</td>
<td>45</td>
</tr>
<tr>
<td>Affected individuals sequenced</td>
<td>26</td>
</tr>
<tr>
<td>Families with mutations identified</td>
<td>9*</td>
</tr>
</tbody>
</table>

*All were clinically confirmed by Sanger sequencing of proband

- Many of the families had only one affected individual
- All the cases had numerous diagnostic tests without receiving a diagnosis prior to exome sequencing.
Nemaline Myopathy
NEB Compound Heterozygous Recessive

Family 74

Phenotype
* Congenital severe global hypotonia
* Trach/vent dependent
* G-tube dependent
* Arthrogryposis
* Bicuspid aortic valve
* Cryptorchidism
* Underdeveloped lung
* Eventrated diaphragm
* Missing 2 ribs
* Deafness
* Mother has history of miscarriages
* Currently ~11 mo. of age
* Muscle biopsy nemaline bodies and myofibrillary disorganization

Pathogenic Variants
* Novel splice variant at exon 59 and previously reported pathogenic inversion splice variant at exon 37

Nebulin (NEB)
* Essential structural component of muscle that stabilizes actin filaments
* Loss of function mutations in NEB cause Nemaline myopathy

Splice Ex-37
Splice Ex-59
Father unaffected
Mother unaffected

Splice Ex-37
Splice Ex-59
Affected Male

NEB
NEB – Nebulin

- Critical muscle structural protein; very large protein
- Many missense mutations occur and are benign
- **Frameshift and splice variants are often pathogenic**
- Cause Nemaline Myopathy
  - Variable phenotypes ranging from early lethality to mild myopathy
  - *Nemaline bodies* are abnormal accumulations of muscle thin-filament proteins caused by mutations in Nebulin (*NEB*) and other genes encoding filament proteins and are characteristic of nemaline myopathy
  - Titin-nebulin filament system stabilizes alignment of thick and thin filaments in skeletal muscle

To Date we have identified 3 unrelated Arizona families with NEB mutations.
Infantile Neuroaxonal Dystrophy

**PLA2G6 Compound Heterozygous Recessive**

**Family 79**

**Phenotype**
- Global neuromuscular delay
- Crawled but regressed
- Progressive cerebellar atrophy
- Generalized hypotonia
- Mild spine deformation
- Bilateral coxa valga
- Weight loss
- Normal CPK
- Mild microcephaly
- Onset ~9mo. of age
- Currently ~3yrs of age

**Pathogenic Variants**

**PLA2G6**
- Calcium-independent phospholipase (A2 group VIA)
- Essential for membrane phospholipid remodeling in axons and synapses
- Mutations in **PLA2G6** cause Infantile neuroaxonal dystrophy, a very rare disorder
ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization
UBA1 Mutations

(Ramser et al 2008 Am J Hum Genet 82:188-93)
Why is UBA1 Important?
Ubiquitin Proteasome System

- UBA1 is the initiating pinnacle enzyme in the Ubiquitin Proteasome System (UPS)

- UBA1 is expressed in every cell with highest expression in the spinal cord

- The UPS is responsible for the degradation of most proteins

- Uses Ubiquitin as a death tag, targeting other proteins for destruction via the proteasome

- Complete loss of UBA1 function is lethal
**UBA1 Conditional Targeted Mouse Design**

The endogenous mouse exon 15 contains the **UBA1 S547G** mutation but it *will not be* expressed until bred with CRE-expressing mouse

1. This partial minigene will express *wt* **UBA1** until removed by breeding with a CRE-expressing mouse

2. After breeding with CRE-expressing mouse the *wt* partial minigene will be removed and the mouse will express **UBA1** with the S547G mutation that causes XL-SMA

We have Southern blot confirmation of heterozygous female mice with the conditional targeted knock-in allele (*see poster*).

Initial development of the UBA1 mouse model was made possible by a *Flinn Foundation Grant* and the *ARBC*.
Investigate Mechanisms of Disease in UBA1 Mouse Model

**UBA1\(^{S547G}\) mouse model expansion and survival**

We will first test whether the \(UBA1^{S547G/y}\) hemizygous (male) mouse mutants have similar perinatal lethality as observed in humans.

**Neuromuscular development in UBA1\(^{S547G}\) mutant mice**

We will evaluate whether \(UBA1^{S547G/y}\) mutant mice exhibit aberrant development or degeneration of the neuromuscular system.

Spinal motor neuron number, axonal outgrowth, and NMJ formation will be evaluated at distinct stages of development using immunolabeling, motor neuron specific reporter mice, and microscopy.

*Image courtesy of J. Newbern ASU collaborator*
ABRC Project Overview

Aim 1. Identification of the genetic causes of undiagnosed neuromuscular disease in Arizona infants and children

Aim 2. We have developed a mouse model to further understand XL-SMA

Aim 3. Exciting finding of a novel neuromuscular disease-causing gene and functional characterization
**SCML2 Mutation Families**

### Family 3

**Phenotype (Neonatal Males)**
- Clear X-linkage
- Neonatal lethality
- Dysmorphic facies
- Arthrogryposis
- Microcephaly
- Hypospadias
- Distal limb deformities
- Subarachnoid hemorrhages

**Potential Pathogenic Variant**
- Novel N76S missense in SCML2

### Family 9

**Phenotype (Neonatal Males)**
- Clear X-linkage
- Neonatal lethality
- Arthrogryposis
- Cerebellar hypoplasia
- Cardiac anomalies

**Pathogenic Variant**
- Novel M1V start loss in SCML2 in obligate carrier and affected son

---

**SCML2**
- Never reported as NMD gene
- Key component of polycomb repressor complex (2)
- Maintains repression of developmental genes
We have developed a collaboration with investigators in Italy, Switzerland, and Germany to study *SCML2* mutations.

- A knock out mouse model with an 11bp frameshift mutation in *SCML2* exon 4 resulting in premature stop codon in the MBT1 domain was generated (confirmed by Sanger Sequencing).
- *SCML2* KO males have fertility issues from hypogonadism and spermatogenesis defects.
- Crossing wild type males with *SCML2* +/- females produced *SCML2* KO males for study.

- Mice exhibited:
  - Impaired inhibitory synapse in spinal motor neuron
  - Impairment of inhibitory currents & synapses
  - Spontaneous cortical hyperexcitability
  - Susceptibility to seizures

Variants in cell line study

Knock out of Scml2 was confirmed by immunostaining

**Portions of these studies are in revision /review at Nature Neuroscience**
ABRC Project Overview

Aim 1. Identification of the genetic causes of neuromuscular disease in Arizona infants and children by whole exome sequencing

Aim 2. Characterization of neuromuscular development in an UBA1 mouse model of XL-SMA

Aim 3. Functional characterization of a novel neuromuscular disease-causing genetic variant

The study of rare diseases provides greater insights into mechanisms which may be relevant to more common related diseases
Acknowledgements

This research was generously supported by the MDA (MDA186435), The Flinn Foundation, and TGen. We would like to thank all members of XL-SMA research team, especially Therese De La Torre (project manager), Jose Ramirez and Stephanie Althoff (Clinical Coordinators) for their dedicated help with this research.

Special thanks to Jesse Hunter, Chris Balak, and Mary Ellen Ahearn (Dr. Saunder Bernes, Phoenix Children’s Hospital—not pictured.)
ACKNOWLEDGEMENTS
An Integrative Personalized Professional Practice using Mobile Technologies for Weight Management

David Jackemeyer, Yulia Abidov, Karen Herbst, NJ Tao, Craig Stump, *Erica Forzani*

eforzani@asu.edu
BRIDGING
THE
GAP

Research World
Data Analysis

Real World
Medical and tracking devices

VOCs

AZRIZONA STATE
UNIVERSITY
Data Analysis

Diabetes Research Program

BRIDGING THE GAP

Research World

Data Analysis

Real World

Medical and tracking devices

VOCs
"No matter how heavy you are, you will significantly lower your blood sugar if you lose some weight"

Cathy Nonas, MS, RD
Spokeswoman for the American Dietetic Association
Professor at Mount Sinai School of Medicine, NY
The Motivation

- 2.1 billion people, or ~30% world’s population, are overweight or obese in 2013
- Obesity is known to cause many chronic diseases, including heart diseases, stroke, diabetes, metabolism syndrome, and some cancers (CDC).
- People spend ~$600 billion per year, yet most are frustrated with the results
The Problem

- Most people know weight management requires balanced diet and exercises, but few know:

  - How much should I eat?
  - How much should I exercise?
  - Why is my weight like yo-yo?
Energy Conservation Law

Caloric Intake = Weight + Total energy expenditure (TEE)

Resting (REE or RMR) ~ 80-90%

Physical Activity < 20 % (sedentary)

Antoine Lavoisier 1743-1794
Total energy expenditure (TEE)

Weight - Caloric Intake = Resting (REE or RMR) + Physical Activity

Resting (REE or RMR) ~ 80-90%
Physical Activity < 20% (sedentary)
Importance of REE or RMR in weight management

Resting Metabolic Rate (RMR) = “Metabolism” = Resting Energy Expenditure (REE)

Total energy expenditure (TEE) = Weight Caloric Intake - Resting (REE or RMR) + Physical Activity

-休息（REE或RMR）
-~ 80-90%
-< 20%（久坐）

最近市场上的工具

经济实惠和易于移动的技术
Energy Conservation Law

**Total energy expenditure (TEE)**

\[ \text{Weight} \quad \text{Caloric Intake} = \quad \text{Resting (REE or RMR)} \quad + \quad \text{Physical Activity} \]

- Resting (REE or RMR) ~ 80-90%
- Physical Activity < 20% (sedentary)

Affordable & Mobile Technologies

Recent tool in the market
Six-month study design

- The participants from the control group had an iPad with My Fitness Pal App to track calorie intake, an activity tracker to track steps and floors, and a weight scale.

- Each participant in the control group was recommended a 500-calorie deficit intake based on the Harris Benedict Equation.

- The intervention group had the same gadgets as the control group, as well as a Breezing Tracker.

- Both groups were followed up with a Standard-of-Care procedure for 6 months, and were reached by e-mail every 2-3 weeks with general health information.

* Most of participants had T2 Diabetes, or were at risk of Diabetes
Clinical study in an overweight and obese population*

Dr. Craig Stump, MD

Characteristics of the population

Table 1. Physical characteristics of recruited study participants. Means +/- (SD)

<table>
<thead>
<tr>
<th>Physical Parameters</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI (kg/m²)</th>
<th>W/H</th>
<th>Fat%</th>
<th>Sys BP</th>
<th>Dias BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG (n=20) F:14, M:6</td>
<td>54 (7)</td>
<td>102 (20)</td>
<td>1.68 (0.08)</td>
<td>36 (6)</td>
<td>0.88 (0.10)</td>
<td>44 (8)</td>
<td>127 (14)</td>
<td>81 (7)</td>
</tr>
<tr>
<td>IG (n=20) F:17, M:3</td>
<td>57 (13)</td>
<td>92 (14)</td>
<td>1.64 (0.10)</td>
<td>34 (6)</td>
<td>0.85 (0.06)</td>
<td>44 (6)</td>
<td>132 (20)</td>
<td>85 (14)</td>
</tr>
<tr>
<td>Normal range</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>18.5–24.9</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>


Table 2. Metabolic and blood parameters of recruited study participants. Means +/- (SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>REE (kCal/d)</th>
<th>Gluc. (mg/dL)</th>
<th>Glyc. Hb (%)</th>
<th>Trigly. (mg/dL)</th>
<th>Chol. (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL/HDL</th>
<th>DHRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG (n=20) F:14, M:6</td>
<td>1420 (300)</td>
<td>109 (33)</td>
<td>6.6 (1.1)</td>
<td>148 (65)</td>
<td>208 (33)</td>
<td>130 (37)</td>
<td>52 (11)</td>
<td>2.8 (1.3)</td>
<td>6/11=54%</td>
</tr>
<tr>
<td>IG (n=20) F:17, M:3</td>
<td>1570 (280)</td>
<td>111 (27)</td>
<td>6.7 (1.5)</td>
<td>120 (42)</td>
<td>200 (36)</td>
<td>130 (37)</td>
<td>51 (9)</td>
<td>2.7 (1.0)</td>
<td>7/12=58%</td>
</tr>
<tr>
<td>Normal range</td>
<td>N/A</td>
<td>70-105</td>
<td>&lt;6.0</td>
<td>0-169</td>
<td>0-200</td>
<td>0-99</td>
<td>&gt;38</td>
<td>1.3-4.7</td>
<td></td>
</tr>
</tbody>
</table>

REE: resting energy expenditure, Gluc.: glucose, Glyc Hb: glycosylated Hemoglobin, Trigly.: triglyceride, Chol: cholesterol., DHRI: Diabetes High Risk Index, percentage a new cases discovered with Glyc Hb levels higher than 6.0%.

* Most of participants had T2 Diabetes, or were at risk of Diabetes
Difference of Calculated REE* – True (measured) REE

Differential Resting Energy Expenditure (kCal/Day)

Study Participant Number

* Predictive Equation (Harris-Benedict)

42% of the cases in the pilot study group (overweight and T2 diabetes) had slower metabolic rates that the predicted from equation.

Dr. Craig Stump, MD
Why track metabolism?

Different people have different metabolisms

Weight: 112 lbs
Height: 5’ 6”
Age: 29 years

Your metabolism = Your energy = Your Fire

1700 kCal/day
1200 kCal/day
Why we can’t use equations to calculate REE?

Data from seminal Harris-Benedict’s work

- An actual REE value (from indirect calorimetry measurement) can differ from an estimated REE value (from the Harris-Benedict calculation).
- The results show that for people of same gender and weight (e.g. men and 63 kg) the difference in actual REE values can be as high as 520 kCal/day.
- If, for instance, subject A’s goal is to maintain weight, and the estimated REE (1640 kcal/day) is higher than the body’s actual REE (1480 kcal/day), a calorie recommendation based on the REE estimate will lead to weight gain.

Therefore, accurately measuring REE is crucial in establishing an effective weight management plan.

Observation: Weight change is accounted from 1\textsuperscript{st} day the participant use MFP (baseline period) up to 6 months after the study.

\textbf{Intervention Group:} 17 of 19 participants (89\%) lost weight, 1 stayed steady and 1 (5\%) gained 1.9 lbs.

\textbf{Control Group:} 11 of 20 participants (55\%) lost weight, 1 stayed steady and 8 (40\%) gained 2+ lbs.

\textbf{Other results:}
Weight loss Greater Than 6 lbs:
\textbf{CG:} 40\% (8/20) vs \textbf{IG:} 68\% (13/19)
Case #2: Weight & Body Mass Index (BMI) changes

The Intervention group’s total weight loss 3x’s Greater than control group

The difference in BMI changes in intervention group was statistically significantly different with respect with control group

Intervention group’s drop of BMI from 35.5 resulted in change from Obese Class II Group to Obese Class I Group

Control group’s drop of BMI from 36.9 was not large enough to move out of Obese Class II Group

Unpaired t test results

P value and statistical significance:
The two-tailed P value equals 0.0296
By conventional criteria, this difference is considered to be statistically significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group One</th>
<th>Group Two</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>-0.5023848000</td>
<td>-1.9214530000</td>
</tr>
<tr>
<td>SD</td>
<td>1.9203068900</td>
<td>1.9965590000</td>
</tr>
<tr>
<td>SEM</td>
<td>0.4293936744</td>
<td>0.4580420482</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>
Case #2: Calorie Intake Completed Days*

* Completed days represent calorie intake values with equal or 25%+ of recommended calorie intake
Case #2: Calorie Intake Complete Days*

- The Intervention group had 70% more entries of completed daily calorie intake than the control group.

* Completed days represent calorie intake values with equal or 25%+ of recommended calorie intake.
Case #2: Calorie Intake Entries

Participants

Control Group

Intervention Group

Number of Entries

My Fitness Pal (MFP)'s Volume Entries (including diet, activity, weight, comments)

Breezing Entries

63 = MFP’s entry average

79 = MFP’s entry average

Intervention Group: 25% more entries vs. control group
Case #2: Benefits of weight loss in blood parameters

HDL change

Intervention group had a better outcome for HDL cholesterol (increased HDL cholesterol with a significant difference of $p = 0.037$ with respect to the control group)

Diastolic Blood Pressure

Intervention group had a better outcome for reduction of diastolic blood pressure: a decrease with a significant difference of $p = 0.07$ with respect to the control group
Summary of facts from the study

1. Breezing users had:
   i) Effectively lost more weight (89% vs 55% controls)
   ii) Completed 70% more calorie intake inputs to Calorie Counter App
   iii) More comprehensive use of calorie counter app via entry volumes of diet, activity, weight, and comments.
   iv) Better HDL cholesterol and Diastolic Blood Pressure parameter outcomes

2. How does knowing Correct Calories Burned relate to Weight Loss?
   89% efficiency of weight loss (IG) vs. 55% efficiency of weight loss (CG)
   5% of weight gain (IG) vs. 40% of weight gain (CG)
General weight loss effect in T2 diabetes

HbA1c reduction

The weight reduction resulted in a reduction of glycated hemoglobin in both groups ($p < 0.1$).

Since both groups had a relatively high rate of weight loss (89%-IG and 55%-CG), there was not significant difference between groups in regard to improvements of glycated hemoglobin (both groups did improved the T2 diabetes parameter).

CONCLUSION: weight loss is a great intervention for decreasing T2 diabetes and risk of Diabetes.

Controls

Between groups: no difference

Intervention

Between groups: no difference
Data Analysis

Research World

Real World

Medical and tracking devices

Type 2 Diabetes

Weight Loss

=  [ ] - [ ] + [ ]
Demo of Metabolism Tracking
• Miscellaneous slides
Six-month study design

- The participants from the control group had an iPad with My Fitness Pal App to track calorie intake, an activity tracker to track steps and floors, and a weight scale.

- Each participant in the control group was recommended a 500-calorie deficit intake based on the Harris Benedict Equation.

- The intervention group had the same gadgets as the control group, as well as a Breezing Tracker.

- Both groups were followed up with a Standard-of-Care procedure for 6 months, and were reached by e-mail every 2-3 weeks with general health information.

- Most of participants had T2 Diabetes, or were at risk of Diabetes
A First Law of Thermodynamics: Energy Conservation Law

Weight = Caloric Intake - Total energy expenditure (TEE)

Resting Metabolic Rate (RMR)

80-90 % (sedentary)

Physical Activity

< 20 % (sedentary)

Affordable & Mobile Technologies

Breezing

Metabolic Rate Tracker

Affordable & Mobile Technologies
What about the variability of REE?

\[ \text{Weight} = \text{Food} - \left[ \text{Resting} + \text{Activity} \right] \]
Energy Balance – How we can modify it?

Total Energy Expenditure (TEE) = Calories burned

Calorie Intake

Food

Physical Activity

REE

REE pr Metabolism (80-90 % in sedentary persons)
Metabolism (RMR) and Physical Activity*

*Speakman et al., Proceeding of the nutrition society, 2003, 62, 621-634 (Fig. 2 reproduction)
Resting Energy Expenditure (REE)  
Resting Metabolic Rate (RMR)

Food + Oxygen → Carbon Dioxide + ATP (heat), storage

• VO₂ (consumed oxygen rate)
• VCO₂ (produced carbon dioxide rate)

Resting Metabolism:
✓ Sustains life
✓ Majority of our daily total energy expenditure

How does it work?
• Indirect calorimetry (Breezing measures consumed oxygen rate and produced carbon dioxide rate)
• Recommended by AND, WHO, ACSM, ADA

33
Indirect Calorimetry Principle

Weir Equation:

\[
\text{REE (kCal/day)} = [3.9 \times \text{VO}_2 + 1.1 \times \text{VCO}_2] \times 1.44
\]

- **VO\textsubscript{2}**: consumed oxygen rate (mL/min)
- **VCO\textsubscript{2}**: produced carbon dioxide rate (mL/min)


Why track metabolism?

Different factors can affect metabolism

Genetics

Hormones

Exercise

Drugs, substances

Diet

Pregnancy

Direct Calorimetry vs. Indirect Calorimetry

Direct Heat Measurement

Indirect Heat Measurement

CO₂

O₂
1900 - Atwater & Rosa’ research: Energy expenditure of 3 men who lived in the calorimeter for 40 days

2717 kCal/day

Error: +/- 0.2 %

* Other researchers’ experiments: Error = +/- 1%

Science demonstrated that direct calorimetry is equivalent to indirect calorimetry
History of Measuring Energy Expenditure

1770’s
Lavoisier & Laplace
Law of the conservation of energy → First Human Calorimetry

1890’s
Atwater & Rosa
Wesleyan Univ.: First important work of Direct Calorimetry

1900’s
Other Direct Calorimetry
Efforts: Airflow, water flow, water storage, and gradient layer calorimeter

1940
Indirect Calorimetry: Open circuit spirometry

1980
Indirect calorimetry: Computer-based Instrumentation (Breath-by-breath)

2000
Portable Breath-by-Breath Instrumentation

2010
First Mobile Metabolism Tracker

2014
$350

Claude Douglas:
Indirect Calorimetry
Gold Standard Method

Indirect calorimetry: Computer-based Instrumentation (Breath-by-breath)

First Mobile Metabolism Tracker

$(10-35)K

CO2

O2
Total Energy Expenditure*

Physical Activity

<30 %

70-90 %

Resting Metabolic rate or Resting Energy Expenditure

*McArdle, Katch & Katch, Ex. Physiology, 2009
Most of daily total energy expenditure (TEE) is spent to maintain basic body functions (energy expenditure at resting state, REE). Work from Arizona State University, 2013.
The risk of using calorie intake recommendations from an equation-based REE value

Weekly Wellness: The sad state of the American metabolism

Our weekly fitness column, "Weekly Wellness," is back again. This week, Matt Gallagher, from MFC Sports Performance in Darien, discusses why Americans weight issues have a lot to do with our poor metabolism.
Tracking Metabolism for Better Health

**Diet**
Changes in diet can significantly change metabolism. For example, a crash diet can cause drastic reduction in metabolic rate, leading to a “weight loss plateau”. See slides in next class.

**Exercise**
Exercise can affect metabolism. For example, muscle-building increases metabolism and High Intensity Intermittent Training (HIIT) creates an “afterburn” effect. See slides in next class.

**Hormones/medication**
Hormonal changes and medications can change metabolism. Monitoring metabolism helps screen for potential hyper- or hypo-thyroidism. See slides in next class.

**Pregnancy**
Metabolism changes significantly throughout pregnancy and after giving birth. Tracking metabolism helps the mother maintain and achieve the proper weight for the baby’s healthy growth. See slides in next class.
Personalized Indirect Calorimeter for Energy Expenditure (EE) Measurement

Abstract

**Background and aims:** A personal indirect calorimeter allows everyone to assess resting and non-resting energy expenditure, thus enabling accurate determination of a person’s total calorie need for weight management and fitness. The aim of this study is to compare the performance of a new personal metabolic rate tracker based on indirect calorimetry, Breezing®, with the Douglas bag method, the gold standard method for energy expenditure (EE) measurement.

**Methods:** Energy expenditures (EE) at rest and during activities, and respiratory quotient (RQ) were measured for 12 healthy subjects, including 7 males and 5 females under different living conditions. A total of 314 measurements were performed with Breezing®, and the results were compared with those by the Douglas bag method.

**Results:** R-squared correlation coefficients (R²) between the data obtained with Breezing® and the Douglas bag method were 0.9976, 0.9986, 0.9981, and 0.9980, for VO₂, VCO₂, EE, and RQ respectively.

**Conclusions:** The EE and RQ values determined by Breezing® are in good agreement with those by the Douglas bag method.
The Tracker for Energy Expenditure (EE) demonstrated ~100% accuracy

Energy management: Cardio-Pulmonary System

- **VO₂**
  consumed oxygen rate

- **VCO₂**
  produced carbon dioxide rate

![Diagram of energy management in the cardio-pulmonary system]

- **CaO₂ - CvO₂ = 5 mL O₂ per 100 mL CO₂**
Case #2: Weight & Body Mass changes

Observation: Weight change is accounted from 1\textsuperscript{st} day the participant use MFP (baseline period) up to 6 months after the study

\textbf{Intervention Group:} 17 of 19 participants (89\%) lost weight, 1 stayed steady and 1 (5\%) gained 1.9 lbs.

\textbf{Control Group:} 11 of 20 participants (55\%) lost weight, 1 stayed steady and 8 (40\%) gained 2+ lbs.

\textbf{Other results:}
\textit{Weight loss Greater Than 6 lbs:}
\textit{CG: 40\% (8/20) vs IG: 68\% (13/19)}
Ventilator Associated Pneumonia

“Genomics and Personalized Medicine”
HonorHealth Research Institute

Charles Hu, MD
Emmanuel Menashi, PhD
Ventilator Associated Pneumonia (VAP)

- Healthcare Associate Infection (HAI)
- High anti-biotic use
- Morbidity/Hospitalization/Cost
- Mortality

Clinical Diagnosis:

- CPIS (Clinical Pulmonary Infection Score) System
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>1 point</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>38.5 to 38.9</td>
</tr>
<tr>
<td>White blood cells/mm³</td>
<td>&lt;4,000 or &gt;11,000</td>
</tr>
<tr>
<td>Secretions</td>
<td>Nonpurulent</td>
</tr>
<tr>
<td>Endo-Tracheal Aspirates (ETA)</td>
<td></td>
</tr>
<tr>
<td>PaO₂/FiO₂</td>
<td>≤240 and no ARDS</td>
</tr>
<tr>
<td>Chest X-ray infiltrates</td>
<td>Diffuse or patchy</td>
</tr>
</tbody>
</table>
Study Goals

a. Early Detection
b. Diagnosis: Accurate Classification of Pathogen(s)

Host/Pathogen interaction

Biospecimens:
1. ETA (Endotracheal Aspirate)
2. BAL (Bronchoalveolar Lavage)
3. Blood

Collection Time:
0 - 24hrs, day 3, 5, 7, 9, 11, 13

Study design

Clinical Pulmonary Infection Score
1. Temp (°C)
2. White blood cells/mm
3. Secretions (ETA)
4. PaO₂/FiO₂
5. Chest X-ray infiltrates

BAL (Bronchoalveolar Lavage) Trap
Methodologies:

1. Early Detection: Host’s Immune Mediators
   - **Flow-Cytometry:** Immune Mediators
   - **HPLC/Mass-Spectroscopy**
     Shotgun Proteomic Approach, LC-MS/MS Peptide Analysis
     LTQ-Orbitrap Velos Mass Spectrometer

2. Diagnostic: Pathogenic Classification
   Next Generation Sequencing: 16s rRNA (V1-V2)
Biospecimens processing:

- **ETA, BAL**
- **MS**
  - shotgun proteomic approach, LC-MS/MS peptide analysis: LTQ-Orbitrap Velos mass spectrometer; Tgen-Proteomic
- **FC**
  - Flow-Cytometry; CBA-based analysis UofA Phoenix campus
- **NGS**
  - 16 srRNA NGS; (V1-V2) Ion Torren: UofA; Tucson campus
Preliminary Data:

- Subject-1: ETA-1, ETA-3, ETA-5, ETA-7 and ETA-9: *No pneumonia*
- Subject-2: ETA-1, ETA-3 and BAL *Pneumonia (Aggressive)*
- Subject-3: ETA-1, ETA-3, ETA-5 and BAL *Pneumonia (Slow)*
Early Detection:

a- Complement System: (Anapylatoxin)

C3a, C4a and C5a

Biospecimens:
1. Tracheal Aspirate
2. Bronchoalveolar Lavage BAL
Anaphylatoxin C5a concentrations in ETA and BAL biospecimens

![Graph showing concentrations of C5a in different subjects and dilutions.](image)

- **Subject**: S1, S2, S3
- **Dilution factor**: 1/100
Early Detection:

b- Pro- and Anti- Inflammatory Immune Responses: (Th1/Th2/Th17)

- Screen seven Cytokines;
  1. TNF-α
  2. IL-6,

Biospecimens:
- Endotracheal Aspirate
- Bronchoalveolar Lavage (BAL)
TNF-α concentrations in ETA and BAL biospecimens

<table>
<thead>
<tr>
<th>Subjects</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concentration (pg/ul)
IL-6 concentrations in ETA and BAL

IL-6: 1/10 dilution

Concentration (pg/ul)

Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution factor</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
</tbody>
</table>

TNF-α

Standards

T1, T3, T5, T7, T9, T1, T3, T5

BAL-S2, BAL-S3
ETAs of subjects 2 and 3 separated by gel electrophoresis
Excised gel bands digested using a tryptic in-gel digestion protocol\(^6\)
Digested peptides analyzed by LC-MS (Waters nanoAcquity UPLC, Thermo LTQ Orbitrap Velos mass spectrometer)
Database identification using MatrixScience Mascot (Uniprot/Swissprot, 2015)
Functional gene enrichment analysis (GEA) performed using ToppFun (toppgene.cchmc.org)
Genes Associated with Immune Responses

- Longitudinal progression from innate immunity to activation of an adaptive immune response.
- Inversed trends suggests shift in homeostatic balance, activation of humoral response, potentially triggered by *Ureaplasma urealyticum* (NGS).

Subject 2

![Bar chart showing Innate and Humoral gene expression over days 1 and 3.]

Subject 3

![Bar chart showing Innate and Humoral gene expression over days 1, 3, and 5.]

- Gradual increase in humoral response, plateau on day 5
- Innate response increases up to day 3, decrease beyond might be related to antibiotic response.
- Overlapping host responses to infection may be caused by multiple pathogens; *Serratia marcescens* at day 3, and *Peptostreptococcus stomatis* at day 5 (NGS).
- Increase in expression of complement components C3, C4 and C5 in both subjects highlight complex relationship between innate and humoral immune response.
- The complement C4 gene generates to classes of polymorphic protein products (protein cleavage of C4 to C4A (~9kDa) and C4B (~190kDa):
  - C4A high binding affinity to –NH2 groups (peptide antigens) and complement receptor CR1, long half-life, role in immuno-clearance and possibly a link between innate and adaptive responses.
  - C4B faster reaction rate toward carbohydrates and –OH, short half-life and propagates complement activation pathways.
  - Absence of C4B during Day 1, but presence of the cleaved C4A may suggest binding to bacterial carbohydrate groups.
Diagnostic: Pathogenic Classification

- 16s Ribosomal RNA: Next Generation Sequencing (NGS)
Subject-1:


T3: Haemophilis spp., **Serratia marcescens**

T5: Morococcus cerebrosus, Neisseria lactamica, Fusobacterium nucleatum

T7: Streptococcus anginosus, Prevotella spp., Gemella morbillorum, Fusobacterium necrophorum, Morococcus cerebrosus, **Serratia marcescens (minor)**

T9: **Serratia marcescens! (nearly all reads are this species)**, Ureaplasma urealyticum

**Prediction:**
Patient developed Serratia Marcescens pneumonia Starting on/or around day 11-12,
Subject-2:

T1: Prevotella spp., Gemella Haemolysans, Strep spp.

T3: *Ureaplasma urealyticum!* (nearly all reads are this species), Mycoplasma hominis


**Prediction:**

Patient developed Infection/pneumonia from *Ureaplasma urealyticum* on Day 3, *expected typically in premature newborns.*

**Clinical Findings: Based on Culture**

Negative; no organism present
Subject-3:

**T1:** Strep sp.

**T3:** Prevotella spp., Neisseria spp., Veillonella parvula, Morococcus cerebrosus, *Serratia marcescens* (minor)

**T5:** Prevotella spp., Strep spp., Veillonella spp., Haemophilus parainfluenzae, *Peptostreptococcus stomatis* (dominant)

**BAL:** *Peptostreptococcus stomatis* (dominant), prevotella spp.

**Prediction:**
patient developed *Peptostreptococcus stomatis* Infection/pneumonia on or about day 5.

**Clinical Findings:**
Negative; no organism present
Summary:

• Defined Immune Mediators, Specific Biological Markers, with Capacity of Early Detection of Infection; 48-72 Hours Prior to Onset of Clinical Symptoms of Pneumonia

1. TNF-α secretions Spiked 48-72 hours prior to development of clinical symptoms of pneumonia
2. Increased in IL-6 Secretions coincided with the development of clinical symptoms of pneumonia and 48-72 hours post spikes in TNF-α secretion
3. The ratio of TNF-α to IL-6 secretion may Provide means for Staging disease progression; early stages of infection to pneumonia development
4. Genomic sequencing, 16s rRNA, provided accurate and complete classification of the Invading Pathogen(s)

• ETA may Present a non-invasive and easy to access Biospecimen Replacing BAL for the Detection and Diagnosis of Pneumonia infections in Intubated Trauma Patients.
Acknowledgement

Flinn Foundation

HonorHealth
Charles Hu, MD.
Emmanuel B. Menashi, MS., PhD
Frederick Zenhausern, MDA., PhD
Denise Filley
Karen Lewandowski
Lori Wood
Jill Lemna

Translational Genomics Research Institute: Center for Proteomic:
Patrick Pirrotte, PhD
Khyati Pathak, PhD
Marrisa Saltzman
Krystine Garcia

University of Arizona:

• Tucson Campus:
Genomic Center: George Watts, PhD

• Phoenix Campus
Flow Cytometry Core: Mrinalini Kala, PhD